

# *BAILEY'S INDUSTRIAL OIL AND FAT PRODUCTS*

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*Sixth Edition*

*Edited by*

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*Memorial University of Newfoundland*

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# *Preface*

Oils and fats are important components of foods, and they, or their derivatives and products thereof, play an important role in non-food applications. In food, oils and fats provide a concentrated source of energy as well as a carrier of fat-soluble components. They also serve as a heat transfer medium for food processing and render desirable texture and flavor as well as mouthfeel to products. Oils and fats originate from plant and animal sources. Although plant sources include oilseeds, tropical fruits, and alga, the latter may originate from land-based animals, fish, marine mammals, and derived sources. The main components of food lipids are triacylglycerols, but minor components are also important for quality characteristics, stability, and application areas. Both the type of fatty acids and their degree of unsaturation as well as the type and content of minor components affect the keeping quality of the oil, and certain minor components such as phytosterols might also be used for fingerprinting and authentication of the source materials.

The physical state of fats and oils and their crystal structures are important for application of such products. In addition, formulation of products for special applications such as bakery, confectionary, frying, salad dressing, margarines, and spreads requires special characteristics that make the products suitable for such purposes. Thus, each source material will be important for its physical and chemical characteristics and hence suitability as a food component.

Recent developments in the area of oils and fats has led to the production of specialty lipids from novel sources such as fruit seeds, nuts, and other minor plant sources. In addition, preparation of structured lipids for a myriad of applications has been of interest. Minor components of oils and fats may be isolated during processing and used as nutraceutical and functional food ingredients. Examples are lecithin, phytosterols, tocopherols, and tocotrienols, among others. Obviously, the health-promoting potential of such products is also of interest.

The processing technologies employed for production of fats and oils, and associated components, to make them shelf-stable with acceptable sensory characteristics and flavor as well as secondary processing technologies for production of specific products are important considerations in this area. Food commodities

may be produced, and some components may also be used in animal feed and other applications. There are many areas where oils and fats are used for non-food purposes. Thus, detergents, soaps, glycerine and polymers, inks, lubricants, and biodiesel may be derived from fatty acids and their derivatives. Many applications would provide alternatives to the use of synthetic material or environmentally friendly substitutes in non-food applications.

The sixth edition of Bailey provides a comprehensive description of topics relevant to the oils and fats industry in six volumes as compared with five volumes in the fifth edition. The additional volume (volume 3) is mainly on specialty oils and fats and their byproducts or minor components as well as on those of low-calorie fat substitutes and structured lipids. An article on fish oils and one on marine mammal oils are also included in this volume. However, the material covered in other volumes is often substantially different from the available in the fifth edition as new articles are introduced, and when the title appears the same, substantial updating of the references and introduction of new material has occurred; new authors in some cases have made these contributions. Thus, the first volume includes three new articles on crystallization and physical properties of oils and fats. There are also new articles on antioxidant theory and regulatory status as well as on mechanisms and measurements of lipid oxidation. A new article has been introduced on quality assurance of oils and fats. Meanwhile, the second volume presents the main sources of food lipids, and new articles on sesame oil and rice bran oil have been introduced. The fourth volume provides a description of application areas, and here again new articles on confectionary lipids as well as on frying oils and snack food production have been added. The fifth volume on processing technologies introduces new articles on supercritical, membrane, and extrusion technologies. Finally, the sixth volume on nonedible uses of fats and oils has new articles on biodiesel, hydrolic fluids, lubricants, inks, as well as pharmaceutical and cosmetic uses of lipids. An article on the use of soybean oil in edible film and adhesive production is also included. Thus, the sixth edition is substantially different from what was available in the fifth edition.

I am indebted to many authors for their state-of-the-art contributions as well as to primary and secondary reviewers for different articles. The advisory committee members served an important role in providing invaluable comments. In addition, staff from John Wiley and Sons provided considerable help in different aspects related to production and assembly of the work. This series serves as a primary source of and as a compendium of information on oils and fats for the industry, academia and government scientists, and technical personnel, and as a reference for senior undergraduate and graduate students in food science, nutrition, dietetics, biochemistry, and related disciplines. An integrated table of contents allows better search of materials of interest, and the last volume has a cumulative index. Extensive bibliography throughout the series also provides the reader with the opportunity to consult primary references for additional information.

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# *BAILEY'S INDUSTRIAL OIL AND FAT PRODUCTS*

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*Sixth Edition*

*Volume 1*

*Edible Oil and Fat Products:  
Chemistry, Properties, and  
Health Effects*

*Edited by*

**Fereidoon Shahidi**

*Memorial University of Newfoundland*

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# 1

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## *Chemistry of Fatty Acids*

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### **1. INTRODUCTION**

Fatty acids, esterified to glycerol, are the main constituents of oils and fats. The industrial exploitation of oils and fats, both for food and oleochemical products, is based on chemical modification of both the carboxyl and unsaturated groups present in fatty acids. Although the most reactive sites in fatty acids are the carboxyl group and double bonds, methylenes adjacent to them are activated, increasing their reactivity. Only rarely do saturated chains show reactivity. Carboxyl groups and unsaturated centers usually react independently, but when in close proximity, both may react through neighboring group participation. In enzymatic reactions, the reactivity of the carboxyl group can be influenced by the presence of a nearby double bond.

The industrial chemistry of oils and fats is a mature technology, with decades of experience and refinement behind current practices. It is not, however, static. Environmental pressures demand cleaner processes, and there is a market for new products. Current developments are in three areas: “green” chemistry, using cleaner processes, less energy, and renewable resources; enzyme catalyzed reactions, used both as environmentally friendly processes and to produce tailor-made products; and novel chemistry to functionalize the carbon chain, leading to new

compounds. Changing perceptions of what is nutritionally desirable in fat-based products also drives changing technology; interesterification is more widely used and may replace partial hydrogenation in the formulation of some modified fats.

The coverage in this chapter is necessarily selective, focusing on aspects of fatty acid and lipid chemistry relevant to the analysis and industrial exploitation of oils and fats. The emphasis is on fatty acids and acylglycerols found in commodity oils and the reactions used in the food and oleochemical industries. The practical application of this chemistry is dealt with in detail in other chapters. Current areas of research, either to improve existing processes or to develop new ones, are also covered, a common theme being the use of chemical and enzyme catalysts. Compounds of second-row transition metals rhodium and ruthenium and the oxides of rhenium and tungsten have attracted particular interest as catalysts for diverse reactions at double bonds. Recent interest in developing novel compounds by functionalizing the fatty acid chain is also mentioned. To date, few of these developments have found industrial use, but they suggest where future developments are likely. A number of recent reviews and books cover and expand on topics discussed here (1–10).

## 2. COMPOSITION AND STRUCTURE

### 2.1. Fatty Acids

Fatty acids are almost entirely straight chain aliphatic carboxylic acids. The broadest definition includes all chain lengths, but most natural fatty acids are  $C_4$  to  $C_{22}$ , with  $C_{18}$  most common. Naturally occurring fatty acids share a common biosynthesis. The chain is built from two carbon units, and *cis* double bonds are inserted by desaturase enzymes at specific positions relative to the carboxyl group. This results in even-chain-length fatty acids with a characteristic pattern of methylene interrupted *cis* double bonds. A large number of fatty acids varying in chain length and unsaturation result from this pathway.

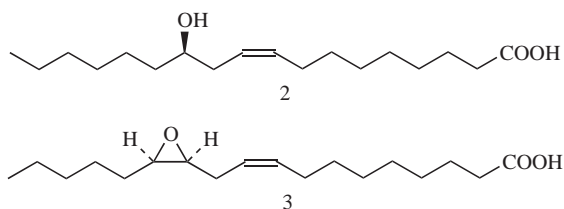
Systematic names for fatty acids are too cumbersome for general use, and shorter alternatives are widely used. Two numbers separated by a colon give, respectively, the chain length and number of double bonds: octadecenoic acid with 18 carbons and 1 double bond is therefore 18:1. The position of double bonds is indicated in a number of ways: explicitly, defining the position and configuration; or locating double bonds relative to the methyl or carboxyl ends of the chain. Double-bond position relative to the methyl end is shown as  $n-x$  or  $\omega x$ , where  $x$  is the number of carbons from the methyl end. The  $n$ -system is now preferred, but both are widely used. The position of the first double bond from the carboxyl end is designated  $\Delta x$ . Common names (Table 1) may be historical, often conveying no structural information, or abbreviations of systematic names. Alternative repre-





Over 1000 fatty acids are known, but 20 or less are encountered in significant amounts in the oils and fats of commercial importance (Table 1). The most common acids are C<sub>16</sub> and C<sub>18</sub>. Below this range, they are characterized as short or medium chain and above it as long-chain acids.

Fatty acids with *trans* or non-methylene-interrupted unsaturation occur naturally or are formed during processing; for example, vaccenic acid (18:1 11*t*) and the conjugated linoleic acid (CLA) rumenic acid (18:2 9*t*11*c*) are found in dairy fats. Hydroxy, epoxy, cyclopropane, cyclopropene acetylenic, and methyl branched fatty acids are known, but only ricinoleic acid (12(*R*)-hydroxy-9*Z*-octadecenoic acid) (**2**) from castor oil is used for oleochemical production. Oils containing vernolic acid (12(*S*),13(*R*)-epoxy-9*Z*-octadecenoic acid) (**3**) have potential for industrial use.



Typical fatty acid composition of the most widely traded commodity oils is shown in Table 2.

**TABLE 2. Fatty Acid Content of the Major Commodity Oils (wt%).**

	16:0 (wt%)	18:1 (wt%)	18:2 (wt%)	18:3 (wt%)	Other [Fatty Acid (wt%)]
butter	28	14	1	1	4:0 (9); 6:0–12:0 (18); 14:0 (14) + odd chain and <i>trans</i>
castor	1	3	4		18:1(OH) (90)
coconut	9	6	2		8:0 (8); 10:0 (7); 12:0 (48); 14:0 (18)
corn	13	31	52	1	
cottonseed	24	19	53		
fish*	14	22	1		16:1 n-7 (12); 20:1 n-9 (12); 22:1 n-11 (11); 20:5 n-3 (7); 22:6 n-3 (7)
groundnut (peanut)	13	37	41		C <sub>20</sub> –C <sub>24</sub> (7)
lard	27	44	11	1	14:0 (2) 18:0 (11) + long and odd chain
linseed	6	17	14	60	
olive	10	78	7		
palm	44	40	10		
palm kernel	9	15	2		8:0 (3); 10:0 (4); 12:0 (49); 14:0 (16)
rape**	4	56	26	10	
sesame	9	38	45		18:0 (6)
soybean	11	22	53	8	
sunflower	6	18	69		18:0 (6)
tallow	26	31	2		14:0 (6) 18:0 (31) + long and odd chain

Typical midrange values shown; the balance are minor components. Data from (9).

\*Cod liver oil.

\*\*Low-erucic-acid rape, e.g., Canola.

Most commodity oils contain fatty acids with chain lengths between  $C_{16}$  and  $C_{22}$ , with  $C_{18}$  fatty acids dominating in most plant oils. Palm kernel and coconut, sources of medium-chain fatty acids, are referred to as lauric oils. Animal fats have a wider range of chain length, and high erucic varieties of rape are rich in this  $C_{22}$  monoene acid. Potential new oil crops with unusual unsaturation or additional functionality are under development. Compilations of the fatty acid composition of oils and fats (6, 9, 11, 12) and less-common fatty acids (13) are available.

The basic structure, a hydrophobic hydrocarbon chain with a hydrophilic polar group at one end, endows fatty acids and their derivatives with distinctive properties, reflected in both their food and industrial use. Saturated fatty acids have a straight hydrocarbon chain. A *trans*-double bond is accommodated with little change in shape, but a *cis* bond introduces a pronounced bend in the chain (Fig. 1).

In the solid phase, fatty acids and related compounds pack with the hydrocarbon chains aligned and, usually, the polar groups together. The details of the packing, such as the unit cell angles and head-to-tail or head-to-head arrangement depend on the fatty acid structure (Fig. 2).

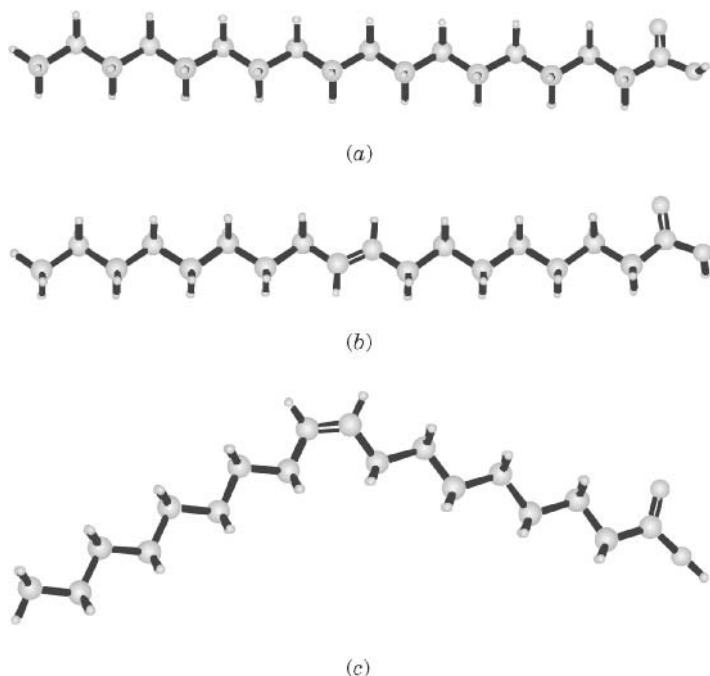
The melting point increases with chain length and decreases with increased unsaturation (Table 3). Among saturated acids, odd chain acids are lower melting than adjacent even chain acids. The presence of *cis*-double bonds markedly lowers the melting point, the bent chains packing less well. *Trans*-acids have melting points much closer to those of the corresponding saturates. Polymorphism results in two or more solid phases with different melting points. Methyl esters are lower melting than fatty acids but follow similar trends.

Fatty acid salts and many polar derivatives of fatty acids are amphiphilic, possessing both hydrophobic and hydrophilic areas within the one molecule. These are surface-active compounds that form monolayers at water/air and water/surface interfaces and micelles in solution. Their surface-active properties are highly dependent on the nature of the polar head group and, to a lesser extent, on the length of the alkyl chain. Most oleochemical processes are modifications of the carboxyl group to produce specific surfactants.

**TABLE 3. Melting Points of Some Fatty Acids and Methyl Esters Illustrating the Effect of Chain Length and Unsaturation.**

Fatty acid	Melting Point (°C)	Fatty Acid	Melting Point (°C)
16:0	62.9 (30.7)		
17:0	61.3 (29.7)		
18:0	70.1 (37.8)		
18:1 9 <sub>c</sub>	16.3, 13.4	18:1 9 <sub>t</sub>	45
18:2 9 <sub>c</sub> 12 <sub>c</sub>	-5	18:2 9 <sub>t</sub> 12 <sub>t</sub>	29
19:0	69.4 (38.5)		
20:0	76.1 (46.4)		

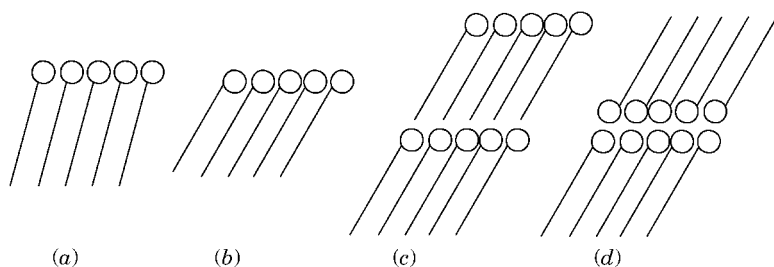
Values for methyl esters in parenthesis.  
Data from (8) and (9).



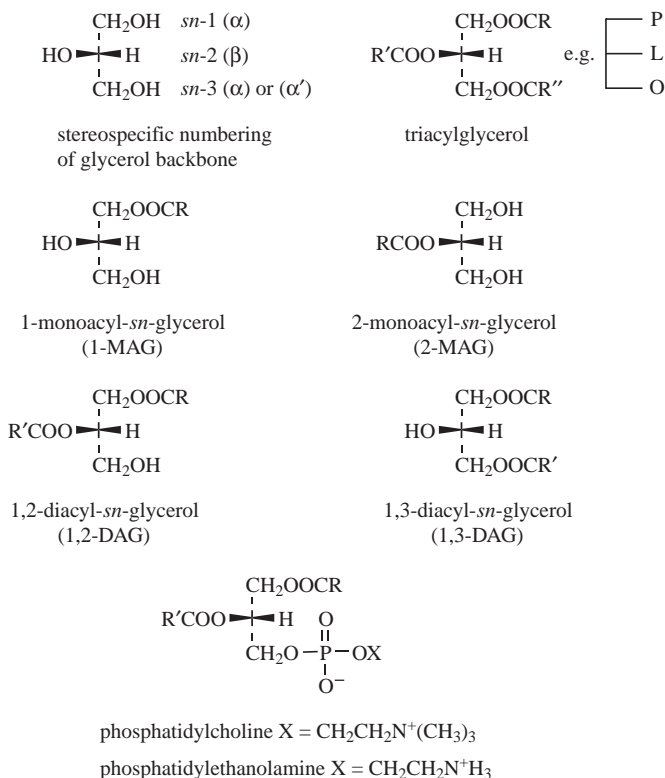
**Figure 1.** “Ball and stick” models of (a) stearic acid, 18:0; (b) elaidic acid, 18:1 9t; and (c) oleic acid 18:1 9c. All three lie flat in the plane of the paper. The cis double bond causes a distinct kink in the alkyl chain of oleic acid.

## 2.2. Acylglycerols

Fatty acids in oils and fats are found esterified to glycerol. Glycerol (1,2,3-trihydroxypropane) is a prochiral molecule. It has a plane of symmetry, but if the primary hydroxyls are esterified to different groups, the resulting molecule is chiral and exists as two enantiomers. The stereospecific numbering system is used to



**Figure 2.** Simplified diagram shows packing patterns of fatty acids in the solid phase. (a) and (b): Hydrocarbon tails (straight lines) aligned at different angles to the line of the polar head groups (circles). (c): Head to tail packing. (d): Head to head packing.



**Figure 3.** Structure and stereospecific numbering of acylglycerols.

distinguish between enantiomers. The Fischer projection of glycerol is drawn with the backbone bonds going into the paper and the hydroxyl on the middle carbon to the left. The carbons are then numbered 1 to 3 from the top (Figure 3). The prefix *sn*- (for stereospecific numbering) denotes a particular enantiomer, *rac*- an equal mixture of enantiomers, and *x*- an unknown stereochemistry. In an asymmetric environment such as an enzyme binding site, the *sn*-1 and *sn*-3 groups are not interchangeable and reaction will only occur at one position. Simplified structures are often used; e.g., 1-palmitoyl-2-linoleoyl-3-oleoyl-*sn*-glycerol is abbreviated to PLO or drawn as shown in Figure 3.

Storage fats (seed oils and animal adipose tissue) consist chiefly (~98%) of triacylglycerols, with the fatty acids distributed among different molecular species. With only two fatty acids, a total of eight triacylglycerol isomers are possible, including enantiomers (Table 4). A full analysis of triacylglycerol molecular species is a major undertaking, and for some oils, there are still technical difficulties to be resolved. More commonly, triacylglycerols are distinguished by carbon number (the sum of the fatty acid chain lengths) or unsaturation, using GC or HPLC for analysis. The number of isomers increases as the cube of the number of fatty acids;

**TABLE 4. Molecular Species of Triacylglycerols Containing only Palmitic and Oleic Acid.**

	PPP	POP	PPO	OPP	POO	OOP	OPO	OOO
enantiomers			*	*	**	**		
carbon number	48	50	50	50	52	52	52	54
double bonds	0	1	1	1	2	2	2	3

Different methods of analysis will give different and often incomplete information about such a mixture. GC analysis will separate molecular species by carbon number (sum of fatty acid chain lengths). Silver-ion HPLC will separate by number of double bonds. Stereospecific analysis measures the proportions of fatty acids at the *sn*-1, *sn*-2, and *sn*-3 positions, but it does not detect individual molecular species.

hence, even in oils with a simple fatty acid composition, many molecular species of triacylglycerol may be present.

Most natural triacylglycerols do not have a random distribution of fatty acids on the glycerol backbone. In plant oils, unsaturated acids predominate at the *sn*-2 position, with more saturated acids at *sn*-1 and *sn*-3. The distribution of fatty acids at the *sn*-1 and *sn*-3 positions is often similar, although not identical. However, a random distribution between these two positions is often assumed as full stereospecific analysis is a time-consuming specialist procedure. In animal fats, the type of fatty acid predominating at the *sn*-2 position is more variable; for example, palmitate may be selectively incorporated as well as unsaturated acids (Table 5).

Only oils that are rich in one fatty acid contain much monoacid triacylglycerol, for example, olive (Table 5), sunflower, and linseed oils containing OOO, LLL, and LnLnLn, respectively. Compilations of the triacylglycerol composition of commodity and other oils are available (8, 9).

The melting behavior of triacylglycerols generally reflects that expected from the fatty acid composition; triacylglycerols rich in long-chain and saturated acids

**TABLE 5. Contrasting Triacylglycerol Composition of Some Commodity Oils [Molecular Species (wt%)].**

Cocoa butter	Coconut	Lard	Olive	Soybean
POP (18-23)	12,12,8 (12)	PPSt (2)	OOL (11)	LnLL (7)
POSt (36-41)	12,12,10 (6)	StPSt (2)	OOO (43)	LnLO (5)
StOSt (23-31)	12,12,12 (11)	PPO (8)	POP (3)	LLL (15)
	12,12,14 (11)	StOP (13)	POL (4)	LLO (16)
unsymmetrical	14,12,8 (9)	POO (5)	POO (22)	LLS (13)
e.g., SSO <1%		StOO (6)	StOO (5)	LOO (8)
		OPO (18)		LOS (12)
		StPL (2)		OOS (5)
		OOO (12)		
		OPL (7)		

L—linoleic; Ln—linolenic; O—oleic; P—palmitic; S—saturate; St—stearic; 8—8:0; 10—10:0; 12—12:0 (lauric); 14—14:0.

Analysis by methods that do not distinguish all isomers; only major components are listed.

Data from (6).

are high melting, and those rich in polyunsaturated acids are lower melting. However, the situation is complicated by the possibility that the fatty acids can be distributed in different molecular species with different melting points. Oils with similar fatty acid composition may have different solid fat content, polymorphic forms, and melting behavior as a result of a different triacylglycerol composition.

Mono- and diacylglycerols (Figure 3) are not significant components of good quality oils, but elevated levels may be found in badly stored seeds, resulting from the activity of lipolytic enzymes. These compounds are produced industrially by partial hydrolysis or glycerolysis of triacylglycerols for use as food grade emulsifiers. Mono- and diacylglycerols readily isomerize under acid or base catalysis and are normally produced as an equilibrium mixture in which 1(3)-monoacylglycerols or 1,3-diacylglycerols predominate.

Phospholipids (Figure 3) are constituents of membranes and are only minor components of oils and fats, sometimes responsible for cloudiness. They are usually removed during degumming, the residue from soybean oil processing being a source of phospholipids used as food emulsifiers. The term “lecithin” is used very loosely for such material, and it may variously mean phosphatidylcholine, mixed glycerophospholipids, or crude phospholipid extracts from various sources. Where possible, more specific nomenclature or the source and purity should be used (14).

### 2.3. Bulk Properties

*Saponification value and iodine value.* Oils and fats are now characterized mainly by their fatty acid composition determined by gas chromatography, replacing the titrimetric and gravimetric assays used previously. However, the saponification value (SV) or equivalent (SE) and iodine value (IV) are still used in specifications and to monitor processes. SE, expressed as grams of fat saponified by one mole of potassium hydroxide, is an indication of the average molecular weight and hence chain length, whereas the IV, expressed as the weight percent of iodine consumed by the fat in a reaction with iodine monochloride, is an index of unsaturation (Table 6). Standard analytical methods are available (15), but these parameters are now often calculated from the fatty acid composition, assuming that the sample is all triacylglycerol (15). Indirect measurement of IV (16, 17) and SV (17) (as well as peroxide and *trans*-content) using FT-NIR spectroscopy have been developed for real-time process monitoring.

*Unsaponifiable matter.* Oils and fats contain variable amounts of sterols, hydrocarbons, tocopherols, carotenoids, and other compounds, collectively referred to as unsaponifiable matter because they do not produce soaps upon hydrolysis (Table 6). The sterol and tocopherol composition of commodity oils is discussed in another chapter. Some of these minor components are removed during refining, and the resulting concentrates may be useful byproducts, for example, tocopherol antioxidants. Characteristic fingerprints of minor components, particularly phytosterols and tocopherols, are also used to authenticate oils and detect adulteration (18).

**TABLE 6. Saponification Equivalent (SE), Saponification Value (SV), Iodine Value (IV), and Unsaponifiable Matter of Some Commodity Oils.**

	SE* (g oil/mol KOH)	SV (mg KOH/g oil)	IV (100 × g iodine/g oil)	Unsaponifiable matter (wt%)
butter	242–267	210–232	26–40	<0.5
castor	300–319	176–187	81–91	
coconut	212–226	248–265	6–11	<1.5
corn	288–300	187–195	107–128	1–3
cottonseed	283–297	189–198	100–115	<2
fish**	292–312	180–192	142–176	<2
groundnut (peanut)	286–300	187–196	86–107	<1
lard	276–292	192–203	45–70	<0.2
linseed	286–298	188–196	170–203	<2
olive	286–305	184–196	75–94	<1.5
palm	268–295	190–209	50–55	<1.3
palm kernel	221–244	230–254	14–21	<1
rape***	291–308	182–193	110–126	<0.2
sesame	288–300	187–195	104–120	<2
soybean	288–297	189–195	124–139	<1.5
sunflower	289–298	188–194	118–145	<2
tallow	281–295	190–200	33–47	<0.5

\*SE = 56108/SV.

\*\*Cod liver oil.

\*\*\*Low erucic rape (Canola).

Data from (11).

### 3. HYDROLYSIS, ESTERIFICATION, AND ESTER EXCHANGE

Reactions converting acids to esters or vice versa and the exchange of ester groups are among the most widely used in fatty acid and lipid chemistry (Figure 4). They find applications from microscale preparation of methyl esters for GC analysis to the industrial production of oleochemicals and biodiesel. The exchange of groups attached to the fatty acid carboxyl is usually an equilibrium process driven to one product by an excess of one reactant or the removal of one product, and it is usually



**Figure 4.** Exchange reactions at the carboxyl group (1) hydrolysis (Chapter xx), (2) esterification (Chapter xx), (3) acidolysis (Chapter xx), (4) alcoholysis (Chapter xx), and (5) glycerolysis (Chapter xx). The starting ester RCOOR' will often be a triacylglycerol. MAG—monoacylglycerol; DAG—diacylglycerol; TAG—triacylglycerol.

carried out with the aid of a catalyst. The catalyst may be an acid, a base, or a lipolytic enzyme. These reactions produce the fatty acids and methyl esters that are the starting point for most oleochemical production. As the primary feedstocks are oils and fats, glycerol is produced as a valuable byproduct. Reaction routes and conditions with efficient glycerol recovery are required to maximize the economics of large-scale production.

There is increasing interest in the use of lipase enzymes for large-scale reactions. Enzyme reactions require milder conditions, less solvent, and give cleaner products—attributes of “green chemistry.” Enzymes can exert regio- or stereospecific control over reactions and may also offer a degree of selectivity for particular fatty acids, not observed with acid or base catalysts. Although the reactions of the carboxyl group are normally independent of those of the double bonds in the fatty acid molecule, the presence of a double bond at the  $\Delta 4$ ,  $\Delta 5$ , or  $\Delta 6$  position often results in slower reaction when a reaction is catalyzed by a lipase. Lipase catalyzed reactions are considered in detail below, following a brief description of the reactions involved.

### 3.1. Hydrolysis

The reaction can be catalyzed by acid, base, or lipase, but it also occurs as an uncatalyzed reaction between fats and water dissolved in the fat phase at suitable temperatures and pressures.

*Base catalyzed hydrolysis.* Historically, soaps were produced by alkaline hydrolysis of oils and fats, and this process is still referred to as saponification. Soaps are now produced by neutralization of fatty acids produced by fat splitting (see below), but alkaline hydrolysis may still be preferred for heat-sensitive fatty acids.

On a laboratory scale, alkaline hydrolysis is carried out with only a slight excess of alkali, typically 1M potassium hydroxide in 95% ethanol, refluxing for one hour, and the fatty acids recovered after acidification of the reaction mixture. This is a sufficiently mild procedure that most fatty acids, including polyunsaturates, epoxides, and cyclopropenes, are unaltered (19).

*Fat splitting.* The industrial production of fatty acids uses the direct reaction between water and fats, which proceeds rapidly at  $\sim 250^\circ\text{C}$  and 2–6 MPa (20–60 bar). Under these conditions, water is moderately soluble in the oil phase, and stepwise hydrolysis of the triacylglycerols proceeds without the aid of a catalyst. The reaction is carried out with a countercurrent of water that removes the glycerol formed, resulting in  $\sim 99\%$  conversion to fatty acids. Glycerol is recovered from the aqueous phase. Sonntag has reviewed industrial fat splitting in detail (20).

### 3.2. Esterification

Fatty acids are converted to esters by reaction with an excess of alcohol using an acid catalyst or a lipase. For the preparation of methyl esters for GC analysis, boron trifluoride, sulfuric acid, or anhydrous hydrogen chloride in methanol are commonly used (19). Reaction is complete in 30 minutes at reflux. Propyl and butyl



esters are prepared in a similar way with the corresponding alcohols. It is not always possible to use an excess of alcohol, for example, in the synthesis of triacylglycerols using a protected glycerol. A more reactive fatty acid derivative such as the acid chloride or anhydride is used, or the fatty acid is reacted directly with the alcohol, using dicyclohexylcarbodiimide (DCC) plus 4-dimethylaminopyridine (DMAP) as a coupling agent, for example, in the synthesis of acylglycerols (21). Some groups in more unusual fatty acids are acid sensitive, for example, epoxides, cyclopropanes, cyclopropenes, and hydroxy compounds, and methods avoiding acids catalysts are needed. Reaction with diazomethane or the less hazardous trimethylsilyl-diazomethane are possibilities (19).

### 3.3. Ester Exchange Reactions

The fatty acid or alcohol groups present in an ester can be exchanged in a number of ways: by reaction with an excess of other fatty acids (acidolysis), alcohols (alcoholysis), or other esters (interesterification). Generally, the starting point will be a triacylglycerol, and these reactions provide routes by which the composition and properties of oils and fats can be modified.

*Acidolysis.* This reaction can be acid or enzyme catalyzed and may be used to modify triacylglycerol composition. Acidolysis of an oil containing only C<sub>16</sub> and C<sub>18</sub> fatty acids with fatty acids rich in lauric acid (e.g., from palm-kernel oil) results in a triacylglycerol enriched in medium-chain fatty acids.

*Alcoholysis.* Methanolysis of triacylglycerols is used to prepare methyl esters for fatty acid analysis, a process frequently referred to as transesterification. This can be acid- or base-catalyzed, the method being chosen to avoid modifying acid- or base-sensitive fatty acids and to minimize reaction times. Sterol esters of fatty acids react more slowly than triacylglycerols, and samples containing them require more vigorous reaction conditions. The preparation of methyl esters from oils and fats for GC and GC-MS analysis has been extensively reviewed (19, 22, 23).

Biodiesel is produced on the industrial scale by methanolysis of vegetable oils (usually rape or soybean) or waste fat, particularly using frying oils. Methanolysis proceeds with modest amounts of base catalyst, provided the levels of free fatty acid and water in the oil are low (24, 25). The fatty acid content may be reduced by physical or chemical treatment before methanolysis but for waste fats, alternative processes that do not use base catalysis may be preferred. Lipase catalyzed methanolysis is less sensitive to fatty acid and water in the oil and has been tested in batch (26) and fixed-bed reactor (27) conversion of waste oil and grease to biodiesel.

Glycerolysis, the treatment of triacylglycerols with glycerol and a basic catalyst (sodium hydroxide or sodium methoxide), is used to produce mono- and diacylglycerols on an industrial scale. Molecular distillation is used to produce MAG, which is 90–95% pure and is widely used as an emulsifying agent in foods and other applications.

*Interesterification.* Interesterification is the intra- and intermolecular exchange of fatty acids on the glycerol backbone of triacylglycerols, although the term is also used more loosely to include acidolysis and other ester exchange reactions. It is applied to either an individual oil or a blend of oils, to produce triacylglycerols with different properties. The molecular species of natural triacylglycerols is not a random mixture of all possible isomers, but it shows greater or lesser selectivity in the distribution of fatty acids between the *sn*-1 and *sn*-3 and the *sn*-2 positions (Table 5). This, as well as the overall fatty acid mixture, determines many of the technically important properties of the oil or fat, for example, solid fat content and melting point. Once subjected to interesterification with a chemical catalyst, the triacylglycerol becomes a random mixture of molecular species. Lipase catalyzed interesterification may alter the distribution of molecular species in a more selective way.

Chemical interesterification (28, 29) is carried out at moderate temperatures (70–100°C), with neat oils and a low concentration (<0.4%) of a base catalyst such as sodium methoxide or ethoxide or Na/K alloy. As the catalyst is destroyed by water and free fatty acids, the oil must be carefully refined and dried before adding the catalyst. Reaction proceeds through sequential fatty acid exchange reactions, following formation of what is believed to be the true catalyst, the alkali metal derivative of a diacylglycerol. There is no observed selectivity for fatty acid or glycerol position, leading to a fully random product. The product composition can be controlled through directed interesterification at lower temperatures. Na/K alloy is used as catalyst as it is active at temperatures below 50°C and cooling the reaction mixture causes high melting trisaturated triacylglycerols to crystallize out, altering the composition of the liquid phase in which reaction occurs. The remaining liquid phase is randomized by further reaction and high melting products continue to crystallize out, eventually leading to solid and liquid products richer in trisaturated and triunsaturated species than the fully randomized fat (29).

Interesterification is used to modify fat properties without recourse to partial hydrogenation. Hardened fats produced by partial hydrogenation contain *trans*-isomers, which are now regarded as undesirable by nutritionists and will be increasingly subject to product labeling regulations. Liquid fats can be hardened by interesterification with fully saturated fats (either stearin fractions or fully hydrogenated oils), raising the solid fat content without isomerizing any of the fatty acids. The use of interesterification to produce margarine and spreads has increased recently, particularly in Europe.

### 3.4. Lipase Catalyzed Reactions

Lipases are enzymes that hydrolyze fatty acids from lipid species (e.g., triacylglycerols or phospholipids) *in vivo*. A number of lipases, mainly of bacterial origin, are now available immobilized onto a solid support for use as industrial scale catalysts.

Immobilized lipases catalyze the whole range of ester exchange reactions described above (alcoholysis, acidolysis, esterification) as well as hydrolysis. There are two significant differences between lipase and chemically catalyzed reactions. First, lipase catalyzed reactions take place at a lower temperature and with fewer side reactions, leading to cleaner products: an environmentally friendly alternative to some existing processes. Second, enzyme catalyzed reactions are more selective, offering control over reactions not possible with a chemical catalyst. Selectivity may be for fatty acids at different positions on the glycerol backbone (*sn*-1 and *sn*-3 rather than *sn*-2) or for particular fatty acids, discriminating by double-bond position or chain length (30, 31). The widely studied Lipozyme RM IM (*Rhizomucor miehei* lipase immobilized onto a weak anion exchange resin) preferentially hydrolyzes short-chain acids relative to medium and long chains from triacylglycerols. Hydrolysis at the *sn*-1 position is somewhat faster than at *sn*-3, and hydrolysis at *sn*-2 is very slow (31).

Lipase catalyzed reactions take place in the neat oil or in a nonpolar (usually hydrocarbon) solvent. The efficiency depends on the amount of water, solvent (if present), temperature, and ratio of reactants. A factorial approach can be used to optimize the conditions (32). In interesterification reactions, 1,3-specific enzymes give control over product composition that is not possible using chemical catalysts. For example, starting with SOS and OOO, chemical interesterification produces all eight possible isomers (see Table 5). Enzymatic interesterification does not exchange fatty acids at the *sn*-2 position, and it will result in only two additional molecular species, OOS and SOO. In more realistic situations, chemical and enzymatic interesterification may produce the same or a similar number of molecular species, but in different proportions (31).

Enzymatic interesterification has most potential for high-value products such as confectionary fats and nutritional products, for example, cocoa butter equivalents prepared from cheap and readily available starting materials. Acidolysis of palm mid fraction, rich in POP, with stearic acid gives a cocoa butter equivalent rich in POSt and StOSt, through exchange at the *sn*-1 and *sn*-3 positions while retaining the oleate at the *sn*-2 position. Tripalmitin treated similarly with oleic acid gives products where the palmitate is retained at the *sn*-2 position, whereas oleate is introduced at *sn*-1 and *sn*-3, producing a human milk fat substitute such as Betapol. In practice, pure starting materials are not used. Feedstocks rich in tripalmitin and oleic acid are reacted in a two step-process: alcoholysis to *sn*-2- monoacylglycerols followed by esterification (33).

Both batch and fixed-bed reactors have been used and tested on the near ton scale (34) for the production of high-value fats. This technology has now progressed to pilot production, using a 1-m<sup>3</sup> fixed-bed plug-in reactor containing the immobilized enzyme Lipozyme TL IM (35). Blends of palm oil or stearin with palm-kernel or coconut oil are interesterified in less than one hour at 70°C, and no downstream processing is required as the enzyme is retained in the reactor. This is a practical, lower energy alternative to hydrogenation and chemical interesterification, free from the *trans*-isomer production of the former and more selective and “natural” than the latter.

Lipases also discriminate between fatty acids with different double-bond positions. The reaction of fatty acids with  $\Delta 4$ ,  $\Delta 5$ , and  $\Delta 6$  double bonds is significantly slower than  $\Delta 9$  acids when catalyzed by some enzymes. This is illustrated by some examples of attempts to concentrate  $\gamma$ -linolenic acid (GLA; 18:3 6c9c12c) from borage oil. Hydrolysis of borage oil with *Candida rugosa* lipase resulted in selective hydrolysis of the  $\Delta 9$  acids (mainly 18:2) increasing the amount of GLA in the remaining acylglycerols (36). The efficiency of the enrichment was influenced by the initial triacylglycerol composition and the extent of hydrolysis. Starting with a borage oil containing 22% GLA, the upper limit of enrichment was to 46%, but higher values resulted from repeated hydrolysis of the recovered acylglycerols. A two-step sequence involving both enzymatic hydrolysis and re-esterification achieved higher enrichment (37). Nonselective hydrolysis with *Pseudomonas* sp. lipase was optimized for high GLA recovery (93%). Esterification with lauryl alcohol, using *Rhizopus delemar* lipase, discriminated strongly against GLA, resulting in enrichment in the unesterified fatty acids from 22.5% to 70.2% with a recovery efficiency of 75.1%. A 92.1% GLA concentrate, obtained by low-temperature crystallization of borage oil fatty acids, was enriched to 99.1% by esterification with butanol, catalyzed by Lipozyme IM-60 (38). The overall recovery was 72.8%. The operating parameters (alcohol, concentration, temperature, and solvent) were systematically investigated.

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA),  $\Delta 5$  and  $\Delta 4$  acids respectively, are discriminated against during lipase catalyzed reactions and reaction of DHA may be significantly slower than EPA. Alcoholysis of tuna oil ethyl esters with lauryl alcohol using *Rhizomucor miehei* lipase enriches the DHA in the unreacted ethyl esters, whereas the concentration of EPA is simultaneously reduced (39). A concentrate containing 60% DHA and 8.6% EPA was alcoholized with excess lauryl alcohol (1:7 mole ratio). The remaining ethyl esters contained 93% DHA in 74% recovery, and EPA was reduced to 2.9%. Both non-regiospecific and *sn*-1,3-specific enzymes incorporate GLA into seal blubber and menhaden oil (3:1 mole ratio of GLA to triacylglycerol) producing an oil rich in both n-3 and n-6 polyenes (40). The highest incorporation was with the nonspecific enzyme.

#### 4. OXIDATION

The fatty acid alkyl chain is susceptible to oxidation both at double bonds and adjacent allylic carbons. Free-radical and photooxidation at allylic carbons are responsible for deterioration of unsaturated oils and fats, resulting in rancid flavors and reduced nutritional quality, but they are also used deliberately to polymerize drying oils. Oxidation of double bonds is used in oleochemical production either to cleave the alkyl chain or to introduce additional functionality along the chain. Enzyme catalyzed oxidation is the initial step in the production of eicosanoids and jasmonates (biologically active metabolites in animals and plants respectively) but is not discussed further here.

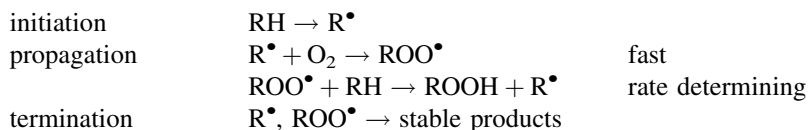
#### 4.1. Autoxidation and Photooxidation

Both autoxidation and photooxidation produce allylic hydroperoxides from unsaturated centers.



During this process, the position and geometry of the double bond may change. The hydroperoxide mixtures produced by autoxidation and photooxidation are not the same, indicating that different mechanisms are involved. Free radical oxidation can be promoted or inhibited. Deliberate promotion speeds the polymerization of drying oils, and strenuous efforts are made to inhibit the onset of rancidity in edible oils. Frankel has recently reviewed this topic in depth (41); see also (1) for an extensive discussion of oxidation of food lipids.

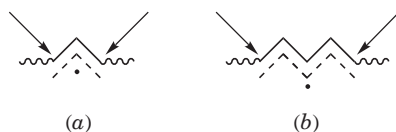
**4.1.1. Autoxidation** Autoxidation is a free-radical chain reaction, involving a complex series of reactions that initiate, propagate, and terminate the chain.



The chain reaction is initiated by abstraction of an allylic hydrogen to give an allylic radical stabilized by delocalization over three or more carbons. The initiator is a free radical, most probably produced by decomposition of hydroperoxides already present or produced by photooxidation. The decomposition may be thermal, but it is more likely promoted by traces of variable redox state metal ions. Autoxidation is characterized by an induction period during which the concentration of free radicals increases until the autocatalytic propagation steps become dominant. During the induction period, there is little increase in oxidation products.

The first step of the propagation sequence is reaction of the allylic radical with molecular oxygen, producing a peroxy radical. This step is much faster than the subsequent abstraction of another allylic hydrogen by the peroxy radical, producing both an allylic hydroperoxide and a new allylic radical that continues the chain reaction. Hydrogen abstraction is the rate-determining step and is therefore selective for the most readily abstracted hydrogen. Methylene-interrupted dienes and polyenes, where the allylic radical can be delocalized over five carbons, are oxidized faster than monoenes where the radical is delocalized over three carbons (Figure 5).

The chain reaction is terminated by reactions that remove radicals that would otherwise produce more allylic radicals by hydrogen abstraction. Examples are the combination of two hydroperoxy radicals leading to nonradical products and molecular oxygen or reaction with a free-radical scavenger (antioxidant) generating a more stable radical.



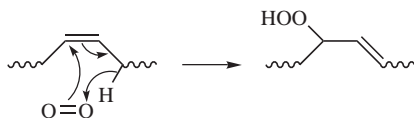
**Figure 5.** Allylic radicals produced during autoxidation. (a) Those from isolated double bonds are delocalized over three carbons. (b) Those from methylene-interrupted dienes or polyenes are delocalized over five carbons. The arrows show the site of attachment of  $O_2$  giving a peroxy radical.

The rate of autoxidation generally increases with increasing unsaturation. Linoleate, as neat methyl or ethyl ester, reacts approximately 40 times faster than oleate, and for higher polyenes, the rate doubles for each additional double bond (42). Trilinolein does not follow the same kinetics as the simple esters and oxidizes somewhat faster. The medium also influences susceptibility to oxidation, and these generalizations may not hold in emulsified systems (e.g., many food formulations) where oxidation occurs at the interface between aqueous and fat phases (43). In aqueous micelles, EPA and DHA are unexpectedly stable (44), oxidizing much more slowly than linoleate. In one experiment, over half the linoleate was oxidized within 50 hours and  $\sim 90\%$  of EPA and DHA was still present after 2000 hours. The stability of the higher polyenes is attributed to their tightly coiled configuration in the aqueous medium, making attack by oxygen or free radicals more difficult.

Mechanistic studies of autoxidation have concentrated on methylene-interrupted fatty acids, but many of the observations are valid for other compounds. Conjugated fatty acids such as CLA also oxidize through an autocatalytic free radical reaction, with the predominant hydroperoxide determined by the geometry of the conjugated diene system (45). Other groups with activated methylenes may be susceptible to oxidation, for example, the ether methylenes of ethoxylated alcohols used as surfactants (46).

**4.1.2. Photooxidation** Light, in the presence of oxygen, promotes oxidation of unsaturated fatty acids. Ultraviolet radiation decomposes existing hydroperoxides, peroxides, and carbonyl and other oxygen-containing compounds, producing radicals that initiate autoxidation (42). Photooxidation by longer wavelength near ultraviolet or visible light requires a sensitizer. Naturally present pigments such as chlorophyll, hematoporphyrins, and riboflavin act as sensitizers as do dyes, including erythrosine and methylene blue. Light excites these sensitizers to the triplet state that promotes oxidation by type I and type II mechanisms. Unlike autoxidation, there is no induction period.

In type I photosensitized oxidation, the triplet state sensitizer abstracts a hydrogen or electron from the unsaturated oil, producing radicals that initiate chain propagation as in autoxidation. However, chain-breaking antioxidants do not stop this reaction as new radicals are produced photochemically. In type II photooxidation, the energy of the triplet sensitizer is transferred to molecular oxygen, converting it



**Figure 6.** Ene reaction between singlet oxygen and an olefinic bond. The hydroperoxide may be attached to either of the initial double bond carbons.

to its excited singlet state. Singlet oxygen is highly electrophilic and reacts rapidly with olefins in an ene reaction, producing allylic hydroperoxides with oxygen attached to one of the original olefinic carbons and the shifted double bond now *trans* (Figure 6).

The ene reaction differs from free-radical oxidation, where oxygen attaches to an outer carbon of the delocalized allylic radical (Figure 5), resulting in a different mixture of hydroperoxides. For example, photooxidation of linoleate produces four isomers: 9-OOH,10*t*12*c*, 10-OOH,8*t*12*c*, 12-OOH,9*c*13*t*, and 13-OOH,9*c*11*t*. The same 9- and 13-hydroperoxides are produced by autoxidation, but the 10- and 12-hydroperoxides are only produced by photooxidation.

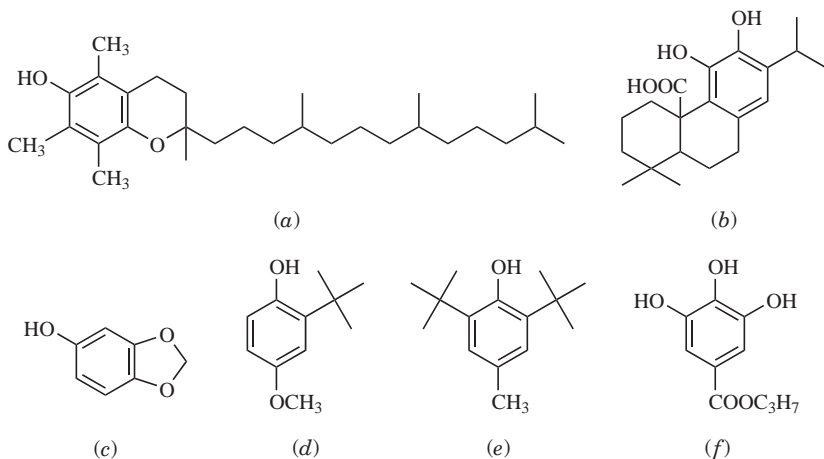
Photooxidation is much faster than autoxidation; the reaction of linoleate with singlet oxygen is approximately 1500 times faster than that with triplet oxygen (47). There is less difference in the rate of photooxidation between monoenes and polyenes than is seen in autoxidation. The relative rates for oleate, linoleate, linolenate, and arachidonate are 1.0, 1.7, 2.6, and 3.1 (48, 49). This contrasts with the 40-fold increase in rate of autoxidation between oleate and linoleate.

**4.1.3. Decomposition of Hydroperoxides** Allylic hydroperoxides are reactive molecules and decompose readily in a complex series of reactions, the course of which depends on the medium and other conditions (1, 41). Cleavage between the oxygens is energetically favored, leading to alkoxy and hydroxyl radicals. Redox metal ions such as  $\text{Fe}^{2+}/\text{Fe}^{3+}$  and  $\text{Cu}^+/\text{Cu}^{2+}$  are particularly effective catalysts. The resulting radicals can initiate further autoxidation and produce a number of stable products, many with undesirable nutritional and flavor properties (Figure 7). Products with the same chain length as the alkoxy radical include epoxides, ketones, and hydroxy fatty acids. The significant products producing off-flavors are those resulting from chain scission  $\beta$  to the alkoxy radical, producing shorter chain aldehydes and hydrocarbons. Alkadienals have particularly low-odor thresholds and a few parts per billion of nonadienals from *n*-3 fatty acids are responsible for a marked fishy taint even when other signs of oxidation are absent (50).

There are a number of analytical measures of oxidative deterioration of oils and fats. The most widely used are the peroxide value (PV) (15), which measures the hydroperoxide content by iodine titration and the anisidine value (AV) (15), which detects aldehydes by a color reaction. As an oil suffers damage because of autoxidation, the hydroperoxide content, and PV rise but do not do so indefinitely. As the hydroperoxides break down, the concentration of aldehydes and AV increase. Oxidation is better assessed by a combination of PV and AV, the Totox value





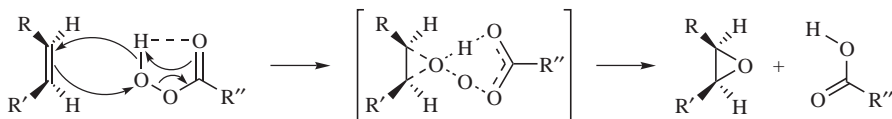


**Figure 8.** Natural antioxidants (a)  $\alpha$ -tocopherol, (b) carnosic acid, and (c) sesamol. Synthetic antioxidants (d) butylated hydroxyanisole (BHA), (e) butylated hydroxytoluene (BHT), and (f) propyl gallate.

Tocopherols are phenolic antioxidants (Figure 8) naturally present in most plant oils (see Chapter X). They are concentrated in the distillate from physical refining, which results in a corresponding decrease in the refined oil. Soybean distillate is a source of tocopherols for antioxidant formulations. Carnosic acid (Figure 8) is isolated from rosemary and other herbs. Sesamol (Figure 8) is a characteristic antioxidant of sesame oil, responsible for its high stability (Chapter xx). Synthetic antioxidants are monocyclic phenols with highly branched substituents (Figure 8). In all of these compounds, the radicals formed by abstraction of the phenolic hydrogen are highly delocalized and unreactive. The antioxidant action of free-radical scavengers is sacrificial, delaying oxidation until the antioxidant is used up. Oxidized tocopherols may be regenerated by ascorbic acid, extending their effective life while keeping their concentration below prooxidant levels.

Photooxidation is not inhibited by free-radical scavengers. Natural pigments that act as sensitizers may be reduced during refining, increasing stability. Singlet oxygen and excited state sensitizers can be deactivated either by competitive reaction or physical energy transfer, for example, to  $\beta$ -carotene. Tocopherols and some amines also act as singlet oxygen quenchers through physical energy transfer.

Redox metal ions, particularly iron and copper, react with hydroperoxides, initiating further autoxidation and producing undesirable decomposition products. Complete removal of these metal ions is not possible, but steps can be taken to reduce their effect. Chelating agents such as EDTA, citric acid, phosphate, and polyphosphates may reduce the effective metal ion concentration. Their efficacy depends on pH, and they may also show prooxidant activity. The role of metal ions in hydroperoxide decomposition in food emulsions has been reviewed recently (52).



**Figure 9.** Epoxidation mechanism proposed by Bartlett (53). The *cis*-olefin gives rise to a *cis*-epoxide.

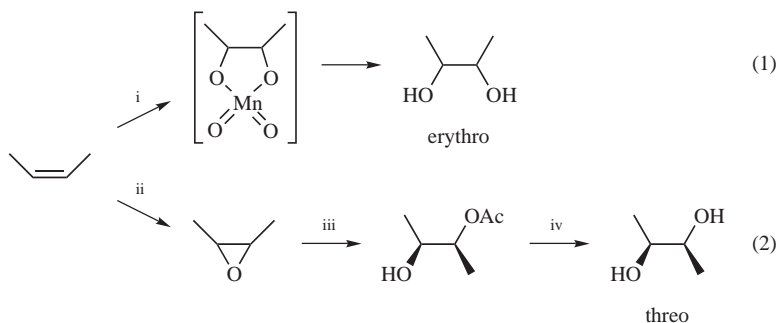
## 4.2. Epoxidation

Epoxides are produced by reaction of double bonds with peracids. This proceeds by a concerted mechanism, giving *cis* stereospecific addition (Figure 9) (53). Thus, a *cis* olefin leads to a *cis* epoxide and a *trans* olefin to a *trans* epoxide. The order of reactivity of some peracids is *m*-chloroperbenzoic > performic > perbenzoic > peracetic; electron withdrawing groups promote the reaction. The carboxylic acid produced is a stronger acid than the strongly hydrogen bonded peracid and may lead to subsequent ring opening reactions especially in the case of formic acid. Small scale reactions are carried out with *m*-chloroperbenzoic acid in a halocarbon or aromatic solvent, in the presence of bicarbonate to neutralize the carboxylic acid as it is formed (54, 55).

Oils, mainly soybean but also linseed, are epoxidized on an industrial scale (100,000 tons per year) as stabilizers and plasticizers for PVC. The reactive epoxide groups scavenge HCl produced by degradation of the polymer. Epoxidation is carried out with performic or peracetic acid produced in situ from formic or acetic acid and high strength hydrogen peroxide (70% w/w). Peracids are unstable, and the reaction is exothermic. The concentration of peracid is kept low by using a low concentration of the carboxylic acid either in the neat oil or in a hydrocarbon solvent. The carboxylic acid is regenerated after epoxidation. Complete epoxidation is not achieved as in the acidic medium ring opening reactions occur producing dihydroxy and hydroxy carboxylates as byproducts.

Recent studies have attempted to improve the efficiency of epoxidation under milder conditions that minimize the formation of byproducts. Chemo-enzymatic epoxidation uses the immobilized lipase from *Candida antartica* (Novozym 435) (56) to catalyze conversion of fatty acids to peracids with 60% hydrogen peroxide. The fatty acid is then self-epoxidized in an intermolecular reaction. The lipase is remarkably stable under the reaction conditions and can be recovered and reused 15 times without loss of activity. Competitive lipolysis of triacylglycerols is inhibited by small amounts of fatty acid, allowing the reaction to be carried out on intact oils (57). Rapeseed oil with 5% of rapeseed fatty acids was converted to epoxidized rapeseed oil in 91% yield with no hydroxy byproducts. Linseed oil was epoxidized in 80% yield. Methyl esters are also epoxidized without hydrolysis under these conditions.

Methyltrioxorhenium (MTO) catalyses direct epoxidation by hydrogen peroxide. The reaction is carried out in pyridine, avoiding acidic conditions detrimental to high epoxide yield and uses less concentrated hydrogen peroxide (30%) than other methods (58). This method epoxidized soybean and metathesized (see Section 7.4)



**Figure 10.** Stereochemistry of hydroxylation reactions: (1) with dilute alkaline permanganate and (2) through epoxide ring opening. (i) KMnO<sub>4</sub>, NaOH; (ii) *m*-chloroperbenzoic acid, NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>; (iii) CH<sub>3</sub>COOH; (iv) base catalyzed hydrolysis.

soybean oil in high yield (59). The epoxidized metathesized oil was more stable to polymerization than that produced using *m*-chloroperbenzoic acid, presumably because it was free of acidic impurities. These and other novel approaches to epoxidation have recently been reviewed (4, 60, 61). None has yet found industrial application.

Epoxides are reactive and readily ring open in acid, following protonation of the epoxy oxygen (Figure 10). This is a route to diols (see Section 4.3), polyols used in polymer production and a range of  $\alpha$ -hydroxy compounds. Ring opening of methylene-interrupted diepoxides leads to 5 and 6 membered ring ethers through neighboring group participation (7).

### 4.3. Hydroxylation

Double bonds are converted to monohydroxy derivatives by acid catalyzed addition of carboxylic acids, followed by hydrolysis. The carbocation intermediate is prone to rearrangement, leading to a mixture of positional isomers. Hydroboration with borane:1,4-oxathiane followed by alkaline hydrolysis a regioselective reaction (62) has been used to prepare hydroxy fatty acids as GC-MS standards in high yield (63).

Hydroxylation reactions leading to diols have much in common with epoxidation and oxidative cleavage reactions (see Section 4.4), the end product depending on the strength of the oxidizing agent. Dilute alkaline permanganate or osmium tetroxide react through cyclic intermediates resulting from *cis* addition of the reagent giving an *erythro* diol. Ring opening epoxides with acid is a *trans* addition, leading to a *threo* product (Figure 10).

An oxygen bridged manganese complex was recently reported to catalyze double-bond oxidation by hydrogen peroxide leading to a mixture of epoxide, *cis*-diol, and hydroxy ketone products (64). This is an interesting model reaction for the efficient use of hydrogen peroxide as a cheap hydroxylating agent if the selectivity can be improved. A number of microorganisms are reported to produce

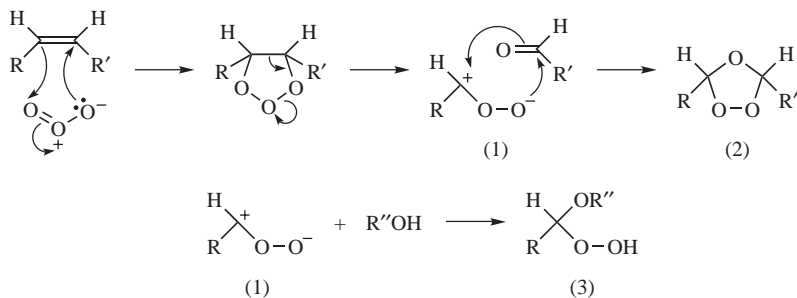
a range of novel di- and trihydroxy fatty acids and are being investigated as potential biocatalysts (65).

#### 4.4. Oxidative Cleavage

Double bonds are cleaved by a number of oxidizing agents, converting the olefinic carbons to carboxylic acids, aldehydes, or alcohols. Fatty acids give a monofunctional product from the methyl end and a difunctional product from the carboxyl end (along with low-molecular-weight products from methylene-interrupted systems).

Although now largely superseded by GC and GC-MS methods for structure determination, oxidative cleavage with ozone or permanganate/periodate and identification of the resulting products is a powerful method for double-bond location, particularly for monoenes (19). Reaction with alkaline permanganate/periodate proceeds through the diol resulting from reaction with dilute permanganate (see Section 4.3). The diol is split into two aldehydes by reaction with periodate, and the aldehydes are subsequently oxidized to carboxylic acids by permanganate. Alternatively, diols derived from double bonds are cleaved to aldehydes by lead tetraacetate or periodate.

Ozone reacts directly with double bonds under mild conditions and is the preferred degradative method for double-bond location (19). The reaction occurs in several steps (64), starting with a 1,3-dipolar cycloaddition (Figure 11). The addition product decomposes rapidly into an aldehyde and a carbonyl oxide. In the absence of solvent or in nonparticipating solvents, these recombine forming a relatively stable 1,2,4-trioxolane or ozonide. The separation into aldehyde and carbonyl oxide during this rearrangement is supported by production of six ozonide species from unsymmetrical olefins. Ozonides can be converted to a number of stable products; oxidation yields carboxylic acids, mild reduction gives aldehydes, and treatment with nickel and ammonia gives amines providing useful synthetic routes to difunctional compounds from fatty acids [e.g., Furniss et al. (67)]. In a carboxylic acid or alcohol solvent, the carbonyl oxide reacts with the solvent producing mainly



**Figure 11.** Ozonolysis reaction mechanism. In nonparticipating solvents, the carbonyl oxide (1) and aldehyde recombine to give the moderately stable ozonide (2). Hydroperoxides (3) are formed in protic solvents, and  $R''$  can be alkyl or acyl.

acyloxy or alkoxyhydroperoxides, respectively, along with other more complex products (68). These hydroperoxides are oxidized or reduced to the same products as the ozonides.

Ozonolysis is the only oxidative cleavage that is used industrially. Around 10,000 tons per year of azelaic acid (nonane-1,9-dioic acid) are produced along with pelargonic acid (nonanoic acid) by ozonolysis of oleic acid. Azelaic acid is used for polymer production and is not readily available from petrochemical sources. Other dibasic acids potentially available by this route are brassylic (tridecane-1,13-dioic) and adipic (hexane-1,6-dioic) acids from erucic (22:1 13*c*) and petroselinic (18:1 6*c*) acids, respectively. High-purity monoenes are required as feedstock to avoid excessive ozone consumption and byproducts. Ozonolysis is a clean reaction, carried out at low temperatures without catalyst. However, ozone is toxic and unstable, as are the intermediates. Industrial scale ozonolysis is carried out in pelargonic acid run countercurrent to ozone at 25–45°C followed by decomposition at 60–100°C in excess oxygen (69). Ozone must be generated continuously on-site by electrical discharge in air, and ozone production is the limiting factor for large-scale production (70).

Ruthenium oxide ( $\text{RuO}_4$ ) catalyzes oxidative cleavage of oleic acid to pelargonic and azelaic acids efficiently in the presence of  $\text{NaOCl}$  as an oxygen donor to regenerate  $\text{Ru(VIII)}$  (71). However, the production of halogen salt byproducts makes this impractical for large-scale production. Hydrogen peroxide and peracetic acid are cheaper and more environmentally benign oxidants, the byproduct from reaction or regeneration of peracid being water, but give very low yields with  $\text{RuO}_4$ . Ruthenium(III) acetylacetonate ( $\text{Ru}(\text{acac})_3$ ) with peracetic acid or  $\text{Re}_2\text{O}_7$  with hydrogen peroxide give moderate yields with internal double bonds, but ~80% conversion with terminal olefins. Terminal olefins, produced from fatty acids with an internal double bond by metathesis with ethylene, are converted to dibasic acids without

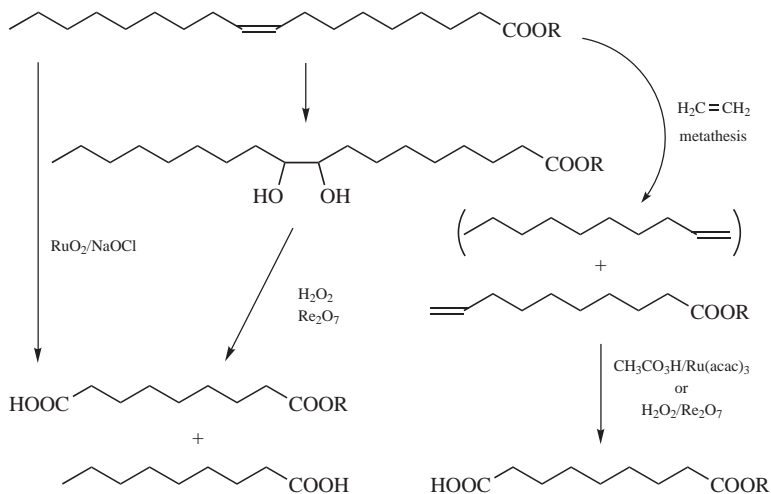


Figure 12. Alternative oxidative cleavage reactions.

concomitant production of monobasic acids. Diols produced by hydroxylation are cleaved by  $\text{Re}_2\text{O}_7$  with hydrogen peroxide to di- and monobasic acids (Figure 12). These reactions offer an alternative to ozonolysis for the production of dibasic acids, but they have still to be optimized for industrial application (71, 72).

## 5. REDUCTION

Both carbon-carbon double bonds and the carboxyl group of fatty acids can be reduced, either together or separately depending on the reaction conditions. Catalytic reduction is an important industrial route to hardened fats, fatty alcohols, and fatty amines, using well-established technologies.

### 5.1. Hydrogenation of Double Bonds

Transition metals such as Co, Ni, Cu, Ru, Pd, and Pt catalyze hydrogenation of double bonds. Palladium on charcoal or Adam's catalyst (platinum oxide) promote saturation of fatty acids at ambient temperature and hydrogen pressure. Hydrogenation is accompanied by exchange and movement of hydrogen atoms along the chain in the region of the double bonds, demonstrated by the large number of isotopomers formed on deuteration. Homogeneous deuteration with Wilkinson's catalyst (tris (triphenylphosphine)rhodium(I) chloride) proceeds without hydrogen movement or exchange (73) and in conjunction with GC-MS analysis is used to locate double bonds. Partial hydrogenation with hydrazine does not isomerize unreacted double bonds and is useful for structural analysis of polyenes and was recently used to examine long-chain metabolites of conjugated linoleic acid (CLA) (74).

### 5.2. Catalytic Partial Hydrogenation

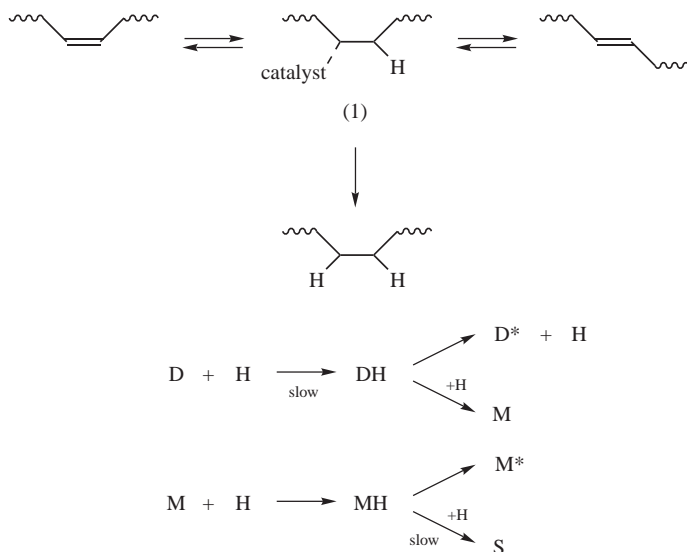
Partial hydrogenation reduces the polyene content of oils while maintaining or increasing the monoene content. Reduction of double bonds is accompanied by a variable degree of *cis*-to *trans*-isomerization. "Brush" hydrogenation of soybean or rape oil reduces linolenic content, improving oxidative stability, whereas more extensive hydrogenation increases solid fat content, producing "hardened" fats for spreads and shortenings. Partial hydrogenation has been used for the past century, in margarine production and remains an important process for edible fat modification (Chapter xx) despite concerns about adverse nutritional properties of *trans*-fatty acids. There are recent reviews of the mechanism (75, 76) and technology (77).

A number of uncertainties remain about the mechanism of the reaction and the factors controlling selectivity between polyenes and monoenes, and the balance between hydrogenation and isomerization. Hydrogenation is a three-phase reaction among liquid oil, gaseous hydrogen, and solid catalysts carried out as a batch process in autoclaves to maintain consistent products. Temperature, hydrogen pressure, amount and formulation of catalyst, and agitation are all carefully controlled.

Supported nickel is invariably used as catalyst. Although other catalysts are equally or more effective, nickel has widespread acceptance from long use, ease of removal, and low cost. Unremoved traces of other metals such as copper might also reduce the oxidative stability of the product.

The reaction mechanism must account for the selectivity of the reaction (polyenes reacting faster than monoenes) and the production of *trans*-monoenes. Hydrogen addition is in two steps with a semihydrogenated intermediate. Addition of the first hydrogen is reversible, regenerating a double bond with potentially altered position or geometry. Addition of a second hydrogen irreversibly produces a saturated bond (Figure 13). Dijkstra (76) proposed that for dienes, the formation of the semihydrogenated intermediate is rate determining and hydrogen concentration dependent, whereas for the conversion of monoene to saturate, the rate-determining and hydrogen concentration-dependent step is the addition of the second hydrogen. At low dissolved hydrogen concentrations, isomerization of monoenes is favored over saturation, allowing control of the product composition by hydrogen pressure, agitation, and reaction time.

Copper catalysts show different selectivity compared with nickel. Copper only catalyzes hydrogenation of methylene-interrupted systems, showing high selectivity for polyenes and no reaction with oleate or other monoenes produced by reduction of polyenes. The first step is production of conjugated dienes that are the species hydrogenated. Dijkstra recently reassessed this reaction, suggesting removal of an allylic hydrogen as the first step in production of the conjugated diene (78).



**Figure 13.** Partial hydrogenation. The partially hydrogenated intermediate (1) may lead to *cis* or *trans* unsaturated or saturated products. D—diene; M—monoene; S—saturate; \* potentially isomerized. Formation of  $M^*$  is favored at a low hydrogen concentration.

### 5.3. Production of Fatty Alcohols

Triacylglycerols, fatty acids, and esters can be reduced to aldehydes, alcohols, or hydrocarbons, the main application being the production of fatty alcohols. On a small scale, lithium aluminum hydride (in excess of stoichiometric requirement) is a convenient reducing agent for the carboxyl group without affecting polyunsaturated chains. Industrially, catalytic hydrogenation is used and has been reviewed (79, 80).

Long-chain alcohols are produced from both oleochemical and petrochemical sources. Oils and fats provide straight chain lengths not readily available otherwise and the possibility of unsaturated chains. The main feed stocks are coconut and palm-kernel oil for  $C_{12}$ – $C_{14}$  alcohols and technical grades of tallow and palm oil for  $C_{16}$ – $C_{18}$  alcohols. The preferred starting material for catalytic hydrogenation is methyl ester. Fatty acids are corrosive and need harsh reaction conditions, leading to unwanted byproducts. Reduction of intact oils leads to loss of glycerol, a valuable byproduct, through over-reduction to propane diol and propanol, as well as excessive hydrogen and catalyst consumption. Methyl esters are reduced to saturated alcohols with copper chromite catalyst (~2%) at 250–300°C and 25–30-MPa (250–300 bar) hydrogen in a suspension system or at 200–250°C with a fixed-bed catalyst. The methanol produced is recycled for methyl ester production. Zinc-based catalysts do not hydrogenate double bonds and are used to produce unsaturated alcohols such as oleyl alcohol.

## 6. PRODUCTION OF SURFACE ACTIVE COMPOUNDS AND OLEOCHEMICALS

The main non-food use of oils and fats is the production of surfactants. The amphiphilic properties of fatty acids, exploited for centuries in the use of soaps, can be modified by changing the carboxyl group into other hydrophilic groupings, giving anionic, cationic, amphoteric, and nonionic surfactants. There is also scope for functionalizing the aliphatic chain, but this has not been widely used commercially. The chain length of the feed stock,  $C_{12}$ – $C_{14}$  from lauric oils,  $C_{22}$  from high erucic rape and fish oils, and  $C_{16}$ – $C_{18}$  from most other sources, can be used to modify solubility. The main starting materials for surfactant production are fatty acids and alcohols with a range of N-containing derivatives produced through amides and amines. Surfactants of oleochemical origin may biodegrade better than petrochemical products, giving an environmental benefit in addition to being derived from renewable resources. Recently, surfactants have been produced from fully renewable resources. Oleochemical surfactant production has been reviewed (81–85).

### 6.1. Nitrogen-Containing Compounds

The presence of nitrogen, either in a neutral or cationic group, gives surfactant properties that are not easily produced with other compounds. A diverse range of nitrogen-containing compounds are produced, for which the starting point is an

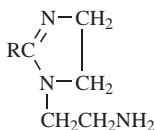


TABLE 7. Routes to Nitrogen-Containing Surfactants.

	Product
$RCH_2NH_2 + CH_2O \rightarrow$ (reduction) $\rightarrow RCH_2NMe_2$	tertiary amine
$RCH_2CONMe_2 \rightarrow$ (reduction) $\rightarrow RCH_2NMe_2$	tertiary amine
$RCH_2OH + Me_2NH \rightarrow$ (catalytic hydrogenation) $\rightarrow RCH_2NMe_2$	tertiary amine
$ROH + CH_2=CHCN \rightarrow RO(CH_2)_2CN \rightarrow$ (reduction) $\rightarrow RO(CH_2)_3NH_2$	etheramine
$RNH_2 + CH_2=CHCN \rightarrow RNH(CH_2)_2CN \rightarrow$ (reduction) $\rightarrow RNH(CH_2)_3NH_2$	diamine
$RNH(CH_2)_3NH_2 + CH_2=CHCN \rightarrow RNH(CH_2)_3NH(CH_2)_2CN \rightarrow$ (reduction) $\rightarrow RNH(CH_2)_3NH(CH_2)_3NH_2$	triamine
$RO(CH_2)_3NH_2 + 2nCH_2(O)CH_2 \rightarrow RO(CH_2)_3N((CH_2CH_2O)_nH)_2$	ethoxylated etheramine
$RNH(CH_2)_3NH_2 + 2nCH_2(O)CH_2 \rightarrow RNH(CH_2)_3N(CH_2CH_2O)_nH)_2$	ethoxylated diamine
$RNH_2 + nCH_2(O)CH_2 \rightarrow H(OCH_2CH_2)_nN(R)(CH_2CH_2O)_nH$	ethoxylated amine
$RN(Me)_2 + (H_2O_2) \rightarrow RN^+(Me)_2O^-$	amine oxide
$RN(Me)_2 + (MeCl \text{ or } Me_2SO_4) \rightarrow RN^+(Me)_3 X^-$	quaternary amine
$R_3N + (\text{benzyl chloride}) \rightarrow R_3N^+Bz X^-$	quaternary amine
$RCOOH + NH_2(CH_2)_2NH(CH_2)_2NH_2 \rightarrow 4$	imidazoline
$2RCOOH + (HOCH_2CH_2)_2NCH_3 \rightarrow (RCOOCH_2CH_2)_2NCH_3 + H_2O$	ester amine

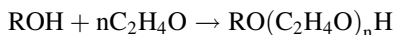
amide or amine. Amides are formed by direct reaction of the fatty acid and ammonia at 180–200°C and 0.3–0.7 MPa (3–7 bar), through dehydration of the initially formed salt. Long-chain amides, e.g., erucamide, are the principle industrial products, used as polythene film additives.

Amines are produced from fatty acids in a reaction sequence in which the nitrile is an intermediate. Nitriles are produced by reaction of the fatty acid with ammonia, giving the amide that is dehydrated in situ at 280–360°C in the liquid phase on a zinc oxide, manganese acetate, or alumina catalyst. Lower temperature and longer reaction times are used with unsaturated fatty acids to avoid polymerization. Hydrogenation with nickel or cobalt catalyst reduces the nitrile to amines via the aldimine ( $RCH=NH$ ). Depending on the reaction conditions, the aldimine reacts with hydrogen or primary or secondary amines, giving primary, secondary, or tertiary amines, respectively, as the major product. Primary amines are produced at 120–180°C and 2–4 MPa (20–40 bar); higher temperature and lower pressure favors production of secondary and tertiary amines with a symmetrical substitution at the nitrogen. The long-chain composition closely reflects the fatty acid composition of the feedstock, although hydrogenation conditions can be adjusted to hydrogenate the alkyl chains or induce *cis-trans*-isomerism. The more widely used unsymmetrical tertiary amines are produced from primary amines, amides, or alcohols (Table 7). Reactions converting amines to other surface-active derivatives and for the preparation of other nitrogen-containing compounds are shown in Table 7. These have appeared in several reviews (2, 82, 84, 86, 87).



## 6.2. Ethoxylation

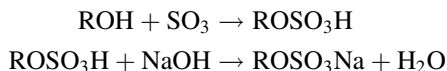
Long-chain molecules with active hydrogen (alcohols, amines, and amides) react as nucleophiles with ethylene oxide usually with a basic catalyst. The product has a hydroxyl group that can react with further ethylene oxide, leading to polyoxyethylene products with a range of molecular weights. The average number of ethylene oxide molecules added depends on the reaction conditions and can be adjusted to alter the solubility and surfactant properties of the product.



Typical reaction conditions are 120–200°C and pressures of 0.2–0.8 MPa (2–8 bar) with potassium hydroxide or sodium alcoholates as catalyst (83). In the reaction with primary amines, both active hydrogens are replaced before further ethylene oxide addition leading to dipolyoxyethylene derivatives. Polyoxyethylenes have a terminal hydroxyl that may be further functionalized under conditions that do not damage the ether linkages, for example, sulfation.

## 6.3. Sulfation

Sulfate esters of alcohols or polyoxyethylene alcohols are prepared by reaction with sulfur trioxide in continuous falling-film plants, immediately followed by neutralization with sodium hydroxide to give the sodium salt (81).



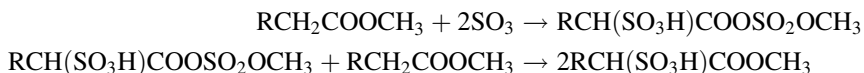
Alcohol sulfates are not stable in acid and are used in alkaline formulations. C<sub>12</sub>–C<sub>16</sub> alcohol sulfates have excellent detergency, high foam, and good wetting properties. Alcohol sulfates are fully biodegradable under aerobic and anaerobic conditions and compete in performance with petrochemical-derived linear alkylbenzene sulfonates (LABS).

Mono- and diacylglycerols are starting materials for sulfate ester surfactants that can be prepared directly from triacylglycerols without reduction to the fatty alcohol. Cocomonooacylglycerol sulfates, used in cosmetic formulations, are produced in a solvent-free process (88). Glycerolysis of coconut oil (mole ratio of glycerol to oil of 2:1) gives the raw material for sulfatization, predominantly mono- and diacylglycerols. Membrane filtration is used to desalt the product.

## 6.4. $\alpha$ -Sulfonates

The methylene adjacent to the carboxyl group is sufficiently activated to react with sulfur trioxide, giving  $\alpha$ -sulfonate products. As allylic methylenes are similarly activated, the reaction is usually carried out with saturated starting materials. The complex reaction involves two moles of sulfur trioxide, giving a disulfonate intermediate that reacts with methyl ester to give the  $\alpha$ -sulfonate ester, or on treatment

with sodium hydroxide the disodium salt (81).  $\alpha$ -Sulfonates have low toxicity and are fully biodegradable.

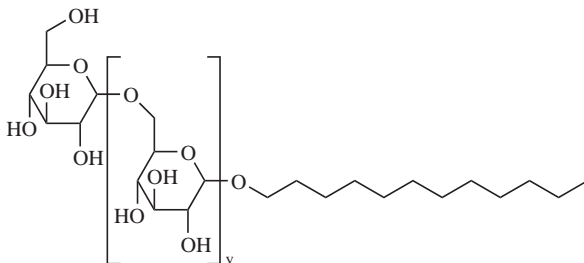


## 6.5. Carbohydrate-Based Surfactants

Carbohydrates and related polyols (as well as amino acids) have attracted attention as the hydrophilic component of nonionic surfactants, particularly as a benign alternative to manufacture using ethylene oxide. Sucrose, glucose, and sorbitol (from hydrogenation of glucose) are available in quantity from renewable resources. Although sorbitol esters have been in use for many years, large-scale synthesis of sugar esters remains difficult because of the similar reactivity of all the carbohydrate hydroxyls, leading to many molecular species in the product. Further difficulties are the insolubility and charring of the carbohydrate in the reaction medium. A more controllable reaction is that between long-chain alcohols and glucose, giving alkyl polyglycosides with the fatty alcohol ether linked only to position C-1 on the glucose ring. Further glucose units are also joined through ether links. Both the alcohol and glucose can be produced from renewable resources (oils and fats and starch, respectively), and the reaction can be carried out in a solvent-free system. In commercial production, glucose is suspended in excess alcohol and reacted at 100–120°C with a sulfonic acid catalyst. The product has an average degree of polymerization of 1.2 to 1.7 glucose units per molecule (Figure 14) and is nonirritant and fully biodegradable (88–91). Alkyl polyglycoside production is currently  $\sim 100,000$  tons per year, which is used in detergent formulations in place of petrochemical-derived products.

## 6.6. Dimers and Estolides

A number of different dimers and oligomers are produced from fatty acids and alcohols. These are branched-chain compounds with significantly lower melting points than straight chain structures of similar molecular weight. Fully saturated dimers



**Figure 14.** Alkyl polyglycoside. Degree of polymerization =  $y + 1$ .

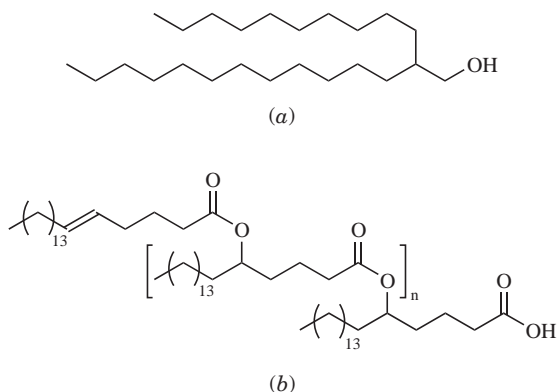
have excellent oxidative stability. This and their extended liquid range are exploited in their use as lubricants and cosmetic additives. Polyfunctional dimers are used in polymer formulations.

*Dimer acids.* Dimer acids are produced by heating monoene or diene fatty acids (e.g., tall oil acids, a byproduct of wood pulping) with a cationic clay catalyst (92). Typical conditions are 4% montmorillonite at 230°C for 4–8 hours. After distillation, the product is a complex mixture of acyclic, cyclic, and bicyclic dimers along with some trimer. Dimer acids are dibasic and react with diamines and triamines to give polyamides. Imidazole derivatives are used as corrosion inhibitors and esters as lubricants.

*Guerbet compounds.* Guerbet alcohols have been known for over a century and are produced by the alkali catalyzed dimerization of aliphatic alcohols with accompanying loss of water. Typical reaction conditions are heating at 200–300°C with potassium hydroxide in the presence of transition metal compounds to catalyze the intermediate reduction step. Dehydrogenation of the alcohol to the aldehyde is followed by aldol condensation and rehydrogenation to give the branched-chain alcohol (Figure 15a).

The alcohols can be oxidized to the corresponding acids. Guerbet alcohols, acids, their esters, sulfates, and ether sulfates are used as lubricants, cosmetic additives, and surfactants. Their synthesis, characterization, and applications have been reviewed (93).

*Estolides.* Estolides are ester-linked branched-chain compounds. They are normally produced under harsh conditions similar to those used to produce dimer acids, but with the addition of around 10% water. Mono- and polyestolides are used as lubricants, greases, and surfactants, and in cosmetic, ink, and plastic formulations. Estolides biodegrade rapidly and completely, at rates comparable with the vegetable oils and fatty acids from which they are derived (94), making them environmentally benign products. The  $\Delta^5$  monoene acids in meadowfoam oil form estolides under



**Figure 15.** (a) Guerbet alcohol from lauryl alcohol (12:0). (b) Estolide from meadowfoam acids (20:1 5c).

mild acid catalysis, neighboring group participation by the carboxyl group facilitating the reaction (Figure 15b) (95). The product from meadowfoam acids shows higher regioselectivity than that from acids with mid-chain olefins where the double bond is further from the carboxyl group. Estolides from mid-chain olefins have significantly lower pour points than the corresponding fatty acids or triacylglycerols, but those from meadowfoam acids show little difference.

## 7. MODIFYING FATTY ACID STRUCTURE

Isomerization and conjugation change the properties of natural methylene-interrupted fatty acids, leading to new applications and potential added value. Chain shortening or extension produces fatty acids not readily isolated from natural sources and is also used to introduce radioactive or stable isotope labels. Metathesis provides a flexible method for modifying the alkyl chain.

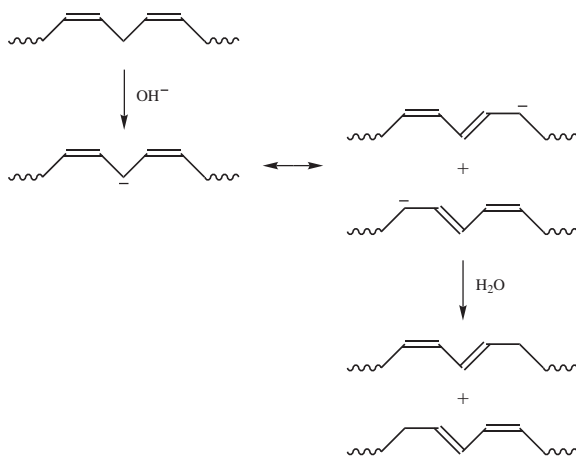
### 7.1. Isomerization

*Trans*-isomers of fatty acids are more stable thermodynamically than *cis*-isomers, because of reduced steric crowding; the equilibrium ratio is approximately 4:1 *trans:cis*. There is a considerable energy barrier to interconversion ( $\sim 125$  kJ/mole). Before the attached groups can rotate about the double bond, it has to be weakened by coordination to a catalyst, high temperature, or temporary conversion to a single bond through addition and elimination reactions. Chemical isomerization agents leading to an equilibrium mixture include selenium (through a  $\pi$ -complex) and nitrogen oxides or thiols (through free-radical addition/elimination).

*Cis*-to *trans*-isomerization accompanies partial hydrogenation (see Section 5.2) and may be exploited to raise the melting point. Unwanted isomerization occurs during physical refining at temperatures above 250°C. More unsaturated acids isomerize faster, making linolenic containing seed oils (e.g., soybean and canola) particularly vulnerable. Conditions for deodorizing rape oil without isomerization have been optimized following a detailed study and development of a model of the isomerization kinetics (96).

### 7.2. Conjugation

Heating with alkali has long been used to produce conjugated drying oils for paints and varnishes. The anion resulting from removal of a bis-allylic methylene rearranges through migration and isomerization, giving a *cis,trans*-conjugated system (Figure 16). Thus, linoleic acid (18:2 9*c*12*c*) gives both 9*c*11*t* and 10*t*12*c* isomers, whereas trienes give a mixture of partially and fully conjugated isomers depending on whether the middle or an outer double bond migrates first. Under the harsh conditions used to prepare drying oils (aqueous alkali at  $\sim 230^\circ\text{C}$ ), a complex mixture of isomers is eventually formed, but under controlled conditions (e.g., KOH in



**Figure 16.** Alkali-induced conjugation of methylene-interrupted olefins.

propylene glycol at  $150^\circ\text{C}$ ), a mixture containing only the *9c11t* and *10t12c* CLA isomers is produced (97). This product and individual isomers prepared from the mixture are used as nutritional supplements.

Thermal isomerization of linoleic acid produces a conjugated isomer mixture that does not contain all possible *cis*- and *trans*-isomers. The absence of the *8c10t* and *11t13c* isomers suggests a concerted pericyclic mechanism that limits the geometrical possibilities for the rearranged double bonds (98).  $[\text{RhCl}(\text{C}_8\text{H}_{14})_2]_2$  in the presence of (*p*- $\text{CH}_3\text{C}_6\text{H}_4$ ) $_3\text{P}$  and  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  is an efficient homogeneous catalyst for the conjugation of linoleic acid, producing conjugated soybean oil with exceptional drying properties and high solvent resistance in high yield (99).

### 7.3 Chain Shortening and Extension

Fatty acids can be labeled at the carboxyl carbon with  $^{13}\text{C}$  or  $^{14}\text{C}$  by chain shortening followed by chain extension with labeled carbon. Chain shortening to the n-halide using the Hunsdieker reaction (decarboxylation of fatty acid silver salts in the presence of halogens) is only suitable for saturated acids, but unsaturation is not altered using the alternative developed by Barton employing *N*-hydroxy-pyridine-2-thione in a halocarbon solvent (100). Chain extension with labeled cyanide followed by hydrolysis or reaction of the derived Grignard reagent with labeled carbon dioxide gives the labeled fatty acid. The Barton decarboxylation was recently used to prepare gram quantities of 1- $^{13}\text{C}$ -linoleic and 1- $^{13}\text{C}$ -linolenic acids for metabolic studies (101).

Two-carbon chain extension at the carboxyl end, mimicking biosynthesis, uses the malonic ester route (102). After reduction of the carboxyl to an alcohol, the readily displaced mesylate is prepared and reacted with sodium diethylmalonate. Saponification and decarboxylation gives the chain extended product in high yield.

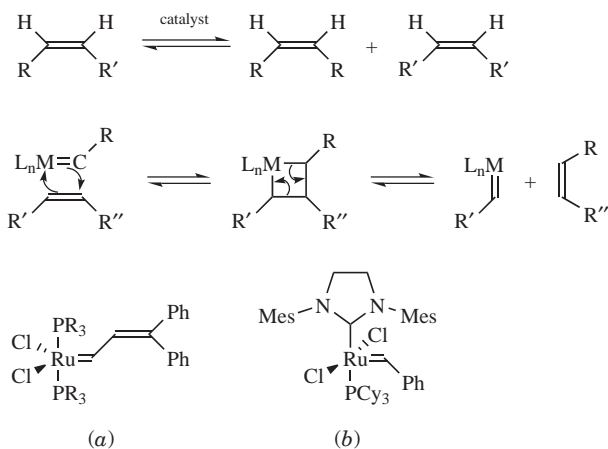


Figure 17. Olefin metathesis reaction and mechanism. (a) and (b) Grubb catalysts.

This is an efficient route to C<sub>20</sub> polyenes, not easily isolated from natural sources, starting from readily available C<sub>18</sub> sources.

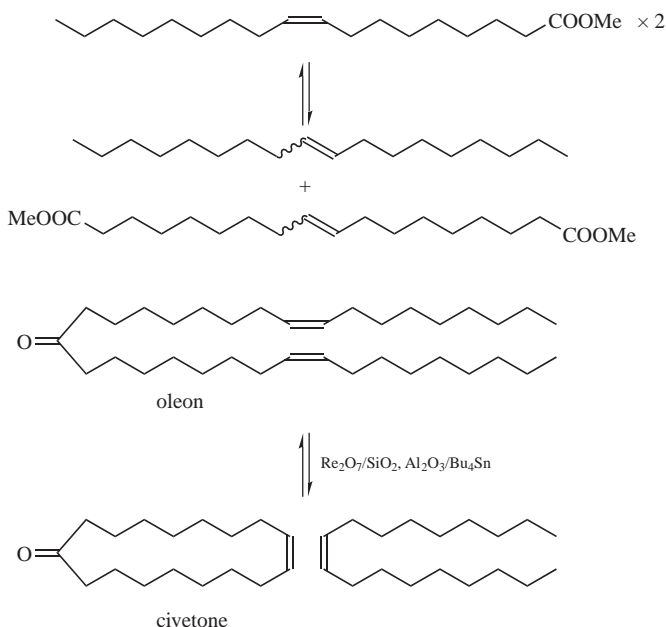
Metathesis (see Section 7.4) provides a flexible route to longer and shorter chains after reaction at a (usually monoene) double bond.

## 7.4 Olefin Metathesis

Olefin metathesis is the catalytic exchange of groups attached to a double bond. It presents a number of interesting possibilities for modifying the alkyl chain of fatty acids (Figure 17).

The mechanism involves a [2,2] cycloaddition between a transition metal alkylidene complex and the olefin, resulting in an intermediate metallacyclobutane (103). The metallacycle breaks in the opposite way to give a new alkylidene and a new olefin. Repeated exchange at the metal results in an equilibrium mixture of olefins, usually as an equilibrium mixture of *cis*- and *trans*-isomers. The reaction is used in the petrochemical industry to modify hydrocarbon structure, using catalysts such as WCl<sub>6</sub>/SnMe<sub>4</sub> or Re<sub>2</sub>O<sub>7</sub>/Al<sub>2</sub>O<sub>3</sub>. These catalysts are less active when other functional groups compete for the active site, and the application of metathesis in oleochemistry has paralleled development of novel catalysts, such as Grubb catalysts, containing sterically hindered metal alkylidenes (Figure 17a,b).

Self-metathesis describes the reaction of an unsaturated fatty acid with itself. For example, methyl oleate gives a mixture of starting material (50%), unsaturated hydrocarbon (25%), and long-chain unsaturated diester (25%), all as a mixture of *cis*- and *trans*-isomers. (Figure 18). The diester can be converted to the musk component civetone, but a more efficient route is through self metathesis of the ketone oleon derived from methyl oleate by Claisen condensation (104) (Figure 18).



**Figure 18.** Self-metathesis reactions.

Cross-metathesis of an unsaturated fatty ester with a normal alkene is a versatile way of producing chain-shortened or chain-extended homologues leading to oleochemicals with chain lengths outside the  $\text{C}_{16}$ – $\text{C}_{22}$  range of most commodity oils. Methyl oleate reacts with hex-3-ene, in large excess to suppress self-metathesis and push the reaction toward the  $\text{C}_{12}$  ester and hydrocarbon products.  $\omega$ -Olefins may be chain extended similarly, the ethene produced being removed to drive the reaction to completion. Cross metathesis provides a route to compounds otherwise difficult to obtain, for example, triacontanol from reduction of the product from methyl erucate and 1-octadecene. Ethenolysis (cross-metathesis with ethene) produces shorter chain  $\omega$ -olefins with a wide range of applications. A high pressure of ethene is used to force the reaction to the desired products.  $\omega$ -Olefins produced either by metathesis or from pyrolysis of castor oil can be coupled to give long-chain dibasic acids (105).

Metathesis of intact oils produces polymeric products resulting from intra- and intermolecular bond formation, and they can be used to produce high-viscosity stand oils from drying oils without the loss of double bonds that occurs on thermal polymerization. Vegetable oils can be metathesized efficiently at low temperature and pressure using Grubb's ruthenium catalyst  $(\text{C}_3\text{P})_2\text{Cl}_2\text{Ru}=\text{CHPh}$ , without the rigorous exclusion of water and oxygen required with  $\text{WCl}_6/\text{SnMe}_4$  (106). Pretreatment of the oil with silica gel may be required.

As a reaction with 100% atom efficiency achieved at moderate temperature ( $<100^\circ\text{C}$ ) using renewable resources, metathesis has potential in a sustainable



chemical industry. A recently developed catalyst (Figure 17b) has an efficiency that justifies industrial application in the production of fine chemicals (106). The hydrocarbon byproducts of metathesis, for example,  $\alpha$ -olefins, are also valuable starting materials. Metathesis in oleochemistry, in the context of green chemistry, has recently been reviewed (107).

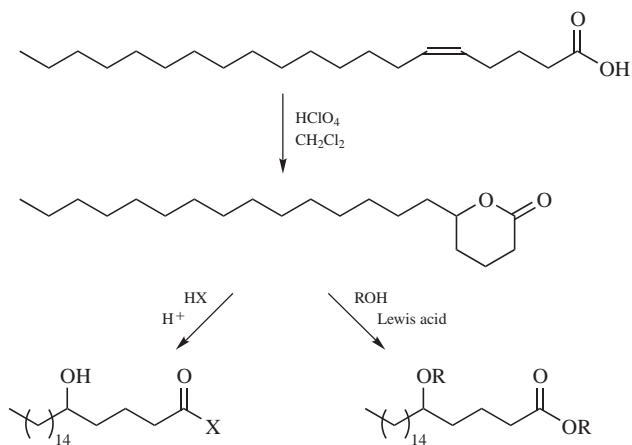
## 8. NOVEL CHEMISTRY FOR FUNCTIONALIZING THE ALKYL CHAIN

Oils and fats are renewable resources for the chemical industry. Increasing the range of oleochemicals that can be produced could add value to existing crops and provide a market for new crops, driving research into novel fatty acid derivatives. Most current oleochemical production involves reaction at the carboxyl group, with the chain length and unsaturation of the alkyl chain chosen to give the desired melting behavior or hydrophobicity. Introducing functionality to the alkyl chain through radical, electrophilic, nucleophilic, pericyclic, and transition metal catalyzed addition to carbon-carbon double bonds leads to novel compounds with commercial potential. Only a small selection of recent research is illustrated here, focusing on three promising approaches: neighboring group participation, Friedel Crafts acylation, and free-radical addition reactions.

Functionalizing the alkyl chain places more emphasis on the structure of the fatty acids used as feedstock. Model reactions use single fatty acids, often monoenes with particular double-bond positions. Large-scale use of these reactions needs oils rich in single fatty acids to maintain the purity of the product and minimize wasteful side reactions. Suitable feedstocks may be current crops such as high oleic or high erucic varieties or new crops with unusual fatty acids (Chapter xx). Petroselenic acid (18:1 6c) from umbelliferae oils and 5-eicosenoic acid (20:1 5c) from meadowfoam oil are of particular interest as distinctive products can result from neighboring group participation. Breeding to increase the monoene content of some oils may be desirable.  $\omega$ -Olefins are useful starting materials; 10-undecenoic acid is available from pyrolysis of castor oil, and others may be produced by metathesis (see Section 7.4). Recent, wide-ranging reviews of this area are available (4, 5, 108)

### 8.1. Neighboring Group Participation

Neighboring group participation is the involvement of a nearby functional group in the reaction of another functional group. It may influence the regioselectivity of the reaction or lead to specific products, often as a result of cyclization to five- and six-membered rings. Neighboring group participation reactions of fatty acids were reviewed recently (109) and can be used to introduce mid-chain functionality, including heterocyclic groups. Double bonds and the carboxyl group usually react independently of each other, but  $\Delta 4$  and  $\Delta 5$  bonds may interact with the carboxyl through neighboring group participation leading to  $\gamma$ - and  $\delta$ -lactones (five- and



**Figure 19.** Neighboring group participation leading to lactones and other products from  $\Delta 5$  acids.  $X = \text{OH}$ ,  $\text{RO}$ , or  $\text{RNH}$ .

six-membered rings, respectively). The  $\Delta 5$  acids from meadowfoam oil readily form lactones when refluxed with perchloric acid. The proportion of  $\delta$ - and  $\gamma$ -lactones depends on the solvent: 6:1 in hexane and 40:1 in dichloromethane. The  $\delta$ -lactone is formed faster, but the  $\gamma$ -lactone is the more thermodynamically stable isomer. High dilution and a nonparticipating polar solvent that stabilizes the intermediate cation favor kinetic control of the reaction (110). The lactones can be ring opened by treatment with water, alcohols, and amines in acid, giving 4- and 5-hydroxy acids, esters, and amides (111); alternatively, treatment with an alcohol and a Lewis acid catalyst under more vigorous conditions results in an alkyl group ether linked to the chain (112) (Figure 19).

## 8.2. Friedel Crafts Acylation

Friedel Crafts acylation with an acyl chloride and Lewis acid catalyst is more often associated with aromatic compounds. Ethylaluminium dichloride ( $\text{EtAlCl}_2$ ) is an effective catalyst for the acylation of aliphatic olefins, including fatty acids and alcohols, giving  $\beta,\gamma$ -unsaturated ketones (113). The reaction occurs with both terminal and internal double bonds, with the acyl group becoming attached to one of the double-bond carbons while the double bond migrates one carbon. Reaction at terminal olefins is regiospecific with addition to the terminal carbon giving a linear product and a predominantly *trans*-double bond. Internal double bonds give an approximately equal mixture of *trans*-regioisomers (Figure 19).  $\alpha,\beta$ -Unsaturated acid chlorides give allyl vinyl ketones that undergo Nazarov cyclization to prostaglandin- and jasmonate-like molecules (Figure 20) (114). Neighboring group participation in petroselenic acid (18:1 6c) leads to intramolecular cyclization (115). Friedel Crafts acylation is a flexible route to new and highly functionalized oleochemicals containing reactive allyl keto functions (115).

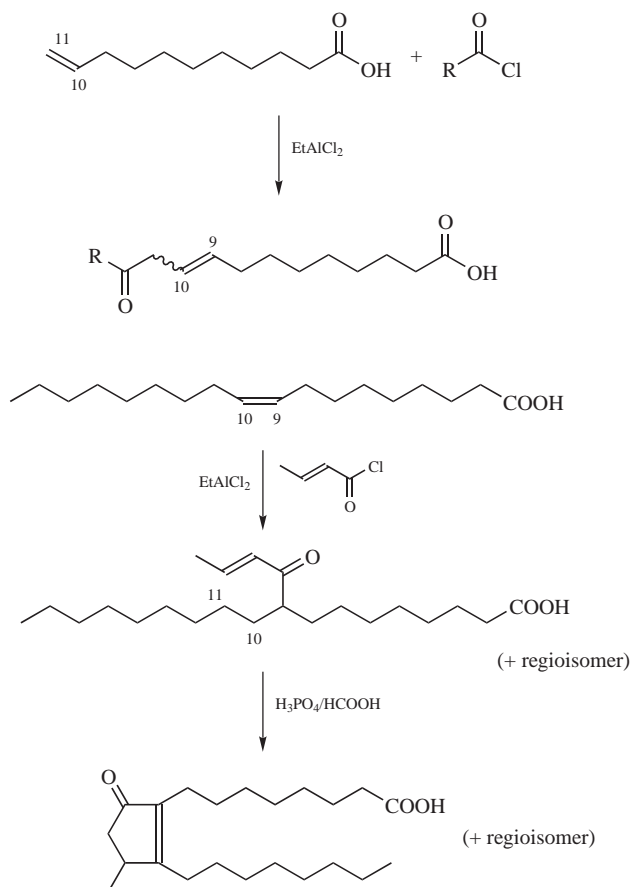
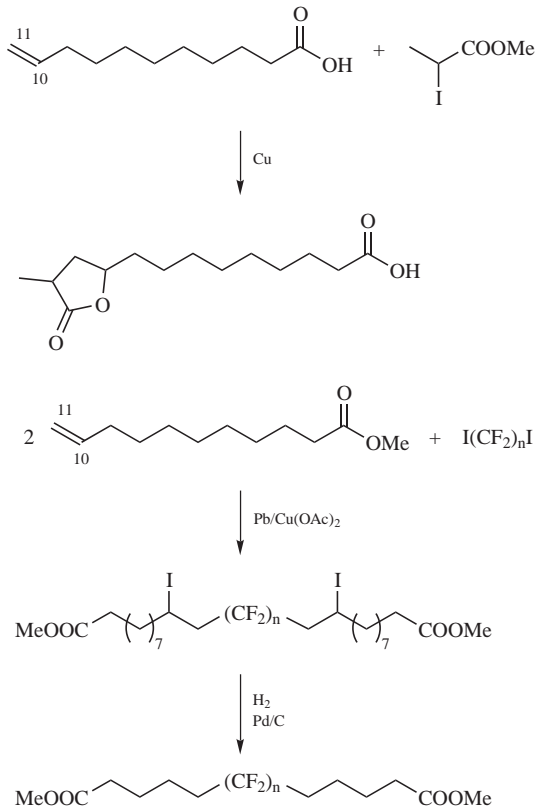


Figure 20. Friedel Crafts acylation reactions.

### 8.3. Free Radical Addition Reactions

Double bonds participate in free radical addition reactions, and these can be of synthetic use in introducing functional groups (116). A particularly simple reaction is the preparation of  $\gamma$ -lactones by solvent-free addition of 2-halocarboxylates to fatty esters, catalyzed by commercial copper powder at 100–130°C (117). Iodides are most reactive and can be prepared in situ from more readily available bromides and sodium iodide (Figure 21).

Perfluoro alkyl iodides add to both terminal and internal double bonds when the reaction is initiated by electron transfer from metals such as finely divided silver, copper powder, and lead with copper acetate. Using an  $\omega$ -olefin and a perfluoro-alkyl- $\alpha,\omega$ -diiodide, a perfluoro group can be inserted into a long-chain compound (118) (Figure 21). Deiodination of the product by catalytic reduction results in highly hydrophobic alkyl chains with interesting surfactant properties.



**Figure 21.** Radical addition reactions.

## REFERENCES

1. C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry, Nutrition, and Biotechnology*, 2nd ed., Marcel Dekker, Inc., New York, 2002.
2. F. D. Gunstone, *Eur. J. Lipid Sci. Technol.*, **103**, 307–314 (2001).
3. F. D. Gunstone and R. J. Hamilton, eds., *Oleochemical Manufacture and Applications*, Sheffield Academic Press, Sheffield, U.K., 2001.
4. U. Biermann, W. Friedt, S. Lang, W. Lühs, G. Machmüller, J. O. Metzger, M. Rüschen, H. J. Schäfer, and M. P. Schneider, *Angew. Chem. Int. Ed.*, **39**, 2206–2224 (2000).
5. G. Knothe and J. T. P. Derksen, eds., *Recent Developments in the Synthesis of Fatty Acid Derivatives*, AOCS Press, Champaign, Illinois, 1999.
6. F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, Inc., New York, 1997.
7. A. Karleskind, ed., *Oils and Fats Manual*, Vols. 1 and 2, Intercept Ltd., Andover, U.K., 1996.
8. F. D. Gunstone, *Fatty Acid and Lipid Chemistry*, Blackie Academic and Professional, London, U.K., 1996.

9. F. D. Gunstone, J. L. Harwood, and F. B. Padley, eds., *The Lipid Handbook*, 2nd ed., Chapman and Hall, London, U.K., 1994.
10. H. Baumann, M. Bühler, H. Fochem, F. Hirsinger, H. Zoebelein, and J. Falbe, *Angew. Chem. Int. Ed.*, **27**, 41–62 (1988).
11. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, Section I, American Oil Chemists' Society, Champaign, Illinois, 1997.
12. E. Ucciani, *Dictionnaire des Huiles Végétales*, Lavoisier Publishing, Paris, 1995.
13. R. O. Adlof and F. D. Gunstone. (2001). Available: <http://www.aocs.org/member/division/analytic/fanames.htm>.
14. M. J. Parnham, *Inform* **7**, 1168–1175 (1996).
15. *Official Methods and Recommended Practices of the American Oil Chemists' Society, Fifth Edition*, Sections Cd 1 and Cd 3, American Oil Chemists' Society, Champaign, Illinois, 1998.
16. H. Li, F. R. van de Voort, A. A. Ismail, J. Sedman, R. Cox, C. Simard, and H. Buijs, *J. Amer. Oil Chem. Soc.*, **77**, 29–36 (2000).
17. H. Li, F. R. van de Voort, J. Sedman, and A. A. Ismail, *J. Amer. Oil Chem. Soc.*, **76**, 491–497 (1999).
18. M. H. Gordon, in M. Jee, ed., *Oils and Fats Authentication*, Blackwell, Oxford, 2002, pp. 143–155.
19. W. W. Christie *Lipid Analysis*, The Oily Press, Bridgwater, UK, 3rd Edition, in press 2002.
20. N. O. V. Sonntag, in R. W. Johnson and E. Fritz, eds., *Fatty Acids in Industry*, Marcel Dekker, Inc., New York, 1988. pp. 23–72.
21. D. R. Kodali, *J. Lipid Res.*, **28**, 464–469 (1987).
22. F. D. Gunstone, in F. D. Gunstone, ed., *Lipid Synthesis and Manufacture*, Sheffield Academic Press, Sheffield, U.K., 1999, pp. 321–346.
23. W. W. Christie. (2002, April 29). *The Lipid Library*. Available: <http://www.lipid.co.uk/infores/select.html>.
24. Y. M. Choo, A. N. Ma, and A. S. H. Ong, in F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, Inc., New York, 1997, pp. 771–773.
25. G. Knothe and R. O. Dunn, in F. D. Gunstone and R. J. Hamilton, eds., *Oleochemical Manufacture and Applications*, Sheffield Academic Press, Sheffield, U.K., 2001, pp. 128–132.
26. K.-L. Lee, T. A. Foglia, and Y.-S. Chang, *J. Amer. Oil Chem. Soc.*, **79**, 191–195 (2002).
27. Y. Watanabe, Y. Shimada, A. Sugihara, and Y. Tominga, *J. Amer. Oil Chem. Soc.*, **78**, 703–707 (2001).
28. A. Rozendaal and A. R. Macrae, in F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, Inc., New York, 1997, pp. 223–245.
29. D. Rousseau and A. G. Marangoni, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry, Nutrition, and Biotechnology*, 2nd ed., Marcel Dekker, Inc., New York, 2002, pp. 301–333.
30. F. D. Gunstone, *J. Sci. Food Agric.*, **79**, 1535–1549 (1999).
31. X. Xu, *Eur. J. Lipid Sci. Technol.*, **102**, 287–303 (2000).
32. C. F. Torres, E. Barrios, and C. G. Hill, *J. Amer. Oil Chem. Soc.*, **79**, 457–466 (2002).

33. C. C. Akoh and X. Xu, in T. M. Kuo and H. W. Gardner, eds., *Lipid Biotechnology*, Marcel Dekker, Inc., New York, 2002, pp. 461–469.
34. M. W. Christensen, L. Andersen, O. Kirk, and H. C. Holm, *Lipid Technol. Newslett.*, **7**, 33–37 (2001).
35. P. M. Nielsen, *Oils and Fats International*, **July**, 18–19 (2002).
36. Y. Shimada, N. Fukushima, H. Fujita, Y. Honda, A. Sugihara, and Y. Tominga, *J. Amer. Oil Chem. Soc.*, **75**, 1581–1586 (1998).
37. Y. Shimada, A. Sugihara, M. Shibahiraki, H. Fujita, H. Nakano, T. Nagao, T. Terai, and Y. Tominaga, *J. Amer. Oil Chem. Soc.*, **74**, 1465–1470 (1997).
38. Y.-H. Ju and T.-C. Chen, *J. Amer. Oil Chem. Soc.*, **79**, 29–32 (2002).
39. Y. Shimada, K. Maruyama, A. Sugihara, T. Baba, S. Komemushi, S. Moriyama, and Y. Tominaga, *J. Amer. Oil Chem. Soc.*, **75**, 1565–1571 (1998).
40. S. A. Spurvey, S. P. J. N. Senanayake, and F. Shahidi, *J. Amer. Oil Chem. Soc.*, **78**, 1105–1112 (2001).
41. E. N. Frankel, *Lipid Oxidation*, The Oily Press, Dundee, U.K., 1998.
42. E. N. Frankel, *Lipid Oxidation*, The Oily Press, Dundee, U.K., 1998, pp. 19 and 43–54.
43. E. N. Frankel, *J. Oleo Sci.*, **50**, 387–391 (2001).
44. K. Miyashita, *Lipid Technol. Newslett.*, **8**, 35–41 (2002).
45. T. I. Hämäläinen, S. Sundberg, T. Hase, and A. Hopia, *Lipids*, **37**, 533–540 (2002).
46. A. Bodin, M. Linnerborg, J. L. G. Nilsson, and A. T. Karlberg, *J. Surfactants Deterg.*, **5**, 107–110 (2002).
47. H. R. Rawls and P. J. Van Santen, *J. Amer. Oil Chem. Soc.*, **47**, 121–125 (1970).
48. F. H. Doleiden, S. R. Farenholtz, A. A. Lamola, and A. M. Trozzolo, *Photochem. Photobiol.*, **20**, 519–521 (1974).
49. D. B. Min, in C. C. Akoh and D. B. Min eds., *Food Lipids: Chemistry, Nutrition and Biotechnology*, Marcel Dekker, Inc., New York, 1998, pp. 289–292.
50. N. Macfarlane, J. Salt, R. Birkin, and A. Kendrick, *Inform*, **12**, 244–249 (2001).
51. P. Schuler, in B. J. F. Hudson ed., *Food Antioxidants*, Elsevier, London, U.K., 1999, pp. 138–141.
52. E. A. Decker and D. J. McClements, *Inform*, **12**, 251–256 (2001).
53. P. D. Bartlett, *Rec. Chem. Prog.*, **11**, 47–51 (1950).
54. S.-P. Chang, *J. Amer. Oil Chem. Soc.*, **56**, 855–856 (1979).
55. K. D. Karlson, R. Kleiman, and M. O. Bagby, *J. Amer. Oil Chem. Soc.*, **71**, 175–182 (1994).
56. S. Warwel and M. Rüschen, *J. Mol. Catal. B*, **1**, 29–35 (1995).
57. M. Rüschen, *J. Amer. Oil Chem. Soc.*, **73**, 1453–1457 (1996).
58. J. Rudolph, K. L. Reddy, J. P. Chiang, and K. B. Sharpless, *J. Amer. Chem. Soc.*, **119**, 6189–6190 (1997).
59. M. D. Refvik and R. C. Larock, *J. Amer. Oil Chem. Soc.*, **76**, 99–102 (1999).
60. M. Rüschen, *Recent Developments in the Synthesis of Fatty Acid Derivatives*, AOCS Press, Champaign, Illinois, 1999, pp. 157–167.
61. G. J. Piazza, in G. Knothe and J. T. P. Derksen, eds., *Recent Developments in the Synthesis of Fatty Acid Derivatives*, AOCS Press, Champaign, Illinois, 1999, pp. 182–195.

62. H. C. Brown and A. K. Mandal, *Synthesis*, 153–155 (1980).
63. R. Wilson, R. Smith, P. Wilson, M. J. Shepherd, and R. A. Riemersma, *Anal. Biochem.*, **248**, 76–85 (1997).
64. J. Brinksma, L. Schmieder, G. van Vliet, R. Boaron, R. Hage, D. E. DeVos, P. L. Alsters, and B. L. Feringa, *Tetrahedron Lett.*, **43**, 2619–2622 (2002).
65. C. T. Hou, T. M. Kuo, and A. C. Lanser, *Inform*, **13**, 307–316 (2002).
66. R. Criegee, *Angew. Chem. Int. Ed.*, **14**, 745–752 (1975).
67. B. S. Furniss, A. J. Hannaford, P. W. G. Smith, and A. R. Tatchell, *Vogel's Textbook of Practical Organic Chemistry*, Longman, Harlow, U.K., 1989, pp. 492–494.
68. L. Rebrovic, *J. Amer. Oil Chem. Soc.*, **69**, 159–165 (1992).
69. L. Rebrovic and F. D. Gunstone, *Lipid Technol.*, **8**, 135–137 (1996).
70. H.-P. Klein, *J. Amer. Oil Chem. Soc.*, **61**, 306–312 (1984).
71. S. Warwel and M. Rüschen, *Lipid Technol.*, **9**, 10–14 (1997).
72. M. Rüschen, S. Warwel, in G. Knothe and J. T. P. Derksen, eds., *Recent Developments in the Synthesis of Fatty Acid Derivatives*, AOCS Press, Champaign, Illinois, 1999, pp. 167–173.
73. B. F. Dickens, C. S. Ramesha, and G. A. Thompson, *Anal. Biochem.*, **127**, 37–48 (1982).
74. J. L. Sébédio, P. Juanéda, S. Grégoire, J. M. Chardigny, J. C. Martin, and C. Ginies, *Lipids*, **34**, 1319–1325 (1999).
75. A. J. Dijkstra, in K. K. Rajah, ed., *Fats in Food Technology*, Sheffield Academic Press, Sheffield, U.K., 2002, pp. 123–141.
76. A. J. Dijkstra, *Inform*, **8**, 1150–1158 (1997).
77. W. T. Koetsier, in F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, Inc., New York, 1997, pp. 265–303.
78. A. J. Dijkstra, *Eur. J. Lipid Sci. Technol.*, **104**, 29–35 (2002).
79. U. R. Kreutzer, *J. Amer. Oil Chem. Soc.*, **61**, 343–348 (1984).
80. R. W. Johnson, in R. W. Johnson and E. Fritz, eds., *Fatty Acids in Industry*, Marcel Dekker, Inc., New York, 1988, pp. 217–231.
81. M. R. Porter, in F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, Inc., New York, 1997, pp. 579–608.
82. A. D. James, in F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, Inc., New York, 1997, pp. 609–631.
83. G. Bogndo, in F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, Inc., New York, 1997, pp. 633–694.
84. R. Franklin, M. D. Hoey, and J. Zachwieja, in F. D. Gunstone and R. J. Hamilton, eds., *Oleochemical Manufacture and Applications*, Sheffield Academic Press, Sheffield, U.K., 2001, pp. 23–54.
85. D. W. Roberts, in F. D. Gunstone and R. J. Hamilton, eds., *Oleochemical Manufacture and Applications*, Sheffield Academic Press, Sheffield, U.K., 2001, pp. 55–73.
86. S. Billenstein and G. Blaschke, *J. Amer. Oil Chem. Soc.*, **61**, 353–357 (1984).
87. L. Dahlgren, *Inform*, **11**, 508–512 (2000).
88. K. Hill, *Agro-Food Industry Hi-Tech.*, **September/October**, 9–15 (1998).
89. K. Hill and O. Rhode, *Fette/Lipid*, **101**, 25–33 (1999).

90. K. Hill, W. von Rybinski, and G. Stoll, eds., *Alkyl Polyglycosides*, VCH, Weinheim, FRG, 1997.
91. W. von Rybinski and K. Hill, *Angew. Chem. Int. Ed.*, **37**, 1328–1345.
92. R. W. Johnson, in R. W. Johnson, and E. Fritz, eds., *Fatty Acids in Industry*, Marcel Dekker, Inc., New York, 1988, pp. 153–175.
93. G. Knothe, *Lipid Technol.*, **14**, 101–104 (2002).
94. S. M. Erhan and R. Kleiman, *J. Amer. Oil Chem. Soc.*, **74**, 605–607 (1997).
95. T. A. Isbell and R. Kleiman, *J. Amer. Oil Chem. Soc.*, **73**, 1097–1107 (1996).
96. G. Hénon, Z. Kemény, K. Recseg, F. Zwobada, and K. Kovari, *J. Amer. Oil Chem. Soc.*, **76**, 73–81 (1999).
97. A. Sæbø, *Lipid Technol. Newslett.*, **7**, 9–13 (2001).
98. F. Destailats and P. Angers, *Lipids*, **37**, 435–438 (2002).
99. R. C. Larock, X. Dong, S. Chung, C. K. Reddy, and L. E. Ehlers, *J. Amer. Oil Chem. Soc.*, **78**, 447–453 (2001).
100. D. H. R. Barton, D. Critch, and W. B. Motherwell, *Tetrahedron Lett.*, **24**, 4979–4982 (1983).
101. O. Loreau, A. Maret, D. Poullain, J. M. Chardigny, J.L. Sébédio, B. Beaufrière, and J. P. Noël, *Chem. Phys. Lipids*, **106**, 65–78 (2000).
102. F. Spener and H. K. Mangold, *Chem. Phys. Lipids*, **11**, 215–218 (1973).
103. R. Toreki. (2002, July 3). *Organometallic HyperTextBook*. Available <http://www.ilpi.com/organomet/olmetathesis.html>.
104. M. F. C. Plugge and J. C. Mol, *Synlett*, 507–508 (1991).
105. S. Warwel, H. G. Jagers, and S. Thomas, *Fat Sci. Technol.*, **94**, 323–328 (1992).
106. M. D. Refvik, R. C. Larock, and Q. Tian, *J. Amer. Oil Chem. Soc.*, **76**, 93–98 (1999).
107. J. C. Mol, *Green Chem.*, **4**, 5–13 (2002).
108. U. Biermann, S. Fürmeier, and J. O. Metzger, in F. D. Gunstone and R. J. Hamilton, eds., *Oleochemical Manufacture and Applications*, Sheffield Academic Press, Sheffield, U.K., 2001, pp. 266–299.
109. F. D. Gunstone, in G. Knothe and J. T. P. Derksen, eds., *Recent Developments in the Synthesis of Fatty Acid Derivatives*, AOCS Press, Champaign, Illinois, 1999, pp. 1–19.
110. T. A. Isbell and B. A. Plattner, *J. Amer. Oil Chem. Soc.*, **74**, 153–158 (1997).
111. T. A. Isbell and B. A. Steiner, *J. Amer. Oil Chem. Soc.*, **75**, 63–66 (1998).
112. T. A. Isbell and M. S. Mund, *J. Amer. Oil Chem. Soc.*, **75**, 1021–1029 (1998).
113. J. O. Metzger and U. Biermann, *Liebigs Ann. Chem.*, 645–650 (1993).
114. J. O. Metzger and U. Biermann, *Fette/Lipid*, **100**, 2–6 (1998).
115. U. Biermann and J. O. Metzger, in G. Knothe and J. T. P. Derksen, eds., *Recent Developments in the Synthesis of Fatty Acid Derivatives*, AOCS Press, Champaign, Illinois, 1999, pp. 80–89.
116. J. O. Metzger, R. Mahler, G. Francke, and A. Hayen, in G. Knothe and J. T. P. Derksen, eds., *Recent Developments in the Synthesis of Fatty Acid Derivatives*, AOCS Press, Champaign, Illinois, 1999, pp. 90–99.
117. J. O. Metzger, R. Mahler, and G. Francke, *Liebigs Ann./Recueil*, 2303–2313 (1997).
118. J. O. Metzger, R. Mahler, and A. Schmidt, *Liebigs Ann. Chem.*, 693–696 (1996).



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## *Crystallization of Fats and Oils*

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### 1. INTRODUCTION

#### 1.1. Control of Lipid Crystallization

In many food products and even some processing operations, it is important to be able to control lipid crystallization to obtain the desired number, size distribution, polymorph, and dispersion of the crystalline phase. In most foods, it is crystallization of triacylglycerols (TAG) that is most important, although, at times, crystallization of other lipids (i.e., monoacylglycerols, diacylglycerols, phospholipids, etc.) may also be important to product quality.

Proper control of the crystalline microstructure leads to products with the desired textural properties and physical characteristics. For example, tempering of chocolate prior to molding or enrobing is designed to control crystallization of the cocoa butter into a large number of very small crystals that are all in the desired polymorphic form. When controlled properly, the cocoa butter crystals in chocolate contribute to the desired appearance (shine or gloss), snap, flavor release, melt-down rate upon consumption, and stability during shelf life (fat bloom). Similar

arguments can be made for other products such as butter, margarine, whipped cream, ice cream, shortening, peanut butter, and a host of others.

During processing of fats, crystallization is often used to modify the properties of the fat. For example, winterization of vegetable oils is needed to ensure that the oil remains a clear liquid even when stored at low temperatures for extended time periods. The process of fractionation of fats to produce components of natural fats with different melting properties also requires control of crystallization to optimize the separation process. Many fats, including palm oil, palm-kernel oil, milk fat, and tallow, are fractionated by crystallization to produce different functional fats.

## 1.2. Crystallization of Natural Fats

There are several aspects of lipid crystallization that make it unique from crystallization of other components in foods (like water, sugars, salts, etc.). These are related to the complex molecular composition of natural fats and the orientation of the triacylglycerol molecules.

Fats are made up primarily of TAGs, approximately 98%, with the remainder of the fat being more polar lipids like diacylglycerols (DAGs), monoacylglycerols (MAGs), free fatty acids (FFAs), phospholipids, glycolipids, sterols, and other minor components. In refined fats, these minor lipids are much lower in concentration than in unrefined fats. Although the TAGs form the main crystalline phase, the minor components, or impurities, can often play a large role in how crystallization occurs and crystallization may be substantially different in a refined oil than in the unrefined starting material.

Natural fats also contain a wide range of TAG species with fatty acids of different chain length and degree of unsaturation. Milkfat, for example, contains hundreds of different TAG species with no single species present at greater than about 5%. TAGs are composed of three fatty acids arranged on a glycerol molecule, and with variations in chain length and degree of saturation of the fatty acids, a wide range of components is possible. This range of composition leads to interesting complexities in crystallization.

The nature of the TAG molecule is such that it can often take multiple forms in a crystal lattice. That is, the same molecule can crystallize into different crystalline forms dependent on processing conditions. The phenomenon is called polymorphism. Although there are numerous molecules that exhibit polymorphism in nature (many in the pharmaceutical field), polymorphism is somewhat unique to lipids in the food industry (although some sugar alcohols also form polymorphs).

In this chapter, the complex nature of lipid crystallization, primarily related to TAG, will be discussed.

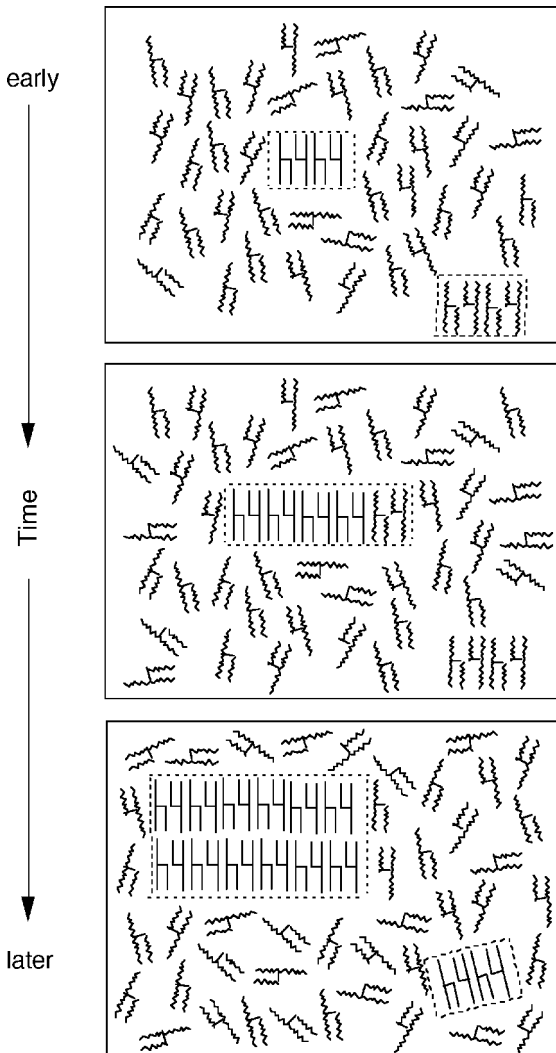
## 2. LIPID PHASE BEHAVIOR

### 2.1. Nature of the Liquid Phase

It is important to understand the nature of the liquid phase prior to crystallization to understand how crystals form. It is widely recognized that lipids retain some degree

of ordering in the liquid phase, with temperatures well above the melting point needed to fully dissociate this ordering. When melting fats, this liquid ordering is termed a crystalline memory effect, where subsequent recooling leads to formation of a different (usually more stable) phase than would occur if the fat was heated to higher temperatures to destroy the liquid memory (1–3).

In nucleation, or the formation of the crystalline phase from the liquid, some organization of molecules is expected. In lipids, the natural ordering of the liquid phase leads to crystal formation. In fact, rapid cooling of liquid lipids results in the formation of a diffuse crystalline phase (low-energy polymorph) because of the ordering structure in the liquid phase. Such rapid cooling of other systems, most



**Figure 1.** Proposed mechanism (highly schematic) for nucleation of triacylglycerols (TAGs). Straight chains indicate crystallized TAGs, whereas bent chains indicate fluid TAGs (4).

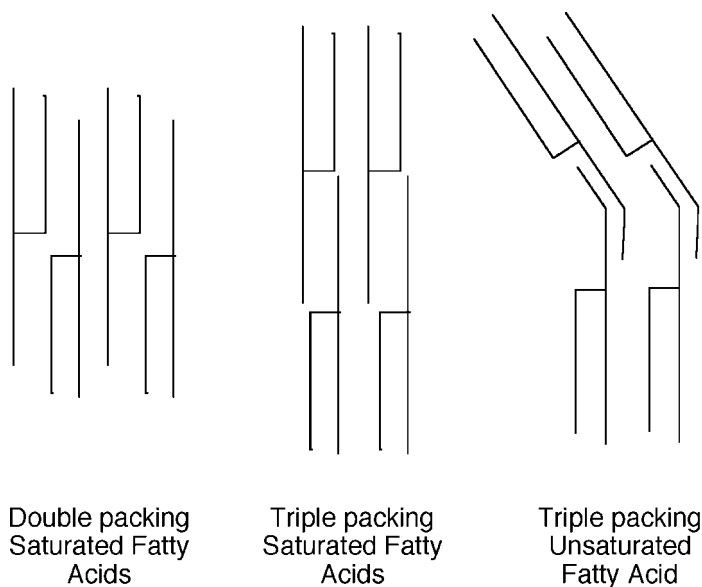
notably sugars and starches, often results in the formation of a glassy state consisting of molecules that are randomly organized together with no long-term ordering.

Upon slower cooling from the liquid, the lipid molecules have time to organize into lamellae (1) and eventually can form coherent, three-dimensional crystals (shown schematically in Figure 1). The arrangement of the molecules into the crystalline state depends on such factors as the cooling rate, the temperature at which crystallization occurs, the agitation rate, and the composition of the lipid phase.

## 2.2. Polymorphism

Polymorphism is the ability of a molecule to take more than one crystalline form depending on its arrangement within the crystal lattice. In lipids, differences in hydrocarbon chain packing and variations in the angle of tilt of the hydrocarbon chain packing differentiate polymorphic forms. The crystallization behavior of TAG, including crystallization rate, crystal size, morphology, and total crystallinity, are affected by polymorphism. The molecular structure of the TAG and several external factors like temperature, pressure, rate of crystallization, impurities, and shear rate influence polymorphism (5).

TAGs are oriented in a chair or tuning fork configuration in the crystalline lattice. The TAG can take either a double or triple chain-length structure as seen in Figure 2. The fatty acids of TAG pairs overlap in a double chain-length structure whereas in triple chain packing, the fatty acids do not overlap. The height of these chair structures and the distance between the molecules in the chair structures are found by using the X-ray spectra as the long and short spacings, respectively.



**Figure 2.** Packing arrangements of triacylglycerol molecules in the crystal lattice (4).

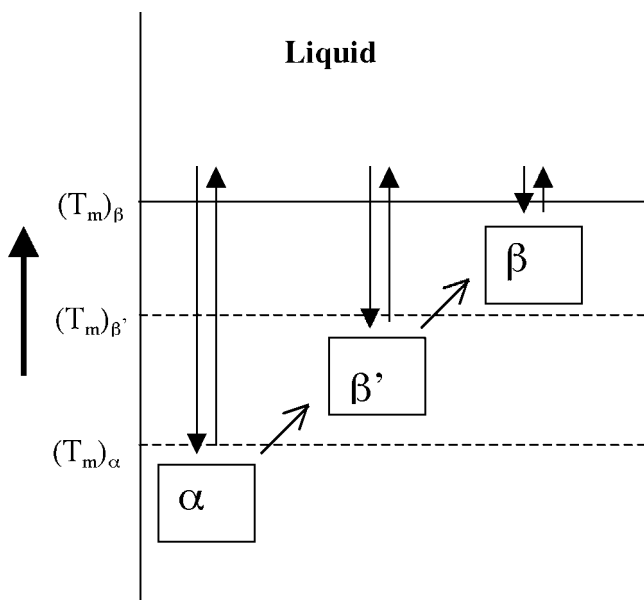
**TABLE 1. Identification of Polymorphic Forms of Fats Based on X-ray Analysis of Short Spacings (6).**

Polymorphic Form	Unit Cell	Lines and Short Spacings ( $\text{\AA}^\circ$ )
$\alpha$	Hexagonal	A single strong and very broad @ 4.15
$\beta'$	Orthorhombic	Two strong lines @ 4.2 and 3.8
$\beta$	Triclinic	A strong line @ 4.6

The polymorphic forms of fats are often simply classified into three categories,  $\alpha$ ,  $\beta'$ , and  $\beta$ , in increasing order of stability. The  $\alpha$  form is the least stable polymorph with the lowest melting point and latent heat of fusion. The  $\beta$  form is the most stable, with the highest melting point and latent heat. Each polymorphic form has distinct short spacings (the distances between parallel acyl groups on the TAG) that are used to distinguish the polymorphic forms based on their X-ray diffraction patterns, as summarized in Table 1. Based on the unique configuration of the molecules within the crystal lattice, each polymorph has a different crystallographic unit cell, also shown in Table 1.

In general, TAGs with three saturated fatty acids crystallize in double chain-length packing, whereas triple chain-length packing is obtained if the TAG contains fatty acids with different structures (chain length and unsaturation). Lutton (7) stated that if the fatty acids of a TAG differ in length by more than four carbons, it forms a triple chain-length structure. Triple chain-length packing is also observed in TAG containing a *cis*-unsaturated fatty acid because this causes a kink in the structure, as seen in Figure 2. *Cis*-unsaturated fatty acids do not mix in one layer with saturated fatty acids, and triple chain-length crystals are formed (8). It should be noted that *trans*-unsaturated fatty acids incorporate into a crystal structure in the same way as the saturated fatty acids (8). The chain-length structure influences the mixing-phase behavior of different types of TAGs in solid phases (5). The triple chain-length structure has greater long spacings than does the double chain-length structure.

Lipids exhibit monotropic polymorphism, where unstable forms are the first to crystallize in a subcooled fat because of their lower energy state, according to the Gibb's free energy (5). Subsequent transformation of unstable polymorphs into more stable forms occurs over time until, eventually, the most stable polymorph for a given lipid is reached. Transformation of unstable to stable polymorphs can be achieved by a slight increase in temperature above the melting point of the less-stable forms. This increase in temperature first causes the melting of the unstable forms and then solidification in a more stable form. Transformation to a more stable form can also take place without melting as seen in Figure 3. The difference in Gibb's, free energy between polymorphs is the driving force for this transformation, as the molecules become more tightly arranged in the crystal lattice. It is assumed that the chair structure is maintained during polymorphic transformations (9). The layer arrangement of the  $\alpha$  polymorph does not change when it is transformed to the  $\beta'$  polymorph, although its lateral chain packing and angle of tilt changes during polymorphic transformation.



**Figure 3.** Monotropic polymorphism of lipids where  $(T_m)_\alpha$ ,  $(T_m)_{\beta'}$ , and  $(T_m)_\beta$  are the melting temperatures of the  $\alpha$ ,  $\beta'$ , and  $\beta$  polymorphs, respectively.

The hydrocarbon chain packing of the  $\beta$  polymorph is denser than that of the  $\alpha$  polymorph. The denser chain packing in the  $\beta$  polymorph gives increased stability compared with the  $\alpha$  polymorph. In addition, stable polymorphs have higher melting point and higher heat of fusion than the less-stable forms. The different polymorphic forms typically crystallize at rates in order of their stability ( $\alpha < \beta' < \beta$ ). Thus, the least-stable polymorphic form typically crystallizes first in a strongly subcooled molten fat because of the lower surface energy (10).

The rate of polymorphic transformation depends on the length of the fatty acid chain and is the greatest for TAGs with short-chain fatty acids (10). Natural fats usually contain a large number of TAGs; thus, the transformation of unstable to stable forms is often very slow. As mentioned previously, the  $\alpha$  form is generally formed first in a rapidly cooled liquid fat, but it is usually very unstable and rapidly transforms to the  $\beta'$  form. The  $\beta'$  form may remain for an extended time (hours to days), although in many fats, it eventually transforms into the  $\beta$  polymorph, which is usually the most stable form. However, in many natural fats, the  $\beta'$  polymorph can exist for long periods of time because of compound or solid solution formation (11). That is, in some mixed-acid TAGs, no  $\beta$  polymorph may form and  $\beta'$  is the most stable. In other cases, two  $\beta$  forms may be present (5). For example, SOS, a mixed-acid TAG, has five polymorphic forms in which two  $\beta$  forms are present. The molecular structures of the five polymorphic forms have been identified using XRD, differential scanning calorimetry (DSC), and Fourier-transformed infrared spectroscopy (FT-IR) techniques (5). In addition, two liquid crystalline phases called LC1 and LC2 were found for SOS using time-resolved synchrotron radiation X-ray

TABLE 2. Polymorphic Forms of Cocoa Butter.

Form		Melting Temperature (°C)	
		Wille and Lutton (13)	Davis and Dimick (13)
I	$\gamma$	17.3	13.1
II	$\alpha$	23.3	17.7
III	$\beta'_2$	25.5	22.4
IV	$\beta'_1$	27.5	26.4
V	$\beta_2$	33.8	30.7
VI	$\beta$	36.3	33.8

diffraction (SR-XRD) analysis (12). The researchers stated that the crystallization properties of SOS polymorphic forms were somehow influenced by the presence of the two liquid crystal phases.

Additionally, more than one subtype within the main polymorphic grouping has been identified in some fats. For example, six different polymorphic forms have been identified in cocoa butter, although there is still some debate whether they are all truly unique polymorphs (Table 2). Two  $\beta'$  and two  $\beta$  forms have been identified for cocoa butter. These polymorphs have slightly different melting points, but they have X-ray spectra that fit within the definition of that polymorph.

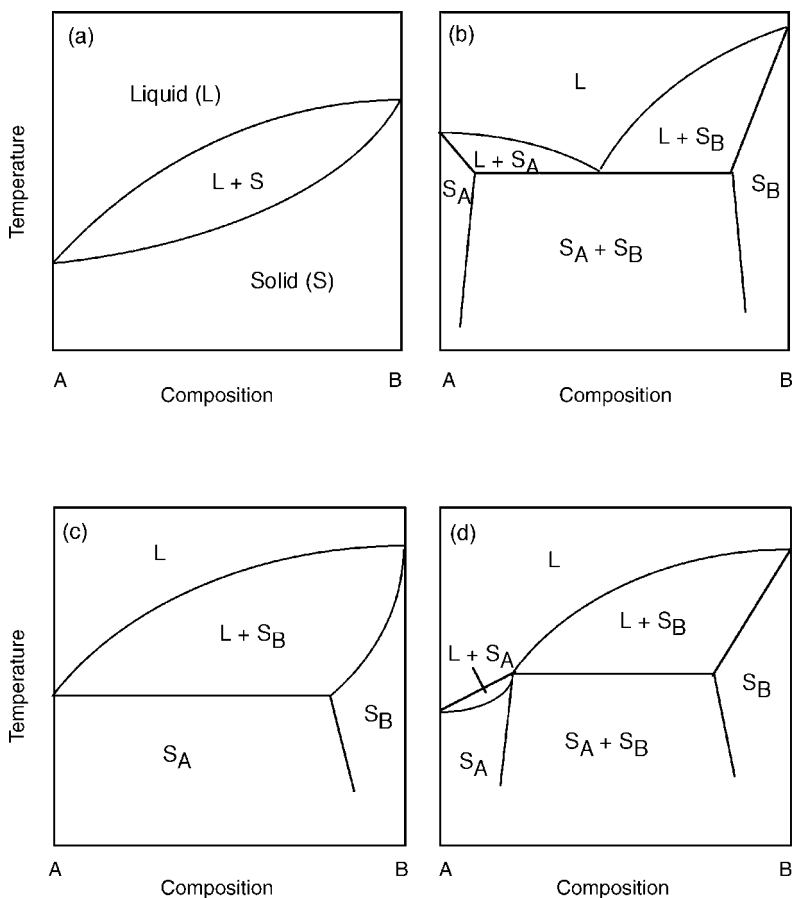
Different nomenclatures have been used for denoting polymorphic forms, as seen in Table 2 for cocoa butter. In the Greek nomenclature, where polymorphs are given a Greek letter, the most stable form within a polymorph type is given the subscript 1, and other polymorphs within that form are ordered in decreasing stability or melting temperature. For example, cocoa butter has two  $\beta'$  forms, with the  $\beta'_1$  form having the highest melting point (most stable). It is also common to see a hyphenated number following the Greek letter, usually 2 or 3, stating the chain-packing arrangement (double or triple chain packing, respectively). Wille and Lutton (13) denoted the different polymorphs of cocoa butter with Roman numerals, ordered in increasing melting point.

The time-temperature relationships governing the polymorphic behavior of cocoa butter (in the temperature range of  $-20^\circ\text{C}$  to  $40^\circ\text{C}$  and a time range of 10 days) were investigated by using real-time XRD (15). The  $\gamma$ ,  $\alpha$ , and  $\beta'$  polymorphs crystallized directly from the melt, and formation of  $\beta'$  is much quicker when it transforms from  $\alpha$  compared with its formation from the melt. The least-stable polymorph  $\gamma$  stayed unchanged at solidification temperatures ( $T_p$ ) below  $-10^\circ\text{C}$  for 10 days. At higher  $T_p$ , the  $\gamma$  polymorph transformed to  $\alpha$  within a short time. The  $\gamma$  always transformed to  $\alpha$  and  $\alpha$  transformed to  $\beta'$ . The  $\alpha$  phase transformed into  $\beta'$  phase within 1 hour or less at temperatures above  $6^\circ\text{C}$ . They noted that two  $\beta$  phases (polymorphs V and VI) were obtained via direct transformation from the  $\beta'$  phase only, not from the melt. Direct  $\beta$  phase formation from melt is only viable if the melt has a memory effect. Their observation of two different  $\beta$  phases from the  $\beta'$  phase is contradictory to the results of the work of Schlichter-Aronhime and Garti (16) who stated that  $\beta$ -V can be directly formed from the melt and that  $\beta$ -VI can be formed only from the transformation of  $\beta$ -V.

### 2.3. Phase Behavior

In order to understand and control lipid crystallization, one should know the thermodynamic driving force for crystallization. In a pure system, like a single TAG, the melting point,  $T_m$ , defines the driving force and a temperature below  $T_m$  is required to induce crystallization. That is, the subcooling or the melting temperature minus the actual temperature ( $T_m - T$ ) defines the driving force for crystallization.

When two TAGs are mixed together, each species can influence the melting properties of the other and a phase diagram is needed to define the crystallization driving force at any condition. Rossell (17) summarized the phase behavior of binary mixtures of various TAGs. Depending on molecular differences (chain length and degree of unsaturation), most binary TAG mixtures had either monotectic, eutectic, or peritectic behavior (Figure 4), where the melting temperatures (liquidus



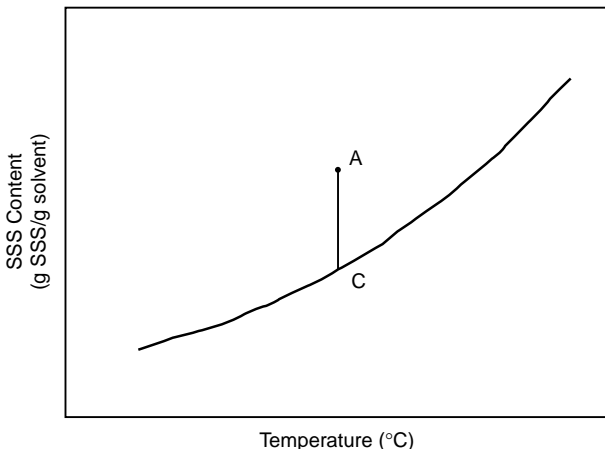
**Figure 4.** Phase behavior in binary systems: (a) monotectic, continuous solid solution; (b) eutectic; (c) monotectic, partial solution; and (d) peritectic (18).



lines) of the species with the higher melting point decreased with increasing addition of the species with the lower melting point. Wesdorp (19) used a thermodynamic approach to predict phase behavior of each of the polymorphs for different binary mixtures of TAGs. At the liquidus line on the phase diagram, the chemical potential of the crystallizing species in the liquid state is equal to the chemical potential of that species in the crystalline state (the definition of equilibrium).

If one of the species in a binary mixture is a liquid (oil or solvent), the other species (higher melting point) will dissolve to some extent into the solvent (the liquid oil can be considered a solvent in this case too). For example, a certain amount of trisaturated TAGs (SSS) dissolves in solvent (either organic solvent like acetone or hexane or a liquid oil), with the solubility concentration increasing with temperature in the normal fashion (as shown schematically in Figure 5). In this case, a binary mixture of SSS and solvent can be supersaturated with SSS once its concentration exceeds the saturation concentration at any temperature, as indicated by line AC in Figure 5. Thermodynamically, the driving force for crystallization is the difference in chemical potential of SSS at point A and the chemical potential at saturation (point C). Often, this crystallization driving force is approximated as the difference in concentrations between points A and C.

When more than two TAG species are mixed together, the phase behavior is significantly more complicated. For mixtures of three TAGs, a ternary phase diagram (sometimes called a triangle diagram) can be used to denote phase behavior at any temperature. The effects of temperature on phase behavior, however, must be taken into account in yet another dimension, and thus, characterizing phase behavior in ternary systems gets very difficult very quickly. The situation is even more complex when there are greater than three TAG components, as occurs when a natural fat is crystallized. Natural fats are mixtures of numerous TAGs, containing perhaps 10 to 12 different TAGs (as in cocoa butter) to well over 100 (as in milkfat). In natural

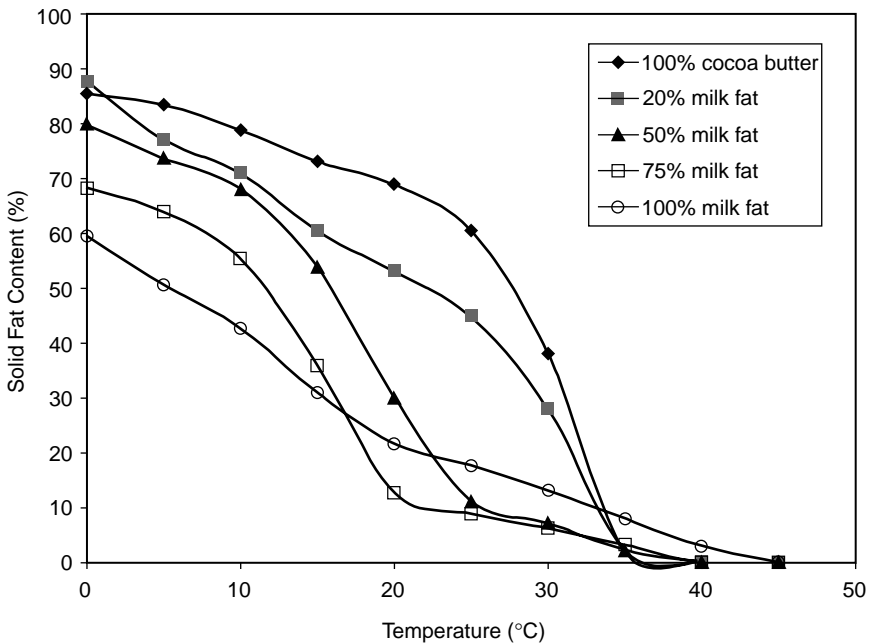


**Figure 5.** Schematic of a solubility diagram for a high-melting fat (SSS) in a liquid oil or solvent. Line AC represents supersaturation for system at point A.

fats, the complex interactions among mixtures of various TAGs with different fatty acids (chain length and degree of unsaturation) and having different melting points result in melting over a range of temperatures. This range of temperatures may be fairly narrow (as for cocoa butter) or may be broad (as for milkfat).

At a temperature above the melting point of the highest melting component, the entire lipid is melted and the natural fat is in a liquid state. This highest melting point, often characterized as the clear point (the temperature at which the last crystal melts under carefully controlled heating conditions), is actually the melting temperature of the TAG with highest melting point in the specific mixture of the other TAG. Some researchers use this highest melting point, or some measure of melting point like the Mettler dropping point, to define the driving force for crystallization when the fat is cooled (20, 21). However, when the natural fat contains a wide range of TAGs with different melting points, cocrystallization of different TAGs into compound crystals is dependent on the temperature of crystallization. Thus, the highest melting point does not necessarily represent the true driving force for crystallization of the TAG species that are cocrystallizing.

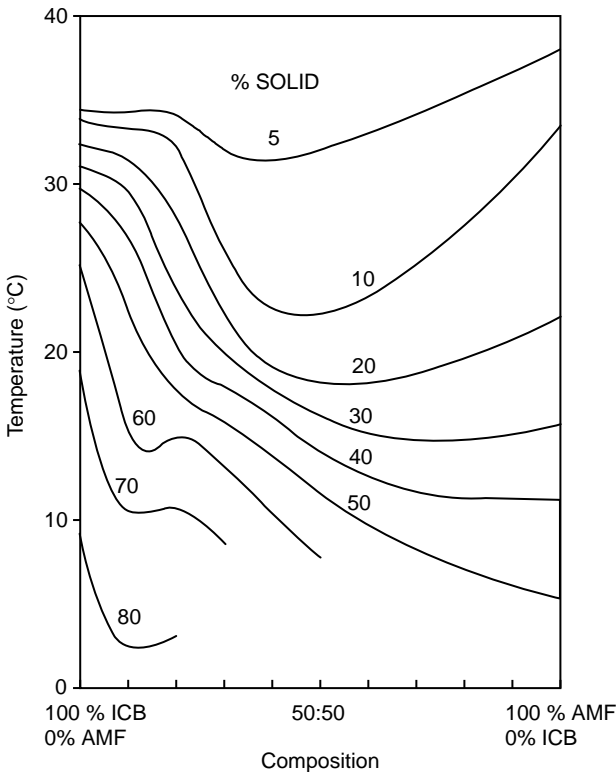
If the fat is cooled to some point below the melting point of the highest melting component and allowed to fully equilibrate (crystallize to the maximum extent in the most stable polymorph), there will be some ratio of solid to liquid fat dependent on the nature of the TAG mixture in the natural fat. This solid fat content (SFC) is often measured by a pulsed nuclear magnetic resonance (NMR) technique. A plot of the maximum amount of fat crystallized (SFC) at sequentially higher temperatures



**Figure 6.** Solid fat curves for milkfat, cocoa butter, and their mixtures (4).

gives a melting profile that represents a type of phase equilibrium for a natural fat. Some fats, like cocoa butter, have a very high SFC at low temperatures (about 90% at 0°C) and then melt very sharply over a narrow temperature range (25–35°C). Other natural fats, like milkfat, have lower SFC at low temperatures (about 50% at 0°C) and melt gradually with increased temperature. These SFC melting curves are dependent on the specific molecular composition of the natural fat, as seen in Figure 6 for cocoa butter and milkfat. Although SFC melting curves denote a certain aspect of phase behavior, they are not true phase diagrams because the composition of the crystalline phase changes as temperature increases. Nevertheless, melting profiles are useful tools for understanding the crystallization behavior of natural fats.

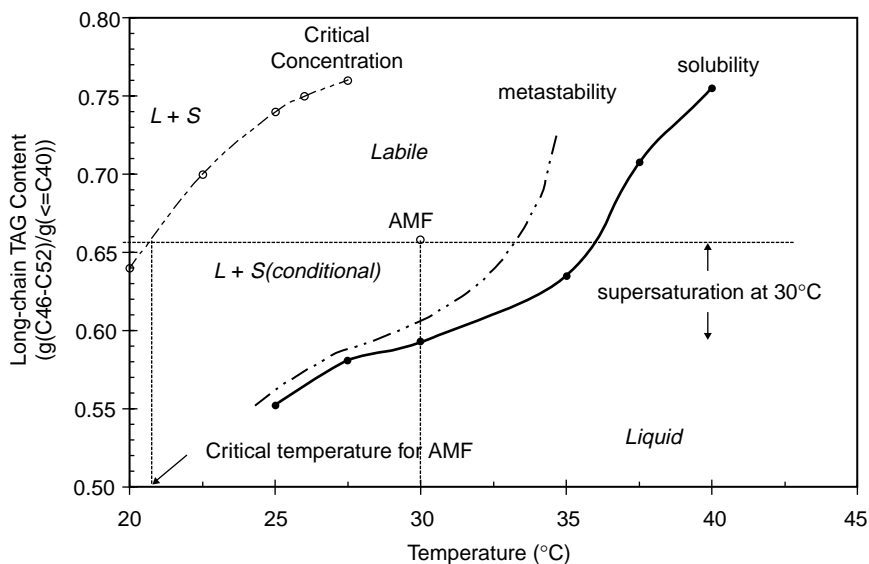
In mixtures of two or more natural fats, as often occurs in processed foods (e.g., milkfat and cocoa butter in chocolate), it is even more difficult to characterize the true phase behavior for crystallization of fats. One approach that has been used to characterize compatibility of fat mixtures is the isosolids diagram (22). SFC melting curves are obtained (by NMR) for various mixtures of the two fats, as seen for cocoa butter and milkfat in Figure 6. Lines of constant SFC for different temperature and composition are calculated and plotted on an isosolids diagram (Figure 7).



**Figure 7.** Isosolids diagram for mixtures of anhydrous milkfat (AMF) and cocoa butter (ICB) (4).

Eutectic behavior is seen where the SFC of a mixture falls below the SFC for either of the two individual components, as seen between 30% and 70% milkfat in Figure 7. Isosolids diagrams allow phase compatibility to be studied (4), but they do not provide a thermodynamic measure of driving force for crystallization. Again, because the crystal phase composition may be different at different temperatures (and mixture ratios), isosolids diagrams do not represent true phase diagrams.

Recently, attempts have been made to characterize the driving force for crystallization of natural fats by considering classes of TAG (high-melting, low-melting, etc.). For example, milkfat contains three primary fractions that crystallize nearly independently. The effective solubility of the high-melting fraction (HMF) in the low-melting fraction (LMF) was found by using a turbidimetry technique (23). Through chemical analysis of the major TAG constituents of HMF, an effective solubility curve in terms of chemical composition of HMF in LMF was developed and used to characterize the driving force for crystallization, as shown in Figure 8. Such an effective solubility takes into account the intersolubility of different TAGs as well as the melt behavior of individual TAGs. Although this approach is still somewhat empirical, it provides a reasonable approximation of the crystallization driving force in complex lipids. Further work is needed in this area to truly define the driving force for crystallization in natural fats.



**Figure 8.** Operational phase diagram for high-melting components of milk fat dissolved in low-melting components of milk fat based on triacylglycerol composition (acyl carbon number) (4).

### 3. CRYSTALLIZATION BEHAVIOR

#### 3.1. Nucleation

Nucleation, or the formation of a crystalline phase from the liquid state, is probably the most important factor in controlling crystallization. The nucleation rate is the major determining factor in the number and size of crystals formed, their polymorphic form, and the ultimate distribution of crystalline solids. Crystallization cannot occur until the phase is supersaturated or subcooled. However, attaining the supersaturated or subcooled state is not necessarily sufficient to promote crystallization because a certain energy barrier exists to formation of nuclei.

A nucleus is the smallest crystal that can exist in a solution at a certain temperature. The formation of a nucleus from the liquid phase, or the nucleation process, requires the molecules to organize into a crystal lattice. There is a free-energy barrier opposing this transition, but when nucleation does occur, there is a release of energy (latent heat of fusion) as the molecules assume the lower energy state in the crystal lattice. Based on these energy considerations, a free-energy maximum exists that must be overcome for nucleation to occur (24). At this maximum free energy, there is a critical size for a stable nucleus. Above this critical size, a stable nucleus is formed that continues to grow, whereas clusters smaller than the critical size can potentially disperse into the liquid state (4, 24, 25).

**3.1.1. Nucleation Theories** Nucleation is generally classified according to primary nucleation, which may occur either homogeneously or heterogeneously, and secondary nucleation mechanisms. The presence of foreign nucleating sites catalyzes the formation of heterogeneous nucleation, whereas homogeneous nucleation occurs without the assistance of outside surfaces. Secondary nucleation occurs when crystals in a subcooled system spawn new nuclei, generally because of contacts between two crystals, or between a crystal and a surface such as a stirrer or a solid wall (4).

**3.1.1.1. Homogeneous Nucleation** Homogeneous nucleation is based on accretion of molecules in the liquid phase. Single species (molecules or ions) come together and form dimers. Dimers become trimers by addition of a molecule, and this accumulation process continues until eventually a stable nucleus forms depending on temperature and supersaturation.

According to the classic nucleation theory, a free-energy barrier must be overcome to form a stable nucleus. The energy needed to form a crystal is proportional to the interfacial tension,  $\gamma$ , and the surface area. However, once a nucleus is formed, there is a release of energy (latent heat) associated with the phase change.

The free-energy change for the formation of the crystal surface is positive and proportional to the surface area ( $r^2$ ) and interfacial tension ( $\gamma$ ) between the crystal and the surrounding fluid. The free-energy change for formation of the bulk of the crystal is negative because energy is released because of latent heat of fusion and proportional to volume ( $r^3$ ). The total free-energy change during nucleation is the

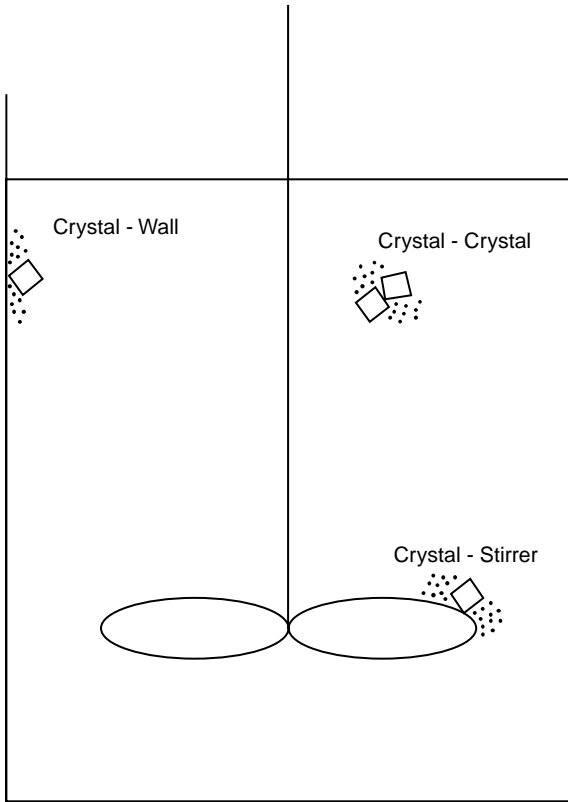
sum of these free-energy terms for the formation of the crystal surface and the crystal volume. Thus, a maximum in free energy occurs during nucleation at some critical nucleus size,  $r_c$ . The critical nucleus size is the minimum size for a stable nucleus. Above this critical size, a stable nucleus is formed, whereas clusters of molecules smaller than this critical size can potentially redisperse into the liquid phase (4, 24–26).

Homogeneous nucleation, however, rarely occurs under commercially important conditions. In practice, nucleation is usually dominated by a heterogeneous mechanism, where a foreign surface serves to reduce the energy barrier to nucleation.

**3.1.1.2. Heterogeneous Nucleation** Typically, nucleation of fats (as well as most other substances) occurs by a heterogeneous process catalyzed by foreign nucleating sites. The presence of these foreign nucleating sites, like dust particles, vessel walls, and other foreign particles in the system, reduces the free energy required for nucleation. Even though the exact mechanisms of heterogeneous nucleation are not clearly understood, it most likely results from the interactions at the interface between the solid particle and the supersaturated fluid. These interactions result in a local ordering of molecules of the crystallizing species; thus, the free energy of formation of a critical size for a stable nucleus is decreased. For example, nucleation on a surface irregularity at a wall results in a decrease in the surface energy required to form a stable nucleus. In general, the capability of a foreign surface to catalyze nucleation is thought to depend on the degree of lattice matching between the solid surface and the crystals of the nucleating species (26), although this trend is not always observed (24). In general, a closer lattice match indicates a greater likelihood that a surface will catalyze heterogeneous nucleation. Because the foreign surface provides some of the energy needed to overcome the formation of the crystal surface, heterogeneous nucleation occurs at lower crystallization driving force (supersaturation or subcooling) than homogeneous nucleation (24, 27). Interestingly, there is an aging effect on the ability of a heterogeneous nucleation site to catalyze nuclei formation (26). That is, the same material nucleated multiple times under identical conditions results in a spread of nucleation capabilities. This variability in heterogeneous nucleation leads to difficulties in controlling lipid crystallization.

**3.1.1.3. Secondary Nucleation** The formation of new nuclei in the presence of existing crystals is called secondary nucleation. Secondary nucleation may occur whenever microscopic crystalline elements are separated from an existing crystal surface (24), although contact secondary nucleation is probably the main mechanism in commercial fat crystallization processes. As a crystal slurry is agitated in a vessel, crystals contacting with other crystals, vessel walls, or stirrer may lead to attrition or fracture of the existing crystal structure, and consequently, secondary nuclei are formed (Figure 9).

Contact secondary nucleation has been explained by two possible mechanisms, namely, the adsorption layer theory and microattrition (4). The adsorption layer theory involves displacement of a surface layer of organized molecules (precrystalline)



**Figure 9.** Potential sources of contact nuclei in a stirred crystallizer (4).

as a result of crystal interactions or collisions. Thus, precrystalline embryos are dispersed into the crystallizing medium, where under conditions of secondary nucleation they survive and develop into stable nuclei (24). Microattrition theory involves the dispersion of broken pieces of a crystal into the fluid, which remain as stable nuclei (28). Production of secondary nuclei may also result from growing crystals containing dislocations, inclusions, or defects (24, 28). As expected, secondary nucleation is also dependent on the crystallization driving force (supersaturation or subcooling), with more stable nuclei being formed at higher supersaturation (25, 27).

Secondary nucleation may also occur in static conditions under certain circumstances (11). In lipid systems, needle-like or dendritic crystals that form under certain conditions may lead to the formation of secondary nuclei. Heat dissipation and/or concentration of noncrystallizing species in certain regions may lead to melting/dissolution at the base of the branches of dendritic crystals and result in the formation of numerous nuclei centers. Although the exact mechanisms for this type of secondary nucleation are not fully understood, it is undoubtedly important for

nucleation in emulsions and in certain cases during seeding of bulk solutions (as in tempering of chocolates).

During fractionation of fats, secondary nucleation is undesired because the small crystals, formed in the presence of larger ones means that subsequent separation is not efficient. Thus, stirring or agitation during fractionation is usually kept to the minimum needed to facilitate heat transfer.

Secondary nucleation is influenced by numerous parameters, including the driving force for crystallization, temperature, additives, impurities, agitator, agitation rate, the number and size of existing crystals, and roughness of the crystallizer surface. The parameters affecting nucleation and nucleation rate will be reviewed in a subsequent section.

**3.1.2. Nucleation Kinetics** Nucleation rate is generally measured as the rate of formation of nuclei (numbers formed per unit volume per unit time). Sometimes the induction time, or the time necessary for the onset of nucleation once the subcooled state has been attained, is used for calculation of nucleation rate because the actual rate is often very difficult to measure. Induction time for nucleation will be reviewed later in this section.

In some cases, as in crystallization of viscous materials from the melt, the Fisher–Turnbull equation (29) is often used to describe nucleation of lipids (20, 30)

$$J = \left( \frac{NkT}{h} \right) \exp \left\{ -\frac{\Delta G_d}{kT} \right\} \exp \left\{ -\frac{16\pi\gamma^3 T_f^2}{3kT(\Delta H_f^2)(T_f - T)^2} \right\}. \quad (1)$$

Here,  $N$  is the number of molecules (monomers) per mole,  $k$  is the Boltzman constant,  $T$  is absolute temperature,  $h$  is Planck's constant,  $\Delta G_d$  is a term denoting the mobility of the lipid molecules,  $\gamma$  is interfacial tension,  $T_f$  is melting temperature, and  $H_f$  is latent heat of fusion. The first exponential term in Equation 1 has been related to the ability of a lipid molecule to attain the necessary conformation to become attached to the crystal lattice, and it is often given as (20)

$$\frac{(\Delta G_d)}{kT} = -\frac{\alpha \Delta S}{R}, \quad (2)$$

where  $\alpha$  is the fraction of molecules with the correct configuration to be incorporated into the crystal lattice,  $S$  is the decrease of entropy associated with incorporation of one mole of lipid, and  $R$  is the ideal gas constant. Kloek (31) determined that 80% of TAG molecules were in the correct conformation for incorporation into a nucleus.

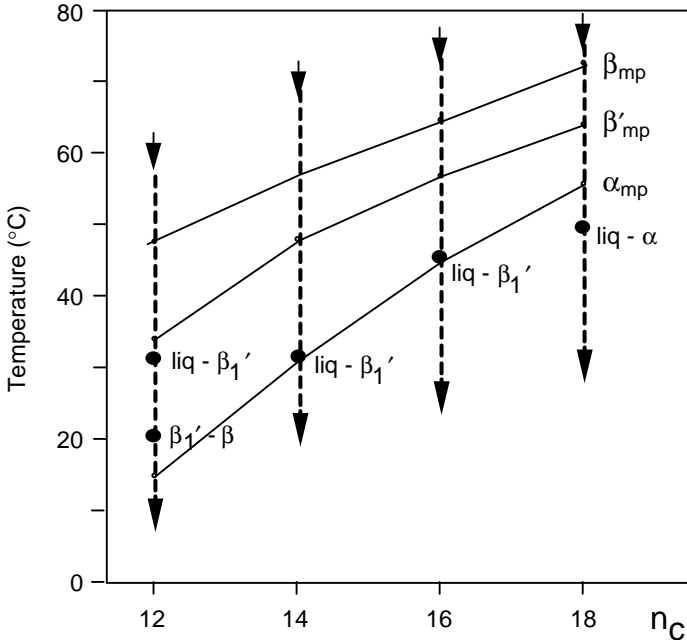
According to the classic theory, nucleation is a very strong function of crystallization driving force. At low driving forces (low supersaturation or high temperatures), nucleation rate is essentially zero. After some critical driving force is attained, nucleation becomes spontaneous and occurs almost instantaneously once the critical driving force has been attained. In natural fats, cooling below a



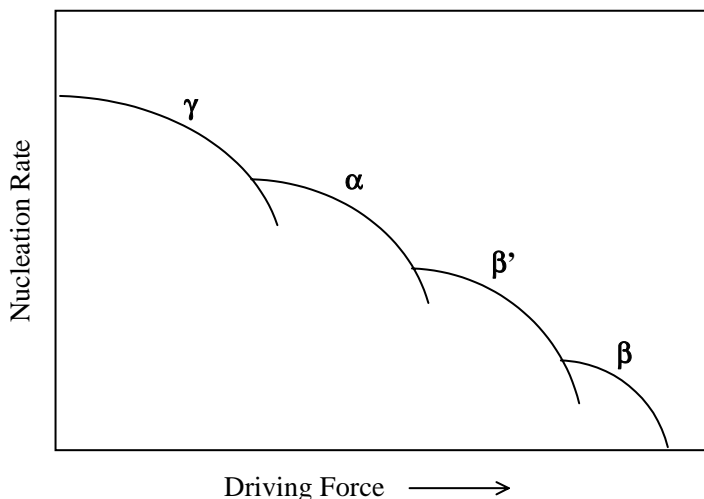
certain temperature results in massive nucleation with numerous nuclei being formed. For fats, the nucleation rate also depends on the type of polymorph formed, because each of the polymorphs has a different melting point and interfacial tension.

The  $\alpha$  polymorph, the least stable of the common polymorphic forms of fats, has the lowest interfacial tension, heat of crystallization, and melting point temperature. The  $\beta'$  and  $\beta$  polymorphs have increasing interfacial tensions, heats of crystallization, and melting point temperatures. Thus, as a liquid fat is cooled, the polymorph that forms first depends on the properties of the different polymorphs. For example, Hernqvist (2) showed that the first polymorph to appear as trisaturated triacylglycerols (with fatty acids from lauric to stearic acid) were cooled was either the  $\alpha$  or  $\beta'$  polymorph, depending on the chain length, even though the nucleation temperature was well below the melting point of the  $\beta$  polymorph (Figure 10). In the case of tristearin, formation of the  $\alpha$  polymorph occurred even though both the  $\beta'$  and  $\beta$  polymorphs were subcooled to a greater extent (higher driving force).

The formation of a less-stable polymorph under conditions where a more stable polymorph is subcooled to a great extent has been explained by the difference in interfacial tensions of the different polymorphs (28). A small difference in interfacial tension can result in a large difference in nucleation rate (25), and this effect



**Figure 10.** Onset temperature of nucleation and polymorphic form of monoacid triacylglycerols with different chain lengths ( $n_c$ ) at slow cooling rate ( $0.4^\circ\text{C}/\text{min}$ ).  $\alpha_{mp}$ ,  $\beta'_{mp}$  and  $\beta_{mp}$  represent the melting temperatures of the different polymorphs (2).

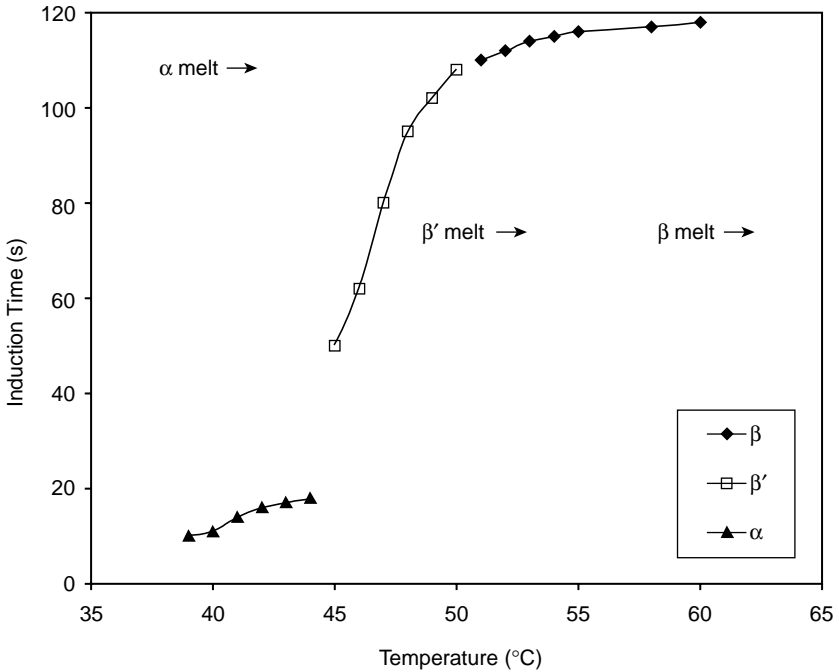


**Figure 11.** Nucleation rate (highly schematic) of lipid polymorphs (4).

generally is greater than the effect of temperature driving force. Thus, nucleation rate of lipid polymorphs is often considered to follow the general trend shown in Figure 11.

Kellens et al. (32) studied the nucleation rate of the  $\beta'$  polymorph of tripalmitin (PPP) by using a microscope counting technique. An increase in temperature from 45°C to 52°C led to a decrease in nucleation rate, as expected. A semilogarithmic plot of nucleation rate versus the inverse of the square of the subcooling, according to the general form of Equation 1, gave a straight line over the range from 45°C to 50°C. Above 50°C, a different straight line was obtained indicative of formation of a different polymorph (confirmed from the change in crystal habit observed microscopically).

Another important kinetic aspect of nucleation is the induction time, defined as the time required for a system to nucleate once a certain subcooling has been attained. That is, induction time for the onset of nucleation is the time required for detection of the first nuclei in a supersaturated or subcooled system. In reality, induction time includes the true time required for nucleation plus the time required for detection of crystallization by the experimental technique. Techniques that have been used for studying lipid nucleation include microscopy, refractive index, light scattering, calorimetry, viscosity, turbidimetry, laser polarized-light turbidimetry, and NMR (4). Each method has its advantages and limitations for studying lipid nucleation (33). Herrera et al. (34) showed that light microscopy could detect a crystal with a minimum size of 0.2  $\mu\text{m}$ , whereas laser polarized-light turbidimetry detected a smaller size of nuclei. Thus, the laser polarized-light turbidimetry technique was more accurate and suitable when size of nuclei is very small. Any method of studying induction time for nucleation must be used with caution (35).



**Figure 12.** Induction time kinetics for onset of nucleation of different polymorphs of tripalmitin. Melting temperatures of each polymorph indicated by straight line (4).

The induction time,  $\tau$ , is a function of subcooling and reflects the time necessary for a critical size of nucleus to be developed in the liquid. The induction time is also dependent on the size at which nuclei are detectable and the growth rate at this early stage. Despite this limitation in measurement methods, induction times are often considered to be inversely proportional to nucleation rate (4)

$$\tau \propto J^{-1}. \quad (3)$$

Induction times for nucleation of a tripalmitin melt at different temperatures are shown in Figure 12 (36). The tripalmitin melt was cooled quickly from 80°C to the different crystallization temperatures indicated on the figure and induction time measured as the first point of detection of crystals on a polarized light microscope. The relative time scales for the onset of nucleation are clearly shown, with the less-stable  $\alpha$  form taking significantly less time to nucleate than the  $\beta'$  polymorph. The induction time for the most stable  $\beta$  polymorph was substantially longer than for either of the less-stable polymorphs.

**3.1.3. Nucleation in Lipid Emulsions** In many foods, the lipid phase appears in emulsion form, or small droplets of fat dispersed in a continuous aqueous phase,

as for example found in cream (37). The nature of the fat crystals in cream plays an important role in determining the physical properties and quality characteristics of butter. Thus, nucleation of fats in emulsion form is an important commercial phenomenon.

When a fat is emulsified, nucleation is substantially altered compared with the same fat in bulk liquid form. This is primarily because of the distribution of heterogeneous nucleation sites among the emulsion droplets. If there are more droplets than heterogeneous nucleation sites, then some of the droplets will nucleate by a homogeneous nucleation mechanism. That is, as a finely dispersed emulsified system is cooled, one population of droplets nucleates at relatively higher temperatures because of heterogeneous nucleation, whereas another population nucleates at substantially lower temperature because of homogeneous nucleation.

It is widely recognized that the size of the emulsion droplets is an important factor in the extent of subcooling (11). Smaller droplet size leads to nucleation at a lower temperature (greater degree of subcooling). Thus, the probability of nucleation within an emulsion droplet is lower than in the bulk fat (38). The dispersity of droplet sizes, however, did not change the critical subcooling required for onset of nucleation (39).

Crystallization from the emulsified state may lead to different nucleation processes than observed for the same fat in bulk liquid form. It has been suggested that nucleation often occurs at the interface of the droplet where surface-active agents are located. The general similarity of the lipophilic components of surfactants oriented at the surface may provide some ordering and structure for the lipid molecules within the droplet and enhance nucleation, as found for example by Kaneko et al. (40) for a hydrocarbon emulsion. Walstra (11) also suggests that formation of compound crystals from emulsions of natural fats may be different than the same fat crystallized from bulk liquid. The initial polymorph formed may also be different, with more stable polymorphs more likely to form in the emulsion (38).

### 3.2. Crystal Growth

Once nuclei have formed, they grow by the incorporation of other TAG molecules from the liquid phase. The incorporation of a new TAG molecule into an existing crystal lattice depends on the probability of it having the correct configuration at the correct site on a crystal surface. When a molecule diffusing from the liquid phase reaches the crystal surface, it may bind into the crystal lattice or return to the supersaturated system, depending on its configuration. Growth continues as long as there is a driving force for crystallization. Eventually crystal growth ceases when the system attains phase equilibrium or the entire system is crystallized (4).

For growth to occur, molecules from the liquid phase must migrate to the surface of the crystal, where rearrangement and orientation takes place. A growth unit (either an individual molecule or a cluster of molecules) then migrates across the crystal surface until it finds an appropriate site for incorporation into the lattice. Once a growth unit has become incorporated, there is a release of latent heat and this energy must be diffused away from the growing surface or else the temperature

will increase to the point where no further growth can occur. General theories of crystal growth have been developed for crystallization of pure substances (4, 24). These theories are based on one or more of the steps in crystal growth being the rate-limiting step. Further details of these theories can be found in the references by Mullin (24) and Hartel (4).

In natural fats, the different TAG species come together to form mixed or compound crystals. The likelihood of two TAG crystallizing together depends on the similarities or differences in molecular configuration (chain length, degree of unsaturation, nature of any double bonds, and arrangement of the fatty acids on the glycerol backbone). TAG species that are similar tend to cocrystallize, but under certain conditions (e.g., very rapid growth), even different TAG species can cocrystallize in a loosely organized crystal lattice ( $\gamma$  or  $\alpha$  polymorphs). In fact, it is this molecular diversity that results in some natural fats remaining in the metastable  $\beta'$  polymorph for extended periods of time.

Growth of TAG crystals is typically very slow (41). There may be several reasons for slow growth rate of TAG crystals:

- The incorporation of a TAG molecule into a crystal lattice requires a very large loss in conformational entropy, and thus, a long time is needed for the TAG molecule to fit into the crystalline lattice. In addition, the TAG molecule may be detached before the crystalline lattice before it is fully incorporated into the crystalline lattice. For example, for growth of tristearin (SSS) in triolein (OOO), linear growth rates of the order of  $10^{-8}$  to  $10^{-7}$  m/s have been observed (41).
- In a multicomponent fat, there is a vigorous competition between similar molecules for a vacant site in a crystal lattice. Multicomponent fats crystallize more slowly than pure TAG at the same crystallization driving force. However, crystal growth in multicomponent fats may be enhanced by the formation of compound crystals. Compound crystals usually occur in the  $\alpha$  or  $\beta'$  forms and rarely in the  $\beta$  form.

According to Timms (25), more stable polymorphs grow faster than unstable ones at any given temperature. This is because of the higher melting point of the more stable polymorphs, which means that the more stable polymorph has a higher degree of subcooling at any given temperature.

### 3.3. Modeling of Crystallization Kinetics of Fats

Crystallization data have typically been treated theoretically using either the Fisher–Turnbull model or the Avrami equation. These analyses not only allow lipid crystallization to be modeled but may also shed some light on the mechanisms of nucleation and growth. However, there is some recent debate about the validity of such models, especially the application of the Avrami equation (42) to accurately depict crystallization of lipids.

Recently, Foubert et al. (43) developed a new, empirical model (Foubert model) to predict the kinetics of fat crystallization. Other authors have used a reparameterized Gompertz equation (Gompertz model) to empirically describe crystallization kinetics of fats (44, 45).

**3.3.1. Avrami Analysis** The Avrami equation, a general approach for description of isothermal phase transformation kinetics originally developed for polymers (46), is often used for describing nucleation and crystal growth in fats. The Avrami equation is given as

$$(1 - X) = \exp\{-kt^n\}, \quad (4)$$

where  $X$  is fraction of crystal transformed at time  $t$  during crystallization,  $k$  is crystallization rate constant that depends primarily on crystallization temperature, and  $n$ , the Avrami exponent, is a constant relating to the dimensionality of the transformation. The values of  $n$  and  $k$  are calculated from the linear form of the Avrami equation (Equation 5) as the slope and intercept at  $\ln t = 0$ , respectively

$$\ln(-\ln(1 - X)) = \ln(k) + n[\ln(t)]. \quad (5)$$

The Avrami exponent ( $n$ ) is a function of the number of dimensions in which growth takes place, and it reflects the details of nucleation and growth mechanisms. For most transformations, the  $n$  is found to be constant over a substantial temperature range (47). Christian (48) tabulated some values of  $n$  expected for various crystallization mechanisms. For example, an  $n$  of 4 indicates heterogeneous nucleation and spherulitic growth from sporadic nuclei, whereas an  $n$  of 2 indicates high nucleation rate and plate-like growth (i.e., two-dimensional growth).

Metin and Hartel (49) applied the Avrami equation to the isothermal crystallization of binary mixtures of cocoa butter with milk fat or milk fat fractions at 15°C. Avrami analysis indicated an  $n$  value of 4 for cocoa butter crystallization, so the suggested mechanism was heterogeneous nucleation with spherulitic growth from sporadic nuclei. For milk fat, the value of  $n$  was 3, suggesting that the crystallization mechanism was instantaneous heterogeneous nucleation with spherulitic growth. For milk fat fractions, the  $n$  value was 2, which suggested that the mechanism was high nucleation rate at the beginning of crystallization decreasing with time, and plate-like growth.

The crystallization rate constant ( $k$ ) is a combination of nucleation and growth rate constants, and is a strong function of temperature (47). The numerical value of  $k$  is directly related to the half time of crystallization,  $t_{1/2}$ , and therefore, the overall rate of crystallization (50). For example, Herrera et al. (21) analyzed crystallization of milkfat, pure TAG fraction of milkfat, and blends of high- and low-melting milkfat fractions at temperatures from 10°C to 30°C using the Avrami equation. The  $n$  values were found to fall between 2.8 and 3.0 regardless of the temperature and type of fat used. For temperatures above 25°C, a finite induction time for crystallization was observed, whereas for temperatures below 25°C, no induction time was

found (crystallization was instantaneous)). Calculation of crystallization rate constant,  $k$ , and half time for crystallization based on the Avrami analysis were in line with the two different behaviors observed in SFC values of the fats.

Even though the Avrami model has been the most frequently used model to describe the isothermal kinetics of fat crystallization, there are some concerns about the use of the model in fat crystallization. Theoretically, integer values should be obtained for the Avrami exponent,  $n$ . However, generally fractional values of  $n$  were obtained in crystallization of fats and oils. Additionally, the linear format of the Avrami equation should give a single slope associated with the value of the Avrami exponent. However, in some studies, two regions of different slopes were obtained. Moreover, secondary nucleation during crystal growth is not considered in the Avrami model, which may in part explain the noninteger values of the Avrami exponent.

**3.3.2. Fisher-Turnbull Analysis** The activation free energy for nucleation,  $G_c$ , may be found from the Fisher-Turnbull equation given in Equation 1. The term in the second exponential of Equation 1 is often given as  $G_c/kT$ . Combination of Equations (1) and (3) allows development of the following equation:

$$\tau T = \left( \frac{h}{Nk} \right) \exp \left\{ \left( \frac{\alpha \Delta S}{k} \right) \right\} \exp \left\{ \frac{16\pi\gamma^3 T_f^2}{3kT(\Delta H_f^2)(T_f - T)^2} \right\}. \quad (6)$$

Based on Equation 11, a plot of  $\tau T$  versus  $\{1/T(T)^2\}$  leads to a straight line for nucleation of a given polymorph. The critical free energy for nucleation,  $G_c$ , is then found from the slope of that straight line,  $s$ , as

$$\Delta G_c = \frac{sk}{(T_f - T)^2}. \quad (7)$$

For a given fat system, although the slope is constant,  $G_c$  varies with crystallization temperature.

The Fisher-Turnbull approach has been used to compare nucleation of various lipid systems. Ng (51) and Herrera et al. (34), for example, have used this approach to characterize crystallization of palm oil and hydrogenated sunflower oil, respectively. The use of the Fisher-Turnbull approach to characterize nucleation leads to a better understanding of the energy changes needed for onset of nucleation and can be used to compare nucleation in different systems. However, this approach is based on a crystallization driving force defined by a single melting point, which may only occur in cases where a single TAG component (or a TAG grouping with narrow range of melting temperature) crystallizes from a liquid oil. It also applies only when the subcooling is low (typically less than 10°C). In cases where massive cocrystallization and compound crystal formation occurs, this approach does not work.

### 3.4. Crystalline Microstructure

The dispersion of the crystalline fat phase in a material determines the physical and textural properties of a lipid-based product. For example, the hardness, snap, and glossy appearance of chocolate is caused by crystallization of cocoa butter in the form of numerous, very small (1  $\mu\text{m}$  or less) crystals of the most stable polymorph ( $\beta$  form). The size distribution (mean size and range of sizes), polymorphic form, and shape of the fat crystals, as well as the network formed among the crystals, all play important roles in determining physical attributes of lipid-based products.

In the case of lipid fractionation, however, a different crystal size distribution is desired. As the fat crystals are to be separated from the liquid phase, uniform crystals of distinct size and shape are needed for the most efficient separation. For the most efficient separation by filtration, reasonably large (200 to 300  $\mu\text{m}$ ) crystals of fairly uniform size (narrow distribution of sizes) are needed. Fractionation technologies carefully control nucleation and growth to produce this uniform distribution of crystals to enhance filtration and separation of the high-melting stearin phase from the low-melting olein phase.

In crystallization of most natural fats, the first crystals formed are often observed as thin and fairly long platelets (41). For example, cooling of melted milkfat leads to initial formation of small  $\beta'$  crystals in needle or platelet shape. As these initial crystals grow, they aggregate into spherulites (52) consisting of the needles arranged radially and ranging in size from a few microns up to about 300  $\mu\text{m}$ . If crystallization is very slow (slow cooling), very large spherulitic crystals form. In contrast, rapid cooling to a low temperature results in the formation of numerous small crystals, often found in a random orientation (53). Thus, cooling rate is one of the most important factors influencing crystalline microstructure. Further details on lipid crystalline microstructure are given in Chapter 4.

## 4. CONTROLLING CRYSTALLIZATION

### 4.1. General Principles of Controlling Crystallization

To truly control crystallization to give the desired crystalline microstructure requires an advanced knowledge of both the equilibrium phase behavior and the kinetics of nucleation and growth. The phase behavior of the particular mixture of TAG in a lipid system controls both the driving force for crystallization and the ultimate phase volume (solid fat content) of the solidified fat. The crystallization kinetics determines the number, size, polymorph, and shape of crystals that are formed as well as the network interactions among the various crystalline elements. There are numerous factors that influence both the phase behavior and the crystallization kinetics, and the effects of these parameters must be understood to control lipid crystallization.



## 4.2. Parameters Affecting Crystallization

Parameters that affect crystallization may influence either the thermodynamic behavior or the crystallization kinetics (or both). Parameters that influence lipid crystallization include chemical composition, subcooling, cooling rate, agitation, minor components of fats (mono- and diacylglycerols, polar lipids, etc.), and scale of operation. The effects of these parameters on lipid crystallization will be reviewed briefly in this section. More detailed information about the effects of these parameters on lipid nucleation and crystal growth may be found elsewhere (4, 24, 28, 54).

### 4.2.1. Compositional Parameters

**4.2.1.1. TAG Composition** Natural fats are composed of a wide range of TAG that contain fatty acids of differing chain length, degree of unsaturation, and positional arrangement on the glycerol backbone. The fatty acid composition of fats may be broad, as in milkfat, or may be limited, as in cocoa butter. It might be expected that a faster nucleation rate occurs in molecularly similar fats compared with the ones with complex structure (wide range of fatty acid species), but this is not necessarily true. Metin and Hartel (55) observed that the induction times for nucleation of milkfat were significantly faster than that for cocoa butter at the same isothermal temperatures (and approximately the same melting point). The faster induction time for milkfat may be a result of a higher driving force (even though the difference between crystallization temperature and final melting point is about the same), or it may be because the TAGs in milkfat more readily come together into mixed crystals. As both are likely to form in a mixture of  $\alpha$  and  $\beta'$  polymorphs, the differences in nucleation rate cannot be attributed to the formation of different polymorphs.

Furthermore, when two fats added together are crystallized from the liquid state, the nucleation rate of the mixture often decreases. For example, the addition of milkfat or milkfat fractions to cocoa butter is widely known to retard crystallization of cocoa butter, with higher addition levels having a greater effect. This effect is commercially important because milk chocolate must be processed at lower temperatures to generate the same level of crystallization as dark chocolate. Metin and Hartel (55) documented the inhibitory effects of milkfat and milkfat fractions on induction time for nucleation of cocoa butter. Martini et al. (56) measured the induction time for nucleation for addition of sunflower oil to a high-melting milkfat fraction. As the level of sunflower oil increased to 40%, the melting point decreased only by a few degrees, but induction time increased by more than a factor of two. This suggests that the effect of sunflower oil on inhibiting nucleation of the milkfat was primarily caused by a true inhibition rather than to a decrease in the driving force for crystallization.

**4.2.1.2. Minor Constituents** Minor constituents in fats that can influence crystallization of TAG include the more polar lipids like DAG, MAG, free fatty acids,

phospholipids, and sterols, although there may be trace amounts of other components that can influence crystallization as well. These constituents have long been considered as active agents for affecting crystallization. In some cases, the presence of these components may enhance crystallization, whereas in other systems, an inhibition is observed.

Nucleation of fats may either be enhanced or inhibited by the presence of these minor components. Dimick (57) has argued that the phospholipids in cocoa butter, with higher melting point than the cocoa butter TAG, crystallize first and subsequently catalyze formation of cocoa butter TAG. The appearance and chemical composition of cocoa butter crystals formed from refined cocoa butter (phospholipids removed) was different from that of the initial crystals formed in nonrefined cocoa butter. Recent studies where these minor components have been separated and then added back to the purified TAG have shown that they invariably inhibit nucleation (21).

There are three potential mechanisms by which addition of minor lipids might affect crystallization. They may limit mass transfer rates of crystallizing TAG to the appropriate site for incorporation into the lattice, they may adsorb on the surface of the growing crystal or cluster and inhibit further incorporation of the crystallizing TAG, or they may actually be incorporated into the crystal lattice as a crystal forms and grows (4). Through any of these mechanisms, the minor constituents in a fat may affect the polymorphic form that is crystallized and often affects the crystal microstructure through preferential inhibition on certain crystal faces (28).

However, in some cases, increased crystallization rate may be observed in the presence of minor constituents. If a macrocrystallizing substance and an additive have a similar structure or form similarly structured complexes to the lattice of the crystallizing substance, then new growth sites on the crystal lattice can be formed by the adsorbed addition. These active sites may be energetically more favorable for incorporating further substances, resulting in an increased crystallization rate (58). For example, Smith et al. (59) found that addition of monolaurin and lauric acid enhanced the crystal growth rate of trilaurin by decreasing facet and crystal size. However, addition of dilaurin decreased the crystal growth rate and altered crystal morphology. They postulated that the varying effects were observed because of the varying sizes and shapes of the additives.

**4.2.1.3. Seeding** At times, crystallization of natural fats may be promoted by the addition of a solid seed material, either of the desired crystallizing species or a foreign particle with nucleating properties. If seeds of the desired crystallizing species are added, they can promote further nucleation and/or provide a surface area for additional crystal growth. Smith (60) reported that addition of  $\beta'$  or  $\beta$  seed crystals to cooled palm oil initiated crystallization at lower degrees of subcooling (higher temperatures) than in the absence of these seeds.

In a sense, tempering of chocolate is done to create a small (<3%) population of seed crystals in the melted chocolate, which catalyze further crystallization of the cocoa butter when the chocolate is subsequently cooled. Through the tempering process, seed crystals in the  $\beta$  polymorph are formed. These stable crystals then

promote formation of numerous small cocoa butter crystals, also in the stable  $\beta$  polymorphic form, as the chocolate is cooled. In this case, the existing seed crystals are thought to spawn additional nuclei through secondary nucleation, although the exact mechanism for this process is not clearly understood. A similar effect is observed upon addition of the high-melting TAG, behenic-oleic-behenic (BOB), to chocolate (61). In this case, the BOB molecules, with very high melting point (53°C), catalyze formation of the  $\beta$  polymorph of cocoa butter crystals, eliminating the need for tempering of chocolate.

## 4.2.2 Operating Parameters

**4.2.2.1. Subcooling or Crystallization Temperature** Arguably, the most important parameter that influences lipid crystallization is subcooling, or the temperature to which the lipid is cooled below the equilibrium point. As subcooling increases, nucleation rate increases and induction time for crystallization decreases. In many natural fats in bulk liquid form (as opposed to emulsified form), only a few degrees of subcooling are necessary to induce crystallization because of the presence of nucleation sites. These sites catalyze nucleation by lowering the energy required for the formation of nuclei.

If subcooling is small, molecules only with the correct configuration (spatial orientation, fatty acid composition, positional arrangement of fatty acids, etc.) are incorporated into a crystal because molecules have sufficient time to orient themselves perfectly. At low subcoolings (crystallization at temperatures within a few degrees of the melting temperature), crystallization rate is slow and only the more stable polymorphs form. When the subcooling is large, incorporation of molecules to the crystal surface is faster, resulting in imperfect attachment of TAG molecules to the surface. Different TAGs can cocrystallize if their chain length and melting points are reasonably close to each other. Consequently, TAGs of different configuration are more easily incorporated into the crystal. The result is more rapid crystallization, but at the cost of formation of compound crystals and lower stability polymorphs.

**4.2.2.2. Cooling Rate** Fat crystallization is greatly influenced by the cooling rate (62). Rapid cooling generally leads to nucleation occurring at a lower temperature than for slow cooling. That is, during slow cooling, the temperature is higher for a longer time and the TAGs have more opportunity to rearrange into a crystal lattice. Cooling rate also affects nucleation rate, which governs crystal size. Rapid cooling to a low temperature promotes a higher nucleation rate, which leads to formation of numerous small crystals (62). When a fat is cooled very slowly, large crystals form. Cooling rate also influences crystalline microstructure. Marangoni and Hartel (53) used confocal microscopy to show that slowly cooled milkfat formed spherulitic crystals, whereas rapidly cooled milkfat formed random crystalline strands.

**4.2.2.3. Agitation (Shear)** The speed of mixing is generally thought to promote both nucleation and crystal growth (4). However, the effects of agitation rate may

be complex because it is sometimes difficult to separate the effects of mixing and cooling rate on crystallization (higher agitation often results in faster cooling rate). Thus, higher agitation rate may influence crystallization time and crystal size without necessarily influencing nucleation and growth (41).

Agitation may promote nucleation because of the mechanical disturbance that supplies energy to overcome the energy barrier for nucleation (24). Agitation aids cooling, crystallization, and formation of small crystals. Slow cooling rate and slow agitation of fats may result in increased number of mixed crystals; thus, melting range is increased. Higher agitation rate results in a higher crystallization rate and formation of small crystals. Agitation also promotes secondary nucleation, primarily by detachment of small particles from crystal structures. Thus, Herrera and Hartel (62) found that higher agitation rates led to the formation of smaller fat crystals in a milkfat model system.

The structure of the crystal network in fats and oils is strongly influenced by cooling and shear rates, the degree of subcooling, and annealing time. For example, crystalline orientation and acceleration of phase transitions induced by shear in different fats (cocoa butter, milkfat, stripped milkfat, and palm oil) were demonstrated using synchrotron XRD (63). The fats were crystallized under static conditions and under shear ( $90 \text{ s}^{-1}$  and  $1400 \text{ s}^{-1}$ ) from the melt ( $50^\circ\text{C}$ ) to  $18^\circ\text{C}$  at a rate of  $3^\circ\text{C}/\text{min}$ . During static crystallization ( $20^\circ\text{C}$  after 1 day), the initial nucleation was characterized by the appearance of platelet-like nuclei far apart from each other. As they grew, the system became a dispersed suspension of rapidly growing crystals. Eventually, clusters of crystals were formed. The introduction of a moderate shear field to the fat system seemed to prevent the formation of these clusters. The presence of shear field resulted in the formation of small asymmetric crystals. Weak or no orientation of the crystals was observed at low shear rates either because of a random distribution of anisotropic crystals or the formation of spherical particles upon aggregation. They also stated that the shear forces accelerated solid-state phase transformations.

The effects of agitation rate on crystallization kinetics of butter fat were studied by Grall and Hartel (64). In a 2 L batch crystallizer, increased agitation rate caused an increase in nucleation rate (more crystals generated per unit time) and an increase in total crystallization (mass deposition) rate. However, the effects of agitation on growth rates of individual crystals were dependent on temperature of operation. At  $30^\circ\text{C}$ , increased agitation led to a decrease in growth rate, whereas for crystallization at either  $15^\circ\text{C}$  or  $20^\circ\text{C}$ , increased agitation caused an increase in growth rate. These results may be related to the different composition effects at the different temperatures (different TAGs cocrystallize).

Garbolino et al. (65) studied the effects of shear rate on crystallization of a confectionery coating fat (hydrogenated and fractionated mixture of soybean and cottonseed oils) using ultrasonic sensors. They hypothesized that primary nucleation is less likely to be affected by shear and suggested that crystal nuclei probably form from heterogeneous nucleation sites (dust particles or other suspended insoluble materials and imperfections in the container walls). They also suggested that

growth of crystals and their interactions are more likely to be affected by stirring because of the occurrence of frequent interparticle collisions.

Thus, from the contradictory results available in the literature, it is clear that our understanding of the effects of heat and mass transfer on crystallization processes is still not complete.

**4.2.2.4. Scale of Operation** The size of the batch being crystallized may influence rate of crystallization. For example, crystallization from an emulsion generally occurs at a lower temperature than for the bulk fat based on the separation of catalyzing nucleation sites. In an emulsion, the catalyzing nucleation sites are more dispersed (spread through the number of droplets) and this leads to nucleation at a lower temperature than the same fat in bulk phase.

Grall and Hartel (64) studied crystallization of milkfat at different scales of operation (2 L and 20 L) and found induction times for nucleation were lower but individual crystal growth rates were higher in the larger scale crystallizer. Other crystallization parameters (total crystal number, mean size, yield, and nucleation rate) were not significantly influenced by this difference in crystallizer size. As scale of operation changes, mixing rates and heat transfer rates change as well, which can influence crystallization processes. Scale up of fat crystallization processes is still somewhat of a trial and error process because of the lack of fundamental understanding of the effects of heat and mass transfer on lipid crystallization.

## 5. SUMMARY

Controlling lipid crystallization in foods has proven to be a technical challenge over the years. Despite a considerable amount of study, controlling the complex interactions between the various lipid components during crystallization remains essentially an empirical process of studying the effects of various operating parameters on crystal formation. Further work on the fundamental principles of lipid nucleation, growth, and polymorphic transformation is needed to truly control crystallization of lipids in foods.

## REFERENCES

1. K. Larsson, *Fette Seifen Anstrichmittel.*, **74**, 136–142 (1972).
2. L. Hernqvist, *Fette Seifen Anstrichm.* **86**, 297–300 (1984).
3. L. Hernqvist, in N. Garti and K. Sato, eds. *Crystallization and Polymorphism of Fats and Fatty Acids*, Marcel Dekker, New York, 1988, pp. 97–137.
4. R. W. Hartel, *Crystallization in Foods*. Aspen Publishers, Inc., Gaithersburg, MD, 2001.
5. K. Sato, *Chem. Eng. Sci.*, 2255–2265 (2001).

6. K. Larsson, in S. Friberg and K. Larsson, eds. *Food Emulsions*, 3rd ed. Marcel Dekker, New York, 1997, pp. 111–140.
7. E. S. Lutton, *J. Am. Oil Chem. Soc.*, **27**, 276–281 (1950).
8. K. Larsson and P. Dejmeck, in S. Friberg and K. Larsson, eds. *Food Emulsions*. 2nd ed. Marcel Dekker, Inc., New York, 1990, pp. 97–125.
9. K. Larsson, *Acta Chem. Scand.*, **20**, 2225–2260 (1966).
10. A. E. Bailey, in *Melting and Solidification of Fats*, Interscience Publishers, New York, 1950, pp. 21–29.
11. P. Walstra, in J. M. Blanshard and P. Lillford, eds. *Food Structure and Behaviour*, Academic Press, Orlando, Florida, 1987, pp. 67–85.
12. S. Ueno, A. Minato, H. Seto, Y. Amemiya, and K. Sato, *J. Phys. Chem. B*, **101**, 6847–6854 (1997).
13. R. L. Wille and E. S. Lutton, *J. Am. Oil Chem. Soc.*, **43**, 491–496 (1966).
14. T. R. Davis and P. S. Dimick, in Proceedings of the Pennsylvania Manufacturing Confectioners' Association (PMCA) Production Conference, Center Valley, PA, 1986, pp. 104–108.
15. K. Van Malssen, A. van Langevelde, R. Peschar, and H. Schenk, *J. Am. Oil Chem. Soc.*, **76**, 669–676 (1999).
16. J. Schlichter-Aronhime and N. Garti, in N. Garti and K. Sato, eds. *Crystallization and Polymorphism of Fats and Fatty Acids*, Marcel Dekker, New York, 1988, pp. 363–393.
17. B. Rossell, *Prog. Lipid Res.*, **23**, 1–38 (1967).
18. R. E. Timms, *Progress Lipid Res.*, **23**, 1–38 (1984).
19. L. Wesdorp, Ph.D. Dissertation, Technical University of Delft, The Netherlands, (1990).
20. W. Kloek, P. Walstra, and T. Van Vliet, *J. Am. Oil Chem. Soc.*, **77**, 643–652 (2000).
21. M. L. Herrera, M. de Leon Gatti, and R. W. Hartel, *Food Res. Int.*, **32**, 289–298 (1999).
22. G. Bigalli, *Manufacturing Confectioner*, **68**, 66–70, 79–80 (1988).
23. R. W. Hartel and K. E. Kaylegian, in N. Garti and K. Sato, eds. *Crystallization Processes in Fats and Lipid Systems*, Marcel Dekker, New York, 2001, pp. 329–355.
24. J. W. Mullin, *Crystallization*. 3rd ed., Butterworth-Heinemann Ltd., Oxford, U.K., 1993, pp. 172–260.
25. R. E. Timms, in R. J. Hamilton, ed. *Developments in Oils and Fats*, Chapman & Hall, Glasgow, U.K., 1995, pp. 204–223.
26. A. G. Walton, in A. C. Zettlemoyer, ed. *Nucleation*, Marcel Dekker, New York, 1969, pp. 225–308.
27. J. Garside, in J. M. Blanshard and P. Lillford, eds. *Food Structure and Behaviours*. Academic Press, Inc., Orlando, FL, 1987, pp. 35–45.
28. R. Boistelle, in N. Garti and K. Sato eds., *Crystallization and Polymorphism of Fats and Fatty Acids*, Marcel Dekker, New York, 1988, pp. 189–226.
29. D. Turnbull and J. C. Fisher, *J. Chem. Phys.* **17**, 71–73 (1949).
30. R. F. Strickland-Constable, in R. F. Strickland-Constable, ed. *Kinetics and Mechanisms of Crystallization*, Academic Press, London, U.K., 1968, pp. 74–129.
31. W. Kloek, Ph.D. Dissertation. University of Wageningen, The Netherlands, 1998.
32. M. Kellens, W. Meeussen, and H. Reynaers, *J. Am. Oil Chem. Soc.*, **69**, 906–911 (1992).

33. A. J. Wright, S. S. Narine, and A. G. Marangoni, *J. Am. Oil Chem. Soc.*, **77**, 1239–1242 (2000).
34. M. L. Herrera, C. Falabella, M. Melgarejo, and M. C. Anon, *J. Am. Oil Chem. Soc.*, **75**, 1273–1280 (1998).
35. S. Martini and M. L. Herrera, *J. Am. Oil Chem. Soc.*, **79**, 411–412 (2002).
36. K. Sato and T. Kuroda, *J. Amer. Oil Chem. Soc.*, **64**, 124–130 (1987).
37. D. Precht, in N. Garti and K. Sato, eds. *Crystallization and Polymorphism of Fats and Fatty Acids*, Marcel Dekker, New York, 1988, pp. 305–362.
38. P. Walstra and E. C. H. Beresteyn, *Neth. Milk Dairy J.*, **29**, 35–65 (1975).
39. D. N. Kaschiev, Kaneko, and K. Sato, *J. Colloid Interface Sci.*, **208**, 167–177 (1998).
40. N. Kaneko, T. Horie, S. Ueno, and K. Sato, *J. Crystal Growth*, **197**, 263–270 (1999).
41. P. Walstra, W. Kloek, and T. van Vliet, in N. Garti and K. Sato, eds. *Crystallization Processes in Fats and Lipid Systems*, Marcel Dekker, New York, 2001, pp. 289–328.
42. A. G. Marangoni, *J. Am. Oil Chem. Soc.*, **75**, 1465–1467 (1998).
43. I. Foubert, P. A. Vanrolleghem, B. Vanhoutte, and K. Dewettinck, *Food Res. Int.*, **35**, 945–956 (2002).
44. W. Kloek, P. Walstra, and T. Van Vliet, *J. Am. Oil Chem. Soc.*, **77**, 389–398 (2000).
45. B. Vanhoutte, K. Dewettinck, I. Foubert, B. Vanlerberghe, and A. Huyghebaert, *Eur. J. Lipid Sci. Tech.*, **104**, 490–495 (2002).
46. M. Avrami, *J. Chem. Phys.*, **8**, 212–224 (1940).
47. J. W. Graydon, S. J. Thorpe, and D. W. Kirk, *J. Non-Crystalline Solids*, **175**, 31–43 (1994).
48. J. W. Christian, *The Theory of Transformation in Metals and Alloys*, 2nd ed., Pergamon Press, London, U.K., 1975.
49. S. Metin and R. W. Hartel, *J. Am. Oil Chem. Soc.*, **75**, 1617–1624 (1998).
50. I. Arvanitoyannis and J. M. V. Blanshard, *J. Food Sci.*, **59**, 197–205 (1994).
51. W. L. Ng, *J. Am. Oil Chem. Soc.*, **67**, 879–882 (1990).
52. H. Mulder and P. Walstra, *Products and Comparable Foods*, Commonwealth Agric. Bureaux, Bucks, England, 1974, pp. 33–52.
53. A. G. Marangoni and R. W. Hartel, *Food Technol.*, **52**, 46–51 (2000).
54. D. Aquilano and G. Sgualdino, in N. Garti and K. Sato, eds., *Crystallization Processes in Fats and Lipid Systems*, Marcel Dekker, New York, 2001, pp. 1–52.
55. S. Metin and R. W. Hartel, *J. Thermal Anal.*, **47**, 1527–1544 (1996).
56. S. Martini, M. L. Herrera, and R. W. Hartel, *J. Agric Food Chem.*, **49**, 3223–3229 (2001).
57. P. S. Dimick, in N. Widlak, ed. *Proceedings of International Conference on Physical Properties of Fats, Oils and Emulsifiers with Applications to Foods*, Am. Oil Chem. Soc. Press, Champaign, IL, 2000, pp. 138–161.
58. J. Nyvlt, O. Sohnel, M. Matuchova, and M. Broul, *The Kinetics of Industrial Crystallization*, Elsevier Science Publishers, New York, 1985, pp. 189–191.
59. P. R. Smith, D. J. Cebula, and M. J. W. Povey, *J. Am. Oil Chem. Soc.*, **71**, 1367–1372 (1994).
60. K. W. Smith, in N. Garti and K. Sato, eds. *Crystallization Processes in Fats and Lipid Systems*, Marcel Dekker, New York, 2001, pp. 357–380.
61. T. Koyano, I. Hachiya, and K. Sato, *Food Structure*, **9**, 231–240 (1990).

62. M. L. Herrera and R. W. Hartel, *J. Am. Oil Chem. Soc.*, **77**, 1177–1187 (2000).
63. G. Mazzanti, S. E. Guthrie, E. B. Sirota, A. G. Marangoni, and S. H. J. Idziak, *Crys. Growth Design*, **3**, 721–725 (2003).
64. D. S. Grall, and R. W. Hartel, *J. Am. Oil Chem. Soc.*, **69**(8), 741–747 (1992).
65. C. Garbolino, G. R. Ziegler, and J. N. Coupland, *J. Am. Oil Chem. Soc.*, **77**, 157–162 (2000).



# 3

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## *Polymorphism in Fats and Oils*

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### **1. INTRODUCTION**

Triacylglycerols (TAGs) are the major components of fats and oils and biologically important organic molecules along with proteins and carbohydrates. In industrial applications, TAGs are the main components in cream, margarine, and confectionery fats in foods and as matrix materials in pharmaceuticals and cosmetics. The physical behavior of TAGs influences the physical properties of fat-based products, such as appearance, texture, plasticity, morphology, and rheology. Most fat-based products are multicomponent TAG mixtures, containing different kinds of fatty acid moieties. Their complex physical properties are ascribed to polymorphism of individual TAG components and their mixing behavior. Therefore, research into the physical properties of the fat-based products usually starts with an understanding of individual TAG molecules and subsequently moves on to an understanding of the mixed systems, while combining this microscopic information with the macroscopic properties of texture, crystal morphology, and rheology. The macroscopic properties of fats and oils will be discussed in other chapters of this volume.

This chapter describes the polymorphism of the principal TAGs with saturated and unsaturated fatty acid moieties and their binary mixtures.

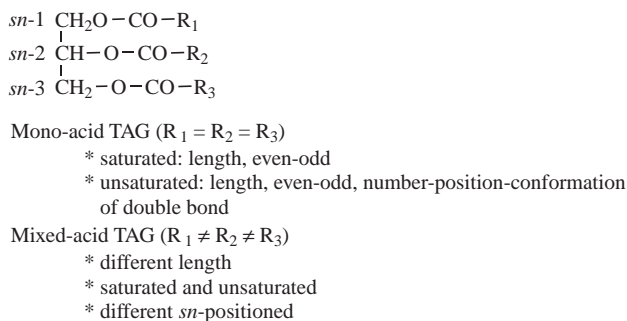
## 2. BASIC CONCEPTS OF POLYMORPHISM OF FATS

TAGs are three-fold esters of glycerol and fatty acids, having the general formula shown in Figure 1. There is a number of fatty acid moiety, as indicated in Figure 1. According to Figure 1, TAGs can be divided into two classes depending on the fatty acid composition. TAGs having only one type of fatty acid are called monoacid TAGs, and those having two and three types of fatty acids are, respectively, called diacid and triacid TAGs, and both are categorized as mixed-acid TAGs. Almost all natural fats and oils are mixed-acid TAGs. In addition, the diacid TAGs can be divided into two types: symmetric and asymmetric TAGs. In the asymmetric diacid TAGs, chiral properties are revealed: For example, *sn*-R<sub>1</sub>R<sub>1</sub>R<sub>2</sub> and *sn*-R<sub>2</sub>R<sub>1</sub>R<sub>1</sub> are stereochemically different from each other, in which *sn* means a stereospecific number. The same chiral properties occur in the triacid TAGs. It is noteworthy that polymorphism of the symmetric TAGs is largely different from that of the asymmetric TAGs.

The physical properties of TAGs are determined by the types of fatty acids that compose them; for example, the number of saturated and unsaturated chains, *cis*- and *trans*-double bonds, short and long chains, chains with even and odd numbers of carbon atoms, and esterified positions of fatty acids with glycerol carbon atoms. Fats are modified by hydrogenation, interesterification, and fractionation to produce desirable physical properties for fat-based products.

### 2.1. Polymorphism of Triacylglycerols

Multiple melting points of fats had already been discovered in the nineteenth century. Clarkson and Malkin showed that this melting behavior resulted from the polymorphism of TAGs (1). In the crystalline state, TAG molecules adopt the ideal

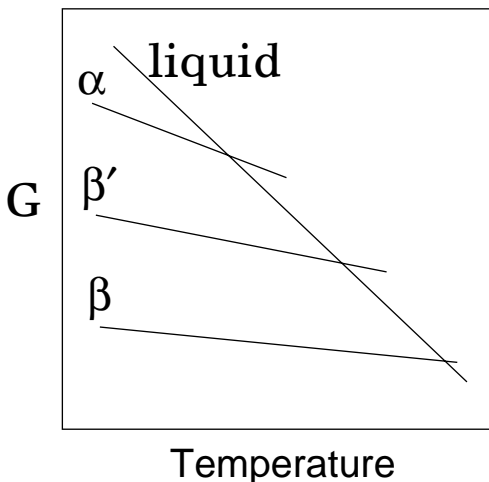


**Figure 1.** A triacylglycerol molecule (*R*: fatty acid moiety, *sn*: stereospecific number).

conformation and arrangement in relation to their neighbors to optimize intramolecular and intermolecular interactions and accomplish an efficient close-packing. On the basis of the structural studies by Larsson (2), the three fundamental polymorphs are called  $\alpha$ ,  $\beta'$ , and  $\beta$ . The significance of the definition of polymorphism of the TAGs lies in unification of otherwise confused nomenclature of the polymorphic forms of the fats differently named by researchers, such as sub- $\alpha$  form, vitreous phase, and so on. In addition, the polymorphic nomenclature makes it convenient to characterize the crystalline properties of fats employed in many applications. For example, the structure and texture of ice cream is caused by a network of partially coalesced  $\alpha$ -form crystals and ice crystals that surround air bubbles to form discontinuous foams (3). The small needle-like  $\beta'$  crystals impart good plasticity that is desirable in products such as margarine, shortening, and baking fats (4). Cocoa butter replacers (CBR) and cocoa butter substitutes (CBS) can crystallize without tempering into their stable  $\beta'$  polymorph upon simple cooling. Tempering is required for  $\beta$  form, which is used for chocolate, cocoa butter, and cocoa butter equivalents (CBE) (5).

One may characterize the polymorphic forms of TAGs by thermal stability, subcell packing, and chain-length structure as described below.

**2.1.1. Thermal Stability** Among the three main polymorphic forms of TAGs and their mixtures, generally,  $\beta$  is the most stable,  $\beta'$  is less stable, and  $\alpha$  is the least stable form (6, 7). A diagram of the Gibbs free energy ( $G = H - TS$ , in which H, S, and T are enthalpy, entropy, and temperature) versus T for TAG polymorphs is shown in Figure 2. The G-T relationship determines the transformation pathways among the polymorphs and liquid (8). The polymorphism of TAGs is monotropic, and the G values are largest for  $\alpha$ , intermediate for  $\beta'$ , and smallest for  $\beta$  in the solid



**Figure 2.** A schematic diagram of Gibbs energy ( $G$ ) and temperature of three polymorphs of a triacylglycerol.

phase domain at low temperature. Each polymorph has its own melting temperature ( $T_m$ ) that is defined as the temperature where the G value of crystal becomes lower than that of liquid. These thermodynamic conditions influence the kinetic aspects of crystallization and transformation of TAGs.

The three basic polymorphic forms shown in Figure 2, which may apply to the saturated monoacid TAGs, are largely modified when the shape of a TAG molecule becomes more heterogeneous. For example, TAGs containing unsaturated fatty acid moieties or saturated diacid moieties exhibit two  $\beta'$  or  $\beta$  forms. In other cases,  $\beta$  does not occur and  $\beta'$  becomes most stable with the highest  $T_m$  instead. These properties will be discussed in Section 3.

A primary concern is polymorphic crystallization in which the Ostwald step rule is very useful (9). This rule predicts that phase changes occur step by step by way of successively more stable phases. For the relative rate of nucleation of polymorphic crystals shown in Figure 2, it follows that nucleation of the metastable forms such as  $\alpha$  and  $\beta'$  occurs first before the most stable  $\beta$  form, when nucleation occurs under a large supercooling or high supersaturation. When the amount of supercooling or supersaturation is decreased, the law is broken and the most stable form tends to nucleate at a relatively slow rate.

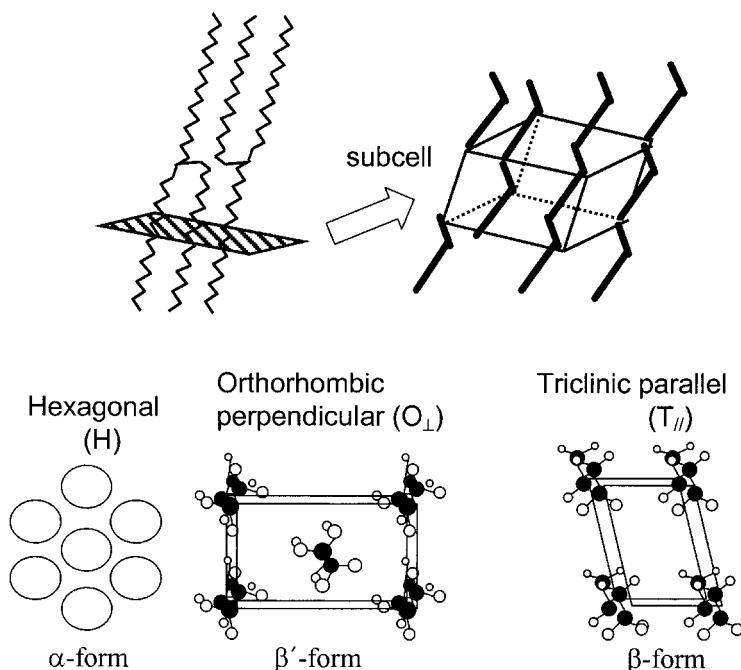
Because of its monotropic nature, the polymorphic transformation occurs irreversibly from the least stable  $\alpha$  form to the most stable  $\beta$  form. The rate of transformation is both time- and temperature-dependent. There are two modes of polymorphic transformation processes: solid-solid and melt-mediated transformations. Solid-solid transformations occur below the melting points of all the polymorphs involved. In contrast, melt-mediated crystallization occurs when the temperature is above the melting points of the less stable forms. Melt-mediated crystallization involves the following processes:

1. Melting of the less stable form
2. Nucleation and growth of the more stable forms
3. Mass transfer in the liquid formed by melting of the less stable form

It has been observed in some TAGs that the rate of melt-mediated crystallization is often much higher than that of solid-solid transformation (10–14).

**2.1.2. Subcell Structure** Subcell structure defines a lateral packing mode of the hydrocarbon chains (2, 15, 16). Three typical subcell structures are shown in Figure 3. The  $\alpha$ ,  $\beta'$ , and  $\beta$  forms have hexagonal (H), orthorhombic perpendicular ( $O_{\perp}$ ), and triclinic parallel ( $T_{//}$ ) subcell structures, respectively (2).

In the hexagonal subcell structure, the two-dimensional lattice is hexagonal and gives rise to a 0.41-nm wide-angle X-ray diffraction (XRD) pattern. The chain packing is loose, and the specific chain-chain interactions are lost because of the ability of the carbon atoms to rotate several degrees and form disordered conformations of hydrocarbon chains. The two-dimensional lattice of an orthorhombic perpendicular ( $O_{\perp}$ ) subcell structure is rectangular, and this represents a tightly

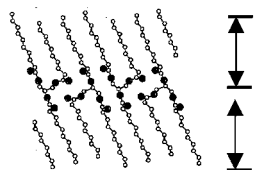


**Figure 3.** Typical subcell structures of TAG polymorphs. The  $\alpha$ ,  $\beta'$ , and  $\beta$  forms have hexagonal (H), orthorhombic perpendicular ( $O_{\perp}$ ), and triclinic parallel ( $T_{\parallel}$ ), respectively.

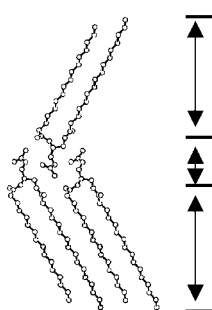
packed lattice with specific chain-chain interactions. The subcell parameters of  $O_{\perp}$  are typically shown in two wide-angle XRD patterns at 0.37 nm and 0.41 nm. Triclinic parallel subcell structure ( $T_{\parallel}$ ) has an oblique two-dimensional lattice and represents tightly packed chains, in which there are specific chain-chain interactions. This subcell structure of  $T_{\parallel}$  is characterized by a strong wide-angle XRD pattern at 0.46 nm and weak patterns at 0.39 nm and 0.38 nm. The values given for these wide-angle XRD patterns of the three polymorphs are typical for the saturated monoacid TAGs; they vary when the fatty acid moieties change from saturated to unsaturated acids.

**2.1.3. Chain Length Structure** The TAG crystals form chain-length structures, in which a repetitive sequence of the hydrocarbon chains is involved in a unit lamellar along the c-axis (Figure 4) (17). One unit layer made up of one hydrocarbon chain is called a leaflet. Several types of chain-length structures can form as shown in Figure 4. The TAGs with the same or very similar fatty acids might form a double chain-length structure. A triple chain-length structure is formed when the chemical natures of one or two of the fatty acids are much different from the others. A quarto-chain-length structure consists of two double chain-length structures, which are combined end-to-end. A hexa-chain-length structure consists of two

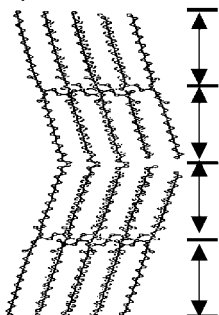
(a) double chain length



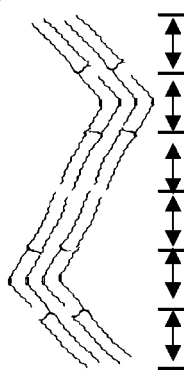
(b) triple chain length



(c) quarto chain length



(d) hexa chain length



**Figure 4.** Typical variations in the chain-length structures of triacylglycerol crystals. An arrow means a leaflet.

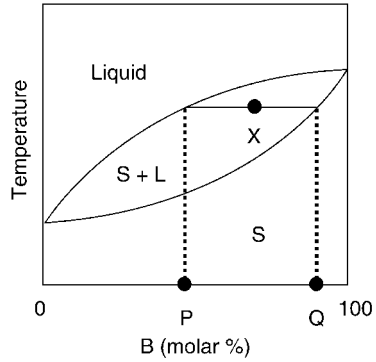
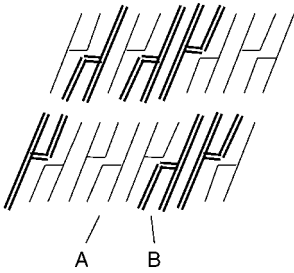
triple chain-length structures. The quarto- and hexa-chain-length structures were observed in asymmetric saturated diacid TAGs, as discussed below.

## 2.2. Phase Equilibria

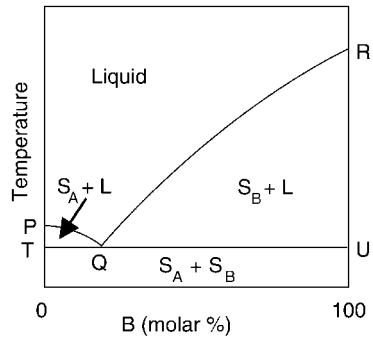
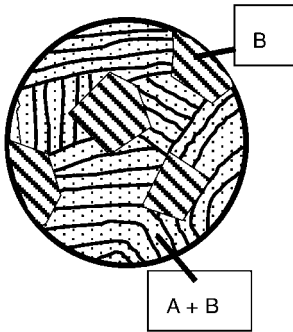
Figure 5 shows three cases that are generally applicable to many materials, and observed in TAG binary mixtures: solid-solution mixture, eutectic mixture, and molecular compound forming mixture (18). Here we summarize basic properties of the three mixture phases. Various binary mixture systems of TAGs will be discussed in Section 4.

**2.2.1. Solid-Solution Mixture** In this system, a binary mixture is cooled but neither component solidifies without containing some of the other component: Both components are deposited simultaneously, and the deposited solid phase is a solid-solution. Only two phases can exist in such a system: a homogeneous liquid-solution and a solid-solution. The equilibrium phase diagram is shown in

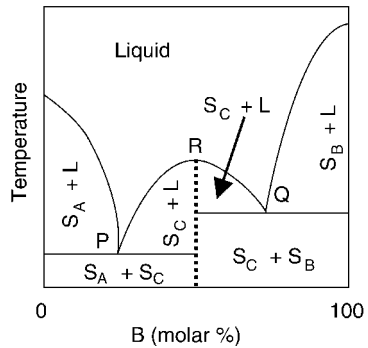
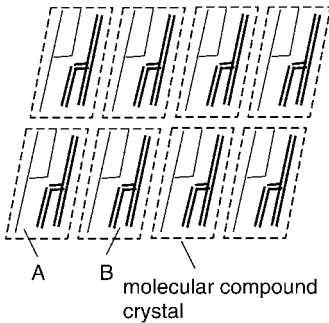
(a) solid-solution mixture



(b) eutectic mixture



(c) molecular compound forming mixture



**Figure 5.** Three typical phase diagrams of binary TAG mixtures represented by A and B fractions.

Figure 5a. All mixtures of the two components have melting points intermediate between the melting points of the pure components.

TAGs that have similar physical and chemical properties, for example, similar melting points, chain-length, polymorphic form, and molecular volume, form solid-solution mixtures.

**2.2.2. Eutectic Mixture** A general example for a eutectic mixture system is shown in Figure 5b, where curves PQ and RQ represent the temperatures at which homogeneous liquid-solutions begin to crystallize. Above the curves P-Q-R, the two components are liquid. Line TQU represents the temperature at which solid mixtures of A-molecule and B-molecule begin to melt, and the two components are completely solid below the line T-Q-U. The small and large areas of PQT and RQU represent mixtures of A-molecule crystals in liquid A/B-molecule and solid B-molecule crystals in liquid A/B-molecule, respectively. It is important to note that the eutectic is a physical mixture, not a molecular compound. Below the eutectic temperature, all mixtures are solid.

Among binary TAG mixtures, the eutectic system is most common. Eutectic systems tend to occur when the TAGs differ in chain-length, molecular volume, shape, or polymorphic form, but they have similar melting points.

**2.2.3. Molecular-Compound-Forming Mixture** A-molecule and B-molecule of a binary system sometimes combine to form a molecular compound. If a molecular compound can coexist in equilibrium with a liquid of the same composition, the compound has a congruent melting point shown by point R (Figure 5c). The phase diagram for this system can be split into two subdiagrams: A-molecule/molecular compound and molecular compound/B-molecule, and each of these subdiagrams may be considered as a eutectic mixture as shown in Figure 5c. Points P and Q are the other two eutectic points of the subdiagrams that are placed in a juxtaposition manner.

A molecular compound-forming mixture can occur in particular combinations of TAGs, being based on the possibility of achieving specific interactions between both molecules in the crystalline state. Even now, there is no general explanation why and how specific combinations of TAGs can result in molecular compound-forming mixtures, but several examples will be provided in Section 4.

## 2.3. Basic Methods for Studying the Polymorphism of Fats

Methodology for studying the polymorphism of fats, among which thermal analysis, most typically, differential scanning calorimetry (DSC), X-ray diffraction (XRD), neutron diffraction, infrared absorption spectroscopy, and nuclear magnetic resonance (NMR), are briefly mentioned here.

DSC analysis provides the data of temperatures, enthalpy and entropy values of melting, crystallization, and polymorphic transitions, which are prerequisites for isolation of individual polymorphic forms and their thermal stability.

Molecular structural information, lamellar distance (long spacing), and subcell structure (the short spacing) are calculated by small-angle and wide-angle diffraction patterns from a powder XRD study using polycrystalline powder sample. Atomic-level crystal structure is revealed by XRD using a high-quality single crystal.

One of the most exciting methodologies that has recently been applied to fat polymorphism is synchrotron radiation XRD (SR-XRD). It has made it possible to perform real-time (in situ) observations of polymorphic transformations at rapid



rates of temperature variation as high as 5°C/min under external stimuli of shear (19) and ultrasonication (20). Furthermore, a combined study of SR-XRD small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) with DSC is now one of the most powerful methods for clarifying the kinetics of the polymorphic transitions of TAGs in single-component and mixture systems. Observing the correspondence of the DSC thermopeaks and variations in the SAXS-WAXS patterns during rapid temperature change has clarified the mechanisms of complicated polymorphic transformations of binary mixtures of TAGs or liquid-crystal to polymorphic crystal conversion, which had been overlooked with conventional laboratory-scale XRD apparatus. Sections 3 and 4 will address these issues.

The use of neutron diffraction provides structural information about fats in liquid and crystalline states through interactions of neutrons with atomic nucleus that is different from the information provided by X-ray diffraction. Neutron diffraction studies with selective deuteration of glycerine and fatty acid chains of a TAG indicated nematic-type liquid crystal organization of the TAG molecules in the liquid phase (21, 22).

For molecular properties of the TAG polymorphs, local molecular structural information such as methyl-end group, olefinic conformation, and chain-chain interaction are unveiled by infrared (IR) spectroscopy, especially Fourier-transformed infrared spectroscopy (FT-IR) (23, 24). Compared with a pioneering work by Chapman (25), great progress has been achieved by using various FT-IR techniques, such as polarized transmission FT-IR, reflection absorption spectroscopy (RAS), and attenuated total reflection (ATR) (26–28).

NMR, especially cross-polarization and magic-angle spinning NMR (CP/MAS NMR), is also a powerful tool for studying the molecular conformations of the TAGs in a crystalline state, because CP/MAS NMR spectra give detailed information about the local environment and mobility of specific carbon sites (29–33).

### 3. POLYMORPHISM OF MONOACID TRIACYLGLYCEROLS

#### 3.1. Saturated Monoacid Triacylglycerol

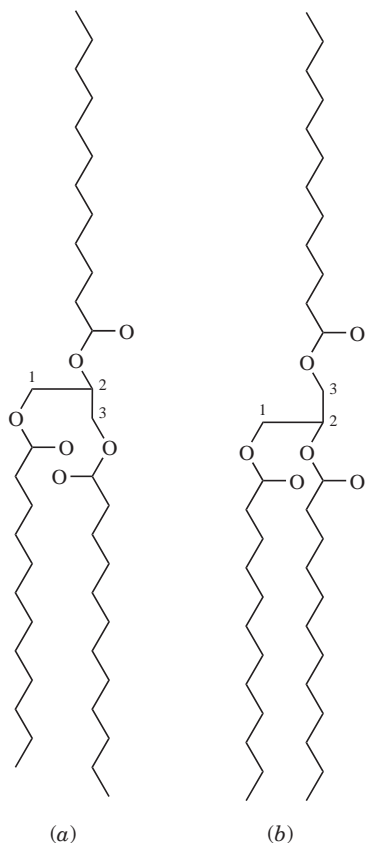
Saturated monoacid TAGs are of the simplest chemical shape and, therefore, have been examined as model substances for the study of the complex fats.

The atomic-level crystal structures of the  $\beta$  form of saturated monoacid TAGs were first clarified almost four decades ago (34–36). Based on these structural data, Lutton postulated the  $\beta$  form structures of saturated monoacid and diacid TAGs (37).

Quite recently, van Langevelde et al. examined the crystal structures of the  $\beta$  form of tripalmitin (PPP) in comparison with the results of tricaprins (CCC) (38, 39), trilaurin (LLL) (36), and predicted the  $\beta$  form structure of trimyristin (MMM) (38). As shown in Table 1, the unit cell parameters, double-chain length structure, and  $T_{//}$  subcell structure of the three TAGs are almost the same, except

**TABLE 1. Unit Cell Parameters of  $\beta$  Form of Tricaprin (CCC), Trilaurin (LLL), and Tripalmitin (PPP) (V; Unit Cell Volume, D; Density).**

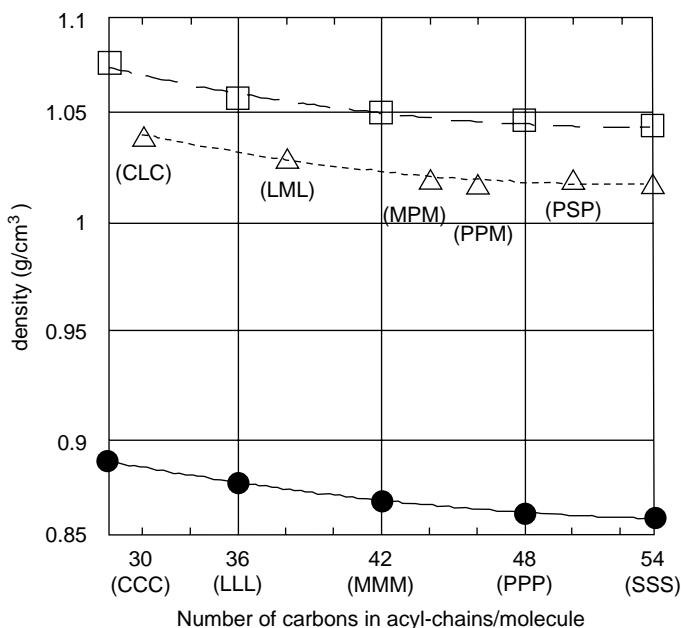
Parameters	CCC	LLL	PPP
Space group	$P\bar{1}$	$P\bar{1}$	$P\bar{1}$
a axis (nm)	1.218	1.208	1.195
b axis (nm)	3.156	3.661	4.684
c axis (nm)	0.549	0.547	0.545
$\alpha$ (deg.)	73.4	73.4	73.8
$\beta$ (deg.)	100.7	100.5	100.2
$\gamma$ (deg.)	119.2	118.7	118.1
V (nm <sup>3</sup> )	1.7613	2.0292	2.5811
D (g cm <sup>-3</sup> )	1.04	1.04	1.04

**Figure 6.** Two types of triacylglycerol conformations in crystal: (a) tuning fork conformation and (b) chair conformation. Numbers correspond to carbon atoms of a glycerine group.

for the chain length parameter (b-axis). Therefore, van Langevelde et al. concluded that, as long as the series of  $C_nC_nC_n$ , in which n means even-number of carbon atoms, all structure models can be predicted by extrapolation of the cell parameters and copying of the atomic coordinates.

There are two types of molecular conformation of TAG molecules in the crystal (39): tuning fork and chair, as shown in Figure 6. In a tuning fork conformation, the two outer acyl chains (*sn*-1 and *sn*-3) point in one direction and the middle acyl chain (*sn*-2) in the opposite direction. In contrast, a chair conformation has the two neighboring acyl chains (*sn*-1 and *sn*-2) pointing in one direction and the third acyl chain (*sn*-3) in the opposite direction. In the  $\beta$  form of CCC, LLL, and PPP, asymmetric tuning fork conformation was revealed.

For  $\beta'$  forms of saturated monoacid TAGs, no crystal structure has so far been determined, because of difficulty in growing single crystals suitable for atomic-level structure determination. However, information about the unit cell can be obtained or calculated. For example, Figure 7 shows the density of liquid,  $\beta'$  and  $\beta$  forms of saturated monoacid and diacid TAGs as a function of the number of carbon atoms (20). The densities of the  $\beta'$  forms of CCC, LLL, MMM (trimyrustin), and PPP could be determined by applying a least-square fitting procedure based on the density of the  $\beta'$  form of tristearin (SSS) (40) and crystal structure data of saturated diacid TAGs (41, 42).



**Figure 7.** Relationship between the density of a TAG and the number of carbon atoms present in the acyl chain of its molecule. Closed circle: liquid state; open triangle:  $\beta'$  form; and open square:  $\beta$  form. Solid and dotted lines are the least-squares fitting curves for each state.

### 3.2. Unsaturated Monoacid Triacylglycerol

Natural fats and oils having  $T_m$  below ambient temperature contain the TAGs with unsaturated fatty acid moieties. Crystallization properties of unsaturated monoacid TAGs were examined six decades ago (43, 44). Compared with the saturated monoacid TAGs, polymorphism of unsaturated monoacid TAGs is more complicated, because of diverse variations in the number and the position of double bonds of their acyl-chain moieties, as clarified by Hagemann et al. (45). The polymorphism of the series of positional isomers of the TAGs having *cis*- and *trans*-octadecenoic acids ( $C_{18}$ , with one double bond) is summarized as follows:

1.  $\alpha$  form occurred except for *cis*  $\Delta 12$ ,  $\Delta 13$ ,  $\Delta 15$ , and *trans*  $\Delta 10$ , where  $\Delta$  means the position expressed as the number of the carbon atom counted from the glycerol backbone at which a double bond is placed,
2. For *cis*-type TAGs, three  $\beta'$  forms were observed for *cis*  $\Delta 7$ ,  $\Delta 9$ ,  $\Delta 11$ , and  $\Delta 13$ , but not for  $\Delta 5$  and  $\Delta 15$ ,
3. For *trans*-type TAGs, two  $\beta'$  forms were observed for *trans*  $\Delta 11$  and  $\Delta 14$ , one  $\beta'$  form for  $\Delta 13$ , whereas  $\beta'$  did not appear for  $\Delta 4$ ,  $\Delta 5$ ,  $\Delta 6$ ,  $\Delta 7$ ,  $\Delta 8$ ,  $\Delta 9$ ,  $\Delta 10$ ,  $\Delta 12$ , and  $\Delta 15$ ,
4.  $\beta$  form was observed in all of the TAGs.

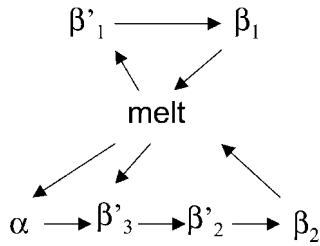
The complexity of polymorphism, especially with regard to the occurrence of multiple  $\beta'$  form, may be ascribed to the variety of positional isomers and *cis-trans*-conformation.

Triolein (OOO) has been studied over many years. Some inconsistency, however, still remains among several reports; for example, Wheeler et al. (43) and Ferguson and Lutton (44) observed an intermediate form, whereas three  $\beta'$  forms,  $\beta'_1$ ,  $\beta'_2$ ,  $\beta'_3$ , were isolated by Hagemann et al. (45). This kind of inconsistency for the presence of  $\beta'$  form may be caused by inconsistency in thermal treatment and purity of the samples employed in the experiments. We have worked on the polymorphism of high-purity OOO (>99 %, supplied from Nippon Oil and Fats Co.) using DSC, X-ray diffraction, and FT-IR (Ueno and Sato, unpublished work). Six polymorphs:  $\alpha$ ,  $\beta'_3$ ,  $\beta'_2$ ,  $\beta'_1$ ,  $\beta_2$ , and  $\beta_1$  were isolated, and thermal and structural properties of the six forms are shown in Table 2. Figure 8 shows the polymorphic transition pathways among the six polymorphs and melt of OOO. Two types of transitions were observed: liquid  $\rightarrow \alpha \rightarrow \beta'_3 \rightarrow \beta'_2 \rightarrow \beta_2$  and liquid  $\rightarrow \beta'_1 \rightarrow \beta_1$ . The former transition occurred after rapid cooling ( $20^\circ\text{C}/\text{min}$ ) to about  $-80^\circ\text{C}$  and subsequent

**TABLE 2. Polymorphism of Triolein (Ueno and Sato, Unpublished).**

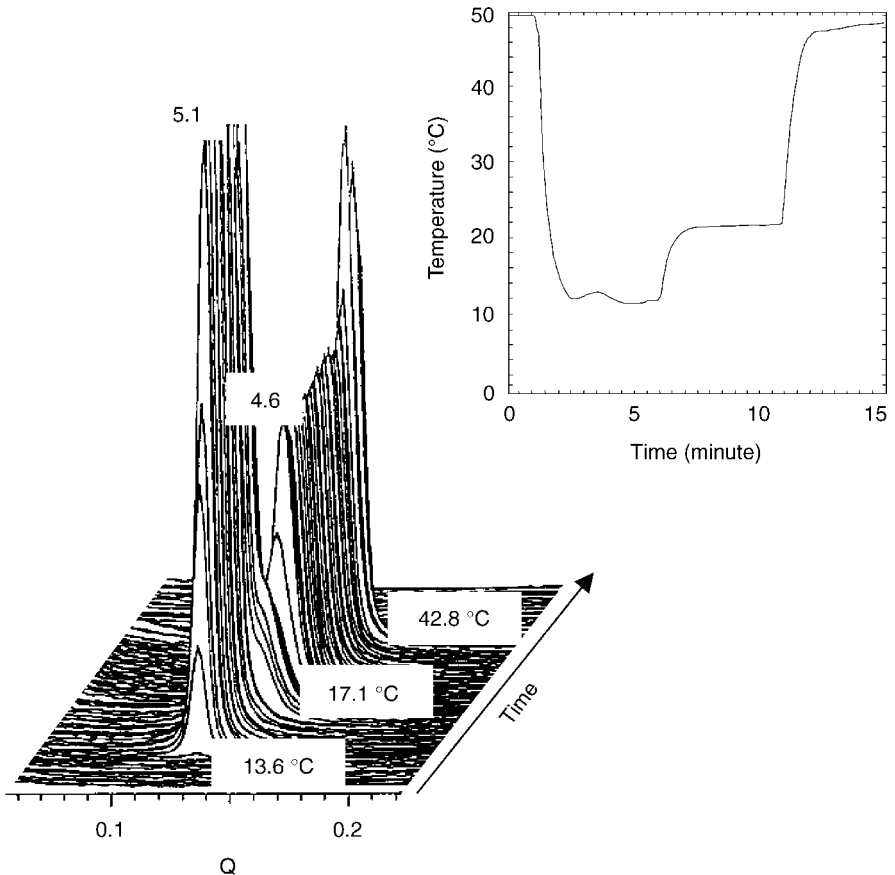
Polymorph	$\alpha$	$\beta'_3$	$\beta'_2$	$\beta'_1$	$\beta_2$	$\beta_1$
$T_m$ ( $^\circ\text{C}$ )	-37.5	-24.9	-15.5	-5.8	4.7	5.9
Subcell	H	$\text{O}_\perp$	$\text{O}_\perp$	$\text{O}_\perp$	$T_{//}$	$T_{//}$
$\Delta H$ (kJ/mol)	—	—	—	—	110	120

Melting point:  $T_m$ , enthalpy of fusion:  $\Delta H$ .



**Figure 8.** Polymorphic transition pathways of triolein.

heating, in which crystallization of  $\alpha$  and successive transformations to  $\beta_2$  through intermediate two  $\beta'$  occurred. By contrast, with slow cooling, the liquid crystallized in  $\beta'_1$  form, which transformed to  $\beta_1$  by subsequent heating. It was interesting to observe that no direct transformation occurred from  $\beta'_2$  to  $\beta'_1$  and from  $\beta_2$  to  $\beta_1$ .



**Figure 9.** Synchrotron-radiation X-ray diffraction patterns of polymorphic transformation of trielaidin taken during temperature variation shown in an inserted figure (unit, nm). Q: wave vector.

It is not easy to understand how such a kind of individual transformation pathway occurs.

As a typical *trans*-monounsaturated TAG, trielaidin (EEE) with *trans*- $\omega$ 9- octadecenoic acid has been examined. Carter and Malkin (46) reported three polymorphs,  $\alpha$ ,  $\beta'$ ,  $\beta$ . Since then, an argument was made to whether the intermediate  $\beta'$  is present or not. In 1990, Desmedt et al. (47) re-examined the existence of the  $\beta'$  form by using DSC and the powder XRD pattern, showing that the  $\beta'$  form was indeed crystallized after the melting of  $\alpha$  form, but soon transformed to  $\beta$ . Therefore, it is difficult to obtain the XRD patterns or FT-IR data of the  $\beta'$  form of EEE at some fixed temperature.

To further investigate the existence of  $\beta'$  form of EEE, we have carried out time-resolved synchrotron radiation X-ray diffraction (SR-XRD) (Ueno and Sato, unpublished). Figure 9 shows the time-resolved small-angle SR-XRD patterns, which reveals the occurrence of a polymorph having the long spacing value of 5.1 nm on cooling, its conversion to the polymorph with the long spacing value of 4.6 nm on heating, and disappearance of the second polymorph during further heating. According to previous reports (46, 47), it was evident that the former polymorph corresponds to the  $\alpha$  form that crystallized when temperature was quenched from 50°C to 13.6°C, and the second form is  $\beta$ . When temperature was jumped to 22°C,  $\alpha$  melted and the  $\beta$  form crystallized, which then melted when temperature increased to 48°C. During this temperature variation,  $\beta'$  form was not observed by the in situ SR-XRD study. Therefore, our conclusion is that trielaidin has the  $\beta'$  form, which is very unstable.

## 4. POLYMORPHISM OF MIXED-ACID TRIACYLGLYCEROLS

Understanding the polymorphism of mixed-acid TAGs is of significance because the fatty acid compositions of natural fats are generally heterogeneous; namely, combinations of fatty acids in TAGs are diverse with respect to carbon number, mixing of saturated-unsaturated chains, the position and the number of double bond of the unsaturated fatty acids, and so on. This group of mixed-acid TAGs is classified to two types: (1) saturated mixed acid type and (2) saturated and unsaturated mixed acid type.

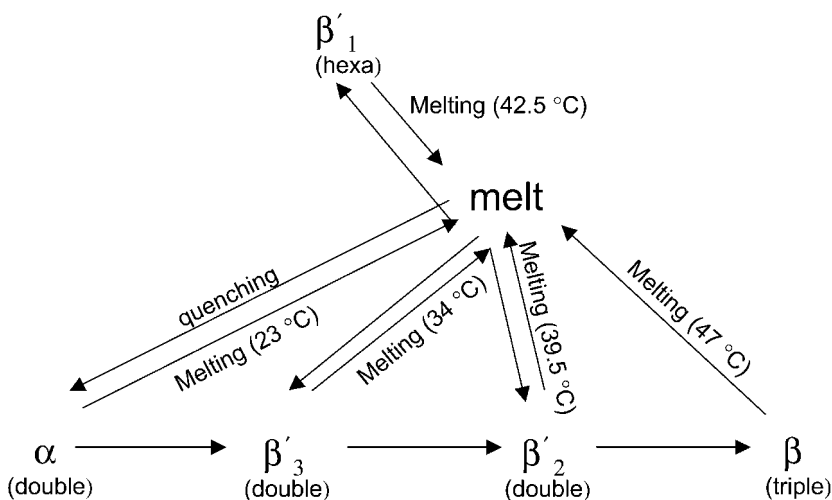
### 4.1. Saturated Mixed Acid Triacylglycerols

**4.1.1. Polymorphic Behavior** In this section, the polymorphism of the  $C_{16}$ - $C_{16}$ - $C_n$ ,  $C_n C_{n+2} C_n$ , and  $C_n C_2 C_n$  TAG series is discussed in terms of their diversity in occurrence of polymorphic structures and their thermodynamic stability and molecular structures.

$C_{16}$ - $C_{16}$ - $C_n$  represents a series of homologous TAGs, in which  $n$ , the carbon number of even-numbered carbon atoms of the *sn*-3 fatty acid chain, varies from 0 to 16. Systematic research on  $C_{16}$ - $C_{16}$ - $C_n$  by using XRD, DSC, and FT-IR

techniques (42, 48–54) showed remarkable diversity in their polymorphism. The  $\alpha$  form was present in  $C_{16}\text{-}C_{16}\text{-}C_n$ . For the  $\beta'$  form, however, there were many variations. One  $\beta'$  form was present in  $C_{16}\text{-}C_{16}\text{-}C_2$  through  $C_{16}\text{-}C_{16}\text{-}C_8$ , but three  $\beta'$  forms ( $\beta'_3$ ,  $\beta'_2$ ,  $\beta'_1$ ) were observed in  $C_{16}\text{-}C_{16}\text{-}C_{10}$ , whereas two  $\beta'$  forms ( $\beta'_2$ ,  $\beta'_1$ ) were isolated in  $C_{16}\text{-}C_{16}\text{-}C_{12}$  and  $C_{16}\text{-}C_{16}\text{-}C_{14}$ . As for  $\beta$  form, one  $\beta$  form was present in  $C_{16}\text{-}C_{16}\text{-}C_2$  through  $C_{16}\text{-}C_{16}\text{-}C_{12}$ . There is, however, no  $\beta$  form in  $C_{16}\text{-}C_{16}\text{-}C_{14}$ , which revealed the most stable form as  $\beta'_1$ . Moreover, chain-length structures of the polymorphic forms in  $C_{16}\text{-}C_{16}\text{-}C_n$  were complicated. For example, single-chain-length structure appeared in the  $\alpha$  form of  $C_{16}\text{-}C_{16}\text{-}C_4$ ,  $C_{16}\text{-}C_{16}\text{-}C_6$ , and  $C_{16}\text{-}C_{16}\text{-}C_8$ , and hexa- and quato-chain-length structures were observed in  $\beta'_1$  of  $C_{16}\text{-}C_{16}\text{-}C_{10}$  and  $\beta'_2$  of  $C_{16}\text{-}C_{16}\text{-}C_{14}$ . Correspondingly, the melting point decreased with increasing carbon number of  $n$  in  $C_{16}\text{-}C_{16}\text{-}C_n$  varying from 0 to 6, whereas it increased with  $n$  from 8 to 16. The unique properties of the series of  $C_{16}\text{-}C_{16}\text{-}C_n$ , in particular for  $C_{16}\text{-}C_{16}\text{-}C_{14}$  have been discussed elsewhere (51, 54). Here we discuss the polymorphic structures and thermal transformation pathways of  $C_{16}\text{-}C_{16}\text{-}C_{10}$  (50), as a typical example of  $C_{16}\text{-}C_{16}\text{-}C_n$ .

Figure 10 shows the polymorphic transformation pathways of five polymorphs of  $C_{16}\text{-}C_{16}\text{-}C_{10}$  together with melt. On quenching of melt, the isotropic liquid produced  $\alpha$  form with a double chain-length structure, which melts at  $22^\circ\text{C}$ . There are two transformation pathways starting from the  $\alpha$  form. The first transformation pathway is  $\alpha \rightarrow \beta'_3 \rightarrow \beta'_2 \rightarrow \beta$ , in which the subcell structure changed from H to two  $O_\perp$ , keeping the double chain length structure in  $\alpha \rightarrow \beta'_3 \rightarrow \beta'_2$ . Then, the chain length structure changed from double to triple, and the subcell structure changed from  $O_\perp$  to  $T_{//}$  when  $\beta'_2$  transformed to  $\beta$ . In the second transformation pathway of  $\alpha \rightarrow \text{melt} \rightarrow \beta'_1$ , the subcell structure and chain-length structure are changed



**Figure 10.** Polymorphic transformation  $\beta$  pathways of  $C_{16}\text{-}C_{16}\text{-}C_{10}$ . Chain-length structure is shown in parenthesis for each polymorph.

drastically. In both cases, many details of the polymorphic transformation mechanisms of  $C_{16}-C_{16}-C_{10}$  remain uncertain. Similarly, interesting phenomena revealed in the polymorphic behavior of  $C_{16}-C_{16}-C_n$  are not understood, e.g., the occurrence of the  $\alpha$  form (single-chain-length structure) and the  $\beta'$  form (tripe-chain-length) in  $C_{16}-C_{16}-C_4$ ,  $C_{16}-C_{16}-C_6$  and  $C_{16}-C_{16}-C_8$ , and disappearance of  $\beta$  in  $C_{16}-C_{16}-C_6$  and  $C_{16}-C_{16}-C_8$ . All of these properties indicate that intramolecular and intermolecular interactions are largely affected by changing the molecular shape of the fatty acid moieties placed at the *sn*-3 position. Understanding the polymorphism of asymmetrical mixed-acid TAGs such as  $C_{16}-C_{16}-C_n$  must be valuable for the formation of structured fats.

In the  $C_nC_{n+2}C_n$ -type TAGs in which *n* is even-numbered and varying from 10 to 16, the most stable form is the  $\beta'$  form, not the  $\beta$  form. The long spacing values and melting points of the  $\beta'$  forms of these TAGs linearly increase with increasing number of *n* (41, 55).

The  $C_nC_2C_n$ -type TAGs, in which *n* is even-numbered varying from 10 to 18, were investigated by Zacharis et al (56). Three polymorphs, the lowest melting, the intermediate, and the highest melting forms, were observed by thermal analysis. As for the highest melting form, the following physical properties were found: (1) The subcell is  $T_{//}$ , (2) enthalpy of fusion, enthalpy of resolidification, and lamellar spacing increased linearly with increasing length of the acyl chains; and (3) the hexa-chain-length structure was formed in  $C_{14}C_2C_{14}$  and  $C_{16}C_2C_{16}$ . Little information has been obtained for the lowest and intermediated melting forms.

**4.1.2. Atomic-Level Crystal Structures of  $\beta'$  Form** We discuss crystal structures of the  $\beta'$  forms of two saturated diacid TAGs,  $C_{10}C_{12}C_{10}$  (41) and  $C_{16}C_{16}C_{14}$  (42). Both are the first  $\beta'$  polymorphs of TAG that have been analyzed at the atomic level by using single crystals. Two different types of the  $\beta'$  structures were found as revealed in the unit cell parameters shown in Table 3.

The unit cell structure of  $C_{16}C_{16}C_{14}$   $\beta'_2$  is shown in Figure 11 (42). The unit cell is stacked in the quarto-chain-length structure, which is constructed by two double layers (I and II in Figure 11) in such a way that the methyl end groups of one double layer are faced with those of another double layer at the center of the unit cell in the a-b plane. The chain axes of the two double layers in the unit cell are alternately

**TABLE 3. Unit Cell Parameters of  $\beta'$  Forms of  $C_{10}C_{12}C_{10}$  and  $C_{16}C_{16}C_{14}$**

	$C_{10}C_{12}C_{10}$	$C_{16}C_{16}C_{14}$
Space group	Iba2	C2
a axis (nm)	5.57368	1.6534
b axis (nm)	2.22783	0.7537
c axis (nm)	0.56945	8.1626
$\beta$ (deg.)	90	90.28
density ( $g\ cm^{-3}$ )	1.04	1.018



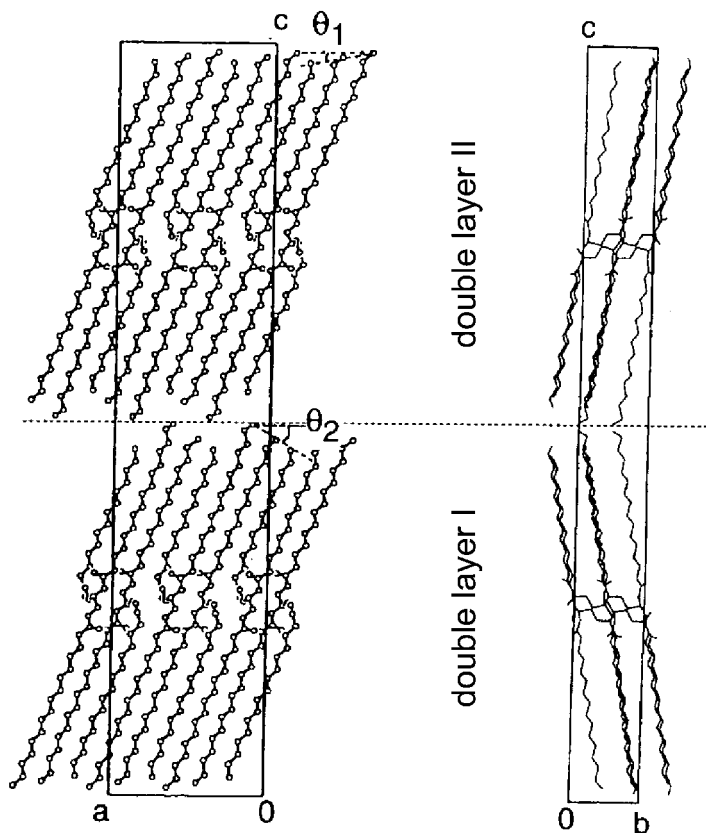


Figure 11. Unit cell structure of  $C_{16}C_{16}C_{14} \beta'_2$ .

inclined against the lamellar interface in the b-c plane. The methyl end groups make zigzag arrangements, but the zigzag angles of the outer interface ( $\theta_1 = 9.6^\circ$ ) and inner interface ( $\theta_2 = 38^\circ$ ) are different. A hybrid-type orthorhombic perpendicular subcell is formed, because of the presence of two asymmetric units in a unit cell. The molecules of two asymmetric units of  $C_{16}C_{16}C_{14}$  have the chair conformation defined in Figure 6.

$C_{10}C_{12}C_{10} \beta'$  crystallizes in a chair conformation with the  $O_\perp$  subcell, having a bend at the glycerol moiety as shown in Figure 12 (41). There is no zigzag methyl end stacking with a flat lamellar interface. Based on the crystal structure of  $C_{10}C_{12}C_{10} \beta'$ , van Langevelde et al. (39) determined the structure of  $C_{14}C_{16}C_{14} \beta'$ , using the powder XRD patterns of polycrystalline samples. They concluded that the two  $\beta'$  forms of  $C_{10}C_{12}C_{10}$  and  $C_{14}C_{16}C_{14}$  are identical, except for the chain-length distance.

The above results of the two  $\beta'$  forms indicate that there is diversity in the crystal structures of  $\beta'$  that are affected by chain-chain interactions of the diacid TAGs.

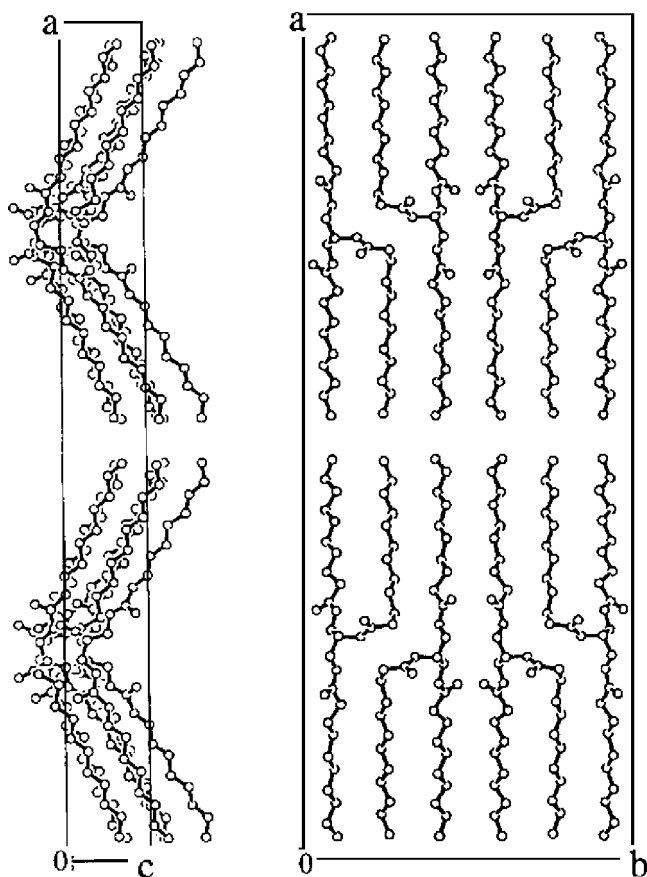


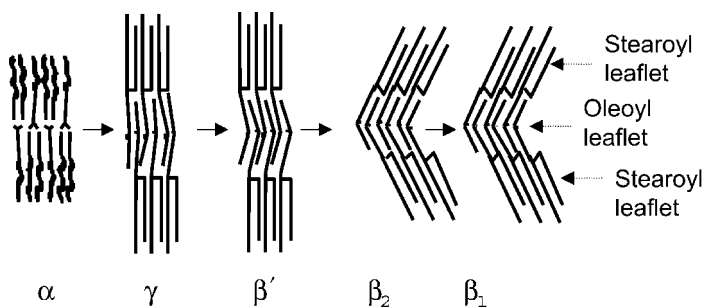
Figure 12. Unit cell structure of  $C_{10}C_{12}C_{10} \beta'$ .

## 4.2. Saturated-Unsaturated Mixed Acid Triacylglycerols

Saturated-unsaturated mixed acid TAGs are the main components of vegetable fats and fish oils. The basic polymorphism of saturated-unsaturated mixed acid TAGs is more complicated than that of the saturated monoacid TAGs (28, 33, 57–65). The chain-chain interactions between the saturated and unsaturated fatty acid moieties are the essential determining factors of this complexity (23, 63).

**4.2.1. 1,3-Disaturated-2-Unsaturated Mixed Acid Triacylglycerols** In this section, we consider the polymorphic behavior of a series of Sat.Unsat.Sat.TAGs, in which the *sn*-2 acid moieties are oleic, ricinoleic, and linoleic acids and the even-numbered saturated acids (palmitic, stearic, arachidic and behenic acids) are placed at the *sn*-1 and *sn*-3 positions.

Figure 13 shows a model of the polymorphic forms of SOS (1,3-distearoyl-2-oleoyl-*sn*-glycerol) (58). Polymorphic transformations occur from  $\alpha$  to  $\beta_1$  through



**Figure 13.** A schematic model of polymorphic transformation of SOS.

$\gamma$ ,  $\beta'$ , and  $\beta_2$ . Compared with the saturated monoacid-type and diacid-type TAGs, the occurrence of the  $\gamma$  form is the unique feature of this group of TAGs. In the case of SOS, the chain-length structure converts from double to triple, and the subcell structures change in different manners between oleic and stearic acid chains. These changes are the result of steric hindrance of the stearic and oleic acid moieties, making the polymorphic transformation of SOS complicated. The main structural properties of SOS are briefly described below;

1.  $\alpha$  form. The double-chain-length structure determined by the SAXS spectra assumes the coexistence of the stearoyl and oleoyl moieties in the same leaflets. The hexagonal subcell shown in the XRD WAXS patterns and FT-IR spectra of  $\delta(\text{CH}_2)$  and  $\nu(\text{CH}_2)$  modes lead to a disordered aliphatic conformation. No specific structure was shown for the olefinic conformation, because no detectable IR band of  $\gamma(=\text{CH})$  was seen (61) and the two carbons adjacent to the *cis*-double bonds were equivalent because of the NMR spectra.
2.  $\gamma$  form. The long spacing value of 7.05 nm assumes a triple-chain-length structure, in which the oleoyl and stearoyl leaflets are separated through the chain sorting during the  $\alpha-\gamma$  transformation. The stearoyl leaflet assumes a specific parallel packing, and the hexagonal subcell structure still remains in the oleoyl leaflets, as verified by FT-IR spectral bands of SOS containing fully deuterated stearoyl and hydrogenated oleoyl chains (61).
3.  $\beta'$  form. The long spacing value 7.00 nm determined by the SAXS peak assumes the triple-chain-length structure, which is constructed by the stearoyl leaflet with the  $\text{O}_\perp$  subcell and the oleoyl leaflet with hexagonal subcell, as shown by FT-IR spectral bands of SOS containing fully deuterated stearoyl and hydrogenated oleoyl chains (61). The  $^{13}\text{C}$  NMR spectra showed clear differences between the two carbons adjacent to the *cis*-double bond and the three glycerol carbons.
4. two  $\beta$  forms. The long spacing values of the triple-chain-length structure were 6.75 nm for  $\beta_2$  and 6.60 nm for  $\beta_1$ . The subcell structures of the stearoyl and oleoyl leaflets are  $\text{T}_{//}$  in  $\beta_1$ . The subcell structure in  $\beta_2$  was very close to  $\text{T}_{//}$  for the two leaflets, but very subtle differences were detectable between the two  $\beta$  forms.

TABLE 4. Polymorphism of POP.

Form	T <sub>m</sub> (°C)	ΔH (kJ/mol)	Long Spacing (nm)	Chain-Length Structure
α	15.2	68.1	46.5	double
γ	27.0	92.5	65.4	triple
δ	29.2	107.5	62.5	triple
β' <sub>2</sub>	30.3	95.5	42.4	double
β' <sub>1</sub>	33.5	98.3	42.4	double
β <sub>2</sub>	35.1	124.4	61.0	triple
β <sub>1</sub>	36.7	130.2	61.0	triple

POP (1,3-palmitoyl-2-oleoyl-*sn*-glycerol) is a homologous substitution of the stearoyl moiety in SOS with the palmitoyl moiety. It was anticipated that POP might show the same polymorphic behavior as SOS. However, a few differences were observed regarding intermediate forms as explained in the following (Table 4) (58):

1. Two β' forms appeared, having a double chain-length structure.
2. During the polymorphic transformation from α to β<sub>1</sub> forms, the chain-length structure changes as double (α) → triple (γ) → double (two β' forms) → tri-triple (two β forms). The alternative variations of the chain-length structure among double and triple are only detected in POP.
3. Another intermediate form with triple chain-length structure, δ, was observed. The cause of complexity in the polymorphism of POP is still open to question.

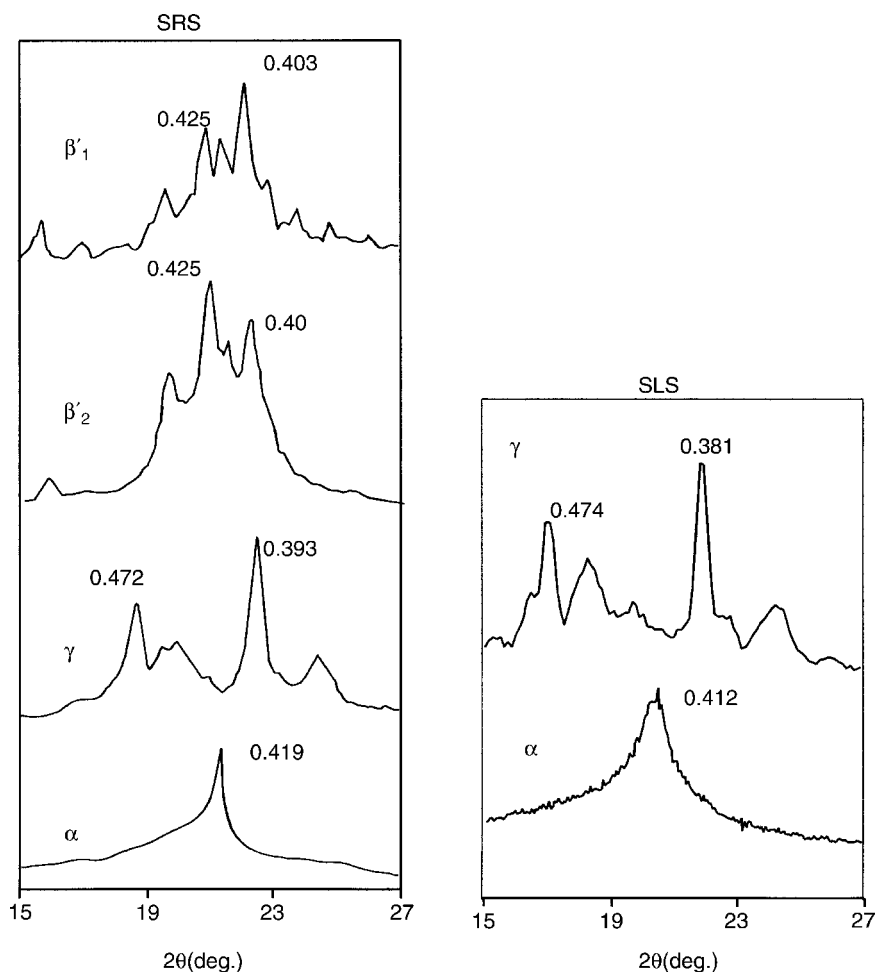
However, the polymorphism of SOS is common to the other Sat.Unsat.Sat.TAGs, as evidenced in the polymorphism of SRS(R, ricinoleoyl) and SLS (L, linoleoyl).

Although SOS, SRS, and SLS share the same polymorphic nature illustrated in Figure 13, remarkable differences are seen in the presence or absence of the stable forms of β' or β among the three TAGs. Namely, SRS has no β form and two β' forms (64), whereas β' and β forms are absent in SLS (65). Thermal data of the

TABLE 5. Thermal Properties of Polymorphism of SOS, SRS, and SLS.

	SOS					SRS				SLS	
	α	γ	β'	β <sub>2</sub>	β <sub>1</sub>	α	γ	β' <sub>2</sub>	β' <sub>1</sub>	α	γ
T <sub>m</sub> (°C)	23.5	35.4	36.5	41.0	43.0	25.8	40.6	44.3	48.0	20.8	34.5
ΔH <sub>m</sub> (kJ/mol)	47.7	98.5	104.8	143.0	151.0	58.1	119.64	171.19	184.76	40.9	137.4
ΔS <sub>m</sub> (J/mol/K)	160.8	319.2	338.5	455.2	477.6	194.35	381.32	539.29	575.31	139.2	448.7

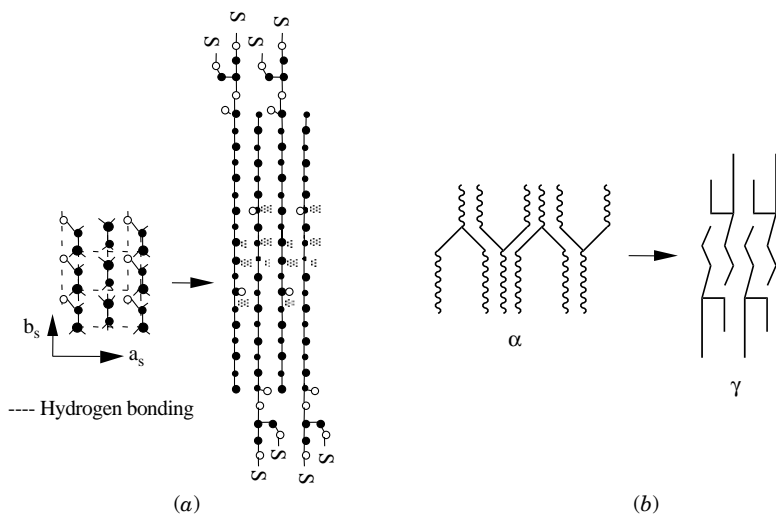
<sup>a</sup>T<sub>m</sub>: temperature of melting; ΔH<sub>m</sub>: enthalpy of melting; ΔS<sub>m</sub>: entropy of melting.



**Figure 14.** Wide-angle X-ray diffraction patterns of polymorphic forms of SRS and SLS (unit, nm).

polymorphic forms of SRS and SLS are shown in Table 5 together with those of SOS. The  $\alpha$  and  $\gamma$  forms present in the two TAGs showed the same molecular structures, as revealed in wide-angle XRD patterns of SRS and SLS shown in Figure 14. It is postulated that hydrogen bonding in the ricinoleoyl chains is so tight that the  $O_{\perp}$  subcell is stabilized through the glycerol groups, probably making  $\beta'$  the most stable in SRS (Figure 15a) (64). The hydrogen bonding in SRS may make the enthalpy and entropy values for melting of the  $\beta'$  forms much higher than  $\beta'$  forms of SOS.

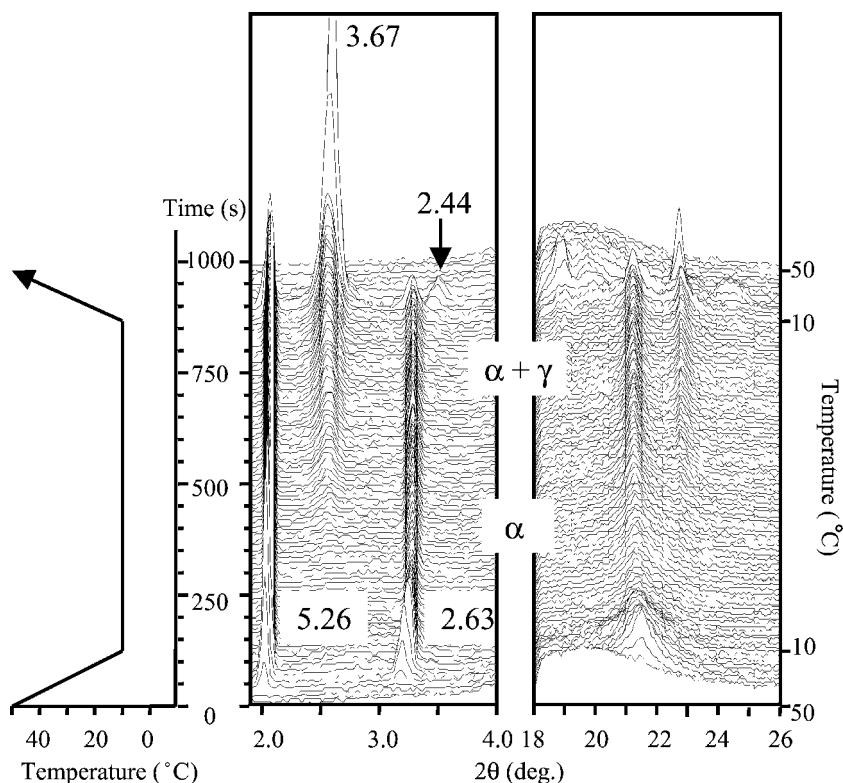
Figure 16 shows SR-XRD patterns of SLS taken during a temperature variation from 50°C to 10°C, kept at 10°C for about 10 min, and heated rapidly to 50°C (65).



**Figure 15.** Schematic model of polymorphic structures of SRS and SLS. (a) A postulated structure of  $\beta'$  forms of SRS, in which a ricinoleic acid leaflet is shown. (b) Polymorphic transformation from  $\alpha$  to  $\gamma$  in SLS.

The occurrence of  $\alpha$  on cooling, transformation from  $\alpha$  to  $\gamma$ , and melting of  $\gamma$  on heating of SLS were clearly observed. In particular, rapid heating from 10°C to 50°C clearly showed the transformation from  $\alpha$  to  $\gamma$  at 20°C. No  $\beta'$  or  $\beta$  forms were detected during further incubation of SLS after melting of  $\gamma$ , or also after long incubation of  $\gamma$  below its melting point. In SLS, the interactions among the linoleoyl chains at the *sn*-2 position, each of which has two *cis*-double bonds, may stabilize the  $\gamma$  form, prohibiting the transformation into more stable forms of  $\beta'$  or  $\beta$ . For this reason, the enthalpy and entropy values for melting of the  $\gamma$  form of SLS are much larger than those of SOS and SRS (see Table 5). The transformation from  $\gamma$  to  $\beta'$  or  $\beta$  in SOS is associated with an inclined chain arrangement with respect to the lamellar interface, which might be prohibited by the linoleoyl chain-chain interactions in SLS.

**4.2.2. 1,3-Diunsaturated-2-Saturated Mixed Acid Triacylglycerols** The polymorphic behavior of symmetric diacid TAGs, 1,3-dioleoyl-2-stearoyl-*sn*-glycerol (OSO), 2-elaidoyl(OEO), and 2-vaccinoyl (OVO) glycerols was studied by Kodali et al. (66). On quenching from the melt, OEO and OVO formed a double-chain-length  $\beta'$  form, whereas OSO formed the  $\alpha$  form. At -7°C,  $\alpha$  of OSO quickly transformed to  $\beta'$ . Long-time incubation of OVO, OEO, and OSO transformed  $\beta'$  form into  $\beta$  form of the triple chain-length structure, in which the two oleoyl chain leaflets are segregated from the vaccinoyl, elaidoyl, and stearoyl chain leaflet. It can be assumed that the driving force to form the triple chain-length  $\beta$



**Figure 16.** Time-resolved SR X-ray scattering patterns of SLS (unit: nm). At left is the temperature change with time.

form of the three TAGs may be the fact that the saturated or *trans*-unsaturated acyl chains at the *sn*-2 position do not pack with the bent oleoyl chains at the *sn*-1 and *sn*-3 positions in the stable polymorphic forms. This mechanism is essentially the same as that present in the Sat.-Unsat.-Sat. TAGs.

The saturated-unsaturated mixed-acid TAGs involving *trans*-unsaturated acids have recently been examined with and without the effects of surfactant additives (67, 68). It is notable that  $\beta'$  is most stable in PEP (1,3-dipalmitoyl-2-elaidoyl-*sn*-glycerol). On the other hand, SES (1,3-distearoyl-2-elaidoyl-*sn*-glycerol) has the most stable form of  $\beta$ . In contrast to the stabilization of  $\beta$  in ESS and SEE, the most stable form of EPP and PEE is  $\beta'$ . The mechanisms for the stabilization of the  $\beta'$  in PEP, EPP, and PEE remain unknown. It seems that the methyl end stacking mode may be a key factor, although further clarification is needed.

By now, we have discussed the polymorphism of mono-acid and mixed-acid TAGs. To summarize, Table 6 shows the number and types of polymorphic forms of principal TAGs.

**TABLE 6. Occurrence of Polymorphic Forms in Representative Triacylglycerols.**

Triacylglycerol	Polymorphic Forms
SSS (tristearoyl-glycerol)	$\alpha, \beta', \beta$
OOO (trioleoyl-glycerol)	$\alpha, \beta'_3, \beta'_2, \beta'_1, \beta_2, \beta_1$
EEE (trielaidoyl-glycerol)	$\alpha, \beta', \beta$
PP14 (1,2-dipalmitoyl-3-myristoyl-sn-glycerol)	$\alpha, \beta'_2, \beta'_1$
PP10 (1,2-dipalmitoyl-3-decanoyl-sn-glycerol)	$\alpha, \beta'_3, \beta'_2, \beta'_1, \beta$
CLC (1,3-dicaproyl-2-lauroyl-sn-glycerol)	$\alpha, \beta'$
SOS (1,3-distearoyl-2-oleoyl-sn-glycerol)	$\alpha, \gamma, \beta', \beta_2, \beta_1$
POP (1,3-dipalmitoyl-2-oleoyl-sn-glycerol)	$\alpha, \gamma, \delta, \beta'_2, \beta'_1, \beta_2, \beta_1$
BOB (1,3-dibehenoyl-2-oleoyl-sn-glycerol)	$\alpha, \gamma, \beta', \beta_2, \beta_1$
SRS (1,3-distearoyl-2-rycinoleoyl-sn-glycerol)	$\alpha, \gamma, \beta'_2, \beta'_1$
SLS (1,3-distearoyl-2-linoleoyl-sn-glycerol)	$\alpha, \gamma$
OSO (1,3-dioleoyl-2-stearoyl-sn-glycerol)	$\alpha, \beta', \beta$

## 5. FAT MIXTURES AND POLYMORPHISM

Fats are multicomponent in two ways: (1) a fat phase contains many different types of TAGs and (2) each TAG molecule involves different types of fatty acid moieties, namely, mixed-acid TAGs. Therefore, it is important to precisely analyze physical and chemical properties of the TAGs in multicomponent systems to understand thermal, structural, and rheological properties of the real food fat systems (69, 70). Particularly, one may note that the kinetic properties of the molecular compound-forming mixture phase are closely related to fat blending and interesterification in food technology (71, 72) and separation of liquid/solid fractions from natural oil resources (73, 74). As a first step in the investigation of multicomponent fat systems, the phase behavior of binary TAG mixture systems has been studied by many researchers (75–84).

The phase behavior of the binary TAG mixtures is classified into three cases: solid-solution, eutectic, and molecular compound formation, as introduced in Section 1. Peculiarities in the mixtures of the TAGs may be explained by the following:

1. The TAGs with similar chemical structures tend to form a solid-solution phase.
2. A eutectic phase is formed between TAGs whose molecular shapes are largely different.
3. Specific interactions result in the formation of a molecular compound as reviewed elsewhere (17, 54).
4. In addition, influences of polymorphism make the phase behavior more complicated.

### 5.1. Binary Mixtures of Saturated-Acid Triacylglycerols

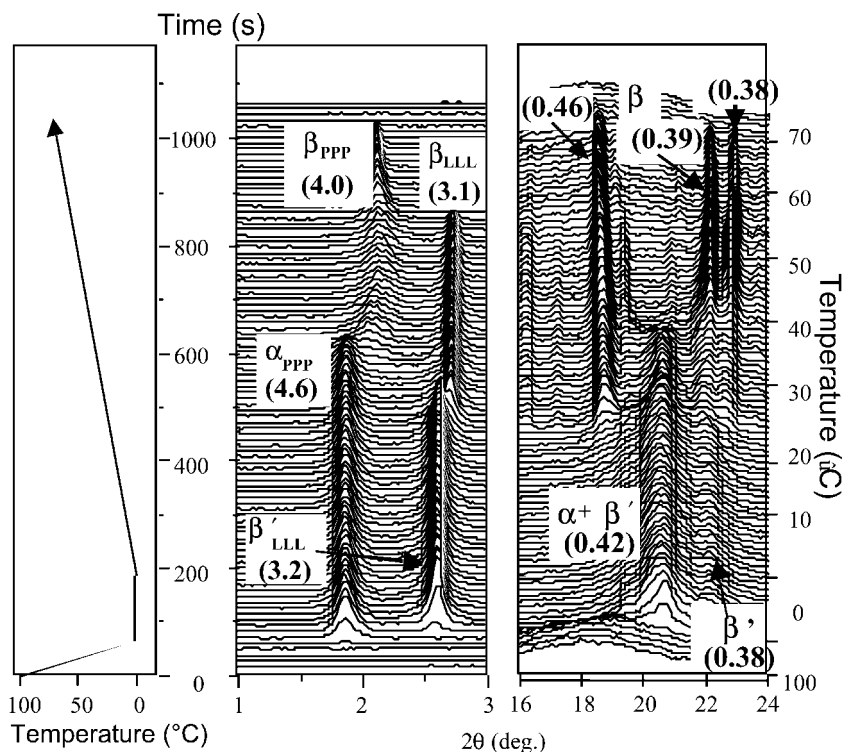
Rossell (85) suggested that a eutectic phase with a limited region of solid solution was formed for the stable  $\beta$  form; yet, the solid solution phase was formed in the



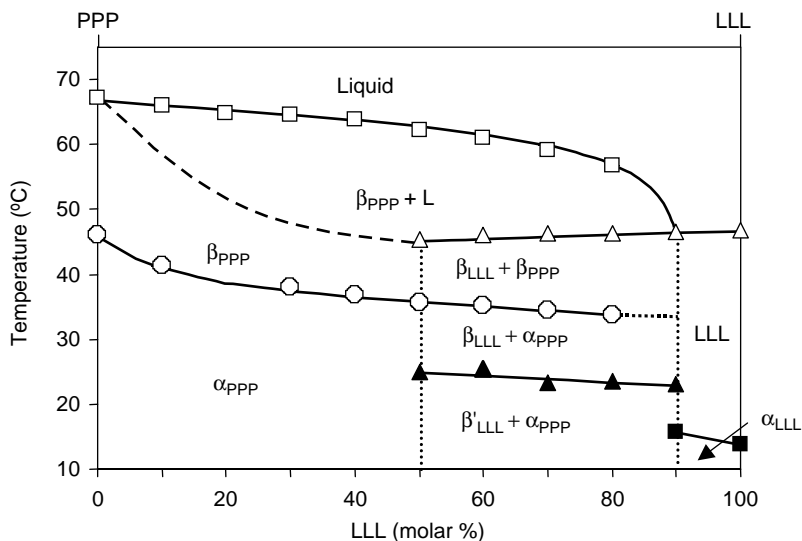
metastable  $\alpha$  and  $\beta'$  in the mixtures of the saturated-acid TAGs. In the 1990s, this behavior was precisely analyzed by a time-resolved SR-XRD study for the PPP/SSS mixtures (78). The 50:50 mixture of PPP/SSS crystallized in  $\alpha$  by quenching the mixed liquid. A single long-spacing peak was the evidence of the solid-solution of the mixture of  $\alpha$ . Upon heating, the  $\alpha$  form transformed to  $\beta'$  and subsequently to  $\beta$ . The miscible  $\beta'$  form also appeared on cooling from the liquid phase. The miscibility was, however, disrupted when the  $\beta'$  transformed to  $\beta$  upon heating, as expressed in a splitting of the long spacing pattern.

Very recently, the phase behaviors of the other types of saturated mono-acid binary mixtures TAGs, LLL (trilauroyl-glycerol)/(trimyristoyl-glycerol), LLL/PPP, and LLL/SSS, were examined by a SAXS/WAXS simultaneous measurement of SR-XRD (86).

As an example, the SR-XRD patterns of the LLL/PPP = 60/40 mixture taken during cooling and heating processes are shown in Figure 17. During cooling, it was clearly shown that the  $\beta'$  form of LLL and the  $\alpha$  form of PPP were crystallized. As for the LLL fraction, direct crystallization of  $\beta'_{LLL}$  with a SAXS peak at 3.2 nm and WAXS peaks at 0.42 and 0.38 nm occurred without the crystallization of  $\alpha_{LLL}$ . Almost at the same time,  $\alpha_{PPP}$  with a SAXS peak at 4.6 nm and a WAXS peak at



**Figure 17.** Time-resolved synchrotron radiation X-ray diffraction patterns of concentration ratio LLL/PPP = 60/40. At left is the temperature change with time (unit: nm).



**Figure 18.** Diagram of the polymorphic occurrence for the LLL/PPP mixtures.

0.42 nm appeared at 35.2°C. Upon heating,  $\beta'_{LLL}$  transformed to  $\beta_{LLL}$  at about 25°C, as identified by the SAXS peak at 3.1 nm and WAXS peaks at 0.46, 0.39, and 0.38 nm. On the other hand,  $\alpha_{PPP}$  transformed to  $\beta_{PPP}$  with a 4.0-nm SAXS peak at 35.2°C. The intensity of the (001) SAXS peak of  $\beta_{PPP}$  (4.0 nm) started to increase soon after the melting of  $\beta_{LLL}$  at 46.0°C. This suggests that the presence of  $\beta_{LLL}$  might hinder the transformation from  $\alpha_{PPP}$  to  $\beta_{PPP}$ . The results were obtained for the LLL concentrations from 50% to 90%. Hence, the immiscible phases were formed in the LLL/PPP mixture system for the three polymorphic forms. Figure 18 shows the phase behavior of the LLL/PPP mixtures, which is subdivided into the three regions:

1. In the LLL concentrations above 90%, the phase behaviors of the LLL/PPP mixtures were mainly governed by LLL.
2. In the LLL concentrations from 50% to 90%, the  $\beta'$ - $\beta$  transformation of the LLL fraction and the  $\alpha$ - $\beta$  transformation of the PPP fraction occurred separately. This indicates that phase separation occurred in the three polymorphic forms.
3. In the LLL concentrations below 50%, the LLL fraction was dissolved in the PPP fraction. Hence, the phase behaviors of the LLL/PPP mixtures were mainly governed by PPP.

Figure 19 shows the phase behavior of the polymorphic occurrence for the LLL/MMM mixtures obtained from DSC and SR X-ray scattering experiments. This diagram indicates the following three points:

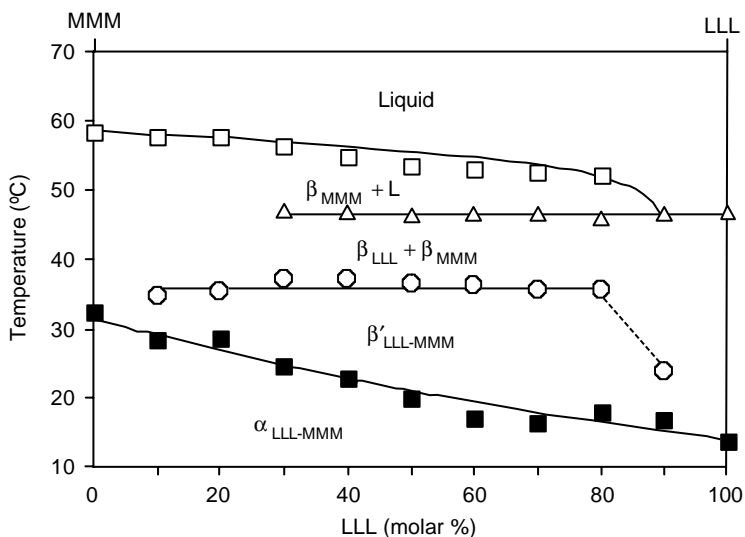


Figure 19. Phase behavior of the polymorphic occurrence for the LLL/MMM mixtures.

1.  $\beta'$  was formed in the mixture system, whereas  $\alpha$  transformed directly to  $\beta$  in LLL and MMM.
2. Miscible solid-solution phases were formed in the metastable  $\alpha$  and  $\beta'$  forms of the mixtures.
3. A eutectic phase was formed in the most stable  $\beta$  form.

These three results are consistent with the results from the PPP/SSS system.

Consequently, it can be concluded for the mixtures of LLL-MMM, LLL-PPP, LLL-SSS, MMM-PPP, and PPP-SSS that the TAG binary mixtures are miscible in metastable polymorphs of  $\alpha$  and  $\beta'$  forms when the difference in the number of carbon atoms of the fatty acid moieties,  $\Delta n$ , equals 2, whereas immiscible mixtures are found in all polymorphic forms when  $\Delta n$  is larger than 2. Results obtained for these mixture systems may indicate a relationship between polymorphism and phase behavior of the binary mixtures of the saturated-acid TAGs in such a way that rotational freedom of hydrocarbon chains and entropy of methyl-end stacking are crucial factors determining the polymorph-dependent phase behavior.

As discussed in Section 1, hexagonal-packed  $\alpha$  has the ability of the carbon atoms to rotate several degrees and form disordered conformations. Hydrocarbon chains of  $\beta'$  and  $\beta$  are all ordered conformations except for near methyl-ends, which have a little rotational freedom. When two types of saturated monoacid TAGs with different fatty acids are mixed, the molecules are arranged in a double chain-length structure because of the interactions among glycerol backbones. Thus, this crystal structure contains the methyl-end stacking gap. For the LLL/MMM mixtures,  $\alpha$  and  $\beta'$  polymorphs form solid-solution phases. These polymorphs contain disordered methyl-end groups so that they can accommodate a methyl-end

stacking gap. In contrast,  $\beta$  polymorph has all-*trans*-hydrocarbon chains and these rigid chains cannot adjust themselves to their circumstance. Therefore,  $\beta$  polymorph shows a eutectic phase. As for the LLL/PPP and LLL/SSS mixtures, eutectic phases occur for all polymorphs. Because of large differences in carbon numbers for fatty acid chains between LLL and PPP ( $\Delta n = 4$ ), and between LLL and SSS ( $\Delta n = 6$ ), there are very large methyl-end stacking gaps in these crystals. Therefore, the increased entropy of methyl-end stacking becomes predominant and phase separation must be favored thermodynamically for all polymorphs.

## 5.2. Binary Mixtures of Saturated-Unsaturated Mixed-Acid Triacylglycerols

In the 1960s, Rossell reported (85) that the binary mixtures of saturated monoacid TAGs and unsaturated monoacid TAGs form an immiscible phases, whereas Moran has suggested (87) that a molecular compound is formed in some binary mixtures of saturated-unsaturated mixed-acid TAGs. It was early 1990s when the formation of molecular compounds was observed in various mixtures of saturated-unsaturated diacid TAGs. Moreover, a miscible mixture phase was discovered in the mixtures of SOS-SLS. Recent studies of various types of the TAG binary mixtures clarified the formation of the molecular compound crystals at the 50/50 concentration ratio: 1,3-distearoyl-2-oleoyl-*sn*-glycerol/ 1,2-distearoyl-3-oleoyl-*rac*-glycerol (SOS/SSO) (77), SOS/1,3-dioleoyl-2-stearoyl-*sn*-glycerol (SOS/OSO) (79), 1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol/ 1,2-dipalmitoyl-3-oleoyl-*rac*-glycerol (POP/PPO) (81), and POP/1,3-dioleoyl-2-palmitoyl-*sn*-glycerol (POP/OPO) (82). These properties are related to molecular-level understandings of the chain-chain interactions occurring in biomembrane lipids containing the saturated-unsaturated mixed acid moieties (16, 23, 88–91). Table 7 summarizes the phase behavior of binary mixtures of saturated-unsaturated diacid and triacid TAGs.

A molecular compound of  $\beta$  form,  $\beta_C$ , was formed at the 1:1 concentration ratio of the binary mixtures of PPO-POP, and SSO-SOS, giving rise to two monotectic

**TABLE 7. Phase Behavior of Binary Mixtures of Saturated-Unsaturated Diacid and Triacid TAGs.**

Phase Behavior	Mixture Systems
Miscible	SSS-SSE POS-SOS SOS-SLS
Immiscible	
Eutectic	POP-PPP
Molecular compound forming	POP-PPO SOS-SSO POP-OPO SOS-OSO

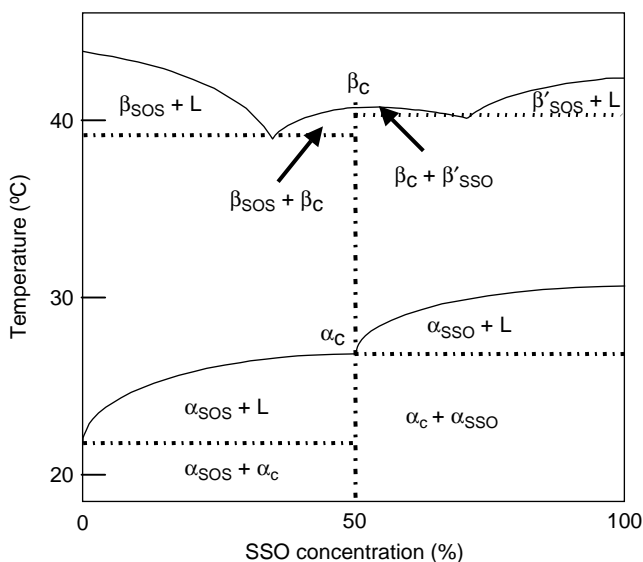
Abbreviations: S: stearoyl, P: palmitoyl, O: oleoyl, L: linoleoyl, E: elaidoyl.

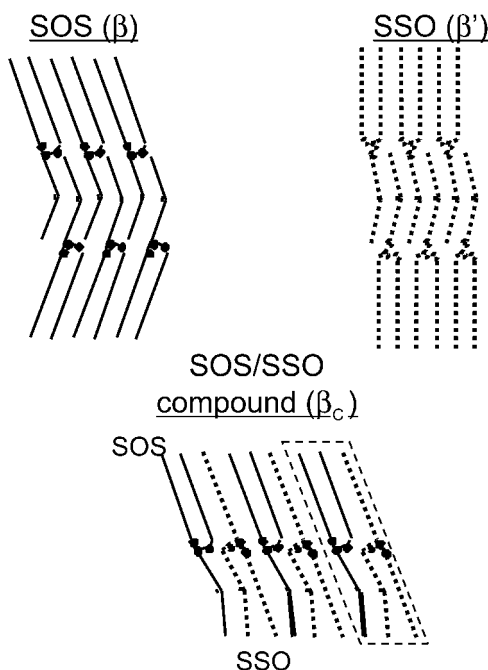
**TABLE 8. Thermal Properties of Polymorphism of Molecular Compound of PPO-POP and SSO-SOS, Together with PPO and SSO.**

	PPO-POP = 50-50			PPO	
$T_m$ ( $^{\circ}\text{C}$ )	$\alpha_c$	$\beta'_c$	$\beta_c$	$\alpha$	$\beta'$
$\Delta H_m$ (kJ/mol)	15.5	28.0	31.2	18.5	35.2
$\Delta S_m$ (J/mol/K)	n.a	90	97	n.a	104
Ling spacing (nm)	n.a	299	319	n.a	337
Chain length	4.6	4.2	4.1	7.8	6.5
	double	double	double	triple	triple
	SSO-SOS = 50-50			SSO	
$T_m$ ( $^{\circ}\text{C}$ )	27.5	34.0	40.6	131.6	41.4
$\Delta H_m$ (kJ/mol)	51.2	73.8	138.2	65.8	101.0
$\Delta S_m$ (J/mol/K)	170.3	240.3	440.5	206.1	321.1
Long spacing (nm)	5.4	5.0	4.5	8.50	7.08
Obtain length	double	double	double	triple	triple

n.a.: not available.

phases of the component TAG and the compound in juxtapositional. Table 8 shows the physical properties of the polymorphs of PPO/POP compound and SSO/SOS together with PPO and SSO. The  $\alpha$  and  $\beta'$  forms of PPO and SSO are triple-chain-length structure, and all the polymorphic forms of POP and SOS except for  $\alpha$  (SOS) or  $\alpha$  and  $\beta'$  forms (POP) are triple-chain-length structure. However, the three polymorphs of the molecular compounds of PPO-POP and SSO-SOS are a double chain-length structure. Figure 20 shows the phase diagram of the stable


**Figure 20. Phase behavior in the SOS/SSO mixtures.**



**Figure 21.** Structure models of stable polymorphs of SOS, SSO, SOS/SSO compound crystal.

$\beta$  form and metastable  $\alpha$  forms of the SOS/SSO mixtures examined by DSC and XRD experiments (92). The most stable forms of SOS and SSO are  $\beta_1$  and  $\beta'$ , respectively. The phase diagrams of  $\alpha$ ,  $\beta'$ , and  $\beta$  polymorphs in the binary mixture of PPO-POP are already explained elsewhere (54, 81). Therefore, the formation of the molecular compound, which is accompanied with the conversion from triple- to double-chain-length structure, is a common feature of the binary mixtures of Sat.-Oleic-Sat. TAGs and Sat.-Sat.-Oleic TAGs. Figure 21 illustrates structure models of SOS, SSO, and SSO-SOS molecular compounds, in which one leaflet is formed of palmitoyl or stearoyl chains and the other leaflet contains the mixture of palmitoyl and oleoyl chains or stearoyl and oleoyl chains.

It should be noted in Figure 20 that  $\beta_c$  form is a congruent-type molecular compound, and its melting point is lower than the most stable forms of SOS and SSO. This raises interesting questions, such as how the molecular compound could be structurally stabilized, and how its crystallization is kinetically favored. The latter question is important, because the supercooling value with respect to  $\beta_c$  is lowest compared with the supercooling values of  $\beta_1$  of SOS and  $\beta'$  of SSO. Nevertheless,  $\beta_c$  is crystallized when the mixture liquid is cooled below its  $T_m$ . Moreover, an in situ XRD study showed that the rate of crystallization of  $\beta_c$  is remarkably higher than  $\beta_1$  of SOS and  $\beta'$  of SSO. When the molecular compound is formed, steric hindrance between saturated and oleic acid chains may be caused. Supposing that the double-chain-length structure is formed in a liquid phase (93, 94), we assume that formation energy of a crystal nucleus of the double-chain-length

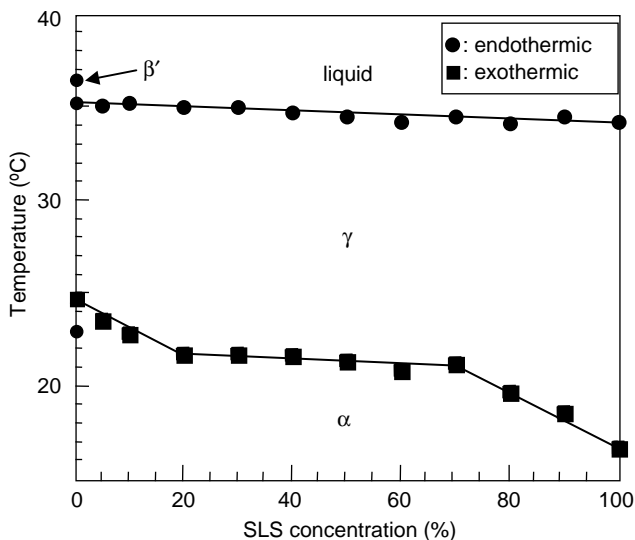
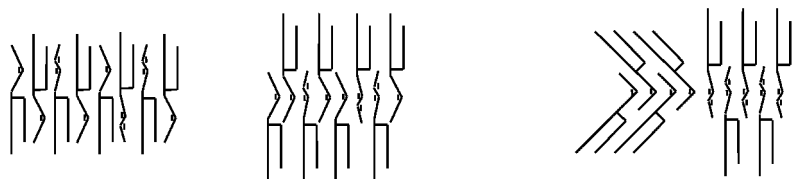


Figure 22. Phase behavior in the SOS/SLS mixtures.

structure may be minimized, compared with those having the triple chain-length structure, and the nucleation frequency of the double-chain-length molecular compound crystals may become higher. In this regard, a small-angle diffraction pattern peak at 4.5 nm without the presence of a wide-angle diffraction pattern occurred, long before the crystallization of  $\beta_c$  from the molten sample of SSO/SOS = 1/3 when the mixture liquid was cooled to 37°C (92). This indicates the presence of the smectic liquid crystalline phase, which may be a precursor of the nucleation of  $\beta_c$ .

The binary mixture systems of SOS-OSO (79) and POP-OPO (83) were examined by DSC, XRD, and FT-IR, giving rise to two monotectic phases of POP (SOS)/compound and compound/OPO(OSO) in juxtaposition.

A new result was obtained for the mixture of SLS/SOS in which a solid-solution mixture was observed in the  $\alpha$  and  $\gamma$  forms in all concentration ranges: the double chain length in  $\alpha$  phase and the triple chain length in  $\gamma$ , as shown in Figure 22 (92). The miscible  $\gamma$  form did not transform to the  $\beta'$  form, when the mixtures were subjected to simple cooling from high-temperature liquid to low-temperature solid phase. Incubation of  $\gamma$  around its  $T_m$  also did not result in transformation to the other polymorph; instead the miscible phase was retained. The  $\alpha$ -melt-mediated transformation, however, caused the disruption of the solid-solution phase, and immiscible phases of  $\gamma_{SLS}$  and  $\beta'_{SOS}$  were formed in concentration ranges of SLS below 30%. By contrast, in SLS concentration ranges above 30%, the  $\alpha$ -melt-mediated transformation caused the crystallization of only the  $\gamma$  form, and  $\beta'$  or  $\beta$  of SOS did not appear. The structural model of solid-solution phases of  $\alpha$  and  $\gamma$  forms and the eutectic phase of the  $\gamma$  of SLS and  $\beta'$  of SOS are shown in Figure 23 (92).

(a)  $\alpha_{\text{SOS/SLS}}$ : miscible      (b)  $\gamma_{\text{SOS/SLS}}$ : miscible      (c)  $\beta'_{\text{SOS}}$  or  $\beta_{\text{SOS}}$ , and  $\gamma_{\text{SLS}}$ : immiscible

**Figure 23.** Structure models of the polymorphic forms of the SOS/SLS mixtures.

The  $\alpha$  form of the SLS/SOS mixture is stacked in the double-chain-length structure, in which the stearoyl and unsaturated (oleoyl and linoleoyl) chains are packed in the same leaflets (Figure 23a). Further thermodynamic equilibration induced the transformation from the double-chain-length  $\alpha$  form to the triple-chain-length  $\gamma$  form. In this transformation, chain segregation occurs during the  $\alpha$ - $\gamma$  transformation in the solid state, because the steric hindrance between the stearoyl and linoleoyl chains is limited. In the  $\gamma$  form, the stearoyl and unsaturated chains are packed in the different leaflets (Figure 23b). Because of olefinic interactions between oleoyl and linoleoyl chains, coexistence between oleoyl and linoleoyl chains in the oleic/linoleic acid leaflet takes place in the  $\gamma$  form having the triple-chain-length structure. This interaction makes the  $\gamma$  form the most stable polymorph of the SLS/SOS mixture, and the transformation from  $\gamma$  to  $\beta'$  or  $\beta_2$  forms does not occur in the mixture during simple cooling and heating processes. Disappearance of the miscible  $\gamma$  form of the mixture can be achieved by melt-mediated transformation from the miscible  $\alpha$  and  $\gamma$  forms to immiscible  $\beta'$  and  $\beta$  forms in the SOS fraction (Figure 23c).

## 6. POLYMORPHISM OF NATURAL FATS

Most natural fats are composed of many different kinds of acylglycerols whose fatty acid compositions are diverse, and the acyl positions esterified at the glycerol groups are complicated. This situation makes the polymorphism of natural fats very complicated.

Take for example, milkfat that consists of TAGs, diacylglycerols (DAGs), monoacylglycerols (MAGs), free fatty acids, phospholipids, sterols, and other polar lipids (95–97). As for TAGs, milkfat is made of about 400 different TAGs containing various kinds of saturated and unsaturated fatty acids whose carbon numbers range from 2 to 24. Because of this fact, milkfat has a wide range of melting temperature from about  $-30^\circ\text{C}$  to  $40^\circ\text{C}$ , and three polymorphic forms of  $\alpha$ ,  $\beta'$ , and  $\beta$  reveal complicated chain-length structures and occurrence behavior that are affected by thermal treatment. Another example is cocoa butter (CB) in which stearic, palmitic, and oleic acids account for about 80 % out of its total fatty acids. This property causes sharp melting behavior of CB. However, polymorphism of cocoa butter is complicated, and its origins are still unanswered.



It is difficult to simply define the polymorphism of natural fats composed of multiple TAGs because of the following two reasons:

1. The polymorphic nature of the multicomponent TAG systems is related to phase behavior that is affected by molecular interactions among the component TAGs. The fat crystals in a miscible phase may exhibit simple polymorphic properties. By contrast, the immiscible eutectic phase may show complicated polymorphic properties as a superposition of the polymorphic forms of the component TAGs. Furthermore, if the molecular compound is formed by specific TAG components, the polymorphic behavior becomes complicated, as shown for the case of POP-OPO (see Section 5.2). Therefore, knowing the phase behavior of the principal TAG components is a prerequisite for precise understanding of the polymorphism of natural fats.
2. The phase behavior of the mixed TAG system is influenced by polymorphism. For example, a miscible phase is formed in  $\alpha$  and  $\beta'$  polymorphs, but it transforms into a eutectic phase in  $\beta$ , as revealed in the SSS-PPP mixture. Then, the polymorphic occurrence is largely affected by cooling rate and temperature fluctuation, and it is therefore necessary to observe the polymorphic properties of the natural fats by varying the rate of cooling or by fluctuating the temperature (so-called tempering).

In this section, the polymorphic properties of natural fats are briefly discussed by highlighting milkfat, cocoa butter, and palm oil fraction based on recent research into the effects of external factors on the polymorphic crystallization such as shear stress, ultrasound stimulation, and addition of food emulsifiers.

### 6.1. Milkfat

Crystallization of milkfat is an important process for fractionation of its contents and production of butter, whipped cream, and ice cream. As the quality of these products strongly depends on polymorphism of milkfat, physical chemical properties of milkfat have been studied by many researchers (98).

As mentioned above, milkfat is characterized as a very complicated mixture of TAGs, and thereby it is almost impossible to clarify how every TAG component crystallizes in a cooperative way with the other TAG components. Instead, milkfat is fractionated in accordance with different melting ranges to obtain three major fractions: high melting fraction (HMF), medium melting fraction (MMF), and low melting fraction (LMF). Marangoni and Lencki concluded that HMF and MMF are fully miscible in the solid state, and mixtures of LMF with HMF and MMF showed monotectic property with nature of partial solid solution (70).

As for the polymorphism of milkfat,  $\alpha$  and  $\beta'$  forms frequently appear, and  $\beta$  form appears under special conditions when HMF and milkfat are stored for long duration (99–101). In regard to the effects of thermal treatment and emulsification on the polymorphic crystallization of milkfat, Lopez et al. recently performed synchrotron radiation X-ray diffraction and DSC studies, using anhydrous milkfat

**TABLE 9. Comparison of Thermal and Structural Behaviors of Anhydrous Milkfat and Cream Observed at a Slow Cooling Rate.**

	Anhydrous Milkfat	Milkfat Globule Cream
Polymorphic occurrence	$\beta$ , then $\beta'$ and $\alpha$	$\alpha$ , then $\alpha + \beta'$
Chain-length structure	4.15 (double): vs*	4.65 (double): m
(unit, nm)	4.83 (double): w	4.0 (double): m
	6.22 (triple): s	7.13 (triple):s
	3.92 (double): m	6.5 (tripl): w

\* vs: very strong, s: strong, m: medium, w: weak.

(AMF) as a bulk fat system and milkfat globule of cream as an emulsion system (102–106).

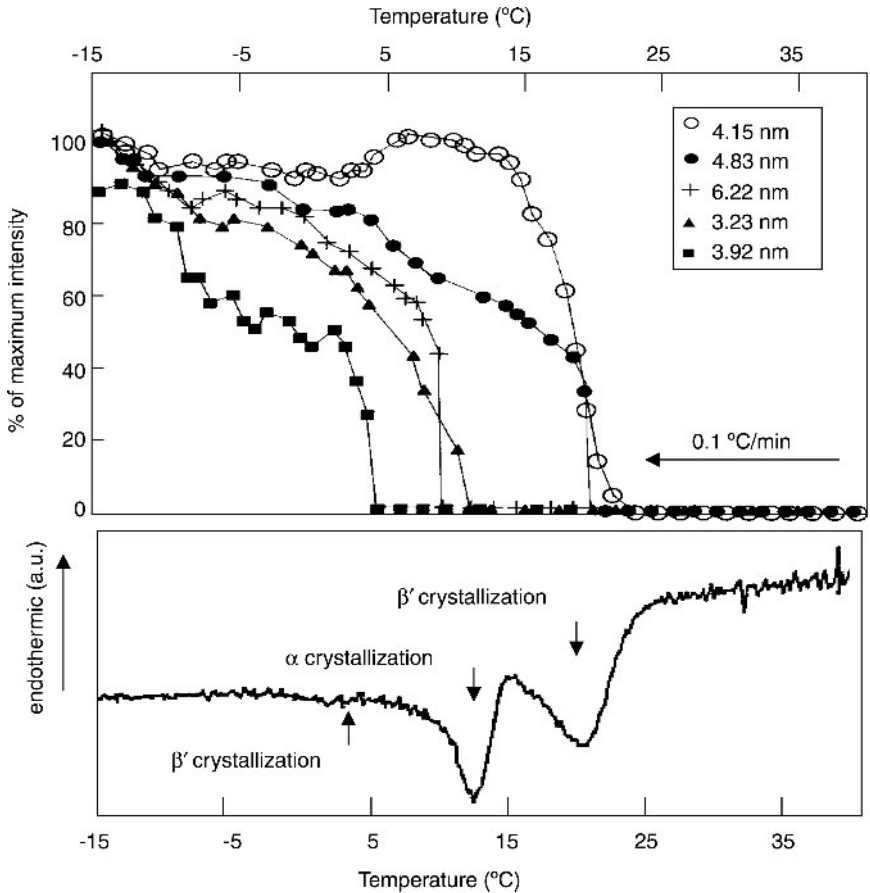
No difference in the polymorphic occurrence was observed between cream and AMF when the samples were rapidly cooled from 50°C to -8°C:  $\alpha$  form first appeared and  $\beta'$  form appeared during subsequent heating after the melting of  $\alpha$  form. On the other hand, crystallization by slow cooling (<0.15°C/min) caused remarkable differences between the emulsion and bulk systems. As summarized in Table 9,  $\beta'$  form first crystallized and  $\beta'$  and  $\alpha$  forms coexisted until the end of cooling in the bulk AMF. By contrast,  $\alpha$  form first crystallized and  $\beta'$  form started to crystallize during further cooling. In the heating process after the crystallization,  $\alpha$  first melted and then  $\beta'$  form melted in both samples.

The chain-length structures largely differed between the bulk AMF and cream samples. Table 9 also shows four different crystals formed in the AMF bulk samples, and four crystals in cream. Quite interestingly, the lamellar spacing values are all different from each other, and double-chain-length and triple-chain-length structures are coexisted. The occurrence domains of the four crystals in AMF during the slow cooling are shown in Figure 24 that shows relative intensity of small-angle X-ray diffraction peaks of the four crystals observed in the bulk AFM and DSC thermopeaks taken during the slow cooling. It is clearly shown that a large exothermic peak around 22°C, a large exothermic peak around 13°C, and a small peak at 4°C are caused by the crystallization of  $\beta'$  form,  $\alpha$  form, and  $\beta'$  form, respectively.

It is assumed that the crystallization behavior of milkfat is different between emulsion and bulk, and the lack of nucleation centers in the emulsion droplets may delay the nucleation, making less stable  $\alpha$  form nucleated in the first. The occurrence of multiple forms of double-chain-length and triple-chain-length structures may be caused by segregated crystallization of multicomponent TAGs exhibiting complicated mixing behavior, but its details are open to future study.

## 6.2. Cocoa Butter

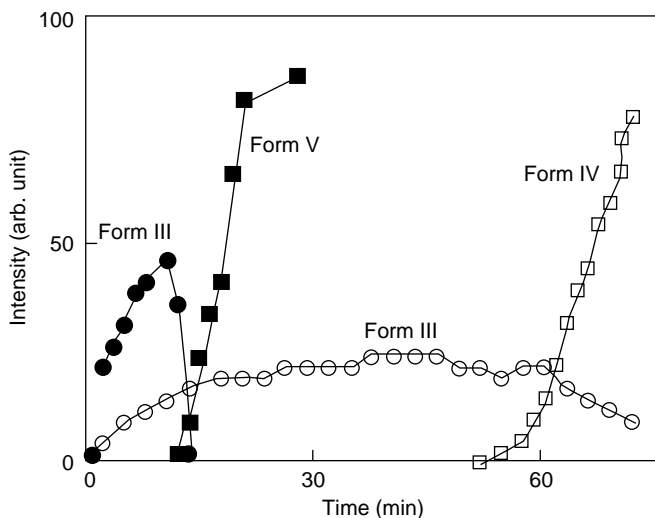
Cocoa butter is the most popular fat used for confectionery. CB consists of three major TAGs, POP, POS, and SOS, and other minor components (107, 108). The three TAGs determine the polymorphic nature of CB that exhibits six polymorphs, Form I through Form VI in accordance with the nomenclature given by Wille and



**Figure 24.** Relative intensity of small-angle X-ray diffraction peaks and DCS thermo peaks of bulk anhydrous milkfat taken during a slow cooling process.

Lutton (109). This section employs this nomenclature, although the other nomenclatures such as  $\beta'_{III}$  and  $\beta_V$ , are used in other researchers (110). As Form V functionally works for chocolate, crystallization of CB in Form V, and preservation of this polymorph during long storage are the prerequisites for quality control of the end products. For this purpose, a tempering method including cooling from a molten state, reheating, and recooling has widely been applied (107, 108). The other technique is to use seed crystals of BOB  $\beta_2$  whose polymorphic structure is identical to that of Form V of CB and whose melting point is higher than Form V of CB (107). The BOB  $\beta_2$  seed crystals can be put in molten chocolate during a simple cooling process without tempering to obtain Form V of CB.

Recently, interesting work has been done to examine the effects of shear stress (19, 110) and ultrasound irradiation on the polymorphic crystallization of CB (111).

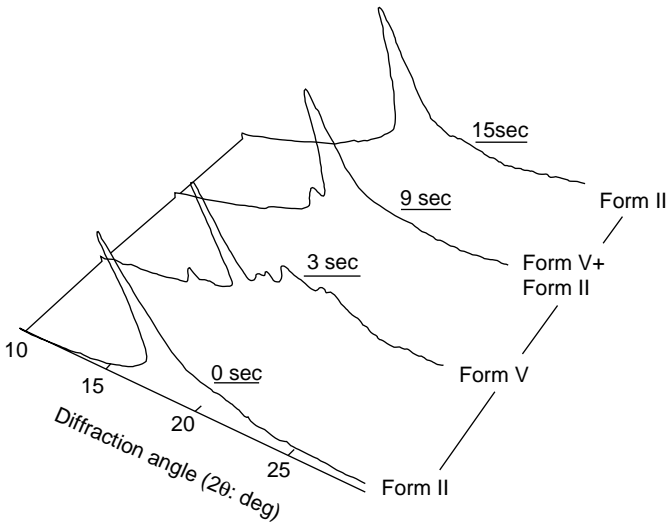


**Figure 25.** Relative intensity of small-angle X-ray diffraction peaks of cocoa butter without shear stress (closed) and shear stress (open).

As for the effects of the shear stress, it was shown by a Synchrotron radiation X-ray diffraction study that transformations from metastable to more stable forms, especially to Form V, were accelerated by high shear stress (110). Figure 25 shows the time variation of relative intensities of X-ray diffraction peaks of CB crystals formed after cooling from 50°C to 18°C at a rate of 3°C/min. In the case of no shear, Form III appeared at first after the temperature reached at 18°C, and then Form IV crystallized at the expense of Form III. On the other hand, applying the shear stress at 1440 s<sup>-1</sup> caused accelerated transformation from Form III to Form V, without the occurrence of Form IV. The same result was observed with lower shear rates (19), and the persistence time of Form III was reduced as the shear rate was increased. Mazaanti et al also observed that the orientation of CB crystals are aligned with the shear flow (110). These results indicated that temperature and shear treatments are the tools for tailoring the desired polymorphic structures of fats.

It was observed that ultrasound stimulation (ultrasonication) also accelerated the crystallization of the more stable polymorphs of CB (111). A fundamental study of the effects of ultrasonication on the polymorphic crystallization of PPP and LLL showed that several factors, such as pressure effect, shear flow, cavitation, and thermal energy caused by absorption of attenuated ultrasound wave, may play concurrent effects of ultrasonication. As a result, there are optimal conditions for temperature and duration of ultrasonication to increase the rate of crystallization and the occurrence of the more stable polymorphs (20). This effect was also observed in CB (111).

Figure 26 shows wide-angle X-ray diffraction profiles of CB with ultrasonication of three durations and without ultrasonication taken after cooling at 20°C from



**Figure 26.** Wide-angle X-ray diffraction patterns of cocoa butter with ultrasonication.

60°C. Ultrasound (200 kHz, 300 W) was stimulated to a 250-mL sample of CB at 32.3°C during cooling before crystallization. Form II occurred without ultrasonication, whereas Form V was observed when ultrasonication was done for 3 seconds. Further ultrasonication for 9 seconds formed a mixture of Form II and Form V, and only Form II was observed by the ultrasonication for 15 seconds. It is assumed that there are conflicting effects by ultrasonication: promotion of nucleation by pressure effect and retardation of nucleation by thermal energy caused by absorption of attenuated ultrasound wave. The former effect may prevail at the ultrasonication for 3 seconds. The temperature rise, however, of the sample caused by absorption of attenuated ultrasound wave was 2°C for 9 seconds and 3.9°C for 15 seconds, and the latter effect may result in the case of cooling from above the melting point of CB.

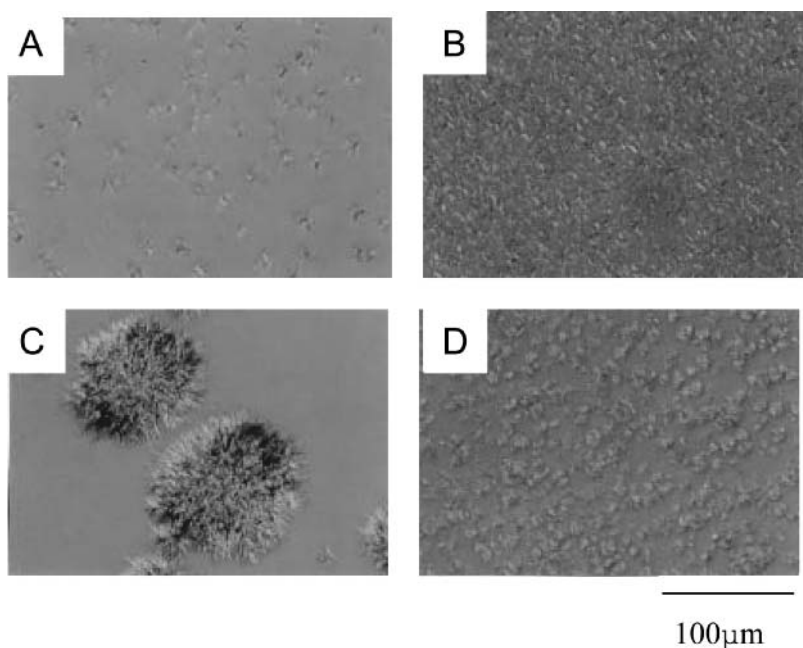
### 6.3. Palm Oil

Palm oil is a common fat and oil resource for many industrial uses. For example, of food applications, it is used as cooking oil, margarine, shortening, and in confectionery products (73, 112). Palm oil has several advantageous properties such as high productivity and high thermal and oxidative stability and plasticity at room temperature. However, the crystallization properties of palm oil are disadvantageous because of a low rate of nucleation and crystal growth of granular crystals (113–114). The granular crystals are easily formed during long storage, causing sandy taste and inhomogeneity of fat crystal morphology of the end products (8, 115–117). Although many TAGs, DAGs, free fatty acids, and so on, are involved in it, palm oil exhibits two polymorphic forms,  $\alpha$  and  $\beta'$  under normal cooling rate, and  $\beta$  form also appears at a very slow crystallization rate. The addition of food

emulsifiers into palm oil has been an efficient external factor to modify the polymorphism crystallization of palm oil.

Polyglycerol fatty acid esters are biograded surfactants that are used widely in industries such as food, cosmetics, toiletries, and pharmaceuticals (118). Advantageous properties of the PGFEs are derived from the easy modification of their hydrophobicity and hydrophilicity by changing the degree of polymerization of glycerol and esterification with fatty acid moieties and by modifying the chemical structures of fatty acid moieties.

It was observed that the addition of polyglycerine fatty acid ester to palm oil affected the polymorphic crystallization and morphological properties of palm oil (119). In particular, polyglycerol behenic acid ester showed a remarkable effect. Optical microscopy observation confirmed that palm oil crystals with the addition of 1 wt.% of polyglycerol behenic acid esters were smaller and the number of palm oil crystals larger than without the additives, as shown in Figure 27. This indicated that the polyglycerol behenic acid ester promoted nucleation and inhibited crystal growth of palm oil. X-ray diffraction patterns of palm oil without the additives revealed that palm oil crystallized in the  $\alpha$  form after rapid quenching of melted palm oil at 10°C. During the heating process from 10°C to 45°C, the  $\alpha$  form transformed to the  $\beta'$  form around 15°C, and the  $\beta'$  form changed to the  $\beta$  form around 40°C. The X-ray diffraction patterns of palm oil with the addition of polyglycerol



**Figure 27.** Optical micrographs of isothermal crystallization of palm oil with and without an additive (1 wt.% of polyglycerine behenic acid ester). (A) 60 min at 20°C without the additive; (B) 60 min at 20°C with the additive; (C) 60 min at 27°C without additive; (D) 60 min at 27°C with the additive. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

behenic acid esters showed that palm oil crystallized in the  $\beta'$  form at 10°C, and it did not transform to the  $\beta$  form during the heating process.

It is generally considered that the nucleation rates of  $\alpha$  and  $\beta'$  are largely different, with  $\alpha$  crystallizing much more rapidly than  $\beta'$  and  $\beta$ . The size of the crystal is associated with the crystal form;  $\beta$  form tends to produce granular crystals, and  $\beta'$  is recommended for producing small crystals. Figure 27 shows palm oil with polyglycerol behenic acid esters added, the  $\beta'$  form is preferentially crystallized, and the crystal does not grow to a granular crystal. One reason for this may be that polyglycerol behenic acid esters, which promote the nucleation of palm oil, have higher melting points than that of palm oil; namely, the emulsifier as an additive may crystallize faster than palm oil, inducing heterogeneous nucleation of palm oil as template.

## 7. SUMMARY

This chapter described polymorphic properties of principal TAGs and natural fats based on recent research work to clarify fundamental aspects of polymorphism of fats and oils. The authors hope that the basic understanding of the polymorphism of the principal TAGs would be useful to elucidate rather complicated polymorphic properties of natural fats and oils that contain TAGs with very heterogeneous fatty acid compositions.

It may be worth noting the following subjects, which are nowadays still open to question and therefore should be worked out in future.

### 1. Polymorphism of diacid and triacid TAGs

Little knowledge of polymorphism of diacid and triacid TAGs have been obtained, because these TAGs have high relevance to natural fats and oils. The main reasons for this must be difficulty in preparing pure materials, which may provide convincing results. It is highly interesting and important to work on diacid and triacid TAGs with an emphasis on the effects of chirality on polymorphism that has rarely been known.

### 2. Polymorphic structures of saturated-unsaturated mixed-acid TAGs

Although much effort has been done to unveil precise structures of saturated-unsaturated mixed-acid TAGs, no atomic-level structure analysis has been successful because of difficulty to obtain high-quality single crystals suitable for X-ray analysis. In particular, SOS and POP are the most representative TAGs that are major components of cocoa butter and palm oil. It is expected that structures data of the stable  $\beta$  forms of SOS and POP may give key ideas to resolve fat bloom phenomena in confectionery fats. Molecular simulations have been done (120, 121), but the experimental results so far obtained are the XRD patterns and FT-IR spectra using powder and single crystals, which do not provide detailed molecular structures, in particular, about olefinic conformation and interfacial structures of oleic acid and saturated acid leaflets.

## 3. Phase behavior of TAG mixtures

As the natural fats and oils are mixtures of different TAGs, their polymorphism is influenced by the phase behavior of the mixture phases. In order to mimic natural fats and oils, the mixture phases of diacid TAGs were studied, as reviewed in Section 4. However, we more need basic research of the mixtures of TAGs whose fatty acid compositions are heterogeneous, e.g., the mixtures of between saturated diacid TAGs of saturated diacid TAGs and unsaturated diacid TAGs. These studies may be significant in understanding the polymorphism of palm oil and its fractions (palm stearin, palm olein, etc.), or milkfat and its fractions (high-melting fraction, etc.).

## REFERENCES

1. C. E. Clarkson, and T. Malkin, *J. Chem. Soc.*, 666 (1934).
2. K. Larsson, *Acta Chem. Scand.*, **20**, 2255 (1966).
3. H. D. Goff, in F. D. Gunstone, and P. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997, pp. 355–368.
4. J. M. de Man, in M. N. Widlak, ed., *Physical Properties of Fats, Oils, and Emulsifiers*, AOCS Press, Champaign, Illinois, 1999, pp. 79–95.
5. R. E. Wainwright, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products* 5th ed., Vol. 3, Wiley, New York, 1996, pp. 353–408.
6. E. S. Lutton, *J. Amer. Oil Chem. Soc.*, **27**, 276 (1950).
7. R. E. Timms, *Chem. Phys. Lipids*, **21**, 793, (1978).
8. K. Sato, in P. B. Padley, ed., *Advances in Lipid Research*, JAI Press, New York, 1996, pp. 213–268.
9. D. Aquilano and G. Spualdino, in N. Garti and K. Sato, eds., *Crystallization Processes in Fats and Lipid Systems*, Marcel Dekker, New York, 2001, pp. 1–51.
10. K. Sato and T. Kuroda, *J. Amer. Oil Chem. Soc.*, **64**, 124 (1987).
11. T. Koyano, I. Hachiya, T. Arishima, K. Sato, and N. Sagi, *J. Amer. Oil Chem. Soc.*, **66**, 675 (1989).
12. M. Kellens, W. Meeussen, and C. H. Riekel, *Chem. Phys. Lipids*, **52**, 79 (1990).
13. M. Kellens, W. Meeussen, R. Gehrke, and H. Reynaers, *Chem. Phys. Lipids*, **58**, 131 (1991).
14. M. Kellens, W. Meeussen, A. Hammersley, and H. Reynaers, *Chem. Phys. Lipids*, **58**, 145 (1991).
15. C. W. Hoerr and F. R. Paulicka, *J. Amer. Oil Chem. Soc.*, **45**, 793 (1968).
16. D. M. Small, *J. Lipid Res.*, **25**, 1490 (1984).
17. D. M. Small, *The Physical Chemistry of Lipids*, Plenum Press, New York, 1986, pp. 475–522.
18. J. W. Mullin, *Crystallization*, 4th ed., Butterworth-Heinemann, Oxford, 2001, pp. 135–180.
19. S. D. MacMillan, K. J. Roberts, A. Rossi, M. A. Wells, M. C. Polgreen, and I. H. Smith, *Cryst. Growth Design*, **2**, 221 (2002).



20. S. Ueno, R. Ristic, K. Higaki, and K. Sato, *J. Phys. Chem. B*, **107**, 4927 (2003).
21. D. J. Cebula, D. J. McClements, and M. J. W. Povey, *J. Amer. Oil Chem. Soc.*, **67**, 76 (1990).
22. D. J. Cebula, D. J. McClements, M. J. W. Povey, and P. Smith, *J. Amer. Oil Chem. Soc.*, **69**, 130 (1992).
23. F. Kaneko, J. Yano, and K. Sato, *Curr. Opin. Struct. Biol.*, **8**, 417 (1998).
24. F. Kaneko, in N. Garti, and K. Sato, eds., *Crystallization Processes in Fats and Lipid Systems*, Marcel Dekker, New York, 2001, pp. 53–98.
25. D. Chapman, *J. Chem. Soc.*, 4489 (1957).
26. J. Yano, F. Kaneko, M. Kobayashi, and K. Sato, *J. Phys. Chem. B*, **101**, 8112 (1997).
27. J. Yano, F. Kaneko, M. Kobayashi, D. R. Kodali, D. M. Small, and K. Sato, *J. Phys. Chem. B*, **101**, 8120 (1997).
28. J. Yano, K. Sato, F. Kaneko, D. M. Small, and D. R. Kodali, *J. Lipid Res.*, **40**, 140 (1999).
29. J. Schaefer, E. O. Stejskal, and R. Buchdahl, *Macromolecules*, **8**, 291 (1975).
30. S. M. Bociek, S. Ablett, and I. T. Norton, *J. Amer. Oil Chem. Soc.*, **62**, 1261 (1986).
31. H. Saito, *Magn. Reson. Chem.*, **24**, 835 (1986).
32. T. Eads, A. E. Blaurock, R. G. Bryant, D. J. Roy, and W. R. Croasman, *J. Amer. Oil Chem. Soc.*, **69**, 1057 (1992).
33. T. Arishima, K. Sugimoto, R. Kiwata, H. Mori, and K. Sato, *J. Amer. Oil Chem. Soc.*, **73**, 1231 (1996).
34. L. H. Jensen and A. J. Mabis, *Nature*, **197**, 681 (1963).
35. L. H. Jensen and A. J. Mabis, *Acta Crystallogr.*, **21**, 770 (1966).
36. K. Larsson, *Arkiv. Kemi*, **23**, 1 (1965).
37. E. S. Lutton, *J. Amer. Oil Chem. Soc.*, **48**, 246 (1971).
38. A. J. van Langevelde, K. van Malssen, F. F. A. Hollander, R. Peschar, and H. Schenk, *Acta Crystallogr.*, **B55**, 114 (1999).
39. A. J. van Langevelde, K. van Malssen, E. Sonneveld, R. Peschar, and H. Schenk, *J. Amer. Oil Chem. Soc.*, **76**, 603 (1999).
40. In ref. 17, pp. 368–369.
41. A. J. van Langevelde, K. van Malssen, R. Driessen, K. Goubits, F. Hololander, R. Pescher, P. Zwart, and H. Schenk, *Acta Crystallogr. B*, **56**, 1103 (2000).
42. K. Sato, M. Goto, K. Honda, J. Yano, D. R. Kodali, and D. M. Small, *J. Lipid Res.*, **42**, 338 (2001).
43. D. H. Wheeler, R. W. Riemenschneider, and C. E. Sandro, *J. Biol. Chem.*, **132**, 687 (1940).
44. R. H. Ferguson and E. S. Lutton, *J. Amer. Chem. Soc.*, **69**, 1445 (1947).
45. J. W. Hagemann, W. H. Tallent, and K. E. Kolb, *J. Amer. Oil Chem. Soc.*, **49**, 118 (1972).
46. M. G. R. Carter, and T. Malkin, *J. Chem. Soc.*, 554 (1947).
47. A. Desmedt, C. Culot, C. Deroanne, F. Durant, and V. Gibon, *J. Amer. Oil Chem. Soc.*, **67**, 653 (1990).
48. D. R. Kodali, D. Atkinson, T. G. Redgrave, and D. M. Small, *J. Amer. Oil Chem. Soc.*, **61**, 1078 (1984).
49. D. R. Kodali, *J. Lipid Res.*, **25**, 1490 (1987).

50. D. R. Kodali, D. Atkinson, and D. M. Small, *J. Phys. Chem.*, **93**, 4683 (1989).
51. D. R. Kodali, D. Atkinson, and D. M. Small, *J. Dispersion Sci. Technol.*, **10**, 393 (1989).
52. D. R. Kodali, D. Atkinson, and D. M. Small, *J. Lipid Res.*, **31**, 1853 (1990).
53. M. Goto, D. R. Kodali, D. M. Small, K. Honda, K. Kozawa, and T. Uchida, *Proc. Natl. Acad. Sci. USA*, **89**, 8083 (1992).
54. K. Sato and S. Ueno, in N. Garti and K. Sato, eds., *Crystallization Process in Fats and Lipid Systems*, Marcel Dekker, New York, 2001, pp. 194–209.
55. A. J. van de Streek, P. Verwer, R. de Gelder, and F. Hollander, *J. Amer. Oil Chem. Soc.*, **76**, 1333 (1999).
56. H. M. Zacharis, D. Mitcham, N. Lovegren, and M. Gray, *Adv. X-ray Analysis*, **18**, 535 (1975).
57. Z. H. Wang, K. Sato, N. Sagi, T. Izumi, and H. Mori, *J. Jpn. Oil Chem. Soc. (Yukagaku)*, **36**, 671 (1987).
58. K. Sato, T. Arishima, Z. H. Wang, K. Ojima, N. Sagi, and H. Mori, *J. Amer. Oil Chem. Soc.*, **66**, 664 (1989).
59. T. Arishima and K. Sato, *J. Amer. Oil Chem. Soc.*, **66**, 1614 (1989).
60. T. Arishima, N. Sagi, H. Mori, and K. Sato, *J. Amer. Oil Chem. Soc.*, **68**, 710 (1991).
61. J. Yano, S. Ueno, K. Sato, T. Arishima, N. Sagi, F. Kaneko, and M. Kobayashi, *J. Phys. Chem.*, **97**, 12967 (1993).
62. S. Ueno, A. Minato, H. Seto, Y. Amemiya, and K. Sato, *J. Phys. Chem. B*, **101**, 3498 (1997).
63. K. Sato, S. Ueno, and J. Yano, *Prog. Lipid Res.*, **38**, 91 (1999).
64. K. Boubekri, J. Yano, S. Ueno, and K. Sato, *J. Amer. Oil Chem. Soc.*, **76**, 949 (1999).
65. M. Takeuchi, S. Ueno, J. Yano, E. Floter, and K. Sato, *J. Amer. Oil Chem. Soc.*, **77**, 1243 (2000).
66. D. R. Kodali, D. Atkinson, T. G. Redgrave, and D. M. Small, *J. Lipid Res.*, **28**, 403 (1987).
67. P. Elisabettini, A. Desmedt, V. Gibon, and F. Durant, *Fat Sci. Technol.*, **97**, 65 (1995).
68. P. Elisabettini, G. Lognay, A. Desmedt, C. Culot, N. Istasse, E. Deffence, and F. Durant, *J. Amer. Oil Chem. Soc.*, **75**, 285 (1998).
69. A. G. Marangoni, and R. W. Hartel, *Food Technol.*, **52**, 46 (1998).
70. A. G. Marangoni and R. W. Lencki, *J. Agric. Food Chem.*, **46**, 3879 (1998).
71. F. B. Padley, in F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997, pp. 391–432.
72. A. G. Marangoni and D. Rousseau, *J. Amer. Oil Chem. Soc.*, **75**, 1265 (1998).
73. R. E. Timms, in F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997, pp. 199–222.
74. K. Sato, *Chem. Eng. Sci.*, **56**, 2255 (2001).
75. R. E. Timms, *Prog. Lipid Res.*, **23**, 1 (1984).
76. V. Givon, A. Durant, and C. Deroanne, *J. Amer. Oil Chem. Soc.*, **63**, 1047 (1986).
77. L. Engstrom, *J. Fat Sci. Technol.*, **94**, 173 (1992).
78. M. Kellens, W. Meeussen, A. Hammersley, and H. Reynaers, *Chem. Phys. Lipids*, **58**, 145 (1991).

79. T. Koyano, I. Hachiya, and K. Sato, *J. Phys. Chem.*, **96**, 10514 (1992).
80. A. Minato, S. Ueno, J. Yano, Z. H. Wang, H. Seto, Y. Amemiya, and K. Sato, *J. Amer. Oil Chem. Soc.*, **73**, 1567 (1996).
81. A. Minato, S. Ueno, K. Smith, Y. Amemiya, and K. Sato, *K. J. Phys. Chem. B*, **101**, 3498 (1997).
82. A. Minato, S. Ueno, J. Yano, K. Smith, H. Seto, Y. Amemiya, and K. Sato, *J. Amer. Oil Chem. Soc.*, **74**, 1213 (1997).
83. A. Minato, J. Yano, S. Ueno, K. Smith, and K. Sato, *Chem. Phys. Lipids*, **88**, 63 (1997).
84. M. Takeuchi, S. Ueno, E. Flöter, and K. Sato, *K. J. Amer. Oil Chem. Soc.*, **79**, 627 (2002).
85. J. B. Rossell, *Adv. Lipid Res.*, **5**, 353 (1967).
86. M. Takeuchi, S. Ueno, and K. Sato, *Cryst. Growth Design*, **3**, 369 (2003).
87. D. P. Moran, *J. Appl. Chem.*, **13**, 91 (1963).
88. L. Di and D. M. Small, *J. Lipid Res.*, **34**, 1611 (1993).
89. L. Di and D. M. Small, *Biochemistry*, **34**, 16672 (1995).
90. M. Goto, K. Honda, L. Di, and D. M. Small, *J. Lipid Res.*, **36**, 2185 (1995).
91. J. Yano, and K. Sato, *Food Res. Int.*, **32**, 249 (1999).
92. M. Takeuchi, S. Ueno, and K. Sato, *Food Res. Int.*, **35**, 919 (2002).
93. K. Larsson, *Fette. Seifen. Anstrichm.*, **74**, 136 (1972).
94. K. Larsson, in *Lipids-Molecular Organization, Physical Functions and Technical Applications*, The Oily Press, Dundee, U.K., 1994, pp 7–46.
95. R. G. Jensen, A. M. Ferris, and C. J. Lammi-Keefe, *J. Dairy Sci.*, **74**, 3228 (1991).
96. W. W. Christie, in P. F. Fox, ed., *Advanced Dairy Chemistry 2: Lipids*, 2nd ed., Chapman and Hall, London, 1995, pp. 1–36.
97. P. Walstra, T. J. geurts, A. Noomen, A. Jellema, and A. J. S. van Boekel, *Dairy Technology*, Marcel Dekker, New York, 1999, pp. 3–105.
98. R. W. Hartel and K. E. Kaylegian, in N. Garti, and K. Sato, eds., *Crystallization Processes in Fats and Lipid Systems*, Marcel Dekker, New York, 2001, pp. 381–427.
99. P. Walstra, in J. M. V. Blanshard and P. Lillford, eds., *Food Structure and Behaviour*, Academic Press, New York, 1987, pp. 67–86.
100. I. L. Woodrow and J. M. de Man, *J. Dairy Sci.*, **51**, 996 (1968).
101. J. E. Schaap, H. T. Badings, D. G. Schmidt, and E. Frede, *Neth. Milk Dairy J.*, **29**, 242 (1975).
102. C. Lopez, P. Lesieur, G. Keller, and M. Ollivon, *J. Colloid Int. Sci.*, **229**, 62 (2000).
103. C. Lopez, F. Lavigne, P. Lesieur, C. Bourgaux, and M. Ollivon, *J. Dairy Sci.*, **84**, 756 (2001).
104. C. Lopez, P. Lesieur, C. Bourgaux, G. Keller, and M. Ollivon, *J. Colloid Int. Sci.*, **240**, 150 (2001).
105. C. Lopez, F. Lavigne, P. Lesieur, G. Keller, and M. Ollivon, *J. Dairy Sci.*, **84**, 2402 (2001).
106. C. Lopez, C. Bourgaux, P. Lesieur, and M. Ollivon, *Lait*, **82**, 317 (2002).
107. K. Sato and T. Koyano, in N. Garti and K. Sato, eds., *Crystallization Processes in Fats and Lipid Systems*, Marcel Dekker, New York, 2001, pp. 429–456.
108. R. E. Timms, *Confectionery Fats Handbook*, The Oily Press, Dundee, U.K., 2003.

109. R. L. Wille and E. S. Lutton, *J. Amer. Oil Chem. Soc.*, **27**, 491 (1996).
110. G. Mazzanti, S. E. Guthrie, E. B. Sirota, A. G. Marangoni, and S. H. J. Idziak, *Crystal Growth & Design*, **3**, 721 (2003).
111. K. Higaki, S. Ueno, T. Koyano, and K. Sato, *J. Amer. Oil Chem. Soc.*, **78**, 513 (2001).
112. K. Smith, in N. Garti and K. Sato, eds., *Crystallization Processes in Fats and Lipid Systems*, Marcel Dekker, New York, 2001, pp. 357–380.
113. H. Ishikawa, T. Mizuguchi, and S. Kondo, *J. Jpn Oil Chem. Soc.*, **29**, 235 (1980).
114. A. Watanabe, I. Tashima, N. Matsuzaki, J. Kurashige, and K. Sato, *J. Amer. Oil Chem. Soc.*, **69**, 1077 (1992).
115. W. L. Ng, *J. Amer. Oil Chem. Soc.*, **67**, 879 (1990).
116. K. Kawamura, *J. Amer. Oil Chem. Soc.*, **56**, 753 (1979).
117. K. Kawamura, *J. Amer. Oil Chem. Soc.*, **57**, 48 (1980).
118. V. K. Babayan, T. G. Kaufman, H. Lehman, and R. J. Tkaczuk, *J. Soc. Cos. Chem.*, **15**, 473 (1964).
119. M. Sakamoto, K. Maruo, J. Kuriyama, M. Kouno, and K. Sato, *J. Oleo Sci.*, **52**, 639 (2003).
120. S. de Jong, T. C. van Soest, and M. A. van Schaick, *J. Amer. Oil Chem. Soc.*, **68**, 371 (1991).
121. J. W. Hagemann and J. A. Rothfus, *J. Amer. Oil Chem. Soc.*, **69**, 429 (1992).

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## *Fat Crystal Networks*

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### **1. INTRODUCTION**

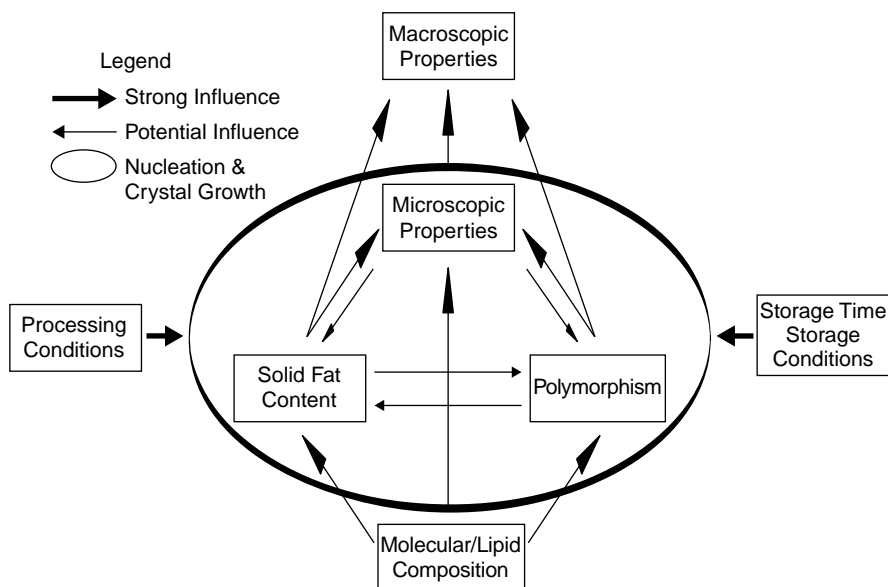
Fats provide fundamental structural and textural attributes to a wide range of consumer products, including lipstick, chocolate, and everyday products such as butter and margarine (1, 2). Within these fat-based products, certain “textural” properties are required to meet desirable sensory attributes to gain consumer acceptance (3). This has led to an increase in research efforts on the physical properties of fats, particularly their rheology.

The goal of this chapter is to investigate the effects of processing conditions on the physical properties of fats, using anhydrous milkfat (AMF) as an example. The approach proposed may lead to an increased understanding of product quality and characteristics, while offering insight into future methods for the determination and prediction of the rheological and textural attributes of fat-based products.

## 2. MECHANICAL PROPERTIES OF MILKFAT

Most mechanical tests developed for fats are empirical in nature and are usually designed for quality control purposes, and they attempt to simulate consumer sensory perception (3, 4). These large-deformation tests measure hardness-related parameters, which are then compared with textural attributes evaluated by a sensory panel (3, 5). These tests include penetrometry using cone, pin, cylinder and several other geometries (3, 6–12), compression (13), extrusion (13, 14), spreadability (15, 16), texture profile analysis (2), shear tests (13), and sectility measurements (14). These methods are usually simple and rapid, and they require relatively inexpensive equipment (3, 4, 17). The majority of these tests are based on the breakdown of structure and usually yield single-parameter measurements such as “hardness,” “yield stress,” and “spreadability,” among others (4, 17–20). The relationship between these mechanical tests and the structure of a fat has, however, not been established. The ultimate aim of any materials science endeavor is to examine the relationship between structure and macroscopic properties.

As a starting point, we need a model that can be used to describe the formation and interaction among structural elements that affect the macroscopic properties of a fat crystal network. One such model is shown in Figure 1. This model suggests that lipid composition, directly under the influence of the processing and storage conditions, will affect the solid fat content, polymorphism, and microstructure of a fat crystal network. The model also suggests that interactions among these



**Figure 1.** Hierarchical model of the factors affecting the macroscopic properties of a fat crystal network.

factors also play an important role, because they all ultimately affect the structure of the fat crystal network, and in turn influence the physical properties and sensory perception of a fat. Each of the factors within the model will be examined in turn.

### 3. LIPID COMPOSITION

#### 3.1. Triacylglycerols

Fats are composed primarily of triacylglycerols (TAG). A TAG consists of three fatty acid residues esterified to a glycerol (three-carbon sugar alcohol) backbone at specific locations known as sn-1, sn-2, and sn-3. Fatty acid residues exist in a wide variety of forms, including short and long chain, saturated and unsaturated, odd or even carbon number, *trans*- or *cis*-, linear or branched, as well as any combination thereof (21). TAG variety in a fat system is very large, as there are potentially hundreds of different fatty acid residues and their isomers available for reaction at any of the three sn-positions.

**3.1.1. Anhydrous Milkfat (AMF) Composition and Properties** AMF is heterogeneous in nature and is the most complex naturally derived fat (22–24). Previous research has revealed greater than 400 different fatty acids in AMF with carbon chain lengths ranging from 4 to 24 with varying degrees of saturation and molecular arrangement (24, 25). In bovine AMF, fatty acids are primarily in the form of TAGs. TAGs make up 96–98% of the total fat and are the primary component in the formation and structure of the fat crystal network (23, 25). The remaining 2–4% are minor components, which include monoacylglycerols, diacylglycerols, phospholipids, free fatty acids, cholesterol, and some protein (26).

Milkfat complexity dramatically increases when one considers the number of combinations for 400 fatty acid species esterified to glycerol at three different bonding sites— $400^3$  (64 million) possible TAG structures are theoretically possible (24, 25). In AMF, however, there are typically only 13 fatty acids present that are in concentrations greater than 1% (w/w), and therefore, a theoretical maximum of  $13^3$  (2197) TAG isomers typically exist (25). However, if locations sn-1 and sn-3 are considered equivalent, the number of possible TAGs is reduced to 455 (25).

For the purpose of investigating the effects of lipid composition on the physical properties of milkfat, we will use the fatty acid concentrations determined using gas-liquid chromatography. Table 1 shows the fatty acid compositions of milkfat and milkfat diluted using canola oil. Dilution with canola oil allows for the controlled variation in solids' content. By diluting with canola oil, AMF composition is altered through the addition of the long-chain fatty acids, primarily 18:1, 18:2, and 18:3, with only small modifications to the saturated fatty acid concentrations (16:0, 18:0, and 20:0). This allows for the study of the effects of lipid composition on physical properties by altering the TAG makeup using a diluent that neither

**TABLE 1. Fatty Acid Composition (wt%) of Anhydrous Milkfat and Dilutions of Anhydrous Milkfat in Canola Oil.**

Fatty Acid	100% AMF	90% AMF	80% AMF	70% AMF
4:0	2.76	2.48	2.21	1.93
6:0	2.18	1.96	1.74	1.53
8:0	1.39	1.25	1.11	0.97
10:0	3.16	2.84	3.01	2.63
12:0	3.76	3.38	2.53	2.21
14:0	12.34	11.15	9.96	8.76
14:1	1.92	1.73	1.54	1.34
15:0	1.42	1.28	1.14	0.99
15:1	0.35	0.32	0.28	0.25
16:0	30.37	27.92	25.46	23.01
16:1	2.93	2.69	2.44	2.20
17:0	0.91	0.82	0.73	0.64
17:1	0.48	0.43	0.38	0.34
18:0	9.48	8.77	8.05	7.34
18:1	21.92	25.69	29.46	33.23
18:2	2.79	4.52	6.24	7.97
18:3	1.31	2.11	2.92	3.72
20:0	0.52	0.61	0.71	0.80
22:0	0.00	0.05	0.09	0.14

significantly solubilizes milkfat TAGs nor crystallizes in the temperature range of interest (usually above 0°C) (27).

AMF complexity gives it unique textural, physical, and sensory properties. The crystallization and melting properties of AMF are directly related to its complex composition. The large variety of TAG species that exist in AMF cause it to have a wide melting range, spanning from -40°C to 40°C. Within this melting range, at least three distinct fractions have been identified and designated as low-melting (LMF), medium-melting (MMF), and high-melting (HMF) fractions (28). These fractions are not well defined; however, the LMF typically contains short-chain (4:0 to 8:0) and long-chain unsaturated (18:1, 18:2, 18:3, 20:1) fatty acids. MMF contains medium-chain (10:0-14:0) fatty acids, and HMF contains longer chain (>16:0) fatty acids. The heterogeneous nature of the material, and its complex phase behavior, causes drastic differences in the solid nature of AMF crystal networks when storage temperature or crystallization conditions are modified. AMF lipid composition is the fundamental property affecting all of the structural characteristics of the crystallized bulk fat (Figure 1), because it will influence nucleation, crystal growth, and the formation of the final crystal network.

There have been various attempts to modify the lipid composition of AMF as a means of producing butter with improved spreadability. These studies have included the fractionation and removal of portions of the HMF region to provide a more spreadable product at refrigeration temperatures (24). Other attempts



have included the modification of bovine diets to incorporate particular fatty acids into MF TAGs, and other work has concentrated on the chemical interesterification of different fatty acids to the TAGs of AMF (9, 29–32). These methods typically affect the flavor of bulk AMF, and labeling the product “butter” is not permitted because of the chemical adulterations performed. For this reason, processors are continually trying to modify crystal structure, and thereby improve textural characteristics solely through the modification of processing conditions.

#### 4. PROCESSING CONDITIONS

The structure of most processed food depends not only on the ingredient formulation, but also on the processing history of the material (33). Processing conditions, such as crystallization, storage temperature, cooling rate, storage time, shear, and tempering, affect the crystal structure and rheological properties of fats. In the model proposed in Figure 1, processing conditions are considered an external factor, and like lipid composition, affect the underlying physical properties of the fat crystal network.

Adjusting processing parameters, such as cooling rate and/or crystallization temperature, will cause fats to exhibit differences in physical properties. Altering cooling rate has been shown to induce the formation of different polymorphs in AMF (34). This research concentrated on determining the initial polymorph present in AMF upon cooling at various controlled rates. AMF was cooled from 70°C to –65°C at different cooling rates, and polymorphism was monitored using differential scanning calorimetry (DSC) and powder X-ray diffraction. At temperatures above 0°C, the  $\alpha$ -polymorphic form was predominant at all cooling rates above 1°C/min. At 1°C/min, a mixture of both  $\alpha$ - and  $\beta'$ -polymorphs was present, and at 0.5°C/min, only the  $\beta'$ -polymorph was detectable. This indicates that the processing conditions affect the nucleation and crystal growth of AMF, as indicated by differences in polymorphism.

Herrera and Hartel (35, 36) demonstrated differences in microstructural size and structure as a result of cooling rate using milkfat and milkfat blends. Cooling a sample more rapidly resulted in smaller and more numerous crystallites. Additionally, they demonstrated differences in SFC and polymorphism. Differences were induced at the nucleation and crystal growth stages. They further demonstrated the effects of cooling rate on the rheological characteristics of the system using the compression storage modulus ( $E'$ ) as an indicator (37). They found that, under shear, the storage modulus decreased with increases in cooling rate (smaller particle sizes), therefore making a link among processing conditions, solid fat content, and microstructure and macroscopic properties (35–37).

These various examples depict the effects of processing conditions on the physical properties of fats and demonstrate that external crystallization conditions, such as cooling rate and storage temperature, can have dramatic effects on the final measurable properties of a fat.

## 5. NUCLEATION AND CRYSTAL GROWTH

Crystal shape, size, and density all affect the physical properties of the final solid fat matrix. Crystal growth, primary nucleation, and secondary nucleation in fat systems are influenced by many factors, including diffusion, molecular compatibility, TAG structure, nuclei composition and surface properties, number of nuclei, and processing conditions (temperature and/or shear) (38, 39). It is during the crystallization process of fats that the template for the final physical properties of the material is created.

### 5.1. Nucleation Mechanisms

Crystallization does not take place until the melt becomes “supersaturated” or “undercooled” (38). Like all substances, fats cannot crystallize until tiny embryonic crystals, known as nuclei, are formed. Supersaturation and consequent homogeneous nucleation is unlikely in MF because MF is of a heterogeneous nature with virtually no pure TAG species in concentrations greater than 2 mol% (39, 40). In the melt, there are two primary mechanisms by which nucleation takes place. These nucleation processes, illustrated in Figure 2, are referred to as homogeneous and heterogeneous, the latter being predominant in AMF crystallization (39, 40). After nucleation, crystal growth takes place on the surface of existing nuclei (38).

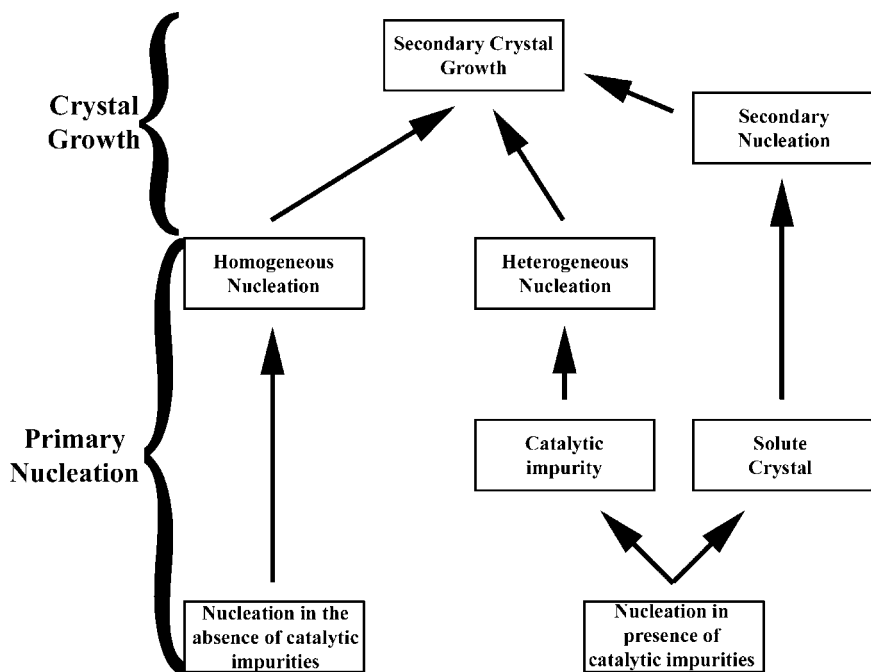


Figure 2. Schematic depicts nucleation and crystal growth processes.

**5.1.1. Homogeneous Nucleation** Homogeneous nucleation, resulting from bimolecular reactions between the TAG species, leads to the formation of nuclei in the absence of foreign particles (39, 40). To achieve homogeneous nucleation, no solid substrate or contaminant can exist in the fat. TAGs interact only with one another, usually at higher degrees of supercooling (in most cases are greater than 30°C below the final melting temperature). AMF typically will not nucleate in a homogeneous fashion because of the following

1. The presence of catalytic impurities such as water, dirt, monoacylglycerols, diacylglycerols, phospholipids, and proteins.
2. The presence of a large variety of different and incompatible TAGs in concentrations of less than 1 mol% of the entire AMF volume (40).
3. The presence of agitation, temperature gradients, inducing inconsistent nucleation, and diffusion-limited gradients (39).

**5.1.2. Heterogeneous Nucleation** Heterogeneous nucleation is the most common process in AMF crystallization (39, 40). Nucleation is induced by the presence of foreign particles or catalytic impurities, and therefore, it requires a far lower degree of undercooling (as low as 3°C) than that required for homogeneous nucleation (39, 40). Therefore, because of the large quantity of structurally incompatible TAGs in AMF, nucleation on catalytic impurities is entropically favored over homogeneous nucleation.

**5.1.3. Secondary Nucleation and Crystal Growth** Secondary nucleation is the process whereby nuclei are created as a result of inhomogeneous growth on primary crystals, or crystal breakage as a result of processing, which yield new interfaces for the creation of secondary nuclei. Secondary growth is the continued solidification of TAGs onto the surface of already existing nuclei, which allows crystals to form larger structures. During crystal growth, fat crystals take shape by forming complex spherulitic or needle-like crystal structures (41). In AMF, spherulitic crystal structures are predominant. Spherulites tend to have a very dense crystal center with decreasing density as the distance from the crystal center increases (41). Spherulites can continue to grow and aggregate to form a three-dimensional network.

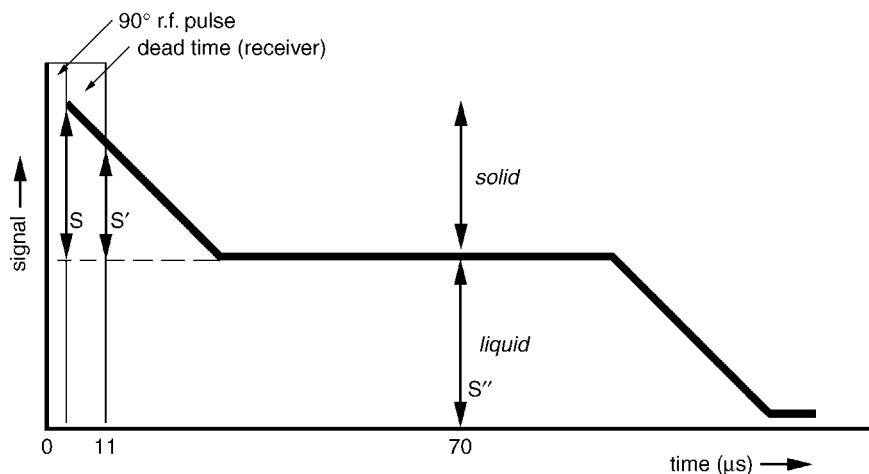
## 5.2. Factors Directly Influenced by Nucleation and Crystal Growth

**5.2.1. Solid Fat Content** Many methods for measuring SFC have been developed. These include dilatometry, calorimetry, and pulsed nuclear magnetic resonance (pNMR). Dilatometry and calorimetry use measurements of volume or heat content ratios between the completely liquid and the completely solid states (42). Dilatometry and calorimetry methods are time consuming and tend to be applicable only when the SFC is less than 50% (42). Therefore, pNMR has become the most commonly used method for SFC determination.

**5.2.1.1. Solid Fat Content by pNMR** NMR techniques allow for a rapid determination of SFC with increased accuracy and precision relative to dilatometry and calorimetry, while offering additional advantages of being noninvasive and rapid (6 seconds per sample) (43). The NMR method for SFC determination is based on the principle that nuclear spins align in a magnetic field. In a pNMR measurement, the realignment of the nuclear spins of  $^1\text{H}$  atoms after a strong electromagnetic pulse is measured (43). Application of a short  $90^\circ$  radio frequency electromagnetic pulse provides excitation to the  $^1\text{H}$  atoms, causing them to leave their equilibrium, aligned state. The pulse is then withdrawn leading to the relaxation of the  $^1\text{H}$  atoms and a return to the aligned, equilibrium state (43). Differences in the time scale for the relaxation process of the solid and liquid protons are used to determine the SFC. Protons, in general, have less mobility in the solid phase than in the liquid phase, therefore leading to large differences in the relaxation times of the  $^1\text{H}$  atoms (43).

A typical excitation and relaxation cycle measured using NMR is shown in Figure 3. The determination of SFC requires measurements at two time periods, one within the short solid response region,  $S'$  ( $11\ \mu\text{s}$ ), and one within the longer liquid response region,  $S''$  ( $70\ \mu\text{s}$ ). The signal intensity  $S'$  provides an estimate of the amount of both solid and liquid protons. Moreover, as the measurement cannot be taken immediately because of receiver "dead time," the true amount of solid and liquid protons cannot be directly measured. For this reason, an extrapolation factor, known as the F-factor ( $F$ ), is used to approximate the maximum response signal ( $S$ ) value (43). SFC is calculated using the terms derived from the curve in Figure 3 and the NMR digital offset ( $D$ ) using the following equation:

$$SFC(Direct) = \frac{(S' - S'')F}{S'' + (S' - S'')F + D}. \quad (1)$$



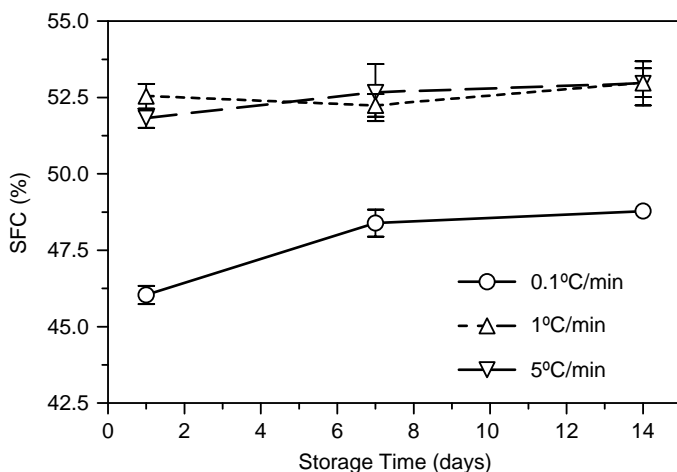
**Figure 3.** Typical signal decay for a partially crystallized fat, following a  $90^\circ$  r.f. electromagnetic pulse. Parameters required for measurements of solid fat content (SFC) are shown.

The value calculated is then compared with the signal obtained from either standard oil or polymer calibration standards to determine the actual SFC value. This method is very reproducible and provides standard deviations of less than 1%, indicating a reliable and rapid method for SFC determination in fats (44).

**5.2.1.2. Solid Fat Content and the Fat Crystal Network** The solids' content of a fat crystal network is of critical importance to the final physical properties of the system. Generally, an increase in SFC leads to an increase in fat firmness. The SFC measurement has been widely used as a determinant quantity for the structural properties of fat systems. Estimations for commercial plastic fats, including butter, predict firmness increases of 10% for every percent increase in SFC (45). As a result, models used to describe the rheological properties of fats incorporate references to SFC values.

The primary factor affecting the SFC of any fat network is molecular composition. In general, longer chain fatty acids will have higher melting and crystallization temperatures than shorter chain fatty acids. Typically, the larger the amount of saturated, long-chain fatty acids, the greater the SFC. Processing conditions, including cooling rate, agitation, and tempering, have the ability to affect the SFC of fats.

**5.2.1.3. Effects of Cooling Rate and Storage Time on SFC in AMF** AMF solid fat content is affected by processing conditions, in particular by cooling rate. The effects of cooling rate and storage time on SFC at 5°C can be appreciated in Figure 4. The effects of cooling rate, storage time, and their interaction have been determined to be statistically significant ( $p < 0.05$ ). Slowly cooling AMF at a rate of 0.1°C/min results in a 4–8% lower SFC than for samples that are cooled more rapidly (1°C/min and 5°C/min). The SFC of slowly cooled samples also

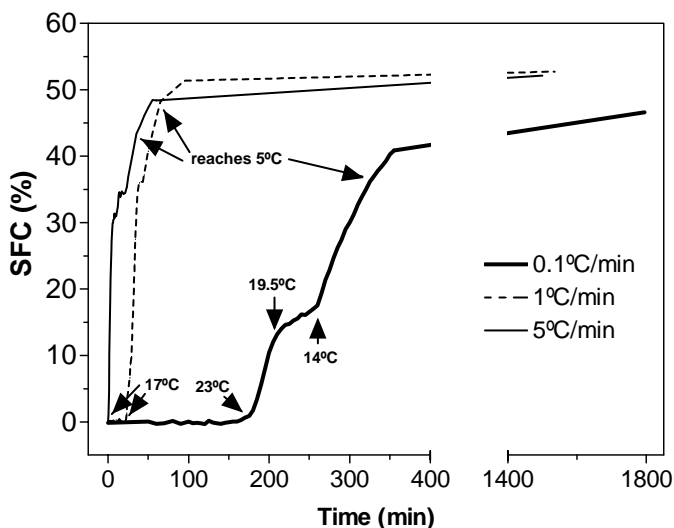


**Figure 4.** Solid fat content of anhydrous milkfat cooled at 0.1°C/min, 1°C/min, and 5°C/min and stored for a period of 14 days at 5°C.

increased slightly in time. On the other hand, at the higher cooling rates of  $1^{\circ}\text{C}/\text{min}$  and  $5^{\circ}\text{C}/\text{min}$ , SFC does not statistically change in time. This demonstrates that cooling rate has a significant effect SFC of AMF, and that small changes in SFC can occur as a result of storage at  $5^{\circ}\text{C}$  for 14 days.

**5.2.1.4. Crystallization Kinetics Monitored using SFC by pNMR** Solid fat content measurements can be used to monitor the kinetics of crystallization. SFC was monitored by pNMR as a function of time while samples were being cooled in a water bath. These results are presented in a plot of SFC versus time in Figure 5. The  $0.1^{\circ}\text{C}/\text{min}$  crystallization curve indicates that crystal growth begins at approximately  $23^{\circ}\text{C}$ , or 170 min, and continues over time once  $5^{\circ}\text{C}$  is reached. Crystal growth continues at a relatively constant rate until 205 min ( $19.5^{\circ}\text{C}$ ). At this point, a plateau-like region occurs in the SFC profile, indicating a significant reduction in crystal growth until a time of 260 min ( $14^{\circ}\text{C}$ ). After this plateau region, crystal growth begins to occur at a more rapid and constant rate until the final temperature of  $5^{\circ}\text{C}$  is reached. At this point, the SFC increases very slowly until an equilibrium SFC is achieved.

The break in the crystallization curve for cooling at  $0.1^{\circ}\text{C}/\text{min}$  possibly indicates that different AMF fractions crystallize at different times, as a result of the slow cooling rate. This fractionation is common in AMF because of the large variety of TAGs and fatty acid species that are present. Within the first crystal growth region, the longer chain saturated HMF triacylglycerols (16:0 to 20:0) crystallize and contribute to crystal growth and subsequent increase in SFC. Within the plateau-like region, TAGs containing both long- and short-chain fatty acids reach their



**Figure 5.** Crystallization and crystal growth of anhydrous milkfat at controlled cooling rates of  $0.1^{\circ}\text{C}/\text{min}$ ,  $1^{\circ}\text{C}/\text{min}$ , and  $5^{\circ}\text{C}/\text{min}$  monitored using SFC by pNMR.

supersaturated state and crystallize. Beyond the plateau region, medium-chain saturated MMF triacylglycerols (10:0 to 14:0) become supersaturated and contribute to crystal growth. The implications of this crystal growth pattern will become more apparent in the discussion of the microscopic and macroscopic properties of the crystallized AMF. Beyond the final temperature of 5°C, any remaining TAGs that can become supersaturated continue to crystallize until an equilibrium state is achieved.

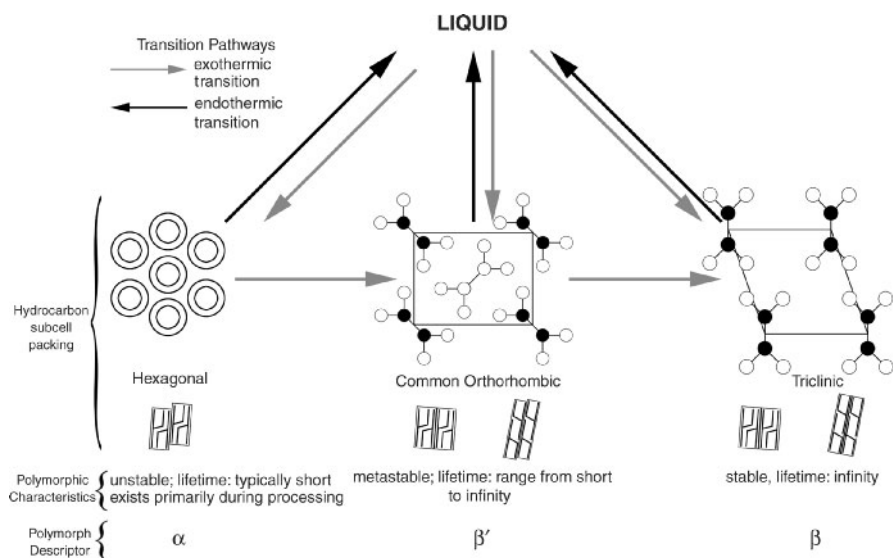
The 1°C/min and 5°C/min crystallization profiles indicate that crystal mass becomes detectable at approximately 17°C in both cases, which occurs after 23 min and 4 min, respectively (Figure 5). Once this temperature is reached, there is a constant and very rapid rate of crystal growth until the final temperature of 5°C is reached. At approximately 12°C, there is an observable fluctuation in the SFC growth curve that is followed by a continued rapid crystal growth until the equilibrium temperature of 5°C is reached. This minor fluctuation in the SFC growth curves may indicate a polymorphic transition or more likely the occurrence of fractionation. The rapid and more spontaneous nucleation and crystal growth that occurs at higher cooling rates leads to less fractionation and the formation of more numerous mixed crystals containing a larger array of TAGs and a final SFC that is 4–8% higher than the samples cooled at 0.1°C/min after 24 hours of storage.

### 5.3. Polymorphism

Polymorphism is another characteristic of fat networks that affects their rheological characteristics. Polymorphic forms are crystalline phases with different structural characteristics, but of identical chemical compositions in their liquid state, when melted (45, 46). Polymorphic forms are usually categorized according to characteristic X-ray diffraction patterns, specific volume, and/or melting points (34, 45, 47). The polymorphic form developed during crystallization of bulk fat can be influenced by several factors, including fat purity, TAG compatibility, temperature, supercooling, cooling rate, catalytic impurities, solvents, and seed crystals (45). Phase transitions from one polymorphic form to the next may also occur during processing and storage depending on many factors (45, 46).

The three common polymorphic forms that exist in fat crystal networks are the sub $\alpha$ ,  $\alpha$ ,  $\beta'$ , and  $\beta$  modifications. These polymorphic modifications and their characteristic crystallization patterns are shown in Figure 6. The sub $\alpha$  and  $\alpha$  forms are metastable (34). Each polymorphic form yields different crystal structures dependent on the magnitude of the crystallization driving force. The polymorphic modifications also have varying thermodynamic stability, which determines their lifetime within a crystal matrix, with the tendency toward greater stability in the order  $\alpha$  to  $\beta'$  to  $\beta$  (24, 34).

**5.3.1. Polymorphism and Milkfat** High cooling rates ( $>1^\circ\text{C}/\text{min}$ ), or high levels of supercooling ( $>15^\circ\text{C}$ ), lead to the rapid formation of metastable  $\alpha$  nuclei (34). The persistence of these unstable nuclei is dependent on thermal treatments that occur after crystallization. These nuclei may remain in the  $\alpha$  form or convert



**Figure 6.** Polymorphic forms of fat crystals, including the possible polymorphic transitions, subcell packing structures, stability characteristic, and triacylglycerol stacking conformations.

to the more stable  $\beta'$  structure. At lower degrees of supercooling or low cooling rates ( $>0^{\circ}\text{C}/\text{min}$  to  $1^{\circ}\text{C}/\text{min}$ ),  $\beta'$  crystals predominate with trace amounts of metastable  $\alpha$  nuclei detectable (34). Many factors influence the formation of different polymorphic forms, including catalytic impurities, agitation, viscosity, TAG concentration, among others (48).

**5.3.1.1. The alpha ( $\alpha$ ) Polymorph** The  $\alpha$  polymorph is most readily formed, but it is very unstable. Under most conditions, it will transform within a short period of time into the  $\beta'$  polymorphic form (34, 40). The  $\alpha$  crystals are composed of TAGs whose long, alkane-like fatty acid chains are packed in a loose hexagonal subcell conformation, as shown in Figure 6 (3, 46). The hexagonal  $\alpha$  subcell has a lower density because of the loose packing of the TAG molecules and is characterized by a single X-ray short spacing (wide-angle reflection) at  $4.15 \text{ \AA}$  (34). This loose packing allows for a large number of reaction sites, the incorporation of a wide variety of TAGs into the solid fat matrix, and ample room for molecular rotation and re-orientation. This allows for transitions from the  $\alpha$  to  $\beta'$  polymorphic form to occur readily (3, 24). This open lattice allows mixed crystal formation, incorporating a mixture of all of the supersaturated TAG present in the melt within the crystals (3). In AMF, the formation of the  $\alpha$  form takes place during crystallizations at high degrees of supercooling or at high cooling rates, and it may persist as the predominant polymorphic form when the fat is stored at subzero temperatures (34). In most situations, the  $\alpha$  form is the first crystal structure formed during crystallization because of the loose packing and lower supersaturation requirements. However,  $\alpha$



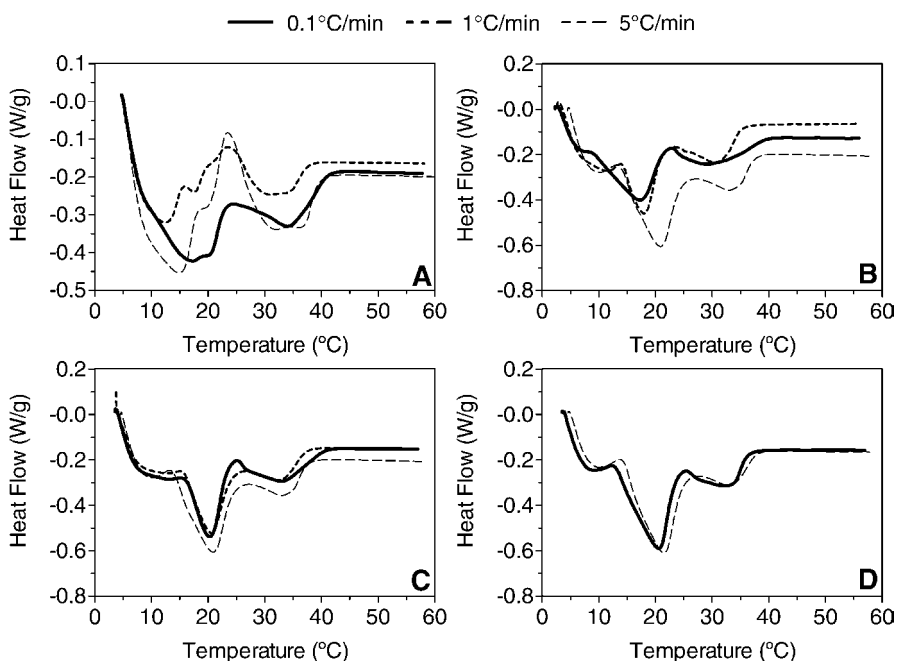
crystals tend to remain small and eventually transform, through the melt or molecular reorientation in the solid state, to the more stable  $\beta'$  modification (3).

**5.3.1.2. The Beta Prime ( $\beta'$ ) Polymorph** The  $\beta'$  polymorph is the most common polymorphic form in AMF. It will form directly from the melt or through transformation of the  $\alpha$  polymorph (Figure 6) (24). The  $\beta'$  polymorphic form exists in either a double- or triple-chain length configuration, the latter being more common in AMF, and orients in a more dense orthorhombic subcell structure (Figure 6), characterized by X-ray short spacings at 3.8 and 4.2 Å (45, 46). Processors prefer this form because it is structurally stable and maintains small- to-moderate crystal sizes allowing for soft and smooth products (3). The  $\beta'$  structure does not allow for the incorporation of a large variety of TAGs within the crystal lattice, compared with the  $\alpha$  structure (3). Lower TAG variety is a direct result of the tighter packing required to form the more stable structure, which requires similar length TAGs. During crystallization in the  $\alpha$  form, dissimilar TAGs will often cocrystallize temporarily; however, in time, the shorter chain, or more unsaturated, less supersaturated molecules will redissolve. This, in turn, allows the remaining, more similar longer chain supersaturated TAGs to convert to the more stable  $\beta'$  form (3, 40). TAGs in AMF under moderate undercooling conditions will crystallize in both the  $\alpha$  and  $\beta'$  polymorphic forms.

**5.3.1.3. The Beta ( $\beta$ ) Polymorph** The presence of the  $\beta$  polymorph is uncommon in AMF, but it has been known to exist in some situations (34). It is the most stable, highest packing density polymorphic form, and it may exist in either double- or triple-chain length conformation. Fatty acid chains within TAGs pack in a hexagonal arrangement (Figure 6), with a characteristic short spacing at 4.6 Å (34, 45). The  $\beta$  form is uncommon in AMF because of the dense packing arrangement of similar TAGs. Because of the wide variety of TAG in low individual concentrations (<1 mol%) in AMF, the conditions required to form  $\beta$  structures are only met when the fat is crystallized at very slow rates, or at low degrees of supercooling (34, 46). These crystallization conditions are unusual in commercial practice; thus, the  $\beta$  polymorph is rare. The formation of  $\beta$  solids is also undesirable in AMF because it leads to the development of large crystals and sand-like texture (3, 34).

**5.3.1.4. Polymorphism in Milk Fat as a Result of Processing Conditions** Work by ten Groetenhuis et al. (34) has shown that AMF typically crystallizes in two fractions. The highest melting group of TAG species represents one fraction, and the middle and low-molecular-weight TAGs form the second. This group also suggests that the application of different cooling rates lead to variations in the initial polymorphic form during crystallization of MF (34).

Two regions of crystallization can be identified from the two major endotherms in the melting profiles of samples subjected to different cooling rates, as shown in Figure 7. Immediate melting profiles showed differences, suggesting that the initial polymorphic forms differ in AMF crystallized at various rates. Figure 7a depicts the melting profile of samples cooled at various rates after 10 minutes of storage at 5°C.



**Figure 7.** Differential scanning calorimetry curves for anhydrous milkfat cooled at rates of 0.1°C/min, 1°C/min, and 5°C/min to 5°C and stored for time periods (A) 10 minutes, (B) 1 day, (C) 7 days, and (D) 14 days.

The sample cooled at 0.1°C/min was expected to have had sufficient time for rearrangement, resulting in more dense packing of the TAGs, and crystallized in the stable  $\beta'$  polymorph (34). For this reason, the peak of the endotherm at 17.5°C is assumed to correspond to the melting temperature of the  $\beta'$  polymorph.

The samples cooled at 1°C/min and 5°C/min (Figure 7a) both have minor inflections in the curve at this same temperature (17.5°C), but they also have a major peak endotherm at around 15°C. This may correspond to an  $\alpha$ -polymorphic melt. It is thus suggested that cooling at 1°C/min results in a mixture of  $\alpha$  and  $\beta'$  polymorphs, and cooling at 5°C/min results predominantly in the  $\alpha$  polymorph. These predictions correspond to the findings of previous studies on AMF employing similar crystallization conditions using DSC and powder X-ray diffraction (34).

The samples cooled at the three cooling rates were also stored at 5°C for 1, 7, and 14 days, yielding the melting profiles shown in Figures 7b, c, and d, respectively. After storage at 5°C, the melting profiles began to look similar irrespective of the cooling rate used during crystallization. As it has been shown that AMF tends to crystallize in the  $\beta'$  polymorph upon storage (some believe that the  $\alpha$  polymorph survives for only minutes), similar shapes and peak melting points would be expected (3, 40).

## 5.4. Microstructure

The textural properties of a fat are influenced by all levels of structure, particularly microstructure. The microstructure includes the spatial distribution of mass, particle size, interparticle separation distance, particle shape, and interparticle interaction forces (49–51). Methods that can be used for the characterization of microstructure in fat systems include, among others, small deformation rheology and polarized light microscopy, employing a fractal approach (49–51).

**5.4.1. Fat Crystal Network Theory** Fat crystal growth is dictated by external processing conditions and TAG composition. TAGs nucleate and grow from the melt into certain polymorphic and polytypic states. These primary crystals then aggregate into larger polycrystalline particles, also known as microstructural elements. Aggregation continues, leading to the formation of larger clusters, or microstructures, until a space-filling three-dimensional network is formed through interactions among microstructures. Crystalline mass within microstructures is distributed in a heterogeneous, disordered fashion, which can be characterized using fractal scaling principles (49–51).

Perfect fractal objects display exactly self-similar at all levels of magnification. Natural systems do not exhibit exact self-similarity, but statistical self-similarity; i.e., the microstructure, on average, is similar in appearance, distribution, and structure within a limited range of magnifications. In particles networks, fractal scaling is usually encountered within the range of magnifications corresponding to the size of microstructural elements to the size of microstructures (51). An example of statistical self-similarity uses the analogy of a tree branch structure. The tree begins as a trunk, the tree trunk has branches, these branches have branches, and so on. When the scale of observation is changed, a statistically self-similar pattern is observed.

This theory was first developed for colloidal aggregate networks and was later adapted to fat crystal networks (52–54). In colloidal systems (with a disordered distribution of mass and statistical self-similar patterns), the mass of a fractal aggregate (or the distribution of mass within a network),  $M$ , is related to the size of the object or region of interest ( $R$ ) in a power-law fashion:

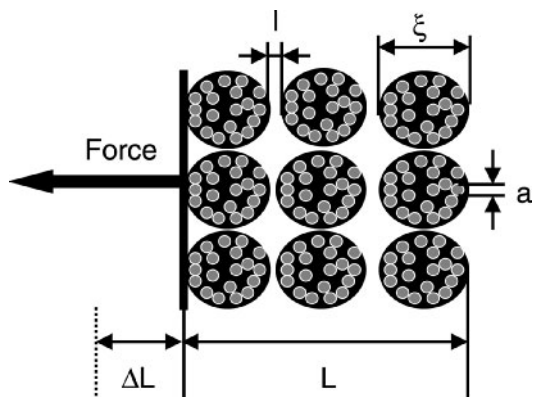
$$M \sim R^D, \quad (2)$$

where  $D$  is the mass fractal dimension of the object, or the distribution of mass within a region of the network.

The elasticity of a fat crystal network is dependent on the microstructure of the fat crystal network, particularly the spatial distribution of mass. The shear modulus ( $G$ ) scales with the volume fraction of solids in a power-law fashion (49–54):

$$G \sim \Phi^\mu, \quad (3)$$

where  $\Phi$  is the volume fraction of solid fat, or SFC/100, and  $\mu$  is dependent on  $D$  (see below).



**Figure 8.** Idealized fat crystal network under extension. Particles ( $a$ ) are packed in a fractal fashion within flocs ( $\xi$ ). A force ( $F$ ) acting on the network causes the links between flocs to yield, and the original length of the system in the direction of the applied force ( $L$ ) to increase ( $\Delta L$ ). Thus, the inter-floc separation distance ( $l$ ), also increases.

The scaling behavior of the elastic modulus was first characterized for colloidal gels within two specific rheological regimes (55) and was later adapted to fat crystal networks (52–54). These two regimes depend on the strength of the links that exist between individual clusters, relative to inner cluster strength. These regimes are referred to as the “strong-link regime” and “weak-link regime” (55). Figure 8 illustrates the behavior of a fat crystal network under extension in relation to these theoretical rheological regimes. The strong-link regime is only applicable at very low solid fat contents (usually below 10%). In the strong-link regime, crystal clusters grow large, and the links between these flocs are stronger than the flocs themselves. The elastic response of the material in this regime is a function of the elastic response of the flocs, and it is thus dependent on internal aggregate structure.

The contrary is true for the weak-link regime, which is observed at high solid fat contents, where flocs are small and stronger than the links between them. In this regime, the elastic response of the material is a function of the elastic response of the links between flocs, and it is not dependent on the structure within the flocs.

In both regimes, the macroscopic elastic modulus ( $K$ ) of a system of size  $L$  is the sum of the elastic constants of the flocs ( $k_\xi$ ) or the links between flocs ( $k_L$ ) in the direction of the applied stress (one-dimensional treatment). In the weak-link regime,

$$K \sim \left(\frac{L}{\xi}\right) k_L \sim \xi^{-1}, \quad (4)$$

where  $\xi$  is the size of a floc. Floc size ( $\xi$ ) scales with the volume fraction of solids ( $\Phi$ ) in a power-law fashion:

$$\xi \sim a\Phi^{\frac{1}{D-d}}, \quad (5)$$

where  $d$  is the Euclidean dimension (usually  $d = 3$ ), and  $a$  is the size of a primary particle. By inserting Equation 5 into Equation 4, we obtain the following relationship:

$$K \sim \Phi^{\frac{1}{3-D}} \sim G. \quad (6)$$

This equation can also be expressed as

$$G = \lambda \Phi^{\frac{1}{3-D}}, \quad (7)$$

where  $\lambda$  is a constant independent of the volume fraction, but dependent on several primary particle structural parameters as well as intermolecular forces.

Our group has derived expressions for the Young's modulus ( $E$ ) of a fat as it relates to the structure of the material (56–58):

$$E \sim \frac{6\delta}{a\varepsilon^*} \Phi^{\frac{1}{3-D}} = \frac{A}{2\pi a\varepsilon^* d_0^2} \Phi^{\frac{1}{3-D}}, \quad (8)$$

where  $\delta$  is the crystal-melt interfacial tension (about 0.01 J/m<sup>2</sup> for TAGs),  $a$  is the primary particle size,  $\Phi$  is the volume fraction of solids (SFC/100),  $D$  is the fractal dimension,  $A$  is Hamacker's constant (about  $5 \times 10^{-20}$  J for alkanes),  $\varepsilon^*$  is the extensional/compressional strain at the limit of linearity, and  $d_0$  is the equilibrium inter-microstructural (flocs or clusters) separation distance. Values for the shear modulus ( $G$ ) could be obtained from knowledge of the Poisson ratio of the material. For a material where no volume change takes place when it is stretched or compressed, the Poisson's ratio is 0.5 and  $E = 3G$ .

Careful scrutiny of Equation 8 would suggest that the stress at the limit of linearity ( $\sigma^*$ ) can be determined from the product of the Young's modulus ( $E$ ) and the strain at the limit of linearity ( $\varepsilon^*$ ),  $\sigma^* = E \cdot \varepsilon^*$ , yielding the expression:

$$\sigma^* = \frac{6\delta}{a} \Phi^{\frac{1}{3-D}}. \quad (9)$$

This expression for the stress at the limit of linearity, proposed in Marangoni and Rogers (58), provides an approximation to the yield stress (and thus hardness) of a fat. This model would allow for the prediction of the yield stress of a fat based on easily determined structural characteristics.

#### 5.4.2. Fractal Dimension Evaluation using Small Deformation Rheology

**( $D_r$ )** To determine  $D_r$  for a particular system by rheological methods, the shear storage modulus ( $G'$ ) must be measured at different solids' volume fractions. The solids' volume fraction, which is equal to the SFC/100, is varied by diluting the fat with an inert solvent that will not cocrystallize, or otherwise alter the behavior of the system. Canola oil has been used as a suitable AMF diluent for these purposes. A log–log plot of  $G'$  versus  $\Phi$  yields a slope  $\mu$  from which  $D_r$  can be

**TABLE 2. Rheologically Determined Fractal Dimensions ( $D_r$ ) and Pre-Exponential Terms ( $\lambda$ ) for Anhydrous Milkfat Crystallized at Various Rates of Cooling and Storage Times at 5°C.**

Processing Conditions		Microstructural Parameters	
Cooling Rate (°C/min)	Storage Time (days)	$D_r$	$\lambda$ (MPa)
0.1	1	2.82	1150
	7	2.78	376
	14	2.79	400
1.0	1	2.67	91
	7	2.59	65
	14	2.60	64
5.0	1	2.57	65
	7	2.50	52
	14	2.47	49

determined. The pre-exponential term  $\lambda$ , which is influenced primarily by particle properties, can be determined from the value of  $G'$ , where  $\Phi = 1.0$  (SFC = 100%).

The effects of cooling rate on  $D_r$  are shown in Table 2. In general, an increase in cooling rate results in a decrease in  $D_r$ . Previous work in our laboratory has also shown that higher fractal dimensions occur in networks that are more ordered; thus, it can be expected that lower cooling rates would display this trend. The higher the  $D_r$ , the more “ordered” the crystal packing. For this reason, the value for  $D_r$  is expected to vary in the order of  $D_{r-0.1^\circ\text{C}/\text{min}} > D_{r-1^\circ\text{C}/\text{min}} > D_{r-5^\circ\text{C}/\text{min}}$ . When observed by time-lapsed microscopy, the samples cooled slowly (0.1°C/min) and yielded less numerous (see Figure 12 below), but more sporadically formed nuclei relative to the more rapidly cooled samples. This allows for a more ordered crystal growth. The 1°C/min sample initially demonstrated sporadic nucleation and controlled crystal growth of more numerous and smaller crystals than the slower cooled sample until reaching 13°C. Beyond 13°C, the AMF began to nucleate spontaneously and fill space very rapidly, leading to a more disordered final system. Finally, samples cooled at 5°C/min demonstrated spontaneous nucleation and rapid crystal growth at 16°C. This resulted in a more disordered system with a fractal dimension lower than for slower cooling rates.

The values for  $\lambda$ , shown in Table 2, are also affected by cooling rate and storage time at 5°C. As with the  $D_r$ , the values for  $\lambda$  decrease with increases in cooling rate. During storage at 5°C, the  $\lambda$  values decrease moderately at each cooling rate and then appear to equilibrate at 7 days, with only minor differences detectable between the 7-day and 14-day values. This decrease may be attributable to continued crystal growth (demonstrated by small increases in SFC) and by the restructuring that occurs during polymorphic transitions and molecular rearrangement.

**5.4.2.1. Fractal Dimension by Microscopy** The use of fractal geometry as a means of characterizing microstructure has been extensive in recent years. Various

applications have included investigating the structure of protein gels (59), depicting the irregular nature of solid adsorbents such as zeolites (60), and predicting optical responses of colloid-adsorbate films (61). Recent work by Kim and Berg (62) has focused on the use of fractal scaling as a link between aggregation kinetics and structure that result from both diffusion and reaction-limited cluster aggregation processes of colloidal materials.

To obtain the fractal dimension of a network of particles, acquiring images of the microstructure is necessary. Many forms of microscopy can be used, including brightfield microscopy, confocal laser scanning microscopy, scanning electron microscopy, and in the case of fat crystal networks, polarized light microscopy.

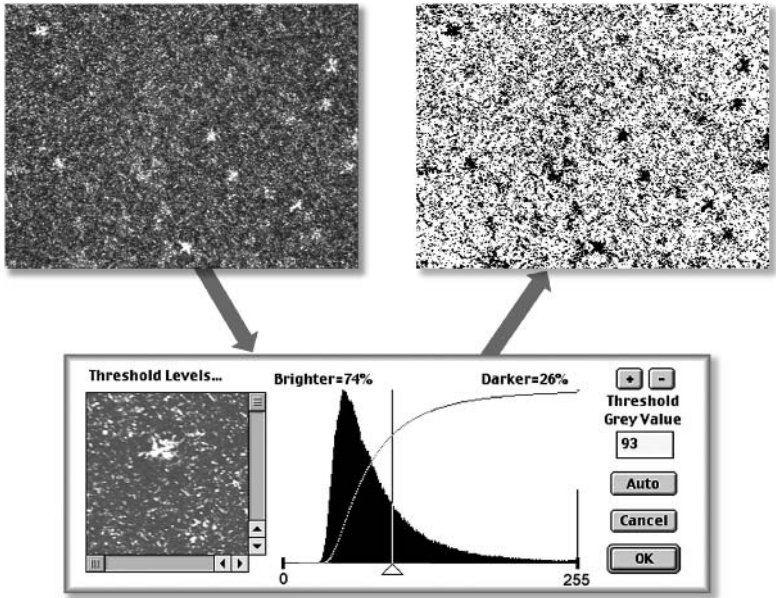
Grayscale images of the network (usually consisting of pixel intensities ranging from pure white to pure black) must be converted to a binary format prior to image analysis. The discriminatory conversion of grayscale images to binary, requires a process termed "thresholding." Images of fat crystal networks are typically obtained as 8-bit (256 color) images and consist of a histogram of pixel values that span the range of 0 (pure white) and 255 (pure black). Thresholding involves the selection of a particular pixel value within the range of 0 and 255 that will result in a binary image that best isolates the features of the network. This process is subject to much controversy because the selection of the cutoff pixel value is subjective. To minimize subjectivity, an autothresholding algorithm was used that provides accurate and reproducible results.<sup>1</sup> This algorithm works by scanning the histogram to find an intensity value where the average moments of the histogram counts about an intensity value are balanced. This means that a threshold value is chosen where the average pixel intensities are equal above and below the threshold (63). The general process of autothresholding can be seen in Figure 9. Once the image is converted to binary and the features isolated, it can be subjected to image analysis. Features are measured, and result in data, which can then be interpreted and related to structure. A flow chart outlining the general process from start to finish is depicted in Figure 10 (64). Emphasis is put on acquiring initial images that are of high quality (high distribution of pixel values, no saturation of white features, and evenly distributed lighting) to avoid unnecessary image processing to enhance features, thereby altering pixel values.

Assuming a statistically constant microstructural element (or particle) size, the relationship between radius and mass (Equation 2) can be used to determine the fractality of crystal networks from two-dimensional PLM images (54).

In this case, the scaling relationship between the number of discrete particles ( $N$ ) and the length of the region of interest containing the particles ( $L$ ) can be expressed as:

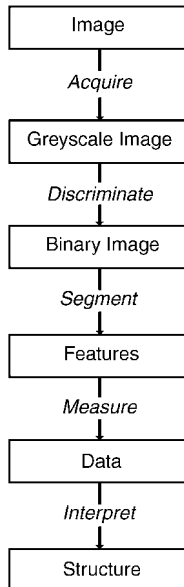
$$N = cL^D. \quad (10)$$

<sup>1</sup>Performed on a Macintosh computer using the public domain NIH Image program, developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>



**Figure 9.** The conversion of 256-color grayscale micrograph to a binary image suitable for image analysis. A threshold value is chosen to isolate the features that will be measured (AdobePhotoshop<sup>®</sup> 6.0).

Image Analysis Flowchart

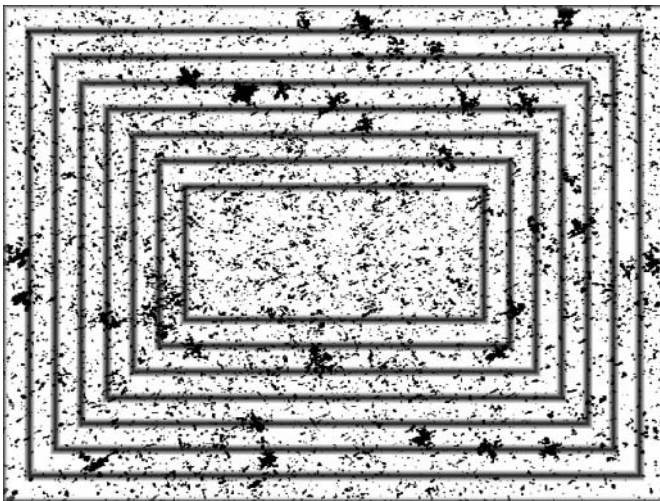


**Figure 10.** Flowchart depicts the steps involved in obtaining and evaluating the microstructure of materials from digitally acquired images.



The first method used was a particle counting algorithm performed using NIH-Image<sup>2</sup> as follows:  $c$  is a constant, and the number of distinct particles ( $N$ ) are first counted within the entire image of known length ( $L$ ). At 5% increments, the image is cropped, thereby making a new, smaller length  $L$ , and the number of particles in each new image are counted. This process is repeated until the length of the image is 35% of the original size. A diagram depicting the overlaying of the boxes of decreasing size can be appreciated in Figure 11. The number of particles counted within each box size of various lengths ( $L$ ) is plotted on a log-log scale, and the slope of the line is determined by linear regression. This slope corresponds to  $D_f$ . This method for calculating  $D_f$  was modified from previous procedures in our laboratory to eliminate artifacts and improve accuracy. This was accomplished by performing these iterative counts twice on each image—once including all particles touching the edge of each region of interest, and once excluding those that touch the edges. By taking the average of these two counts, a  $D_f$  that best represents the spatial distribution of mass is obtained. This improved method is equivalent to well-established methods that involve counting features that touch two sides of the region of interest (64).

The fractal dimension arrived at by this method reveals information on the degree of order in the packing of the microstructural elements within the microstructures. Systems that display a high degree of order have characteristically lower



**Figure 11.** Schematic diagram shows the incremental decreases in box size used for the particle counting method for the determination of the microscopic fractal dimension  $D_f$ .

<sup>2</sup>Analysis performed on a Macintosh computer using the public domain NIH Image program, developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>

fractal dimensions than those of a more disordered network (54). Previous simulations and studies employing mass-radius techniques for the determination of  $D$  have found that fast, diffusion-limited cluster aggregation (DLCA) typically results in  $D$  values of 1.75–1.8, whereas slow, reaction-limited cluster aggregation (RLCA) processes result in  $D$  values of 2.0–2.1 (60).

Another method used to examine microstructure is the box counting or “grid dimension” analysis. This dimension is referred to as a grid dimension because for mathematical convenience, the boxes are usually part of a grid that is laid over the image (65). The box dimension is defined as the exponent  $D$  in the relationship:

$$N(L) \sim \frac{1}{L^D}, \quad (11)$$

where  $N(L)$  is the number of boxes of linear size  $L$  necessary to cover a data set of points distributed in a two-dimensional plane. If the network is indeed fractal, plotting the logarithm of  $N(L)$  versus the logarithm of  $L$  results in a linear plot with a negative slope equal to  $-D$  (65). This analysis appears to be sensitive to the degree of fill of the solids in a crystal network. It can be expected that a network that is more empty (many large void spaces) will result in a lower proportion of full boxes counted, and thus, it will result in a lower fractal dimension and vice versa.

It is important to note that in some cases, the fractal dimensions estimated by different methods are not the same. It is therefore necessary that one be aware of the particular method used and its interpretation; this is mandatory for comparisons of dimensions of different data sets (65). One of the future goals within our laboratory is to continue investigating and quantifying the link between the fractal dimensions determined by rheology and microscopy.

**5.4.3. Polarized Light Microscopy** Microscopy allows for the visual observation of crystallization, and crystal growth of fat networks in realtime and subsequent image analysis can be used to quantify particle size, degree of order, and space-filling mass. Polarized light microscopy was used to examine crystal network properties of AMF during nucleation and crystal growth, and over time as a result of the processing conditions and storage time.

Cooling at 0.1°C/min, 1°C/min, and 5°C/min resulted in detectable birefringent crystal mass at onset temperatures of 26.8°C, 20°C, and 16°C, respectively. The onset temperatures of the 0.1°C/min and 1°C/min samples do not correspond exactly to those determined by DSC or NMR. This is because of the small quantity of matter crystallizing at the onset of nucleation, which although visible by PLM, does not release enough heat to be resolved by the DSC or enough solid mass to be detected using pNMR. The sample cooled at 5°C/min, on the other hand, shows visual signs of crystallization at 16°C, which corresponds closely to the values obtained in the DSC and pNMR experiments. This similarity in measurement

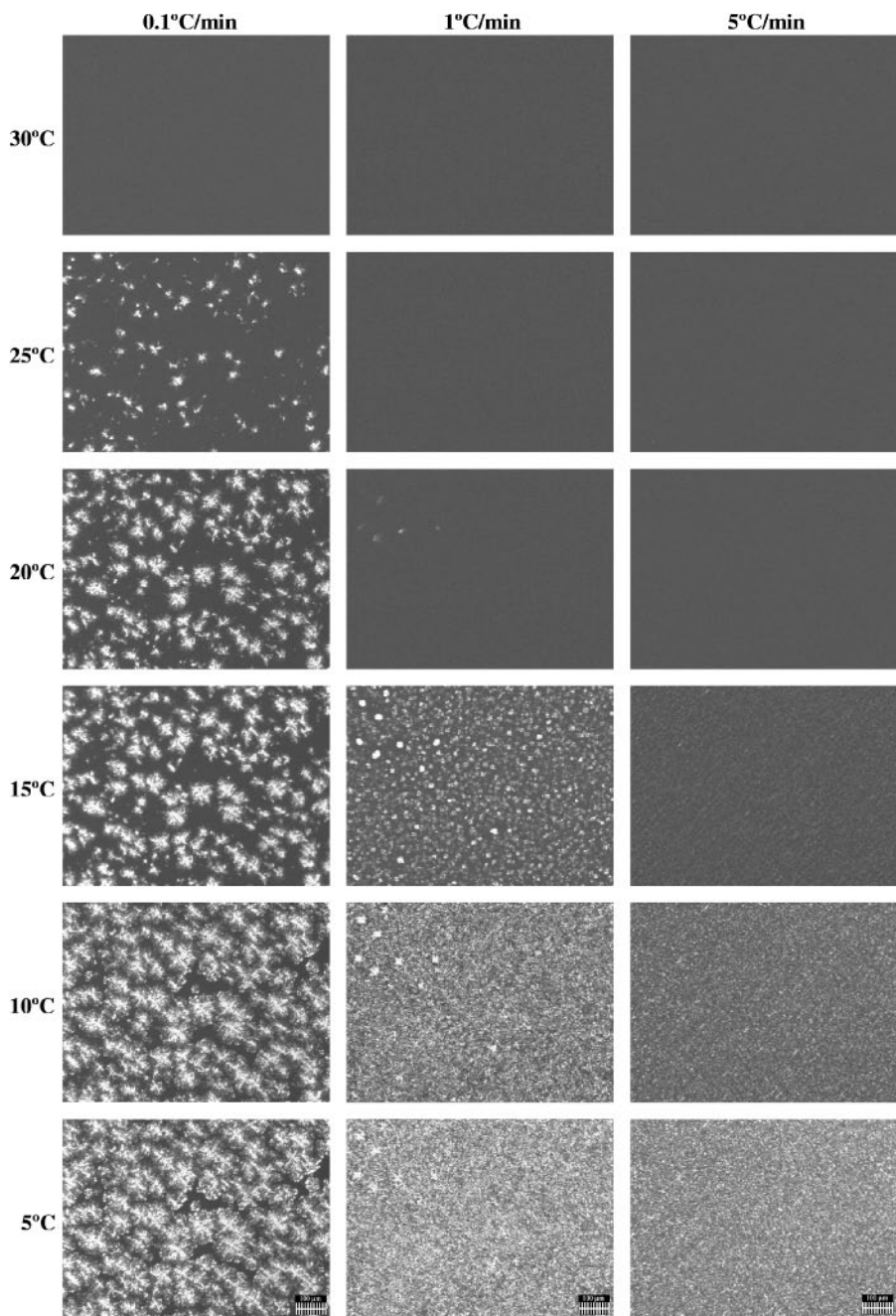
and resolution by each of the methods is attributable to the large change in state that occurs at higher cooling rates.

Microscopy also allows for the observation of dynamic changes that occur during nucleation and crystal growth. Figure 12 depicts still images of crystallizations at cooling rates of 0.1°C/min, 1°C/min, and 5°C/min. The images shown are at 5°C intervals in the range of 30°C to 5°C and portray dramatic differences in kinetics, and crystal properties, as a result of altering the cooling rates.

The crystallization profile for AMF cooled at 0.1°C/min shown in Figure 12 spans a total time period of 250 minutes. At 26.8°C, sporadic nucleation begins, and as time progresses, these early nuclei intermittently grow outward in a radial fashion as the temperature decreases. Until late in the crystallization period (at 5°C), there is no visible evidence of secondary nucleation. Between the temperatures of 26.8°C and 23°C, there is a discrete and continuous growth region where the microstructures increase dramatically in size as a function of time. This is followed by a period of very little growth (little evidence of changing microstructure sizes) until 14.5°C, when significant growth occurs once again up until a temperature of 12°C is reached. From approximately 8°C until 5°C, minor space-filling crystal growth occurs. These intermittent, temperature-dependent-related growth regions are likely attributable to the crystallization of the AMF fractions of HMF (26.8°C–24°C), followed by MMF (14.5°C–12°C), and then the space-filling crystallization of some of the LMF. This is made possible by the very slow change in supercooling that occurs over time when cooling AMF at 0.1°C/min, which permits adequate time for diffusion-related interaction to occur, leading to the fractionation-mediated growth of the network. The resulting fat crystal network is made up of a small number of very large crystal structures and relatively large regions of void space as seen in Figure 12.

The crystallization profile for AMF cooled at 1°C/min shown in Figure 12 spans a real-time period of 25 minutes. The first sign of crystal structure occurs at 20°C and is followed by a large amount of spontaneous nucleation and radial crystal growth until a temperature of 16°C is reached. After the rapid growth period, there is minimal visible growth until approximately 12°C is reached and a very rapid, spontaneous, space-filling secondary nucleation and growth occurs and continues until 5°C is attained. This rapid period of space filling crystallization leads to the masking of the larger microstructures formed during the earlier stages of crystallization. Like the system cooled at 0.1°C/min, AMF cooled at 1°C/min demonstrates fractionation-mediated growth where most of the HMF and some MMF likely crystallizes during the early stages, followed by crystallization of the MMF and LMF during the later stages. The resulting microstructure consists of a large number of small crystal structures; however, previous micrographs collected at the higher temperatures indicate the presence of somewhat larger structures (Figure 12).

The crystallization profile of AMF cooled at 5°C/min spans a real-time period of just 5 minutes (Figure 12). The first visible sign of a crystal structure occurs at a temperature of 16°C, after which very spontaneous, and rapidly space-filling, nucleation and crystal growth occurs. The progression of growth until 5°C shows

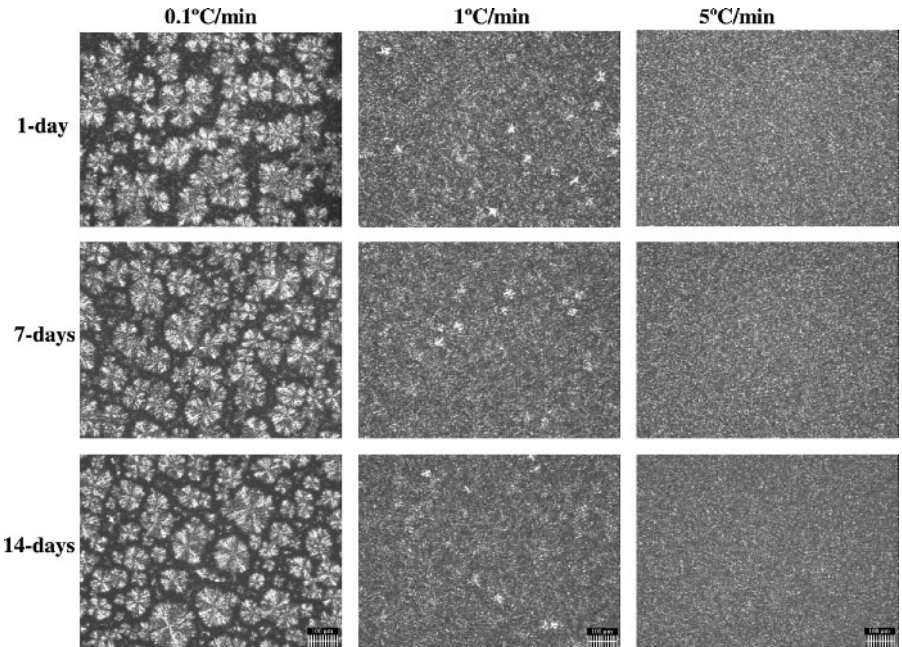


**Figure 12.** Polarized light micrographs of anhydrous milkfat cooled at 0.1°C/min, 1°C/min, and 5°C/min. Images were acquired during crystallization in the range of 30°C to 5°C at intervals of 5°C.

minimal differences over time with the exception of the occurrence of significantly increasing birefringence with decreases in temperature. This is an indicator of the densification and continued crystal growth of the microstructural elements within the crystal network. The resulting network is a densely packed, space-filling matrix of very small crystal structures. In general, as cooling rate increases, the number of crystal aggregates also increases, and the size of the constituent particles decreases.

The effects of storage time on microstructure at 5°C are represented in Figure 13. For each cooling rate, the network structure and particle size established during crystallization remain relatively unchanged during storage. Minor changes in the appearance of the 0.1°C/min sample occur as a result of the filling of void space, during storage time, by small crystal structures similar to those present in the originally formed crystal matrix. Rapidly cooled samples demonstrate no significant change in network or particle appearance as a function of storage time at 5°C.

The effects of cooling rate and storage time on mean particle size, as determined by image analysis, are shown in Table 3. Cooling at 0.1°C/min resulted in average particle sizes that were approximately two times larger than those resulting from cooling at 1°C/min, and 5°C/min. Also, the minor changes in the appearance of samples cooled at 0.1°C/min can be further characterized by a slight decrease



**Figure 13.** Polarized light micrographs of anhydrous milkfat cooled at 0.1°C/min, 1°C/min, and 5°C/min followed by storage for 1 day, 7 days, and 14 days at 5°C.

**TABLE 3. Particle-Counting Fractal Dimension ( $D_f$ ), Box-Counting Fractal Dimension ( $D_b$ ), and Mean Microstructural Element Area (MEA) for Anhydrous Milkfat Crystallized at Various Rates of Cooling and Storage Times at 5°C.**

Processing Conditions		Microstructural Parameters (n = 10–12)		
Cooling Rate (°C/min)	Storage Time (days)	$D_f$	$D_b$	MEA( $\mu\text{m}^2$ )
0.1	1	1.98 ± 0.11	1.72 ± 0.01	24.76 ± 7.83
	7	1.98 ± 0.09	1.73 ± 0.01	15.71 ± 1.43
	14	1.98 ± 0.08	1.73 ± 0.01	15.72 ± 1.42
1.0	1	1.92 ± 0.08	1.85 ± 0.01	4.12 ± 0.47
	7	1.88 ± 0.04	1.85 ± 0.01	3.91 ± 0.22
	14	1.86 ± 0.07	1.85 ± 0.01	3.44 ± 0.17
5.0	1	1.91 ± 0.03	1.87 ± 0.01	3.88 ± 0.40
	7	1.91 ± 0.04	1.88 ± 0.00	3.50 ± 0.19
	14	1.89 ± 0.04	1.88 ± 0.00	3.26 ± 0.07

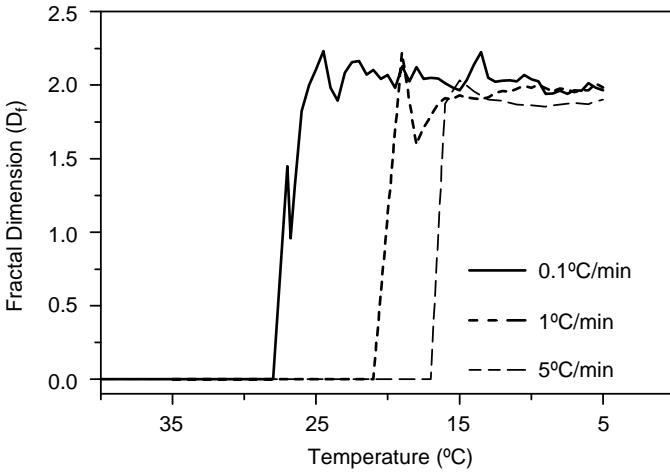
in mean particle area (MEA = total crystal area/no. of particles) as a function of time. Samples cooled at higher rates of 1°C/min and 5°C/min remain relatively constant in time.

Fractal dimensions of micrographs obtained at different cooling rates, determined using the particle counting method, reveal information about the degree of order in the resulting networks (Table 3). The  $D_f$  values decreased in the order of  $D_{f-0.1^\circ\text{C}/\text{min}} > D_{f-5^\circ\text{C}/\text{min}} \geq D_{f-1^\circ\text{C}/\text{min}}$ , with no significant changes detectable over time.

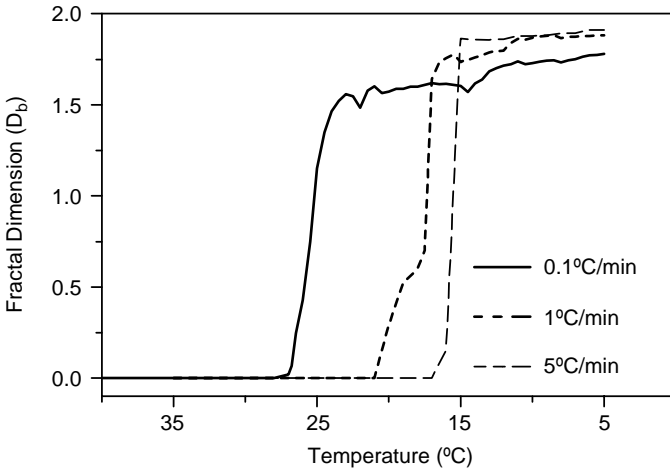
Fractal dimensions determined via the box-counting or grid dimension method are also shown in Table 3.  $D_b$  values decrease in the order of  $D_{b-1^\circ\text{C}/\text{min}} > D_{b-5^\circ\text{C}/\text{min}} > D_{b-0.1^\circ\text{C}/\text{min}}$ , and they do not significantly change in time.  $D_b$  is sensitive to the degree of fill within the network; therefore, higher values indicate an increase in space-filling mass (i.e., the smaller, more-numerous particles of the 1°C/min sample fill more space relative to the larger, less-numerous 0.1°C/min particles).

**5.4.4. Importance of Nucleation and Crystal Growth Kinetics on the Final Crystal Properties and Network** As stated in the hierarchical model (Figure 1), the processing and storage conditions affect the physical properties, including the SFC, polymorphism, and microstructure during nucleation and crystal growth. Therefore, processing conditions exert their most dramatic effect during crystallization, during which the final crystal network properties are developed. Scrutiny of the micrographs in Figure 12 demonstrates that the crystal structures formed during the early stages of nucleation and crystal growth dictate the final fat crystal network structure. The samples cooled at 0.1°C/min show fewer, more sporadic nuclei, which appear at higher temperatures. These nuclei then grow slowly to form

large crystal structures that continue to grow until the final temperature of 5°C is reached. Similar trends are seen in the faster cooled samples, where spontaneous nucleation occurs, leading to a large number of small crystals. This distribution remains at the final temperature of 5°C/min. Analysis of these micrographs using the fractal dimensions ( $D_f$  and  $D_b$ ) also indicates that the crystal network structure is determined before the final temperature is reached. These results can be seen in Figure 14. These results demonstrate that at 10°C–15°C prior to the final temperature of 5°C, the final equilibrium value (or close to the final value) for



(a)



(b)

**Figure 14.** Cooling profile analysis of polarized micrographs of 100% AMF collected during the static crystallization process at the cooling rates of 0.1°C/min, 1°C/min, and 5°C/min depicting the microscopic properties (A)  $D_f$  and (B)  $D_b$  monitored against changing temperature.

microscopically determined fractal dimensions are reached. This indicates that the final structure is predetermined far before the final equilibrium state is reached.

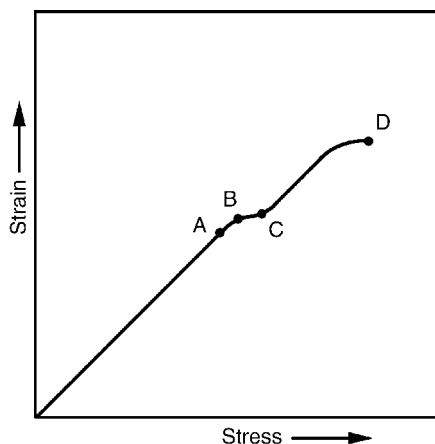
## 6. MECHANICAL PROPERTIES

### 6.1. Small Deformation

Small deformation rheometry refers to testing procedures that do not cause structural damage to the sample. Constant stress rheometers, such as dynamic mechanical analyzers or oscillatory constant stress rheometers, are often used.

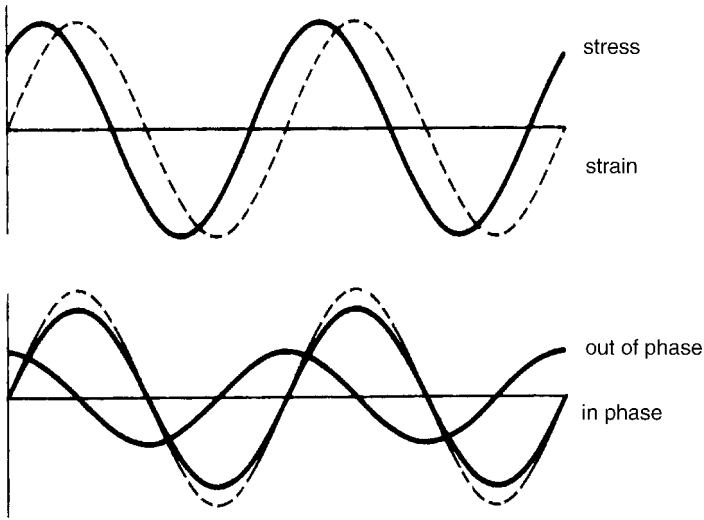
There have been a number of studies that demonstrate that crystallized AMF and butter exhibit linear (ideal) viscoelastic behavior at low levels of stress or strain (4), where the strain is directly proportional to the applied stress. For most materials, this region occurs when the critical strain (strain where structure breaks down) is less than 1.0%, but for fat networks, the strains typically exceed 0.1% (4, 66). Ideally, within the LVR, milkfat crystal networks will behave like a Hookean solid where the stress is directly proportional to the strain (i.e.,  $\sigma \propto \gamma$ ), as shown in Figure 15 (66, 68). Within the elastic region, stress will increase linearly with strain up to a critical strain. Beyond that critical strain (strain at the limit of linearity), deformation of the network will occur at a point known as the yield point. The elastic limit quickly follows, beyond which permanent deformation and sample fracture occurs. Beyond these points, the structural integrity of the network is compromised and the sample breaks down.

From small-deformation oscillatory methods, several useful parameters can be obtained to describe the mechanical properties of a material. These measurements



**Figure 15.** Stress-strain behavior of a typical elastic system, including (A) yield point, (B) elastic limit, (C) irreversible deformation, and (D) fracture.





**Figure 16.** Time profile of an applied sinusoidal stress wave and the corresponding resulting sinusoidal strain wave as they apply to small deformation rheological testing.

include the complex modulus ( $G^*$ ), the shear storage modulus ( $G'$ ), the shear loss modulus ( $G''$ ), and the tangent of the phase shift or phase angle ( $\tan \delta$ ).

These rheological parameters can be determined using controlled stress rheometers using dynamic oscillatory testing within the LVR region (68). The oscillatory method collects strain information by applying a controlled stress via the application of a sinusoidal stress wave. The rheometer measures the variation in strain as a function of the applied stress, in terms of the magnitude of the strain and the phase angle ( $\delta$ ) between the applied stress wave and the resulting strain wave. A typical stress-strain sinusoidal relationship is shown in Figure 16. The vectorial resolution, shown in Figure 17, of the stress-strain ratio is used to calculate the complex modulus  $G^*$ , which is derived from the following equation:

$$|G^*| = \sqrt{G'^2 + G''^2}. \quad (12)$$

The strain response can be broken down into its elemental components of stress, which are in phase or out of phase, to derive the values for  $G'$  and  $G''$ . The storage modulus  $G'$  is the ratio of the applied stress that is in phase with the strain ( $\delta = 0^\circ$ ). This means that  $G'$  is an expression of the magnitude of the energy stored in the material, recoverable per deformation cycle (68). The loss modulus  $G''$  is the ratio of the applied stress that is out of phase with the strain ( $\delta = 90^\circ$ ), meaning that it is a measurement of the energy lost as viscous dissipation per deformation cycle (66–68). These two moduli are dependent on the phase angle of the system and are

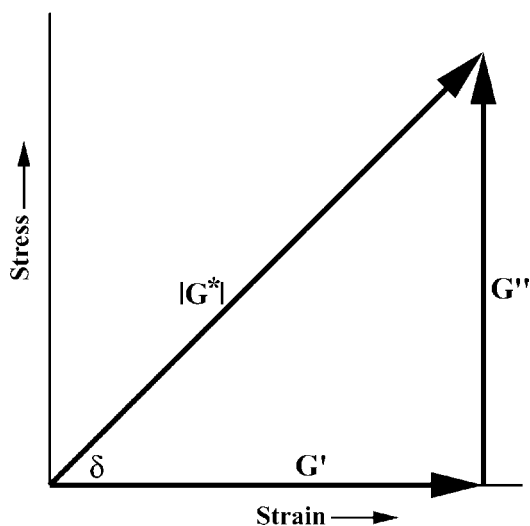


Figure 17. Vectorial resolution of the complex modulus components.

derived from the vectorial components of  $G^*$  and are calculated using the following relationships:

$$G' = \left( \frac{\sigma_0}{\gamma_0} \right) \cos \delta, \quad (13)$$

$$G'' = \left( \frac{\sigma_0}{\gamma_0} \right) \sin \delta. \quad (14)$$

The tangent of the phase angle ( $\tan \delta$ ) can be expressed as the ratio of the loss to the storage moduli, and it represents the relative balance of elastic to viscous components in a material:

$$\tan \delta = \frac{G''}{G'}. \quad (15)$$

These rheological parameters have been successfully correlated to textural attributes of hardness and spreadability and provide information pertaining to the fat crystal network (69). The value of  $G'$  is useful in assessing the solid-like structure of the fat crystal network. Increases in the value of  $G'$  typically correspond to a stronger network and a harder fat (66). Alternatively,  $G''$  represents the fluid-like behavior of the fat system. This value can be related to the spreadability of a fat system, because increases in  $G''$  indicate more fluid-like behavior under an applied shear stress. The  $\tan \delta$  is the ratio of these two values. As the value of  $\delta$  approaches  $0^\circ$  (stress wave in phase with strain wave), the  $G''$  value approaches zero, and therefore, the sample behaves like an ideal solid and is referred to as perfectly elastic (68). As  $\delta$  approaches  $90^\circ$  (stress is completely out of phase relative to the strain),

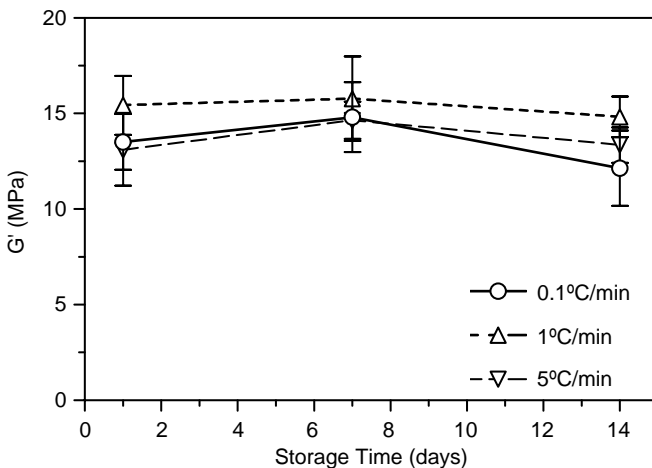
the  $G'$  value approaches zero; all of the energy will be dissipated as heat, and the sample will behave predominantly as a fluid (68). At intermittent values, the samples are considered to be viscoelastic in nature. Therefore,  $\tan \delta$  is an excellent indicator of the structural integrity of the sample, indicating the proportion of the material structure attributable to the crystal network and to the liquid phase.

**6.1.1. Effects of Processing Conditions—Small Deformation Rheology** The effects of cooling rate and time on small deformation rheological measurements of  $G'$ ,  $G''$ , and  $\tan(\delta)$  are shown in Figures 18, 19, and 20, respectively.

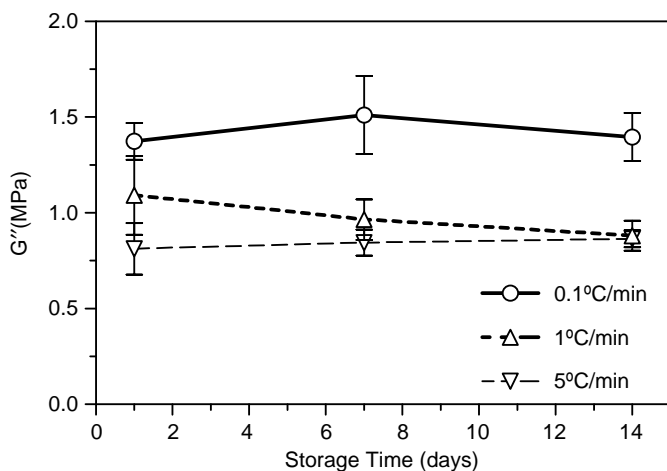
Figure 18 illustrates the effects of cooling rate and storage time on the storage modulus ( $G'$ ) measured using small deformation rheology at  $5^\circ\text{C}$ . There are no significant effects of cooling rate or storage time on the  $G'$  of AMF samples, indicating the absence of differences in solid-like properties and potentially hardness.

Figure 19 illustrates the effects of cooling rate and storage time on the loss modulus ( $G''$ ), measured using small deformation rheology at  $5^\circ\text{C}$ . Results show lower  $G''$  for samples cooled at  $5^\circ\text{C}/\text{min}$  and  $1^\circ\text{C}/\text{min}$  than at  $0.1^\circ\text{C}/\text{min}$ . Additionally, the  $G''$  values for the  $5^\circ\text{C}/\text{min}$  and  $0.1^\circ\text{C}/\text{min}$  cooling rates do not change significantly with time, and the values for the  $1^\circ\text{C}/\text{min}$  samples decrease slightly with time, which may be indicative of time-dependent hardening.

The tangent of the phase angle ( $\tan \delta$ ) offers a better indicator of structural integrity than either the  $G'$  or  $G''$  measurements do individually. The phase angle ( $\delta$ ) or its tangent ( $\tan \delta$ ) are an indicator of the system's structural behavior or integrity because it indicates whether the system behaves predominantly as a solid, liquid, or viscoelastic material. The greater the  $\tan \delta$ , the more liquid-like the samples will behave, and conversely, low values indicate more solid-like properties. A  $\tan \delta$  value of 1 is indicative of a viscoelastic material.



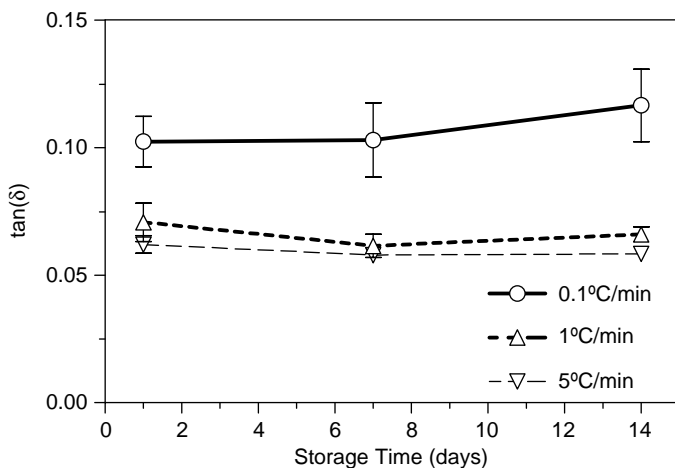
**Figure 18.** Storage moduli ( $G'$ ) of anhydrous milkfat cooled at rates of  $0.1^\circ\text{C}/\text{min}$ ,  $1^\circ\text{C}/\text{min}$ , and  $5^\circ\text{C}/\text{min}$  followed by storage for 14 days at  $5^\circ\text{C}$ .



**Figure 19.** Loss moduli ( $G''$ ) of anhydrous milkfat cooled at rates of  $0.1^\circ\text{C}/\text{min}$ ,  $1^\circ\text{C}/\text{min}$ , and  $5^\circ\text{C}/\text{min}$  followed by storage for 14 days at  $5^\circ\text{C}$ .

The graphs in Figure 20 demonstrate the effects of cooling rate and storage time on  $\tan \delta$  at  $5^\circ\text{C}$ . Values of  $\tan \delta$  increase in the order of  $\tan \delta_{5^\circ\text{C}/\text{min}} < \tan \delta_{1^\circ\text{C}/\text{min}} \ll \tan \delta_{0.1^\circ\text{C}/\text{min}}$ . The  $\tan \delta$  value for AMF cooled at  $0.1^\circ\text{C}/\text{min}$  is significantly higher than the values at  $1^\circ\text{C}/\text{min}$  and  $5^\circ\text{C}/\text{min}$ . The values do not show large increases with time; therefore, the changes during storage at  $5^\circ\text{C}$  are minimal.

The data from small deformation rheology indicate that at higher cooling rates, the fat samples are more solid-like in nature. Conversely, the low cooling rate of  $0.1^\circ\text{C}/\text{min}$  results in a softer, less elastic system.



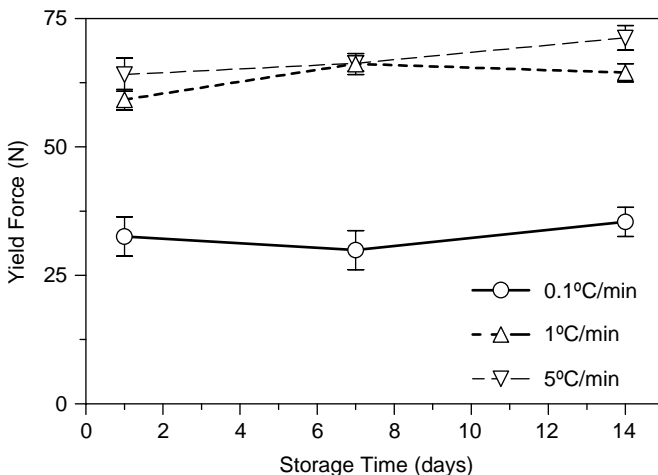
**Figure 20.** Values of  $\tan \delta$  of anhydrous milkfat cooled at rates at  $0.1^\circ\text{C}/\text{min}$ ,  $1^\circ\text{C}/\text{min}$ , and  $5^\circ\text{C}/\text{min}$  followed by storage for 14 days at  $5^\circ\text{C}$ .

## 6.2. Large Deformation

Large deformation methods usually are based on the determination of the amount of applied force required to induce a change in a sample. Parameters determined include hardness, spreadability, cutting force, or yield force (4). One such method involves the compression of a sample between two parallel plates to determine relative hardness values via the measurement of yield force.

The compression of uniform samples to the point where the force exceeds the structural capacity causes it to permanently deform and essentially break (4). A typical load-deformation curve can be used to derive values for yield stress, yield strain, and compressive yield work, and depending on the linearity of the onset of compression, a compressive modulus may be obtained (4). These measurements can be used to provide an index of hardness for fats, which have been successfully correlated to the textural attributes of hardness and spreadability obtained through sensory evaluation (4). Unfortunately, these tests are destructive in nature and yield minimal information about the native microstructure of the system.

**6.2.1. Effect of Processing Conditions—Large Deformation Rheology** The effects of cooling rate and time on the yield force of AMF and its dilutions are shown in Figure 21. Yield force measurements ( $F_y$ ) demonstrate that cooling rate and, to a lesser extent, storage time affect the hardness of AMF. In general, the  $F_y$  increases with increasing cooling rate; however, the difference between  $1^\circ\text{C}/\text{min}$  and  $5^\circ\text{C}/\text{min}$  is relatively small compared with the difference observed between  $0.1^\circ\text{C}/\text{min}$  and  $1^\circ\text{C}/\text{min}$ . AMF samples cooled at  $0.1^\circ\text{C}/\text{min}$  have yield force values (30–35 N) that are approximately half that of the higher cooling rates of  $1^\circ\text{C}/\text{min}$  (58–63 N) and  $5^\circ\text{C}/\text{min}$  (64–72 N). Over time, the yield force increases slightly, by

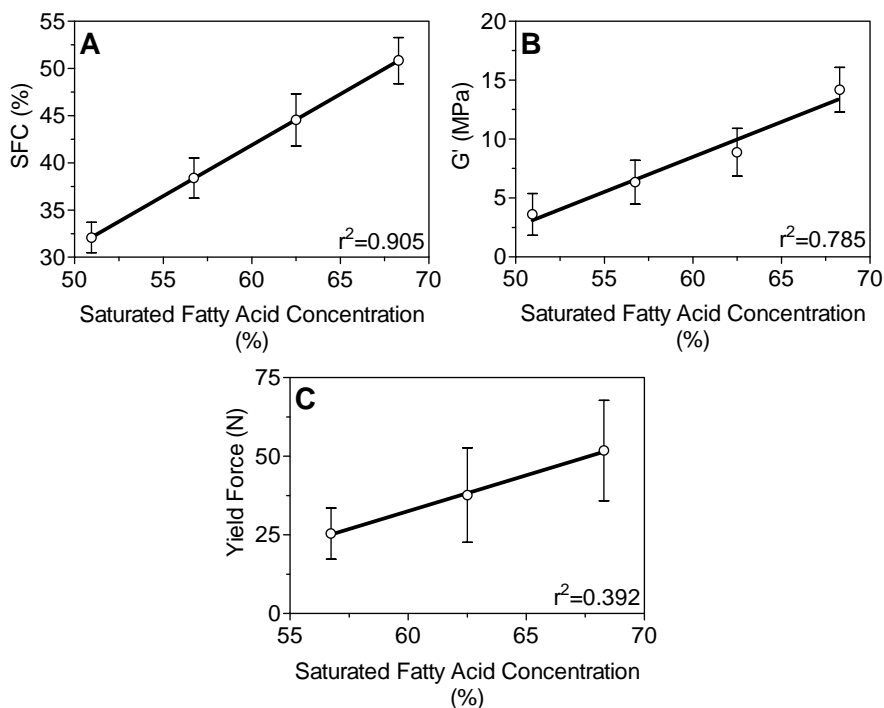


**Figure 21.** Yield force ( $F_y$ ) of anhydrous milkfat cooled at rates of  $0.1^\circ\text{C}/\text{min}$ ,  $1^\circ\text{C}/\text{min}$ , and  $5^\circ\text{C}/\text{min}$  followed by storage for 14 days at  $5^\circ\text{C}$ .

a few Newtons, at each of the cooling rates. This indicates that the samples may be hardening slightly; however, the yield force does not significantly increase ( $p > 0.05$ ) during the 14-day storage period.

## 7. ASSESSING THE VALIDITY OF THE MODEL: CORRELATING EXPERIMENTALLY DETERMINED PARAMETERS

In order to further demonstrate the existence of inter-relationships between parameters that influence macroscopic properties, linear correlations were performed on data obtained from the study of cooling rate and storage time (Table 4). As the physical properties of a fat system are dictated primarily by lipid composition, it is interesting to observe the effects of composition of other measured parameters. For example, a significant correlation exists between the saturated fatty acid content (SFAC) and the final SFC attained (Figure 22a). This agrees with expectations, because the saturated FAs generally have higher melting points, and thus, they are likely to crystallize and contribute to the measured SFC at any given temperature. Significant positive correlations also exist for relationships between SFAC and



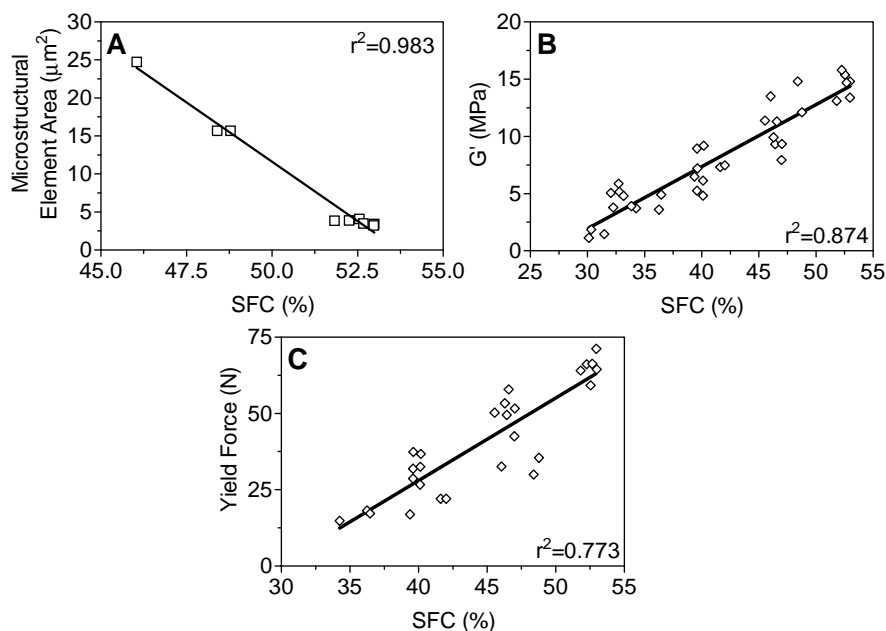
**Figure 22.** Linear correlations between saturated fatty acid content (%) and (A) solid fat content, (B) storage modulus, and (C) yield force. Data shown represent all points collected at all cooling rates and storage times.

**TABLE 4. First Order Linear Regression Coefficients (+2) for Various Physical Properties of Anhydrous Milkfat. Correlations were Performed After Pooling all Results from Experiments Involving Various Cooling Rates and Storage Times at 5°C.**

	SFAC	SFC	Df	Db	Dr	MEA	$\lambda$	G'	G''	tan ( $\delta$ )	Yield Force
SFC	<b>0.91 (+)</b>										
Df	0.37 (+)	<b>0.80 (-)</b>									
Db	0.62 (+)	<b>0.89 (+)</b>	<b>0.78 (-)</b>								
Dr	na	<b>0.78 (-)</b>	<b>0.70 (+)</b>	<b>0.90 (-)</b>							
MEA	0.31 (-)	<b>0.98 (-)</b>	0.77 (+)	<b>0.90 (-)</b>	<b>0.77 (+)</b>						
$\lambda$	na	<b>0.86 (-)</b>	<b>0.54 (+)</b>	<b>0.60 (-)</b>	<b>0.54 (+)</b>	<b>0.90 (+)</b>					
G'	<b>0.78 (+)</b>	<b>0.87 (+)</b>	0.19 (-)	0.11 (+)	0.04 (-)	0.15 (-)	0.11 (-)				
G''	<b>0.71 (+)</b>	<b>0.57 (+)</b>	<b>0.80 (+)</b>	0.92 (-)	<b>0.87 (+)</b>	0.76 (+)	<b>0.50 (-)</b>	<b>0.90 (+)</b>			
tan ( $\delta$ )	0.08 (-)	0.23 (-)	<b>0.81 (+)</b>	<b>0.95 (-)</b>	<b>0.87 (+)</b>	<b>0.79 (+)</b>	<b>0.52 (+)</b>	<b>0.34 (+)</b>	0.10 (+)		
<i>Yield Force</i>	<b>0.39 (+)</b>	<b>0.77 (+)</b>	<b>0.87 (-)</b>	<b>0.97 (+)</b>	<b>0.90 (-)</b>	0.86 (-)	<b>0.61 (-)</b>	<b>0.61 (+)</b>	0.02 (+)	<b>0.63 (+)</b>	
<i>Work</i>	<b>0.58 (+)</b>	<b>0.81 (+)</b>	0.66 (-)	<b>0.48 (-)</b>	0.28 (-)	0.53 (-)	0.36 (-)	<b>0.63 (+)</b>	0.14 (+)	<b>0.31 (+)</b>	<b>0.76 (+)</b>
<i>Strain</i>	0.04 (+)	0.02 (+)	<b>0.58 (+)</b>	<b>0.72 (-)</b>	<b>0.73 (-)</b>	0.61 (+)	0.44 (+)	0.01 (+)	<b>0.29 (+)</b>	<b>0.52 (+)</b>	<b>0.23 (-)</b>

Legend

- na comparisons unachievable due to method of calculation
- bold  $p < 0.05$
- SFAC saturated fatty acid concentration (%)
- (+) or (-) indicates positive or negative correlation



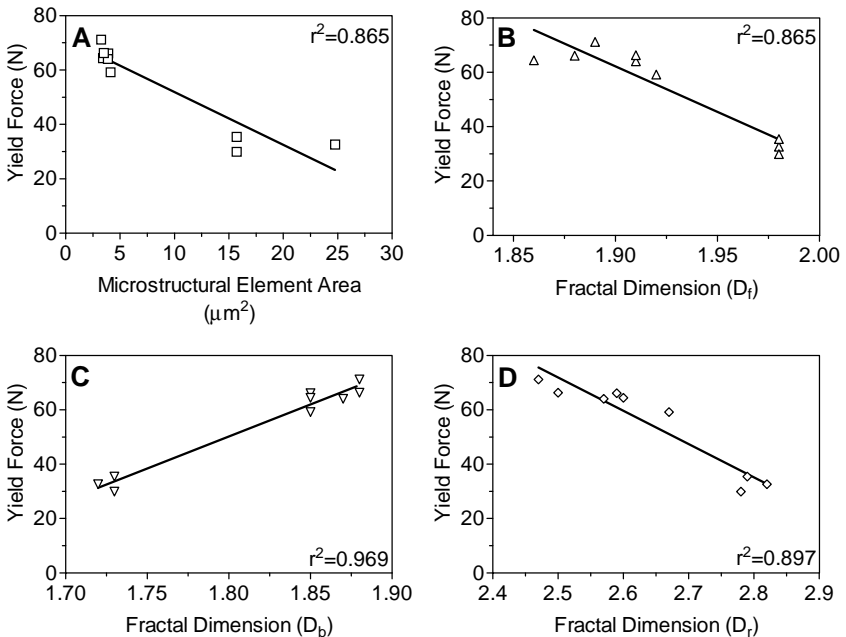
**Figure 23.** Linear correlations between solid fat content and (A) microstructural element size, (B) storage modulus, and (C) yield force. Data shown represent all points collected at all cooling rates and storage times.

measures of apparent hardness. SFAC is linearly correlated with both the storage modulus ( $G'$ ) and yield force ( $F_y$ ) (Figure 22b,c). Higher saturate levels, their subsequent crystallization, and contribution to the solids content of the network translate into increases in the materials' hardness.

SFC, which is commonly used as a primary indicator of hardness, shows a high positive correlation to both the storage modulus ( $G'$ ) and yield force ( $F_y$ ) in milkfat (Figure 23a,b). This relationship forms the basis of methods used to determine the fractal dimension of fat crystal networks. Evidence of a relationship between SFC and microstructural element size was also established (Figure 23c). Smaller crystal aggregates are a result of higher cooling rates, which also dictate the final SFC. This relationship between factors further exemplifies the interdependence of processing conditions, microstructure, and SFC.

Correlations shown in Figure 24 highlight the relationships between various microstructural parameters and yield force. As stated previously, microstructure strongly influences macroscopic hardness. Networks consisting of smaller crystal aggregates are generally harder than those made up of larger microstructures (Figure 24a). Correspondingly, networks consisting of particle distributions that are more disorderly (lower  $D_f$  and  $D_r$ ) and more space filling (higher  $D_b$ ) are also harder, as indicated by higher yield force values (Figure 24.b, c, and d).





**Figure 24.** Linear correlations between yield force and (A) microstructural element area, (B) fractal dimension by particle-counting, (C) fractal dimension by box-counting, and (D) fractal dimension by rheology. Data shown represent all points collected at all cooling rates and storage times.

## 7.1. Summary of the Effect of Processing Conditions on the Physical Properties of AMF

The schematic illustration of the combined effects of the majority of the physical properties examined can be appreciated in Figure 25.

Figure 25a illustrates the effect of rapid cooling ( $5^\circ\text{C}/\text{min}$ ) on the physical properties of AMF. The diagram depicts a large number of small, randomly packed crystal structures. These structures are highly space filling and therefore have a larger number of particle–particle interactions. The result is a crystal network with an even higher SFC (51–53%), high  $D_b$  (highly space filling), and low  $D_r$  and  $D_f$  values, resulting in an even harder fat crystal network.

Figure 25b illustrates the effects of the midrange cooling rate ( $1^\circ\text{C}/\text{min}$ ) on the physical properties of AMF. This diagram shows a combination of both small- and medium-sized crystal structures that are more randomly distributed in space. The increased number of smaller crystal structures lead to increases in the number of particle–particle interactions and a decrease in void space. This resulted in a system with a higher SFC (51–53%), higher  $D_b$  (more space filling), and decreased  $D_r$  and  $D_f$  (less packing order), which all translated into a harder fat.

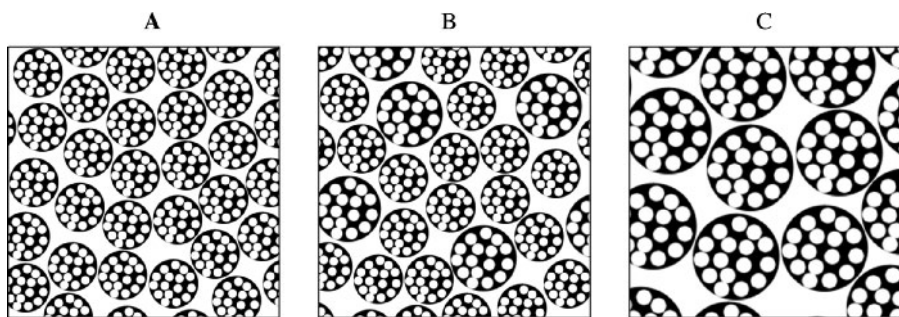


Figure 25.

Finally, Figure 25c depicts an illustrative model for the effects of the slow ( $0.1^{\circ}\text{C}/\text{min}$ ) cooling rate on the physical properties of AMF. As shown, the crystal network is composed of a small number of large highly ordered crystal structures that are homogeneously distributed in space. The decrease in crystal structure numbers and increased size lends results in fewer particle–particle interactions and a larger amount of void space. The final result was a lower SFC (46–48%), lower values for  $D_b$  (less space filling crystal mass), and increases in  $D_r$  and  $D_f$  (increased structural packing order), which all resulted in a softer fat (50% lower yield force than samples cooled at  $1^{\circ}\text{C}/\text{min}$  and  $5^{\circ}\text{C}/\text{min}$ ).

Therefore, through experimentation using AMF as a model fat, it has been shown that processing conditions affect the underlying physical properties of a fat, and it is these physical properties and their interrelationships that ultimately effect the final macroscopic properties of a fat crystal network.

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## REFERENCES

1. M. Kalab, *J. Dairy Sci.*, **68**, 3234–3248.
2. A. L. Halmos, *Food Australia*, **49**(4), 169–173 (1997).
3. W. D. de Bruikne and A. Bot, in A. J. Rosenthal, ed., *Food Texture Measurement and Perception*, Aspen, Gaithersburg, Maryland, 1999 pp. 185–227.
4. A. J. Wright, M. G. Scanlon, R. W. Hartel, and A. G. Marangoni, *J. Food Sci.*, **66**(8), 1056–1071 (2001).
5. D. Rousseau and A. G. Marangoni, *Food Res. Int.*, **31**, 381–388 (1998).
6. J. H. Prentice, *J. Texture Studies*, **3**, 415–458 (1972).

7. L. de Man, J. M. deMan, and B. Blackman, *J. Amer. Oil Chem. Soc.*, **66**, 128–132 (1987).
8. J. M. deMan and A. M. Beers, *J. Texture Studies*, **18**, 303–318 (1987).
9. D. Rousseau, A. R. Hill, and A. G. Marangoni, *J. Amer. Oil Chem. Soc.*, **73**, 983–989 (1996).
10. A. M. Fearon and D. E. Johnston, *J. Food Quality*, **12**, 23–38 (1989).
11. A. J. Haighton, *J. Amer. Oil Chem. Soc.*, **36**, 345–348 (1959).
12. K. R. Davey and P. N. Jones, *J. Texture Studies*, **16**, 75–84 (1985).
13. M. Kawanari, D. D. Hamann, K. R. Swartzel, and A. P. Hansen, *J. Texture Studies*, **12**, 483–505 (1981).
14. H. Rohm and F. Ulberth, *J. Texture Studies*, **20**, 409–418.
15. V. R. Huebner and L. C. Thomsen, *Amer. Dairy Sci.*, **40**, 834–838 (1957).
16. C. Pompei, E. Casiraghi, M. Lucisano, and B. Zaroni, *J. Food Sci.*, **53**, 597–602 (1988).
17. H. T. Lawless and H. Heymann, in *Sensory Evaluation of Food*, Aspen, Gaithersburg, Maryland, 1999, pp. 379–405.
18. B. K. Mortensen and H. Danmark, *Milchwissenschaft* **36**, 393–395 (1982).
19. H. Corey, *CRC Crit. Rev. Food Technol.*, May, 161–198 (1970).
20. A. S. Szczesniak, *Food Technol.*, October, 52–58 (1966).
21. D. M. Small, in D. J. Hanahan, ed., *The Physical Chemistry of Lipids, from Alkanes to Phospholipids*, Vol. 4, Plenum Press, New York, 1966, pp. 475–522.
22. A. Kuksis, L. Marai, and J. J. Myher, *J. Amer. Oil Chem. Soc.*, **50**, 193–201 (1973).
23. J. Gresti, M Bugaut, G. Maniongui, and J. Bezard, *J. Dairy Sci.*, **76**, 1850–1869 (1993).
24. B. Breitschuh and E. J. Windhab, *J. Amer. Oil Chem. Soc.*, **75**, 897–904 (1998).
25. H. E. Swaisgood, in O. R. Fennema, *Food Chemistry*, 3rd ed., 1996, pp. 841–878.
26. W. W. Christie, in P. F. Fox, ed., *Advanced Dairy Chemistry*, Vol. 2, 2nd ed., Chapman & Hall, London, 1995, pp. 1–36.
27. A. J. Wright, S. E. McGauley, S. S. Narine, R. W. Lencki, and A. G. Marangoni, *J. Agric. Food Chem.*, **48**, 1033–1040 (2000).
28. A. G. Marangoni and R. W. Lencki, *J. Agric. Food Chem.*, **46**, 3879–3884 (1998).
29. D. Rousseau and A. G. Marangoni, *J. Agric. Food Chem.*, **46**, 2368–2374 (1998).
30. D. Rousseau and A. G. Marangoni, *J. Agric. Food Chem.*, **46**, 2375–2381 (1998).
31. A. G. Marangoni and D. Rousseau, *J. Amer. Oil Chem. Soc.* **75**, 1633–1636 (1998).
32. A. G. Marangoni and D. Rousseau, *Food Res. Int.*, **31**, 595–599 (1998).
33. A. G. F. Stapley, H. Tewkesbury, and P. J. Fryer, *J. Amer. Oil Chem. Soc.*, **6**, 677–685 (1999).
34. E. ten Groetenhuis, G. A. van Aken, K. F. van Malssen, and H. Schenk, *J. Amer. Oil Chem. Soc.*, **76**, 1031–1039 (1999).
35. M. L. Herrera and R. W. Hartel, *J. Amer. Oil Chem. Soc.*, **77**, 1177–1187 (2000).
36. M. L. Herrera and R. W. Hartel, *J. Amer. Oil Chem. Soc.* **77**, 1197–1204 (2000).
37. M. L. Herrera and R. W. Hartel, *J. Amer. Oil Chem. Soc.* **77**, 1189–1195 (2000).
38. J. Garside, in J. M. V. Blanshard and P. Lillford, eds., *Food Structure and Behaviour*, Academic Press, London, 1987, pp. 35–49.
39. D. S. Grall and R. W. Hartel, *J. Amer. Oil Chem. Soc.*, **69**, 741–747 (1992).
40. P. Walstra, T. van Vliet, and W. Kloek, P. F. Fox, eds., *Advanced Dairy Chemistry*, Vol. 2, 2nd ed., Chapman & Hall, London, 1995, pp. 179–211.

41. A. G. Marangoni and D. Rousseau, N. Widlak, ed., *Physical Properties of Fats, Oils and Emulsifiers*, AOCS Press, Champaign, Illinois, 1999, pp. 96–111.
42. B. L. Madison and R. C. Hill, *J. Amer. Oil Chem. Soc.*, **55**, 328–331 (1978).
43. J. van Duynhoven, G. J. Goudappel, M. C. M. Gribnau, and V. K. S. Shukla, *Inform*, **105**, 479–484 (1999).
44. L. van Putte, I. Bruker minispec documentation.
45. W. W. Nawar, in O. R. Fennema, ed., *Food Chemistry*, 3rd ed., 1996, pp. 226–319.
46. J. M. deMan and L. deMan, in N. Widlak, R. Hartel, S. Narine, eds., *Crystallization and Solidification Properties of Lipids*, AOCS Press, Champaign, Illinois, 2001, 225–235.
47. C. W. Hoerr, *J. Amer. Oil Chem. Soc.*, **37**, 539–546 (1960).
48. P. Walstra, T. J. Geurts, A. Noomen, A. Jellema, and M. A. J. A. van Boekel, in P. Walstra, ed., *Dairy Technology: Principles of Milk Properties and Processes*, Marcel Dekker, New York, 1999, pp. 50–70.
49. S. S. Narine and A. G. Marangoni, *Food Res. Int.*, **32**, 227–248 (1999).
50. S. S. Narine and A. G. Marangoni, *Adv. Food Nutr. Res.*, **44**, 33–145 (2002).
51. A. G. Marangoni, *Trends Food Sci. Technol.*, **13**, 37–47 (2002).
52. R. Vreeker, L. L. Hoekstra, D. C. den Boer, and W. G. M. Agterof, *Food Hydrocolloids*, **6**, 423–435 (1992).
53. A. G. Marangoni and D. Rousseau, *J. Amer. Oil Chem. Soc.*, **73**, 991–994 (1996).
54. S. S. Narine and A. G. Marangoni, *Phys. Rev. E*, **59**, 1908–1920 (1999).
55. W. H. Shih, W. Y. Shih, S. I. Kim, J. Liu, and I. A. Aksay, *Phys. Rev. A*, **42**, 4772–4779 (1990).
56. S. S. Narine and A. G. Marangoni, *Phys. Rev. E*, **60**, 6991–7000 (1999).
57. A. G. Marangoni, *Phys. Rev. B*, **62**, 13951–13955 (2000).
58. A. G. Marangoni and M. A. Rogers, *Appl. Phys. Lett.*, **82**, 1–3 (2003).
59. T. Hagiwara, H. Kumagi, and K. Nakamura, *Food Hydrocolloids*, **12**, 29–36 (1998).
60. M. Tather and A. Erdem-Senatalar, *J. Phys. Chem. B*, **103**, 4360–4365.
61. K. Solecka-Cermakova, B. Vlckova, and F. Lednicky, *J. Phys. Chem.*, **100**, 4954–4960 (1996).
62. A. Y. Kim and J. C. Berg, *Langmuir*, **16**, 2101–2104 (2000).
63. G. Joss. 2002. Available: <http://list.nih.gov/cgi-bin/wa?A2=ind0101&L=imagej&D=0&P=5222>.
64. J. C. Russ, in D. B. Williams, A. R. Pelton, and R. Gronsky, eds., *Images of Materials*, Oxford University Press, New York, 1991, 338–373.
65. Trusoft International Incorporated. (2000). Available: <http://www.trusoft.com>.
66. H. Rohm and K. H. Weidinger, *J. Texture Studies*, **24**, 157–172 (1993).
67. J. F. Steffe, in *Rheological Behaviour of Butter at Small Deformations*, 2nd ed., Freeman Press, East Lansing, Michigan, 1996, pp. 294–349.
68. M. A. Rao, in M. A. Rao, ed., *Rheology of Fluid and Semisolid Foods: Principles and Applications*, Chapman & Hall, Gaithersburg, Maryland, 1999, pp. 59–152.
69. S. S. Narine and A. G. Marangoni, *Lebensmittel Wissenschaft und Technologie*, **34**, 33–40 (2001).

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## *Animal Fats*

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### **1. INTRODUCTION**

The use of animal fats by humans may well predate civilization. As the depot fats of animals are readily noticed during the butchering of a slaughtered animal, are easily harvested, and are available in the absence of plant domestication and the adoption of established agriculture, it is probable that animal fats were the first lipids employed as “industrial” and as distinct edible lipids by humans. This is evidenced by the fact that the paints used in prehistoric cave paintings were animal fat-based, as were the fuels in the lamps that illuminated the cave artists at their work. Despite a tremendous diversification to include other lipid types over the intervening centuries, animal fats still play a prominent role in our diets, industry, and commerce.

Lipids are biological materials that are insoluble in water but soluble in nonpolar organic solvents. Here the term will be used interchangeably with “acylglycerol,” the primary component of animal lipids. These are classified as either fats or oils. The former are predominantly solid at room temperature (24°C), and the latter are liquid. The depot lipids of animals are generally fats. The major animal fats (also termed meat fats) of contemporary commerce are produced from pigs (*Sus scrofa*), in which case they are termed lard and rendered pork fat, from the fat of cattle (*Bos taurus*) or sheep (*Ovis aries*) and termed tallow, or from poultry (primarily chickens, *Gallus gallus*) and termed poultry fat. Tallow

from domestic cattle is known as beef tallow, whereas that from sheep is termed mutton tallow.

Animal depot lipids are used in edible applications, sometimes being consumed directly but more often in such applications as baking, cooking, and deep fat frying. They are also used in industrial applications, primarily in soap production, as an energy and nutrient source in animal feeds, in lubricants, and as a source of industrial fatty acids. These applications are discussed in detail elsewhere in this series.

This chapter will consider contemporary aspects of the classification, composition, properties, production, processing, and consumption of the depot lipids of land animals. For a more detailed discussion, the reader is referred to the recent comprehensive animal fats text (1). Additional discussions of the material covered here, and related topics, are found in excellent contemporary texts (2–4). Some consideration of greases will also be included here, because these are largely handled by and traded within the animal fats sector of the lipid industry. Discussions of milk-fats and fish oils can be found elsewhere in this series.

In addition to the citations accompanying this chapter, the reader will find numerous valuable websites on the Internet. These can be located by the use of search engines such as [www.google.com](http://www.google.com) and [www.dogpile.com](http://www.dogpile.com).

## **2. SOURCES, FATTY ACID CONTENT, AND ACYLGLYCEROL STRUCTURE**

### **2.1. Acylglycerol Components**

The major fat depots of animals include subcutaneous fat (located under the skin and overlying superficial muscles) and intermuscular fat (located between muscles). Appreciable amounts of fat also are deposited in the abdominal cavity and other internal sites. The distribution of fat between different sites varies somewhat with animal species, breed, and degree of finish.

On the larger species, some of the fat deposits, for example, those located around the kidneys, heart, intestines, and greater omentum, are stripped from the animal when it is slaughtered. These are called the killing-floor or killing fats. Additional fat separated from the carcass when it is cut apart to give wholesale or retail cuts, or for processed meat products, is called cutting-floor or cutting fat.

White, yellow, and brown greases are also defined and traded in the industry (5). White grease is derived primarily from the rendering of pork offal. Yellow grease is composed largely of spent deep fat fryer lipids. Brown grease can be any lipid that does not meet the specifications for yellow grease. All are specified to contain at least 90% lipid. Poultry fat is composed of 100% poultry offal from slaughter operations. Formal subtypes or classifications are not identified. Individual purchasers may make specifications as to its quality or content.

Animal and plant depot lipids consist primarily of triacylglycerols: triesters of glycerol and three fatty acids. Lipids differ from species to species with regard to the types and amounts of fatty acids they contain. Table 1 presents the fatty acid

**TABLE 1. Typical Fatty Acid Compositions: Subcutaneous Adipose Tissue of Selected Animals, Greases, and Selected Vegetable Oils.**

Fatty Acid	Trivial Name	Beef Tallow <sup>a</sup>	Mutton Tallow <sup>a</sup>	Lard <sup>a</sup>	Chicken Fat <sup>a</sup>	Choice White Grease <sup>b</sup>	Yellow Grease <sup>b</sup>	Soy <sup>c</sup>	Rape <sup>c</sup>
10:0	capric	0–0.1	0.1–2	0.1		0.2			4
12:0	lauric	0.1	0.1–0.5	0.1		0.2			
14:0	myristic	2.7–4.8	2.8–4.9	1.4–1.7	1.3	1.9	1.9	0.1	0.1
14:1	myristoleic	0.8–2.5	0.7–0.8	0–0.1	0.2			0	0
16:0	palmitic	20.9–28.9	19.5–21.3	23.1–28.3	23.2	21.5	16.2	11	2.8
16:1	palmitoleic	2.3–9.1	1.4–2.3	1.8–3.3	6.5	5.7	2.5	0.1	0.2
17:0	margaric	1	1	0.5	0.3			0	0
18:0	stearic	7.0–26.5 (19) <sup>d</sup>	17.6–28.9	11.7–24 (14) <sup>d</sup>	6.4	14.9	10.5	4	1.3
18:1	oleic	30.4–48.0 (43) <sup>d</sup>	33.2–40.4	29.7–45.3 (44) <sup>d</sup>	41.6	41.1	47.5	23.4	23.8
18:2	linoleic	0.6–1.8	1.2–3.4	8.1–12.6	18.9	11.6	17.5	53.2	14.6
18:3	linolenic	0.3–0.7	1.4–1.9	0.7–1.2	1.3	0.4	1.9	7.8	7.3
20:0	arachidic	tr.–0.9	tr.–0.3	0.2–0.3		1.8 for all > C20	1.0 for all > C20	0.3	0.7
20:1	gadoleic	0.3–1.7	0.2–0.3	0.8–1.3					12.1
20:2	eicosadienoic	0–0.1	0	0.3–0.5					0.6
20:4	arachidonic								
22:0	behenic	0–0.1	0	0–0.4				0.1	0.4
22:1	erucic	0–tr.	0	tr.–0.1				0	34.8
24:0	lignoceric	0–tr.	0	0–0.5					1
24:1	nervonic	0	0	0–0.6					
<i>Trans</i> -fatty acids		1.3–6.6	11.0–14.6	1.1–1.4					

<sup>a</sup>Data from (1)

<sup>b</sup>See (6).

<sup>c</sup>See (7).

<sup>d</sup>Typical value for U.S. product: From (8).

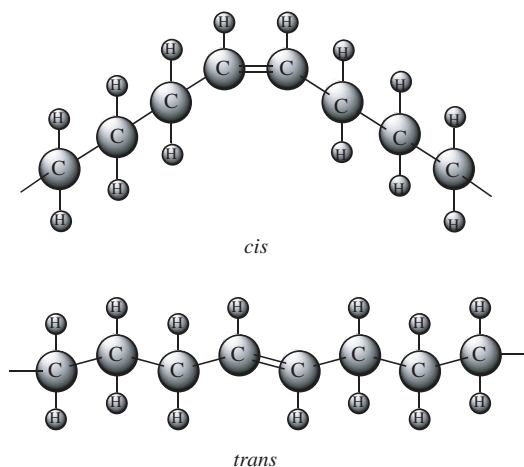
compositions of the major industrially important animal lipids, with data for some plant lipids for comparison. Data are also included for white and yellow greases. In countries such as the United States where animal fats are no longer used in substantial quantities for deep fat frying, yellow grease consists largely of hydrogenated vegetable oil, whereas elsewhere it may contain a substantial portion of beef tallow. Thus, the fatty acid content and physical properties of yellow grease may depend on country or region of origin and can change over time as food industry practices change. Note that there is a wide range of values stated for some entries in Table 1, such as a stearic acid (18 carbons, no double bonds, i.e., 18:0) content between 7% and 27% for beef tallow. This reflects the fact that a number of factors impact the composition of an animal fat, and thus any general statement of composition must have a broad range.

Examination of Table 1 shows that the prevalent fatty acids in animal depot lipids are either 16 or 18 carbons in length and are either fully saturated or contain one or two double bonds. Animal lipids generally contain a higher proportion of saturated fatty acids than do the lipids of temperate zone plants.

The physiological role of lipids is to serve as a carbon and energy reserve. To be biochemically accessible for these purposes, it is best that a lipid be liquid, or at least semiliquid. Thus, at body temperatures, depot fats are semisolids. They solidify when cooled to room temperature. The largest factor contributing to the freezing or melting temperature of an acylglycerol is its fatty acid composition, specifically with regard to the chain lengths and number of double bonds. A double bond in a fatty acid has the effect of introducing a kink into the linear run of carbons (Figure 1). This kinking interferes with the tendency of the acyl chains of the fatty acids in the lipid to align and pack with one another. As a consequence, more energy must be removed from the system to induce crystallization. This is manifested as a reduced freezing or melting point relative to saturated fatty acids, which can adopt straighter chain configurations, pack more readily, and crystallize (freeze) at higher temperatures. Differences in the content of unsaturated fatty acids play the largest role in determining melting point differences between various animal and temperate zone vegetable lipids. Thus, it is the relatively low content of unsaturated fatty acids that renders fats solid at room temperature, whereas the liquidity of oils is due to their higher content of unsaturates. Note also (Figure 1) that the extent of kinking introduced into a fatty acyl chain by a double bond depends on the configuration of that bond, with *cis*-bonds introducing a considerable kink and *trans*-bonds barely perturbing the linear run of the carbon chain. This leads to substantially different physical and biochemical properties for the two types of unsaturated fatty acids. The great preponderance of naturally synthesized unsaturated fatty acids contain *cis*-double bonds. The industrial hydrogenation of oils to produce more fully saturated lipids can result in the production of a substantial proportion of *trans*-double bonds. Differences in the physiologic effects of *cis*-vs. *trans*-fatty acids in dietary lipids have led to concerns regarding consumption of the latter, as discussed below (see Recent Developments).

It is energetically more costly to synthesize an unsaturated fatty acid than a saturated one of the same chain length. The body fats of warm-blooded animals need to





**Figure 1.** The configurations of *cis*- and *trans*-carbon-carbon double bonds. (Used with the kind permission of the Institute of Shortening and Edible Oils, Inc. Washington, D.C.)

contain only a sufficient proportion of unsaturated fatty acids to render them semi-solid at body temperature, which is approximately constant and generally higher than ambient temperature. The resulting relatively high proportion of saturated fatty acids causes these lipids to be fats: to have melting points above room temperature. As plants have no notable mechanism of temperature control, their lipids must stay fluid over the typical range of temperatures encountered in the field. Plants thus synthesize and incorporate a higher proportion of unsaturated fatty acids, typically oleic (18:1), linoleic (18:2), and linolenic (18:3) acids into their lipids, allowing them to remain fluid at relatively low ambient temperatures. These tendencies are so general as to allow the term “fat” alone to be used in some contexts to refer to animal lipids, whereas “oil” is used to refer to plant lipids. Tropical plants experience higher ambient temperatures than those of temperate climates, and thus their lipids generally contain a higher proportion of saturated fatty acids.

Among animals, another feature that bears on the fatty acid content of the depot lipids is whether an animal bears a rumen. The rumen harbors a dense, diverse, and metabolically very active microbial community. By conducting the biochemical hydrogenation of unsaturated fatty acids, it is this community that modifies the unsaturate-rich lipid diet of a grazing animal to produce the saturated fatty acids that are incorporated into the body fat. The biohydrogenation pathway has several intermediates, and these can be substrates for other reactions within the rumen or the whole animal. As a result, the depot fats of ruminants have a very diverse fatty acid composition. Tallow contains hundreds of different fatty acid structures, most present only in small or trace amounts. The saturated fatty acids of ruminant acylglycerols contain normal and methyl-branched components; the latter may be of the *iso* (terminally branched) or *anteiso* (subterminally branched) form. Odd- and even-numbered carbon chain lengths are found, and both geometrical and positional isomers of the unsaturated fatty acids usually are present. This presence of

*trans*- and positional unsaturated fatty acid isomers is characteristic of ruminants and animals with ruminant-like digestive systems.

The degree of rumen-mediated fatty acid modification varies from species to species. For example, the biohydrogenation of dietary unsaturates is greater in sheep than in cattle, and thus mutton tallow contains 5% to 10% more stearic acid, and a correspondingly lower amount of oleic acid, than beef tallow. Table 1 illustrates this trend, although it is somewhat obscured by the necessarily wide ranges of values reported.

In general, the concentration of saturated fatty acids, especially stearic, is higher in ruminant than in nonruminant fat. Additionally, the polyunsaturates, especially linoleic acid, the most prevalent natural polyunsaturate, are lower in ruminant fat than in that from monogastric (i.e., nonruminant) species. Thus, the fat of cattle and sheep is, in general, firmer than that from pigs.

The fatty acid structural diversity seen in the fats of rumen-containing animals is not found in plant lipids. Extensive microbial modification of dietary fatty acids is also absent in monogastric species (e.g., pig, poultry). Consequently, the fatty acid content of their lipids is not only simpler than that of ruminants, but also more closely mirrors the dietary fatty acid intake, displays a greater sensitivity to alterations in dietary fatty acid content, and can be intentionally manipulated to a greater degree by adjustments to the diet. Enser (9) has summarized work regarding the relationship between dietary and depot lipid fatty acid contents, among other things, pointing out a linear correlation between dietary and depot linoleic acid levels in the pig. Table 2 illustrates the ability of dietary unsaturated oils to increase the degree of unsaturation of pork fat. The fatty acid content of the pork fat reflected that of the dietary lipids. In contrast, the fatty acid composition of beef fat varied

**TABLE 2. Influence of Diet on the Fatty Acid Compositions (% of total) of Pork Fat and Beef Adipose Tissue.**

Fatty acid	Pork Fat <sup>a</sup>		Pork Fat <sup>b</sup>		Beef Fat <sup>b</sup>	
	Tallow Diet <sup>c</sup>	Soy Oil <sup>c</sup>	Corn & Soy Meal	Canola Oil	Corn & Cottonseed Meal	Rapeseed & Cottonseed Oil
14:0	1	0.8	1.3	0.5	3.9	3.6
16:0	26.6	22.1	25.6	10.8	26.7	24.3
16:1	4.1	2.5	0.9	0.4	2	2
18:0	12.1	11.3	12.9	4.3	20.2	20.5
18:1	40.5	33.2	46.9	56.1	41.2	43
18:2	11.2	24.4	11.5	21.5	4.8	5.5
18:3	4	4.9	0.9	6.5	—	—
20:4	0.3	0.5	—	—	—	—
22:6	0.2	0.2	—	—	—	—
Total saturates	39.6	34.2	39.8	15.6	50.8	48.4

<sup>a</sup>See (10).

<sup>b</sup>See (11).

<sup>c</sup>Diets contained 3% tallow or soy oil.

little between animals on a corn-cottonseed diet and those on a diet also containing rapeseed oil, which is enriched in erucic acid (22:1) (Table 2). It has been reported that the back fat of pigs on a diet containing soy oil had an elevated content of linoleic acid (the most prevalent fatty acid in soy oil), although the altered fat did not pose any problems with regard to carcass appearance or softness during processing (12). In other studies, pigs were fed a corn-soybean meal diet with added tallow, safflower oil, or a combination of tallow and safflower oil (13). The increased levels of dietary safflower oil resulted in a decrease in the contents of stearic and oleic acids and an increase in linoleic acid (the predominant acid in safflower oil), 20:2, and 20:3 in the subcutaneous fat.

For ruminant fat to become directly responsive to dietary unsaturated fats, it is necessary to protect the lipids against saturation by rumen microorganisms. The alteration of the lipid content of mutton by the feeding of such protected oil supplements has been described (14). Also, it has been shown that a diet of extruded soybeans increased the linoleic acid and linolenic acid contents of steer adipose tissue (15).

A number of factors other than diet can influence the fatty acid composition of the depot lipids of a given species. These include genetic and sex effects (16, 17). Physical environment also plays a role. For example, in sheep, colder temperatures result in softer body fats with lower melting points and higher iodine numbers (18). In general, diet has a more marked effect on fat quality than do breed or sex, especially in nonruminants, which are susceptible to alteration of tissue fatty acids by dietary modification.

Location within the body also influences the degree of unsaturation in a fat. The temperature of a warm-blooded animal is not constant throughout the body. Fats located near the skin experience colder temperatures than those within a carcass. To remain semifluid and thus metabolically accessible in the face of this thermal challenge, fats near the surface tend to have lower melting points and be softer, traits resulting from an elevated content of unsaturated fatty acids relative to fats from the interior of the carcass. For example, the saturated fatty acid content of beef tallow located just below the hide has been reported to be 48.7% of the total fatty acid content, whereas that from the kidney region, deep within the carcass, has a saturated fatty acid content of 57.9% (19). In general, the fatty deposits increase in hardness from surface subcutaneous locations through the inter- and intramuscular fat to deep abdominal and kidney fats in cattle, sheep, and pigs. Thus, the internal fats from these species, especially those surrounding the kidney, tend to be harder than those from fats near the surface of the carcass. Products made from kidney fat will be firmer. They will also have better flavor stability, because rancid flavors are the result of oxidation of the double bonds of unsaturated fatty acids. Although relevant in some food applications, this feature is of restricted overall importance because it is rare for animal fat to be segregated on the basis of anatomical origin.

Whether for food or nonedible industrial applications, the choice of raw material lipid is dependent on a match between the physical properties of the lipid and the desired performance properties. Physical and performance properties are largely a

function of fatty acid composition. Thus, for example, the higher saturated fatty acid contents of animal fats, by rendering them solid at room temperature, makes them desired feedstocks for the production of bar soaps. It also affects their performance in baking, and for decades made them the optimal choice for such applications. Also, because their lower content of polyunsaturated fatty acids confers greater oxidative stability, animal fats are suggested for oxidation-prone applications such as deep fat frying and as lubricants in high-speed metal working.

## 2.2. Nonacylglycerol Components

Animal fats consist mainly of triacylglycerols, containing only minor amounts (<0.05%) of other compounds such as phospholipids, tocopherols, and carotenoids (9). However, one minor component of considerable note is cholesterol. Typical cholesterol levels for animal fats of industrial importance are on the order of 850–1100 mg/kg, with poultry fats at the lower end of this spectrum. These levels are at least an order of magnitude greater than those for vegetable oils. Cholesterol is a necessary component of the human body. It is both synthesized *de novo* and obtained in the diet. In most individuals, the consumption of moderate amounts of cholesterol poses no health risk, as it is balanced by a reduction in endogenous synthesis. However, concern over the role of cholesterol as a dominant factor in the genesis of atherosclerosis (20), a major cause of mortality in developed countries, has led to government advisories to restrict the intake of cholesterol (and of saturated fatty acids, which exacerbate its atherosclerotic effects) (21, 22). As a result, since the 1980s, there has been a substantial replacement of animal fats by vegetable oils in many food applications, greatly reducing the utilization of animal fats in edible applications. To perform properly, the vegetable oils are often hydrogenated to convert their unsaturated fatty acids to saturates, thereby increasing solid fat content, conferring desirable performance properties, and increasing oxidative stability.

Methods are available to remove cholesterol from animal fats (23), and a line of low cholesterol edible lipids, termed "Appetize," was marketed in the United States in the 1990s (24). The product consisted of 70–90% tallow whose cholesterol had been reduced by steam distillation to only 8 mg/100 g. Despite the fine rationale underlying its production, however, high production costs resulted in the withdrawal of the Appetize line from the market. Supercritical fluid extraction can also be used to reduce the cholesterol content of animal fats (25), although to this author's knowledge, this approach has not been commercially implemented.

## 3. ACYLGLYCEROL STRUCTURE AND ITS RELATIONSHIP TO FUNCTIONALITY AND USE

For nonedible uses, fatty acid content, availability, and price play the largest roles in determining which lipid is employed in a particular application. In cases where both animal fats and vegetable oils are able to perform a desired function, animal fats can be attractive raw materials because their bulk prices have historically been

substantially below those of the least expensive vegetable oils. For example, in the United States, edible tallow prices are typically 40% to 60% of that of soybean oil.

For edible uses, however, aspects of lipid structure other than simply fatty acid content come into consideration. Chief among these are the pattern of distribution of fatty acids on the glycerol chain, because the melting profile, plastic range, and other properties of a fat are affected by this feature, known as "acylglycerol structure." These properties affect performance parameters such as mouthfeel and melting range. The carbons of the glycerol molecule are not chemically equivalent. They are identified, based on position and stereochemical numbering conventions, as *sn*-1, -2, and -3, with the -2 position being the secondary hydroxyl in the middle position of the molecule. The distribution of fatty acids among these three carbons in natural fats (and oils as well) is not random, and varies with species and fatty acid. Thus, a lipid will contain an array of acylglycerol structures including those consisting entirely of saturated fatty acids (SSS); those containing saturated, mono-unsaturated (M), and polyunsaturated (U) fatty acids (SSM, SSU, UM S, etc.) and all-unsaturated species (MMM, UMU) (Table 3). As expected, a lipid with a higher content of unsaturated fatty acids will have a higher amount of multiply unsaturated (polyunsaturated) triacylglycerols (Table 3). Within these groups is further species-dependent diversity, e.g., content of SSU vs. SUS (Table 3). Recent advances in mass spectral methods have greatly facilitated this speciation of natural lipids (28–31). Differences in fatty acid content and distribution cause differences in the physical properties of fats, and thus they can determine the uses of a fat. In the first instance, fully saturated acylglycerols have high melting points, whereas those of partially or fully unsaturated ones are much lower. (The difference in melting points between tristearin and trilinolenin is nearly 100°C.) The melting profile,

**TABLE 3. Positional Distribution of Fatty Acids in Triacylglycerols of Animal Depot Fats.<sup>a</sup>**

Fat	Position	Fatty Acid, mol%						
		14:0	16:0	16:1	18:0	18:1	18:2	18:3
Pig (outer back fat)	1	1	10	2	30	51	6	
	2	4	72	5	2	13	3	
	3	—	tr.	2	7	73	18	
Cattle (subcutaneous fat)	1	4	41	6	17	20	4	1
	2	9	17	6	9	41	5	1
	3	1	22	6	24	37	5	1
Sheep (perineal fat)	1	1	35	2	47	4 <sup>b</sup>	—	
	2	4	14	2	15	52 <sup>b</sup>	5	
	3	3	16	1	42	26 <sup>b</sup>	2	
Chicken <sup>c</sup>	1	2	25	12	6	33	14	2
	2	1	15	7	4	43	23	3
	3	1	24	12	6	35	14	3

<sup>a</sup>See (26).

<sup>b</sup>Results for 18:1 *cis* isomers only. 18:1 *trans* was present in positions *sn*-1, *sn*-2 and *sn*-3 as 5, 2 and 6% respectively.

<sup>c</sup>See (27).

plastic range, and other properties of a fat are affected by the range of its acylglycerol structures. For example, lard is plastic over only a narrow temperature range, has a grainy texture, and does not cream well. This is in large part a result of its fatty acid content and pattern of acylglycerol structures, especially to a high content of acylglycerols with palmitic acid (16:0) in the *sn*-2 position (Table 3). In lard, 72% of the residues at this site are palmitic acid, whereas this fatty acid constitutes only 10% of the residues at the *sn*-1 position and is found in only traces in the *sn*-3 position. In beef tallow (and sheep and chicken fat as well), palmitic acid is located at all three positions of the triacylglycerol molecule: In the *sn*-2 position, 17 mol% is palmitic; at the *sn*-1 position, palmitic acid makes up 41%; and in the *sn*-3 position, 22% (Table 3). The greater uniformity of lard triacylglycerol structures leads to a shorter plastic range, a sharper melting point, and larger crystals in the solid phase. The latter are largely responsible for the poor creaming abilities and grainy mouthfeel of lard. Although their fatty acid contents are not widely different, lard and beef tallow do exhibit some substantial differences in triacylglycerol structure. Thus, lard contains about 7 mol% trisaturates and 32 mol% disaturates, whereas beef tallow contains 15 mol% trisaturated triacylglycerols and nearly 40 mol% disaturates. Lard has a greater proportion of triacylglycerols with two double bonds than does beef tallow and about half as many mol% of triacylglycerols with three double bonds. Differences such as these confer different physical properties on fats.

In contrast to the high proportion of saturated fatty acids in the *sn*-2 position of lard, the tallows contain predominantly an unsaturated fatty acid (about 60% relative abundance), generally oleic (18:1), in this position. This has been exploited to develop a method to detect the adulteration of beef fat with lard (32). This involves first the isolation of the acylglycerols containing one saturated and two unsaturated fatty acids, followed by determination of the fatty acid population at the *sn*-2 position of this fraction. The sensitivity is reported to be sufficient to detect the presence of 1% lard in the tallow.

Lard is unique among the common fats and oils in having a preponderance of palmitate at the *sn*-2 position. This has led to a unique use of this lipid: Human milkfat also contains primarily palmitate at the *sn*-2 position of its acylglycerols, and lard has been employed in infant formulas in attempts to produce a material more closely resembling human milk. Approaches taken in this work have included the direct addition of lard (33, 34) as well as the enzymatic restructuring of its acylglycerols. The latter approach employs lipases that specifically interesterify (see below) the *sn*-1 and *sn*-3 positions of acylglycerols, thus retaining the palmitate at the *sn*-2 position, to introduce fatty acids from soybean oil into the terminal positions of the fat molecules, creating structures closely mimicking those in human milkfat (35).

Texture is one property affected by the content and arrangement of fatty acids in the acylglycerols of a lipid. When a melted lipid cools and crystalizes to form a solid, its acylglycerols will generally adopt one of three predominant crystal lattice forms,  $\alpha$ ,  $\beta$ , or  $\beta'$ , depending on acylglycerol content and the kinetics of cooling (36, 37). This ability to adopt more than one crystal form is termed polymorphism. Crystals of the  $\beta$  type are large and coarse and confer an undesirable grainy

mouthfeel. Crystals of the  $\beta'$  form are smaller and result in a smoother, more desirable, mouthfeel. Tallow tends to adopt the  $\beta'$  configuration. Lard has a relatively large proportion (27%) of disaturated acylglycerols, mostly oleoylpalmitoylstearin, which has a propensity to crystallize in the  $\beta$  form. This gives lard a grainy texture and poor creaming ability. Through interesterification (see Section 7.5) a more random distribution of fatty acids can be achieved, yielding a product that crystallizes in the  $\beta'$  form, which is more desirable for such edible applications as margarines.

The physical structure of the  $\beta'$  form is less stable than the  $\beta$  form, and can convert to the latter over time. Margarines typically contain mixtures of vegetable oil and solid fat of  $\beta'$  form. The small crystal structure of the latter aids in keeping the mixture plastic and uniform. The spontaneous conversion of the  $\beta'$ - to a  $\beta$ -dominated hard fat population over time results in replacement of the fine crystals by larger coarse one. These are less effective at carrying the oil phase, and the result can be separation, yielding an undesirable two phase liquid/solid product. Appropriate choices of feedstocks, processing conditions, and crystal stabilizers can reduce the rate of product deterioration due to changes in crystal structure.

#### 4. QUALITY INDICATORS FOR EDIBLE FATS

Raw fat is susceptible to defects caused by (1) oxidation of the double bonds of fatty acids, which creates degradation products that confer undesirable odors and flavors; (2) ester bond hydrolysis (lipolysis) by contaminating microbes, which releases free fatty acids; and (3) the formation of off colors. Best quality is obtained if contaminants such as blood and manure are kept out of the raw material, and if it is then kept cold, processed within a few days of slaughter with adequate attention to process controls, and handled and stored properly. If these conditions are not achieved, deterioration can occur. Waste greases are especially subject to such degradation, because they are typically stored for long periods of time without refrigeration. Because the resulting chemical changes can impact performance and acceptability, various analytical parameters and specifications have been developed to identify lipid quality. Some of these also provide basic information on the properties of a lipid, and they can be used to assess its suitability for a given application. Among the properties, analyses, and terms by which lipid samples are characterized are as follows:

- **Color:** Excessive color in a lipid sample can prevent its use in some applications or necessitate the application of color reduction technologies. Several methods for expressing the color of a fat or oil exist, but all rely on the direct comparison of the color of a sample to that of a series of color standards. For raw samples, especially of tallow and grease, which can be deeply colored, this value is often measured by comparing a sample of filtered liquid fat with a set of 26 color standards designated by the Fat Analysis Committee (FAC) of the American Oil Chemists' Society (AOCS), and assigning a number from 1 (lightest) to 45 (darkest), sometimes referred to

as simply FAC. Conduct of this measurement is described in AOCS Official Method Cc13a-43 (38). Color reduction, when necessary, is generally achieved through the use of bleaching clays. In the United States, after bleaching, color is typically measured and expressed in terms of the Wesson color method, described in AOCS Official Method Cc13b-45 (39). In most other countries, the color after bleaching is determined by means of the Lovibond Method, AOCS Official Method Cc13e-92, which is the accepted international standard for the measurement of color in animal and vegetable fats and oils (40). Particularly for use in bar formulation soaps for hand and body washing, white color is desired in the feedstock lipids.

- Free fatty acid content: A measure of the amount of acylglycerol hydrolysis. Free fatty acids can reduce the palatability, acceptability, and performance of a lipid and are therefore generally considered a negative trait. As with all parameters described here, acceptable levels vary depending on application.
- Iodine number or value (IV): A measure of the degree of unsaturation of a lipid, as measured by its iodine absorption, a trait proportional to double bond content. Expressed as the number of grams of iodine absorbed by 100 g of fat. As fatty acid oxidation occurs at double bonds, a high IV can indicate a fat sample that will have marginal oxidative stability.
- Moisture, impurities, and unsaponifiables (MIU): A summation of the non-acylglycerol materials in the product. Moisture is undesirable because it will support microbial growth and facilitate lipid hydrolysis. Unsaponifiables are any materials that will not saponify (form soap) when incubated with sodium hydroxide. These include sterols, pigments, and hydrocarbons. These are natural components of both animal and plant lipids, but excessive amounts can indicate a sample that will not perform comparably with a sample richer in acylglycerols, and they can indicate adulteration or contamination by petroleum products.
- Peroxide value (PV, also referred to as initial peroxide): The presence of fatty acid hydroperoxides, formed by the oxidative degradation of fatty acids, is a measure of oxidative abuse and degradation of the lipid. Products generated by hydroperoxide degradation will confer a rancid note in edible applications.
- Refined and bleached (R&B) color: A measure of the amount of red color in the rendered fat is an indicator of the quality of both the starting material and the rendering techniques. This value is measured using the Lovibond 5.25-inch scale according to procedures described in AOCS Official Method Cc 13b-43 (39). The result is referred to as the AOCS Wesson color, the AOCS Lovibond color, or simply Lovibond color. The lower the value, the less colored the sample.
- Saponification value (SV): Defined as the number of milligrams of potassium hydroxide required to hydrolyze (saponify) 1 g of fat. The higher the SV, the lower the mean chain length of the component fatty acids of an acylglycerol.



- Titer (titre): The solidification temperature of the free fatty acids derived from a lipid. The higher the value, the greater the unsaturated fatty acid content. An especially important characteristic in fats used to produce bar soap or fatty acids, where degree of hardness is important. The melting point of an intact fat is not a good indicator of its firmness, because this value depends on the crystal form adopted by the fat. The titer value does not suffer from this defect, and thus, it is a much more reliable estimate. Trade practice in the U.S. rendering industry is to designate animal fats with titers of 40°C and up as tallow, and those below 40°C as grease. Tallow titers can be as high as 59–61°C, although 40–50°C is more common. Lard exhibits a slightly higher value, because of its greater content of high-melting stearic acid.

Formal classification standards for fats and oils have been defined by many bodies and organizations. Those established by the *Code of Federal Regulations* (United States) and the *Codex Alimentarius* are widely employed to guide commerce. The U.S. *Code of Federal Regulations* (41) specifies that lard should have the following quality characteristics: free of foreign odors and flavors; maximum free fatty acid value of 0.5% (as oleic acid equivalents) or an acid value of 1.0 mg of KOH consumed per gram of sample; maximum peroxide value (as milliequivalents of peroxide per kilogram of fat) of 5.0; moisture and volatile matter at a maximum of 0.2%; insoluble impurities no greater than 0.05%; and white in color, with a maximum reading of 3.0 red units in a 5¼-inch cell on the Lovibond scale.

In the *Codex Alimentarius* (42), maximum free fatty acid levels are specified as 0.65% for lard, 1.00% for premier jus, and 1.25% for rendered pork fat and edible tallow. For all these, a peroxide maximum of 10-milliequivalents active oxygen per kilogram fat is specified. The *Codex* standards also specify levels for antioxidants and antioxidant synergists and maximum allowed amounts of impurities, soaps, and certain metals.

Adulteration is another quality rating factor of commercial lipids. Methods for the detection of tallow adulteration with lard were discussed above. Tests have also been developed to detect the presence of beef fat in lard. The best known of these tests is the Bömer test, which is based on the difference between the melting points of acylglycerols and the fatty acids they contain (43). This difference is large for unhydrogenated pork fat and small for tallow. The test is invalidated by the presence of hydrogenated fat in the lard. As another means of detecting adulteration, it has been suggested that more than 0.01%, 0.05%, and 0.05% of branched chain 14, 15, and 16 carbon fatty acids, respectively, in lard indicates the presence of tallow (44). However, when pigs are fed tallow, they incorporate some of the branched fatty acids into their depot fat (45).

Triacylglycerol profiles, determined by high-performance liquid chromatography (HPLC), may also be a tool for the detection of the adulteration of pork by beef fat (44). The presence of 5% or more of pork fat in beef or mutton tallow can be detected and quantified by HPLC analysis of fatty acids in the *sn*-2 position of the triacylglycerols, because the ratio of 16:0/18:1ω9 at this position is about 5.0

for lard, whereas for edible tallow, it is about 0.4. Gas liquid chromatography can also be employed to make such a determination of the purity of a lipid.

## 5. REGULATORY AND COMMERCIAL CLASSIFICATIONS OF ANIMAL FATS

Although Table 1 lists the fatty acid compositions of various lipids, this is not the only or the final arbiter of their classification. As opposed to vegetable fats and oils (other than olive oil), where only one oil is generally identified as originating from an oilseed (e.g., corn oil), a diversity of definitions and specifications is used in the identification of and trade in animal fat products. These often include statements of the allowed limits of any number of quality parameters.

In the case of tallow, two broad categories are defined: edible and inedible. Edible tallow originates from cattle or sheep that are judged by a competent regulatory authority to be healthy, sound, and fit for consumption at the time of slaughter. Tallow obtained from the inedible offal resulting from slaughter, from animals unfit for consumption, or from outdated meats returned from commercial outlets is classified as inedible.

The *Code of Federal Regulations* (41) of the United States concerns itself with only one pure animal fat, e.g., lard, which is defined as the fat rendered from clean and sound edible swine tissues. Tissues to be used for lard are to be reasonably free from blood and shall not include stomachs, livers, spleens, kidneys, brains, or settlings and skimmings. "Leaf Lard" is prepared from fresh leaf (abdominal) fat. Lard (when properly labeled) may be hardened by the use of lard stearin (a lard fraction rich in acylglycerols containing saturated fatty acids) or hydrogenated lard or both and may contain refined lard and deodorized lard, if so labeled. A detailed compilation of the killing and cutting fats to be used in producing lard and rendered pork fat has been provided (46).

The *Codex Alimentarius* (42) contains international standards for four main products from animal sources: lard, rendered pork fat, premier jus, and tallow. "Lard" is defined as the fat rendered from fresh, clean, sound edible-grade fatty tissues from swine. These tissues must lack bones, detached skin, head skin, ears, tails, organs, windpipes, large blood vessels, scrap fat, skimmings, settlings, pressings, and be reasonably free of muscle tissues and blood. "Rendered pork fat" is defined similarly to lard, with the exception that the tissues forbidden in lard production are allowed. "Premier jus" (or oleo stock) is the product obtained by low-temperature rendering of the fresh fat of heart, kidney, greater omentum, and mesentery of bovines, collected at slaughter, as well as cutting fats. This fat has a creamy white-to-light yellow color, a characteristic mild flavor, and a very low free fatty acid content (1.0% maximum). "Edible tallow" ('dripping') is the product obtained by rendering the clean, sound fatty tissues, including trimming and cutting fats, but also the attendant muscles and bones of bovine animals and/or sheep. It is distinguished from premier jus by the allowance of sheep tissues and of a greater diversity of materials from which it can be obtained, by the specification of a higher

**TABLE 4. Codex Alimentarius Standards for Lard, Rendered Pork Fat, Premier Jus, and Edible Tallow.<sup>a</sup>**

Characteristic	Lard	Rendered Pork Fat	Premier Jus	Edible Tallow
Relative density (40°C/water at 20°C)	0.896–0.904	0.894–0.906	0.893–0.904	0.893–0.904
Refractive index ( $n_{\frac{40}{D}}$ )	1.448–1.460	1.448–1.461	1.448–1.460	1.448–1.460
Titre (°C)	32–45	32–45	42.5–47	40–49
Saponification value (mgKOH/g fat)	192–203	192–203	190–200	190–202
Iodine values (Wijs)	55–56	60–72	36–47	40–53
Unsaponifiable matter (maximum, g/kg)	10	12	10	12

<sup>a</sup>See (42).

maximum free fatty acid content (1% to 1.25%), and by a higher allowed peroxide level. Lard, rendered pork fat, and edible tallow may contain certain further processed forms of the rendered fat, such as refined or hydrogenated product, or stearines, as long as labeling regulations are followed.

The *Codex* descriptions specify that all edible animal fats must come from animals determined to be in good health at the time of slaughter and fit human consumption as judged by a competent authority recognized in national legislation. The main *Codex* analytical identity standards for lard, rendered pork fat, edible tallow, and oleo stock are given in Table 4. The ranges for fatty acid composition specified in the *Codex* standards for lard, rendered pork fat, edible tallow, and oleo stock are given in Table 5. Note that these values are not necessarily constant over time, having undergone revision since the previous edition of this chapter.

In terms of trade in animal fats, another important classification system is the Specifications for Tallow and Greases established by the American Fats and Oils Association. These specifications (Table 6) establish 13 categories of lipids, and guide U.S. industry and commerce. One of the specified categories is for edible lard, 11 are for various grades of tallow, and the remaining 2 provide specifications for white and yellow grease. The categories are identified in terms of both species of origin and the composition-related parameters minimum titer, maximum free fatty acid content, maximum color, maximum refined and bleached color, and maximum moisture, insolubles, and unsaponifiables. The numerous designations of tallow are necessary because in various locations not only cattle but also swine and/or poultry products are rendered together, giving products of varying content depending on the species mix. Choice white grease is a pork product, consisting of pork lipids other than leaf lard. Accordingly, its fatty acid composition is very similar to that of lard (Table 1). “Yellow grease” is a term given to used fat from deep fryers. It is defined in terms of its free fatty acid content, with no specification made as to biological origin. The displacement of tallow by hydrogenated vegetable oils in deep fat frying in recent years has led to yellow grease that is presently in many

**TABLE 5. Ranges of Fatty Acid Composition (%) Specified by the Codex Alimentarius Standards for Lard, Rendered Pork Fat, Premier Jus, and Edible Tallow.<sup>a</sup>**

Fatty acid	Lard, Rendered Pork Fat	Premier Jus, Edible Tallow
<14	<0.5	<0.5
14:0	1.0–2.5	2–6
14:ISO	<0.1	<0.3
14:1	<0.2	0.5–1.5
15:0	<0.2	0.2–1.0
15:ISO	<0.1	} <1.5
15:ANTI ISO	<0.1	
16:0	20–30	20–30
16:1	2.0–4.0	1–5
16:2	<0.1	<1.0
16:ISO	<0.1	<0.5
17:0	<1	0.5–2.0
17:1	<1	<1.0
17:ISO	<0.1	} <1.5
17:ANTI ISO	<0.1	
18:0	8–22	15–30
18:1	35–55	30–45
18:2	4–12	1–6
18:3	<1.5	<1.5
20:0	<1.0	<0.5
20:1	<1.5	<0.5
20:2	<1.0	<0.1
20:4	<1.0	<0.5
22:0	<0.1	<0.1
22:1	<0.5	Not detected

<sup>a</sup>See (42).

places largely a vegetable oil product. Accompanying this change have been increases in the degrees of unsaturation and of *trans*-fatty acids in yellow grease. Brown grease can be anything that does not meet the minimum specifications for yellow grease, irrespective of origin (Table 6). Only one grade of poultry fat is included in Table 6. Other poultry fat lipid products are sold, but they are not detailed here because the standards for poultry fat are generally set by the individual customer. Potential users should be acquainted with these specifications when contemplating performance or purchase needs. Commercial renderers and brokers of fats are familiar with these terms and specifications and the trading rules associated with the purchase of these products.

In the United Kingdom, trade is also governed by a multiplicity of definitions for tallow. Premier jus is the term applied to the highest, edible grade of tallow, and its specifications conform to that of the *Codex Alimentarius* (42). For inedible tallows, British Standard 3919 (49) specifies six grades and grease (Table 7). Again, these grades of inedible tallow are defined on a compositional basis, including a specified

**TABLE 6. Specifications for Some Commercial Grades of Tallows, Animal Fats, and Greases.<sup>a</sup>**

Grade	Specifications <sup>b</sup>				
	Titer (°C, min.)	FFA (%, max.)	FAC (max.)	R & B (max.)	MIU (%, max.)
Lard (edible)	38	0.5	<sup>c</sup>	None	<sup>d</sup>
Edible tallow	41	0.75	3	None	<sup>d</sup>
Top white tallow	41	2	5	0.5	1
All Beef Packer Tallow	42	2	None	0.5	1
Extra fancy tallow	41	3	5	None	1
Fancy tallow	40.5	4	7	None	1
Bleachable fancy tallow	40.5	4	None	1.5	1
Prime tallow	40.5	6	13–11B	None	1
Special tallow	40	10	21	None	1
No. 2 tallow	40	35	None	None	2
'A' Tallow	39	15	39	None	2
Choice White	36	4	13–11B	None	1
Grease					
Yellow Grease	<sup>e</sup>	<sup>e</sup>	39	None	2
Brown Grease <sup>f</sup>	n.s. <sup>g</sup>	>15	n.s.	n.s.	n.s.
Poultry Fat <sup>h</sup>	28–35	15	19	n.1. <sup>i</sup>	2

<sup>a</sup>As issued by (47).

FFA: free fatty acids, FAC: color as per Fat Analysis Committee, R&amp;B: refined and bleached color, MIU: moisture, impurities, and unsaponifiables.

<sup>c</sup>Lovibond color for 5.25-inch cell: maximum 1.5 red. Lard peroxide value: 4.0 M E/K maximum.<sup>d</sup>Moisture maximum 0.20%. Insoluble impurities maximum 0.05%.<sup>e</sup>When required, to be negotiated between buyer and seller on a contract-by-contract basis.<sup>f</sup>See (5).<sup>g</sup>n.s.: not specified.<sup>h</sup>Provided by (48).<sup>i</sup>n.l.: not listed.**TABLE 7. Trading Grades for Technical Tallows and Animal Greases According to British Standard 3919.<sup>a</sup>**

Grade	FFA (max.) (% m/m)	Bleached Colour, red (max.)	Moisture and Dirt (basis) (% m/m)	Unsaponi- fiable (max.) (% m/m)	Titre (°C) (min.)	Iodine Value (max.)	Plastics (max.) (mg/kg)
Tallow 1	3	0.5, 5 1/4 in. cell	0.5	0.5	40	55	200
Tallow 2	5	1.0, 5 1/4 in. cell	1	1	40	55	200
Tallow 3	8	3.0, 5 1/4 in. cell	1	1	40	55	200
Tallow 4	12	4.0, 1 in. cell	1	1.5	40	58	200
Tallow 5	15	12, 1 in. cell	1	1.5	40	58	200
Tallow 6	20	No limit	1	2	40	58	200
Grease	20	No limit	2	2	36.0–40.0	61	200

<sup>a</sup>See (49).

**TABLE 8. Naming Conventions for Inedible Tallow and Grease Products.<sup>a</sup>**

Region	Inedible Beef Tallow	Rendered Tallow (Standard Grade)	Rendered Tallow (Lower Grade)	Inedible Grease	Recovered Cooking Oil
U.S.	Bleachable Fancy Tallow Packer Grade	Bleachable Fancy Tallow Rendered Grade	Prime, Special Tallow	Choice White Grease	Yellow Grease
E.U.	Technical	—	Bonefat, 4%	Tierkupper	RO <sup>b</sup> , RVO <sup>b</sup> , RTO <sup>b</sup>
U.K.	UK 2	—	UK 6	—	RO, RVO, RTO
Eire	Irish 2	Tallow 3/4 Irish 6 (High FFA)	—	—	RO, RVO, RTO
Italy	—	Tallow S	Tallow A	—	RO, RVO, RTO

<sup>a</sup>Provided by D. Dempsey, Unichema, Chicago, IL.

<sup>b</sup>RO: recovered oil, may be either vegetable or animal; RVO: recovered vegetable oil; RTO: recovered tallow oil.

maximum for plastic content. This material makes its way into inedible fats via the inclusion of outdated commercial meats and fats, still in their wrapping containers, into the rendering process. These standards are also often applied to edible tallow.

Table 8 provides some insight into the naming conventions of several countries for fat products. Poultry fat is used virtually exclusively as an animal feed. For feed grade fats, the accepted U.S. industry definitions are those established by the Association of American Feed Control Officials (Oxford, Indiana, [www.aafco.org](http://www.aafco.org)), an organization primarily concerned with issues related to animal feeds. It defines “fat product, feed grade” as “any fat product which does not meet the definitions for animal fat, vegetable fat, or oil, hydrolyzed fat or fat ester.” The Association defines three categories of material:

1. Animal fat, and within this category, “poultry fat,” which is fat obtained from poultry tissues via commercial rendering or extracting. It consists primarily of acylglycerols and contains no additions of free fatty acids or other materials. The total fatty acid content exceeds 90%, with unsaponifiables and insoluble impurities making up no more than 2.5% and 1%, respectively. The presence of any added antioxidants must be stated.
2. “Hydrolyzed animal fat” is animal fat obtained via the procedures commonly used in edible fat processing. Its free fatty acid content is not less than 85%, with not more than 6% unsaponifiables and not more than 1% insoluble impurities. A maximum moisture level must be guaranteed, and the presence of any added antioxidants must be stated. Its source must be stated, e.g., “hydrolyzed poultry fat.”
3. “Fat Product, Feed Grade” is any fat product that does not meet the definitions for fat and hydrolyzed fat. It is sold on the basis of its individual

specifications regarding total fatty acid content, unsaponifiables, insoluble impurities, free fatty acids, and moisture. Again, added antioxidants must be declared. Around these definitions, individual purchasers of poultry fat typically set their own standards with regard to fat quality, as measured by such parameters as free fatty acid content. Thus, for example, pet food manufacturers will generally specify a higher quality fat than for other animal feeds. Presently, most poultry fat is consumed as poultry feed, although there is a trend toward increased use in other animal feeding applications as well.

## **6. PATTERNS AND TRENDS IN THE PRODUCTION AND USE OF ANIMAL FATS**

Domesticated animals are grown primarily for their meat. Carcass fat is a minor coproduct, contributing less than 10% of the total market value of an animal. Therefore, fat production does not drive producer decisions regarding the number of animals to raise. In this aspect, animal fats are a different type of commodity than most oilseeds, for which the oil value constitutes a sizeable portion of the value of the crop, causing producer decisions to be influenced by oil demand and price. Lipids are desired dietary components, contributing energy and essential dietary nutrients. Both a continuing increase in world population and increases in the standard of living have led to increased lipid consumption in many regions of the world. However, the increased demand for fats and oils has largely been met by an increase in vegetable oil production.

Representative data for worldwide annual production of the major fats and oils at selected intervals from 1968 to 2001, with a projection to 2008–2012, are presented in Table 9. It can be seen that in 1968, global vegetable oil production was roughly 23 million metric tons and grease and animal fat production was approximately 9 million metric tons, for a total global lipid production of roughly 32 million metric tons. Animal fats and grease constituted about 28% of global lipid production. In the subsequent 32 years to the turn of the millennium, global production of the major vegetable oils rose at an annual rate of about 13%, nearly four fold overall, to 90 million metric tons. In contrast, the sum production of tallow, grease, and lard increased at only about 1.5% annually to 15 million metric tons. At the turn of the century, then, greases and animal fats had fallen to constitute about 14% of the global fats and oils production of approximately 105 million metric tons. This percentage drop was not a result of decreased animal fat production but of tremendous increases in the production of vegetable oils, especially palm-based lipids, which increased about 14-fold, and soybean oil, which registered a roughly 5-fold increase. Projections for the near future (Table 9) suggest continued slow growth and approximately constant market share for animal fats and oils.

In addition to large increases in vegetable oil production in recent decades, a trend toward lower carcass fat contents at slaughter has also held down the rate of growth of animal fat production. This trend has been a result of two factors: consumer preferences for lean meat and economic pressure to produce animals more

**TABLE 9. Worldwide Production of Fats and Oils (1000 metric tons).<sup>a</sup>**

Commodity	1968	1978	1988–1989	1997–1998	2000–2001	2008–2012 <sup>b</sup>
<b>Edible vegetable oils</b>						
Cottonseed	2415	3195	3628	3701	3510	5900
Olive	1479	1582	1502	2526	2558	2100
Peanut	3505	3136	3729	4180	4296	5700
Rapeseed	1880	2693	7599	11425	13174	15600
Soybean	5540	11283	14574	23665	27029	25100
Sunflower	3975	4717	7263	8289	8333	12000
Corn	265	436	n.r. <sup>c</sup>	1,680 <sup>b</sup>	n.r.	2749
<b>Total</b>	<b>19059</b>	<b>27042</b>	<b>~38,295</b>	<b>55466</b>	<b>~58,900</b>	<b>69149</b>
<b>Tropical Oils</b>						
Coconut	2260	3148	2580	3285	3417	3300
Palm	1480	3578	9467	16973	23676	
Palm kernel	395	569	1238	2202	2906	29800
<b>Total</b>	<b>4135</b>	<b>7295</b>	<b>13285</b>	<b>22460</b>	<b>29999</b>	<b>33100</b>
<b>Animal Fats</b>						
Tallow and grease	4655	5866	6603	8342	8312	8100
Lard	4440	3663	n.r.	5,800 <sup>d</sup>	n.r.	7700
<b>Total</b>	<b>9095</b>	<b>9529</b>		<b>13342</b>		<b>15800</b>

<sup>a</sup>See (50).<sup>b</sup>Predicted annual average production for the stated time period (51).<sup>c</sup>n.r.: not reported<sup>d</sup>See (4).

efficiently. As a result, for example, lard production per 100 pounds of pig live weight was 13.9 pounds in 1959, 10.8 pounds in 1965, and 4.6 pounds in 1983 (46). Such reductions have been achieved by changes in animal breeding and nutrition, and by a movement to younger ages at slaughter, which results in a carcass with less depot fat. In the more economically developed countries, there has also been a trend to reduce lipid consumption in an effort to reduce obesity. Furthermore, an increasing awareness over the past decade of the correlation between the dietary consumption of saturated fats and cholesterol and the incidence of coronary heart disease (52–54) has led to the replacement of animal fats by vegetable oils in many edible applications. Thus, in the United States, for example, animal fats comprised 2.1% of the margarine and 21.2% of the shortening produced in 1984, whereas by the year 2000, animal fat usage in these items had fallen to 0.7% and 7.9%, respectively (50, 55).

Surprisingly, despite increased public awareness of, and stated dedication to, the value of low fat diets, annual per capita edible lipid consumption in the United States has grown in recent years. Between 1991 and 2000, for example, this value rose from 65.5 to 74.6 pounds per person annually. In addition, the sum of lard and tallow consumption over this period rose from 3.2 to 5.9 pounds per person. These trends are attributable to an increase in the consumption in the home of commercially prepared foods, which have a higher fat content and animal fat component, and to an increase in dining in restaurants. The greater use of animal fats in these



**TABLE 10. Prices (cents U.S., per pound) of Selected Commodity Lipids at Randomly Chosen Times in the Period 2000–2003.**

Commodity	March 27, 2000 <sup>a</sup>	October 15, 2001 <sup>b</sup>	June 23, 2003 <sup>c</sup>
Soy oil, refined	18.9	18.6	28.3
Palm, refined	18.0	14.8	32.3
Tallow, edible	12.5	13.0	19.0
Tallow, inedible	10.5	12.0	18.5
Lard	12.3	13.3	19.0
Grease, white	10.0	11.0	18.0
Grease, yellow	7.5	9.3	14.5

<sup>a</sup>See (56).<sup>b</sup>See (57).<sup>c</sup>See (58).

sectors is the result of their historically lower price compared with even the least expensive refined edible vegetable oils, and the belief that in some applications, such as deep fat frying, animal fats impart superior flavor to foods. Thus, it may be that societal factors accompanying affluence can result in unexpected increases in the consumption of animal fats.

Table 10 presents representative recent U.S. price data for lipids of interest here. U.S. values are presented because in many cases they constitute the only, or the most complete, data sets for areas of interest here, and because the United States is among the top producers and consumers of animal fats. Table 10 shows that in the United States, edible tallow and lard are typically priced at 60–70% the price of palm and soy oils. Table 10 also shows that in recent years, inedible tallow has offered a price discount of up to 15% relative to edible tallow over this time period, although the price gap has nearly disappeared recently. Inedible tallow is used in industrial applications. As the price difference between it and edible tallow narrows, it is common to see inedible tallow displaced by the edible material, which often is of higher quality and thus requires less cleanup of starting material or product. When the U.S. fast food industry switched from tallow to vegetable oils for deep fat frying in the mid-1980s, substantial amounts of edible grade tallow flowed into uses that had previously consumed inedible material. Table 10 also illustrates the significantly lower costs of yellow and white grease compared with refined vegetable oils, the latter being nearly twice the price of the greases.

For tallow, although industry identifies and trades in many grades of raw material, cumulative production and use data are listed in terms of only edible vs. nonedible tallow, at best. The United States accounts for approximately half of the annual world production of tallow, with the balance coming primarily from Australia, Canada, New Zealand, Argentina, and Brazil. In the United States, the production of inedible tallow typically is slightly more than double that of edible tallow. Thus, in the year 2000, the U.S. inedible tallow production was 1.7 million metric tons, and edible tallow production was estimated at 0.76 million metric tons (59). In the year 2000, estimated lard production, for use mostly in cooking, was estimated at

only 0.24 million metric tons (59). Grease production rivals that of inedible tallow, and in that year was estimated at 1.5 million metric tons (59).

Edible tallow is used primarily in shortening (i.e., baking and frying fats) and margarine, with additional uses in the chemical, soaps, and personal care products areas. About 30% of U.S. inedible tallow is exported. The remainder is used in animal feeds (48% of U.S. usage in 2002 and rising), for the production of bar soap for washing (6% ), as a source of industrial fatty acids (32%), and for other uses such as textile sizing, leather processing, metalworking, lubrication, and paint production. Substantial growth is occurring in the use of tallow in animal feeds.

In 1999, the top five tallow exporting countries were the United States, with just over 50% of the 2.3-million-ton export market, Australia, Canada, New Zealand, and Germany (60). The top five lard exporting countries were the United States, Germany, Hong Kong, Argentina, and Hungary, with the United States and Germany accounting for nearly 80% of lard exports (60). Although the United States exported 80,000 to 130,000 metric tons of tallow and lard to the EU annually near the turn of the century, none of this was destined for the European food market. Hormones are used in American meat production, and products so produced are banned from edible applications in Europe. Cultural and religious practices and regulations can influence the use of tallow and other animal fats. This may be a result of the existence of prescribed methods of slaughter, as for those following the Islamic and Jewish faiths, or to other dictates. Jewish law forbids the use of pork products, for example, and in Islamic countries, the use of fatty acids from animal sources is forbidden in toiletries (e.g., toothpaste).

Poultry fat production rates are difficult to determine, because there are no formal governmental tallies for this commodity. U.S. industry sources estimate current annual production of rendered poultry fat in that country to be 900,000 metric tons, or about one-tenth of global tallow production (61). This probably represents a sizeable proportion of global poultry fat production. Presently most of this goes into animal feeds. Historically this was the poultry feed market, although recently there has been an increase in use in other animal feeds as well.

Grease production data are often lumped with that for inedible tallow. Greases constitute about 45% of this total and are used exclusively in nonedible applications. In the United States, the use of yellow grease in animal feeds is increasing yearly and in 2000 accounted for 36% of total feed fat usage (62).

## **7. PROCESSING OF ANIMAL FATS**

### **7.1. Rendering**

The fatty tissues separated from meat animals at slaughter and during cutting consist of fat deposited in a connective tissue matrix containing protein and water. To separate the fat from other components, a technology known as rendering (63) has been developed that is based primarily on the melting of the fat and its removal from the nonfat matrix surrounding it. The aim of rendering is to obtain

as complete a separation of the fatty tissue components as is possible. Most rendering systems rely on heat to release fat from the cells of the fatty tissues, in either the absence (dry rendering) or presence (wet rendering) of added water/steam. In other methods, the heat is kept low and the fat is released by mechanical rupture of the cells.

In dry rendering, the chopped fatty tissues are heated to about 121°C, usually in a horizontal steam-jacketed vessel, to disintegrate the fat cells, release the melted fat, and drive off moisture. Most batch cookers for dry rendering are equipped with rotating agitators that may be steam-heated. Agitation aids in heat transfer. After 4 to 5 hours, the fat is released and sufficient moisture is removed. The material is pressed or expelled to recover the lipid, which is strained or filtered to remove cooked proteinaceous residue (the cracklings). Dry rendering can be carried out at atmospheric pressure, under vacuum, or at elevated pressure. The atmospheric pressure method is the most common, with operating temperatures of 115°C to 140°C. It is the method of choice for inedible animal lipids. It offers the advantages, compared with wet rendering, of reductions in water requirements and air and water pollution, and a reduction in odors. In the United States, it is no longer approved for use by the U.S. Department of Agriculture for the production of edible grade fats. Rendering for the production of inedible lipids can use raw materials such as diseased or condemned animals and fats picked up from butcher shops as trimmings or scraps or outdated meat products. The latter can arrive in the polyethylene containers in which they were offered for edible sale. These are often thrown directly into the rendering vessel. If not removed downstream, this polyethylene can later pose a problem to the operation of continuous fat splitters. For this reason, some fat specification sheets list a maximum polyethylene content.

In the low-temperature continuous (dry rendering) process, finely ground fatty material is heated to approximately 38°C and fed to a centrifuge that separates a fat sludge stream from the remaining material, which is largely undenatured protein. The fat stream is heated to approximately 99°C and centrifuged again to recover the majority of the fat. Wet rendering is then used to recover residual fat remaining in the solids stream. The final lipid product of the continuous low-temperature process is very light in color and low in free fatty acids.

In wet rendering processes, the fatty tissues are heated in the presence of water, usually at lower temperatures (82°C to 96°C) than in most dry rendering. Fats rendered at lower temperatures typically have less color and a milder flavor than those that are dry-rendered. Wet rendering is the method of choice for edible fat rendering, often using a steam rendering process. In this method, live steam is injected into a closed vessel containing the fatty tissues, and the rendering takes place under pressure to shorten the cooking time. Lard produced by this process is called "prime steam lard."

Continuous processes have been developed for both wet and dry rendering. They offer the advantages of higher throughput rates and lower operating costs, although batch systems offer better control of product quality. Nonetheless, high-quality edible products can be produced by continuous low-temperature rendering systems. In these systems, the fat is separated from the protein fraction at low temperatures

in a relatively short time. The resulting products are light in color, mild in flavor, and low in free fatty acids. The features and operation of a low-temperature system used for edible fat processing have been described in detail (64).

If edible-grade fatty tissues are handled and rendered properly, the resulting product is suitable for use as a food fat without further treatment. For the better grades of tallow and greases, air is excluded from the melting operation in order to maintain the color and reduce oxidation of the unsaturated fatty acids. Alkali refining can be employed if the free fatty acid content is greater than about 0.3% or if collagen or proteinaceous material are present (65). Edible animal fats also may be subjected to bleaching, hydrogenation, deodorization, interesterification, or fractional crystallization to improve their characteristics or produce fats for specialized use. For the finer grade products, such as bleachable tallows, care is taken to use high-quality raw material from the packing plant operation. Materials such as floor sweepings, catch basin contents, and the carcasses of diseased and condemned animals go to lower grade tallows.

## 7.2. Bleaching

Bleaching is conducted to remove components that give fats an undesirable color, and to decompose peroxides. Most lards do not require bleaching, whereas tallows may be bleached to remove colored materials. Bleaching is usually accomplished by adding natural or acid-activated clays (montmorillonite) and, to a lesser extent, activated carbons. These adsorb the color bodies and certain degradation products in the fats. The clays have a high density of negative charges and act via cation exchange interactions to remove colored compounds, which are generally polar or charged. They also remove metal ion contaminants such as iron and copper, which can serve as pro-oxidants. Specific recommendations for bleaching lard and tallow have been reported (66). For lard, a contact time of 15 minutes at 95–100°C is suggested, with a maximum of 0.5% mildly activated clay or 0.25% moderately activated earth. For top grades of tallow, up to 1% mildly activated clay or about 0.3% well-activated clay is suggested, and a contact time of 20 minutes at 95–100°C is normal. For animal fats, activated carbon tends to be less frequently employed because of its higher cost, although it is often efficacious as an admixture with clay in the treatment of particularly dark samples or to reduce bleaching earth consumption.

Color and peroxide values (PV) have been compared for tallows bleached at atmospheric pressure and under vacuum at several temperatures (67). At 90°C, atmospheric bleaching gave better color than bleaching under vacuum, but peroxide values were lower for the vacuum bleached product (PVs of 2 versus 16, respectively). At higher temperatures, color was better and peroxide values were lower. A bleaching temperature of 90°C to 110°C was recommended for beef tallow.

Treatment of lard with bleaching earth decomposes the peroxides and increases the content of conjugated trienes, which absorb at 268 nm. This characteristic has been the basis of a quality control procedure for determining whether lard has been bleached (66).

### 7.3. Hydrogenation (67, 68)

In some applications, a relatively hard fat is desired. In the process known as hydrogenation, a metal catalyst is employed to add hydrogen to fatty acid double bonds, reducing them to single bonds. As the melting point and solid fat content of lipids increase with the degree of saturation of their fatty acids, this increases firmness. In addition, the elimination of oxidation-prone double bonds increases oxidative stability. The hydrogenation process can be adjusted and controlled, and it is then termed "partial" or "selective," so that various degrees of hydrogenation can be attained. Hydrogenation is also employed in the fatty acid industry to reduce fatty acid nitriles to amines and to produce fatty alcohols from fatty acids, esters, or acylglycerols. These technologies will not be considered here.

As an intermediate product, hydrogenation isomerizes *cis*-double bonds to the more thermodynamically stable *trans*-configuration. It also catalyzes double-bond migration along the fatty acid chain. As *trans*-double bonds confer higher melting points than do their *cis*-counterparts, this isomerization again contributes to increased firmness. *Trans*-unsaturated acylglycerols are desirable in the context of margarine, confectionary, and similar applications because, although solid at room temperature, they have lower melting points than acylglycerols composed of saturated fatty acids. Thus, they can give a more desirable spreading and melting performance than lipids hardened by the presence of saturated acylglycerols. Process technology optimized for the production of *trans*-fatty acids while reducing the yield of fully saturated fatty acids is therefore often employed. However, substantial concern regarding the medical implications of *trans*-fatty acids in the diet has led to questions about the role and future of hydrogenation in edible lipid technology (see discussion in Recent Developments).

Because of their higher contents of unsaturated fatty acids relative to animal fats, vegetable oils are more commonly subjected to hydrogenation technology. This particularly improves their performance as margarines and in baking and frying applications. Being naturally firm, tallows are seldom hydrogenated for edible uses as intact fats, and then only very slightly. Mild hydrogenation of edible tallows delays or prevents the development of undesirable flavors thought to be caused by the peroxidation of trienoic and tetraenoic fatty acids (46). However, for use in the production of mono- and di-acylglycerols, which are widely used as food emulsifiers, animal fats are generally fully hydrogenated, achieving final iodine values less than 1.

Unhydrogenated lard can lack desired melting point or plastic range properties, especially because the melting range characteristics of lard from different animals or animals fed different diets can vary greatly. A consistent product can be obtained by blending lards from different sources or by blending them with lards that have been hydrogenated to almost complete saturation ( $IV < 5$ )(46).

For edible applications, lipids are often used intact. For such uses, the whole fats and oils are subjected to hydrogenation. For industrial uses, lipids are the feedstocks for free fatty acid production. When firmer fractions are needed for industrial applications, the free fatty acids obtained by lipid hydrolysis are subjected to

hydrogenation. Inedible tallows have traditionally been used as an industrial source of saturated fatty acids, and for this application, their fatty acids are extensively hydrogenated. When the economics are favorable, usage can shift to edible tallows because their greater purity makes for easier processing.

Industrial hydrogenation may be conducted in batch or continuous modes. In either case, the engineering challenge is to achieve efficient contact among liquid lipid, solid metal catalyst, and gaseous hydrogen. Batch mode is by far the most common approach in industrial edible oil hydrogenation, being used in more than 90% of applications, although in the future a shift to continuous processes may be seen. Batch reactors can have capacities up to 35 metric tons. To increase turbulence and mixing, they contain rotating central shafts bearing agitators, and they may also have baffles built into the reactor walls. Agitation also aids in temperature control, and it keeps the catalyst suspended. Continuous reactors, with typical outputs of 50–200 tons per day, may move a slurry of oil and catalyst along a pipe, with vigorous introduction of hydrogen at several sites to provide turbulent mixing. Alternatively, hydrogen and oil may be mixed prior to addition of catalyst and then passed through the reactor vessel. Fixed bed systems also exist, in which the catalyst is packed in a column through which the reactants flow.

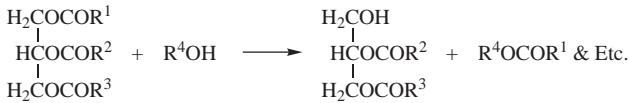
Hydrogenation is conducted under pressure, typically 300–400 psi (20–27 atmospheres [atm]), and at temperatures of 120–200°C. Nickel is most commonly employed as catalyst, and it is produced by the decomposition of nickel salts on either silica or alumina carriers. Platinum is used less extensively and is more costly. These metal catalysts are sensitive to poisoning by such agents as peroxides, water, polyethylene, phosphorus, sulfur, nitrogen, halides, and free fatty acids and their soaps. Inactivation losses increase the cost of hydrogenation. In some cases, impurities in the starting material can be successfully and economically removed by screening or filtration (polyethylene) or treatment with bleaching earth. In other instances, it is expedient to employ a higher quality, cleaner, starting material in order to extend catalyst lifetime.

The reduction of double bonds is an exothermic reaction. Heat control is important during hydrogenation to avoid damage to the product and the equipment. Also, the capture of heat and its reuse to bring subsequent hydrogenation runs up to reaction temperatures is a significant aspect in overall process economics.

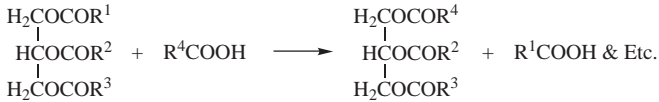
#### **7.4. Deodorization**

Animal fats are subjected to deodorization when a very bland or essentially flavorless fat is desired, such as in margarines or cooking fats. The fats are heated at 200°C to 260°C in the absence of air (to prevent oxidation) and treated with dry steam under a vacuum of 5–10 milliatmospheres. Off-flavor compounds are volatile under these conditions and are captured and removed in the steam stream. In addition to flavor components, free fatty acids, which can also contribute undesirable flavors, and other minor constituents such as peroxides, sterols, sterol esters, tocopherols, and other natural antioxidants are partially or completely removed from the fat by this treatment.

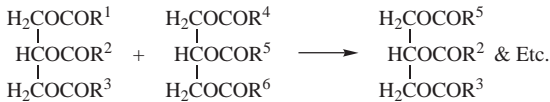
## a. Alcoholysis



## b. Acidolysis



## c. Transesterification (ester interchange, ester rearrangement, interesterification)



**Figure 2.** Types of interesterification reactions of acylglycerols.

## 7.5. Interesterification

The ester bonds of fats and oils are not immutable. Under appropriate conditions, they can be broken and reformed. This allows the replacement of the acid and alcohol components in an ester bond, a class of reactions generally termed interesterification (69–71). Three general types of interesterification reactions can be identified (Figure 2):

1. Alcoholysis, as in “lysis by alcohol,” in which a new alcohol species (R-OH) is exchanged into the ester bonds of the lipid. This converts an acylglycerol into simpler fatty acid esters, as in the reaction of a lipid with methanol to form fatty acid methyl esters. A subset of this reaction type is glycerolysis, where the incoming alcohol is glycerol and the products are predominantly mono- and di-acylglycerols, which see extensive use as emulsifiers. In a sense the lysis of ester bonds by water, hydrolysis, is a form of alcoholysis in which the role of the generic alcohol, R-OH, is played by H-O-H.
2. Acidolysis, lysis by acid, in which a new acid group (R-COOH) is introduced into the ester bonds of the lipid. This results in replacement of the fatty acids within a lipid by other fatty acids. An example would be the acidolysis of tripalmitin with oleic acid to generate a product containing monooleoyldipalmitin, dioleoylmonopalmitin, and triolein.
3. Transesterification, in which fatty acid exchange occurs between esters. For example, the transesterification of tripalmitin (PPP) and triolein (OOO) would produce all positional isomers of POO and PPO. The reaction can be conducted using a single intact fat as the reactant, in which case shuffling

of fatty acids within the component acylglycerols will occur. As the simplest example, if a homogenous sample of POP was subjected to transesterification, the product would contain a mixture of PPO, OPP, and POP as well as PPP, OOO, OOP, POO, and OPO. This reaction can also be conducted with mixtures of natural lipids, as in the interesterification of tallow and sunflower oil. This type of reaction is sometimes referred to as ester interchange, ester rearrangement, or confusingly, interesterification. Of the types of interesterification reactions, transesterification is the one most commonly conducted on edible fats and oils.

Although interesterification will occur in the absence of added catalysts at sufficiently high temperatures, catalysts are employed by industry to speed this reaction, reducing reaction time and the sample degradation that occur at elevated temperatures. The most commonly used inorganic catalysts are alkaline ones such as sodium methoxide, sodium ethoxide, sodium or potassium metal, and alloys of sodium and potassium. Catalyst concentrations of 0.05% to 0.1% are employed. As the catalysts will react with water, free fatty acids, and oxidized compounds, it is important to use clean, dry feedstocks. Reaction temperatures are generally kept below 100°C. The reactions can be run in batch or continuous formats. In batch mode, the reaction times are typically less than an hour.

In the simplest interesterification reaction, termed “nondirected,” fatty acids are rearranged among the acylglycerol species to achieve a fully random, statistically determined population distribution. This can be useful in modifying the performance properties of a natural fat or oil that contains a preponderance of one acylglycerol species, and for this reason has been useful in improving the utility of lard in baking applications. Shortenings for cakes and related products must cream readily to facilitate incorporation of air into the batter. Unmodified lard has a large proportion (64%) of acylglycerols with palmitic acid at the *sn*-2 position, and a relatively high proportion (approximately 27%) of disaturated acylglycerols, mostly oleoylpalmitoylstearin (OPS). These features cause the lard to adopt a  $\beta$  configuration upon crystallization. The resulting large crystals confer a grainy texture and give poor creaming performance. The uniform acylglycerol population also results in an undesirable narrow plastic range. Randomization of lard, in the absence of any additional fats or oils, is the most common application of interesterification technology to animal fats. It decreases the proportion of palmitic acid in the *sn*-2 position of the lard triacylglycerols to 24% (72, 73) and produces a mixture of disaturated acylglycerols with a substantially lower melting point than the original OPS-rich material. Randomized lard is an improved baking fat. It crystallizes in the  $\beta'$  form, which is characteristic of hydrogenated vegetable shortenings, has smaller crystal structures, lacks graininess, and exhibits improved performance as a creaming agent.

In “directed” interesterification, the course of the reaction is shifted away from production of a population of acylglycerols with a fully random fatty acid distribution. This is achieved by a modification of reaction conditions to selectively remove from reaction some of the produced acylglycerol species as they are formed. Most



typically this involves conducting the reaction at a sufficiently low temperature that formed acylglycerols with melting points above that temperature solidify as they are formed and precipitate from the reaction. This approach is used to remove the high-melting trisaturated species from the reaction liquid. It is not that these species are themselves actually isolated and removed as they form. Rather, their precipitation simply prevents the further participation of their fatty acids in the interesterification event. The remaining ester pool is enriched in unsaturated fatty acids. As additional saturated acylglycerols are formed by continued interesterification, they too will be removed from reaction. Thus, as the interesterification-precipitation reactions continue, the acylglycerol population in the fluid phase of the reaction mixture becomes increasingly unsaturated. The final acylglycerol distribution will depend on such variables as the temperature and duration of reaction. The reaction is not run to such an extent as to drive all saturated fatty acids into triunsaturated acylglycerols, as this is both undesirable and time consuming. Rather it is conducted until a sufficient distribution of acylglycerol species is achieved to confer desired performance properties.

Directed interesterification can be employed to produce lard with an increased solids content at high temperatures, because of production of a fully saturated acylglycerol population. Such a product would be plastic over a greater range of temperature. Lard produced by nondirected interesterification requires the addition of stearin for high-temperature stability.

Intesterification reactions can also be conducted between two different natural lipids, generating a product with an acylglycerol content representing the statistical random population predicted by the content of the starting materials. As it provides a means of introducing saturated fatty acids into the acylglycerols of liquid vegetable oils, interesterification of oils with hard fats represents an alternative to partial hydrogenation for the production of plastic fats for margarine. With increasing concerns regarding the negative health implications of the consumption of the *trans*-unsaturated fatty acids generated by conventional hydrogenation, it is possible that this approach will be implemented at the industrial scale for margarine production. The interesterification of sunflower oil with lard and tallow has been described (74). Also, soybean oil has been interesterified with beef tallow to produce a plastic fat suitable for use in making tub-type margarine (75). The interesterified blend of 60% soybean oil and 40% tallow contained 3–3.4% *trans*-fatty acids from the tallow, substantially less than in commercial margarines produced by hydrogenation hardening of vegetable oils.

Biological catalysts can also be used to conduct interesterification reactions. Lipases are enzymes produced by nearly all living organisms to catalyze hydrolysis of the ester bonds of fats and oils, the first step in their metabolism. Lipases can be used as applied catalysts for lipid hydrolysis. However, in low water (microaqueous) systems, with water contents of a few percent or less, they will also catalyze the various interesterification reactions. Lipase catalysis offers several advantages over nonenzymatic catalysts, among them the fact that because they are active at ambient temperature and pressure, lipases reduce energy needs, minimize degradation of the feedstock, and allow reactions on labile polyunsaturated lipids. Various

known lipases exhibit substrate specificity, including specificity regarding the length or degree of unsaturation of the fatty acid they will accept as substrate, or the position on the glycerol molecule at which they will act. These can be exploited to perform directed interesterifications involving positional or fatty acid selectivity.

Considerable recent research has defined conditions for successful use of lipases and other enzymes in numerous lipid modification reactions, including a variety of types of interesterifications (69, 71, 76). For edible applications to date, they have been employed at industrial scales for the production of (1) cocoa butter substitutes, for which disaturated, monounsaturated acylglycerols with the unsaturated fatty acid in the *sn*-2 position are desired (77); (2) to produce human milkfat analogues, where 2-palmitoyl acylglycerols are desired (77); (3) in the synthesis of 1,3- diacylglycerols (78); and in the production of diacylglycerols for edible applications. These reactions employ vegetable oils as feedstocks.

The use of enzymatic catalysis for the interesterification of animal fats has also been reported. For example, blends of tallow and rapeseed oil (79) were interesterified using a commercial immobilized 1,3-specific *Rhizomucor miehei* lipase as a catalyst. The altered composition of triacylglycerols in the interesterified product was reflected in significant changes in solid fat content in the temperature range of 0–45°C. The degree of melting point reduction achieved depended on the mass fraction of the substrates: the lower the mass fraction of tallow, the larger the decrease. Similarly, the solid fat content of tallow was altered by interesterification with high-oleic sunflower oil or soybean oil using lipases with either positional or fatty acid selectivity (80, 81).

Combinations of physical fractionation and enzyme-catalyzed interesterification have been employed to modify lipids. With regard to animal fats, Bhattacharyya et al. (82) employed low (12°C and 15°C) and intermediate (25°C) temperatures in acetone to prepare a high-saturates hard “stearin” and a softer “olein” fraction from tallow. Chemical or enzymatic interesterification of these fractions was then conducted to produce various samples with melting points and solid fat contents similar to shortening, margarine, and vanaspati (a plastic fat, usually prepared by hydrogenation, popular in India, Pakistan, Bangladesh, and some Eastern countries). Similarly, tallow was subjected to directed lipase-catalyzed self-interesterification, in either a batch or continuous mode, to reduce its content of saturated fatty acids (83). Trisaturated acylglycerols synthesized by enzyme action were removed from the mixture by a low-temperature step, resulting in the production of an unsaturate-enriched tallow derivative. However, the process succeeded in elevating the content of unsaturates from 45% to only 57% and required a 14-day incubation for this purpose.

Human milk lipids are unique in that they contain predominantly palmitate at the *sn*-2 position. To produce an infant formula whose lipids resemble those of mothers milk, Yang et al. (84) employed *Rhizomucor miehei* lipase to catalyze the acidolysis of lard, which is rich in 2-palmitoyl acylglycerols, with soybean fatty acids. As the enzyme had tight specificity for reaction at the *sn*-1 and *sn*-3 positions of the

acylglycerols, the 2-palmitoyl structure was preserved, creating a product whose fatty acid composition and acylglycerol structure resembled that of human milk. A process similar to this, but using palm stearin as the source of 2-palmitoyl residues, has been commercialized.

The term "structured lipid" (SL), very broadly defined, refers to acylglycerols whose fatty acid composition or distribution has been altered by enzymatic or non-enzymatic catalysis, or any of a number of biological or physical methods (85). The products of acidolysis and transesterification are thus structured lipids. More specifically, this term is often applied to acylglycerols in which some of the long-chain fatty acids at the *sn*-1 and -3 positions have been replaced by medium-chain length ones, generally caprylic acid (8:0) or capric acid (10:0). In these positions, medium-chain fatty acids are readily released from fats and oils in the gut by the action of (1,3-positionally specific) pancreatic lipase and absorbed. Medium-chain fatty acids are directly metabolized for energy rather than deposited as depot fat. Thus, structured lipids containing them provide readily available energy and have a reduced tendency to foster obesity. They are desirable dietary components for those requiring high-density energy sources, such as athletes and individuals recuperating from burns. The presence of some degree of long-chain fatty acid content in SLs is desirable to provide a source of essential fatty acids, especially linoleic acid (18:2). Substantial work has been conducted to investigate and optimize SL production from vegetable oils. Animal fats have also been shown to be substrates for SL production. Thus, the introduction of caprylic acid into the *sn*-1 and *sn*-3 positions of an unsaturate-rich fraction of tallow (86) or chicken fat (87) and of unfractionated chicken fat (88) have been described.

Cocoa butter is the premier confectionary fat. Its desirable property of sharp melting near the temperature of the tongue, which imparts a cooling sensation, is largely a result of its high content of 1,3-disaturated, 2-monounsaturated triacylglycerols. In an attempt to find economical replacements for cocoa butter, substitutes have been produced from several types of less-expensive lipids by several investigators, some at the commercial scale. Osborn and Akoh (89) enzymatically randomized the fatty acid distribution of tallow and conducted the lipase-catalyzed acidolysis of tallow with a twofold molar excess of stearic acid. The physical properties of the resulting products suggested their potential usefulness as cocoa butter replacements in chocolate type coatings.

Other uses of lipases as catalysts for animal fat modification are considered in the discussion of biodiesel. Despite this variety of research-scale investigations, enzymatic catalysis has not yet been implemented for animal fat modification at industrial scales. This may be a result of the recent general decline in the food use of animal fats or a result of the disadvantages of lipases as catalysts. Among these are the facts that they are relatively expensive compared with conventional catalysts, can tend to be unstable, and require relatively expensive process technologies. Although enzymes can be appropriate catalysts for the production of high-value food, nutraceutical and pharmaceutical products, these factors have to date largely prevented their use for the production of bulk food lipids.

## 7.6. Fractionation

Natural fats are heterogenous in composition, containing acylglycerols with different fatty acid compositions. Each acylglycerol exhibits unique chemical properties, among them melting temperature and solubility in organic solvents, that depend on the size and degree of unsaturation of these fatty acids and their position on the glycerol backbone. Physical fractionation relies on these differences in chemical behavior to isolate specialty subfractions with desirable compositions and performance properties.

The most frequent use of fractionation (90–92) is to separate a natural fat into two general categories: (1) fractions whose acylglycerols are enriched in saturated fatty acids, are firm or solid at room temperature, and are referred to as “stearin”; and (2) fractions whose acylglycerols are relatively rich in unsaturated fatty acids, are liquid at room temperature, and are referred to as “olein.”

The two predominant types of fractionation are termed “dry” and “wet.” Dry fractionation is the oldest, simplest, and most widely practiced approach. In very general terms, it involves melting the lipid, cooling it to some desired temperature below the melting point of the more saturated acylglycerols, and collecting the crystals of this stearin fraction when they form. Contemporary industrial scale crystallizers have capacities between 5 and 50 tons. To operate effectively, substantial attention is required to such details as the triacylglycerol composition and purity of the materials (the latter influences crystal formation), the nature of any pretreatment of the fat, temperature differential between cooling surfaces, and the melt, cooling rate, and degree of agitation employed during cooling and holding. The goal is to obtain large crystal sizes, as these are most readily removed in downstream recovery operations and will carry over the least amount of entrained olein. This method is also termed fractionation from the melt, simple fractionation, or natural fractionation. Recovery of the solid fraction can be by centrifugation or by vacuum or membrane filtration.

The relationship among composition, melting point, titer, and solid fat index of beef tallow and its liquid and solid fractions obtained by dry fractional crystallization has been described (93). This study was conducted with Uruguayan tallow, which has been reported to have a higher titer (43.2–47.8°C) and melting point (45.0–48.8°C) than is average for beef tallow (94).

Alternatively, in a commercial process termed “Lipofrac,” an aqueous solution containing a surfactant and an electrolyte are added such that the solid fat crystals partition into the aqueous phase. This is isolated and heated to melt this stearin fraction, allowing its recovery by centrifugation (95, 96). The Lipofrac method results in higher stearin yields than obtained by dry fractionation using vacuum filtration for product recovery. However, the introduction of more efficient means of recovering the stearin, especially the use of membrane filter presses introduced in the 1980s, to dry fractionation technology has increased the yield of stearin to such a degree that the use of Lipofrac technology has declined.

In wet fractionation, the fat is dissolved in organic solvents, most generally hexane or acetone, and the solution is brought to a temperature suitable to allow

crystallization of a higher melting acylglycerol fraction of desired composition. The crystals are separated by decanting, filtering, or centrifuging. The solvent acts to dilute and reduce the viscosity of the lipid, allowing more effective removal of the included liquid lipid from the solid fat fraction. Thus, this method gives a cleaner solid fat fraction. Crystal nucleation and growth are faster, heat transfer is easier, and the recovered stearin fraction can be washed with fresh solvent to reduce the amount of entrained liquid fraction. However, wet fractionation is more costly than dry fractionation, and it is not presently being implemented at the industrial scale for the fractionation of animal fats.

Multiple fractionation steps at various temperatures can be conducted. Thus, an olein fractionation can be chilled to further isolate a solid component enriched in the least saturated of the unsaturated lipid components of a lipid. Tallow can be fractionated to yield several fractions with different melting ranges. Tirtiaux (97) has described a process for fractionating beef tallow to give products ranging from a very hard stearin (melting point [m.p.] 56°C) to an oil (m.p. less than 20°C). The wet fractionation of beef tallow using acetone has also been described, producing five fractions using a multistep crystallization process (98). Two of the five fractions were crystalline, one was a plastic solid, and two were liquid. The properties of the plastic solid were similar to those of cocoa butter, and this fraction was reported to have excellent compatibility with cocoa butter. However, the process was never commercialized, probably because of complexity, a decline in animal fat food use, and competition from superior fractions produced from palm oil.

Fractionation technologies are much more frequently employed in the processing of vegetable oils, especially palm oil. An estimate of the magnitude of the difference in scopes of the applications can be obtained by examination of the reported new fractionation capacities installed worldwide in the period 1991–1993: >8000 tons per day for palm oil vs. 320 tons per day for all other oils and fats combined (92). Nonetheless, the use of tallow fractions as cost-effective ingredients to substitute for, or extend, cocoa butter has been investigated (99), production of a pourable tallow oil shortening for deep-fat frying has been reported (100), and tallow fractions have been reported to perform well in producing french-fried potatoes (101).

## 7.7. Lipid Hydrolysis (“Fat Splitting”)

Fats and oils are the feedstocks for the industrial production of fatty acids (102). In addition to a major use in the production of bar soaps, these are also employed as starting materials for the production of fatty amines, amides, alcohols, polyoxyethylene esters, and other derivatives widely used in the nonfood industrial sector.

Water alone will hydrolyze fatty acid esters, and the greater its concentration in the lipid phase, the greater its effectiveness in this regard. At temperatures below boiling, the lipid solubility is too low to achieve the concentrations necessary to promote hydrolysis. Under elevated pressures and with the addition of heat, conditions are achieved where the lipid solubility of water is sufficient to achieve acceptable rates of hydrolysis. Accordingly, high-temperature, high-pressure hydrolysis reactions are the prevalent method of industrial fat splitting. In the United States,

the predominant technology, termed the Colgate–Emery process, involves the continuous countercurrent flow of water and fat or oil at pressures of 48–51 atm, and approximately 260°C. Heated liquid lipid is introduced at the bottom of a vertical cylindrical reactor. Heated water enters at the top. As the lipid charge rises through the falling water charge under pressure, a continuous zone of high water solubility in oil forms, below the bulk lipid layer and above the bulk aqueous layer, wherein hydrolysis occurs. Effluent from the column is recovered, free fatty acids from one outlet and an aqueous glycerol stream from the other. Although its high temperatures can degrade polyunsaturated fatty acids, the Colgate–Emery process is useful for the hydrolysis of lipids such as most animal fats that have iodine values less than 120.

Lipases, developed by nature for the hydrolysis of fatty acyl ester bonds, have also been explored for the hydrolysis of fats and oils. However, because of issues of cost, stability, and productivity, they are not presently employed in industrial lipid hydrolysis.

## 8. ANTIOXIDANTS IN ANIMAL FATS

The oxidation of fatty acid double bonds is responsible for the generation of off flavors and performance defects in lipids. Oxidation can be enzymatically catalyzed by, for example, lipoxygenases produced by microbial contaminants. However, nonenzymatic oxidation initiated by nonlipid contaminants is a more frequent danger, as it initiates free radical reactions leading to oxidative decomposition of the lipid. Light can also initiate lipid oxidation, by pathways that may or may not involve free radical mechanisms. In this process, metals and other initiators trigger the removal of a hydrogen from the allylic (i.e., adjacent to a double bond) position of unsaturated fatty acids. The resulting fatty acid free radical can react with oxygen to generate a peroxy radical that can react with other fatty acids, forming hydroperoxides. Breakdown of these generates aldehydes, hydrocarbons, ketones, and alcohols. In edible applications, these are perceived as rancid flavor. More important to industrial applications, further reaction can lead to polymer formation and deposition. To prevent such undesired degeneration, steps such as the removal of air and blanketing with nitrogen are adopted to reduce fatty acid oxidation.

In the context of free radical oxidation mechanisms, antioxidants are compounds able to quench lipid radicals, thereby terminating autooxidation (103). The most popular natural antioxidants are the tocopherols (Vitamin E), which are commercially available for use in this application. Their endogenous levels in animal fats are low, generally two to three orders of magnitude, than in vegetable oils and fats, even after refining and hydrogenation of the latter (104). The tocopherol contents of tallow and lard range from 7 to 27 mg/kg (105).  $\alpha$  Tocopherol is the prevalent isomer present, representing 90.7%, 94.6%, and 69.8% of the total tocopherols in the body fat of beef, lamb, and pork, respectively (104, 106). Increasing the level of tocopherols fed to meat animals has increased the tissue levels of tocopherol, and it has provided some protection against fatty tissue lipid oxidation, even in pig fat,

with its relatively elevated levels of unsaturated fatty acids compared with beef tallow (10).

Lard and tallow respond well to the addition of antioxidants, and numerous studies have been conducted to evaluate the protection afforded to lard by various antioxidants and metal sequestering agents, such as citric acid. These involved either tocopherols or synthetic antioxidants. In the latter category, ethoxyquin, butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) and tertiary-butylhydroquinone (TBHQ) are effective in animal fats. The latter is approved for use in the United States and Canada, but not in Japan, the EU, and elsewhere. In lard, the tocopherols increase in effectiveness in the order:  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  (107). A concentration of 0.02%  $\gamma$  tocopherol was reported to be more effective than the same concentration of BHA or BHT in chicken, pork, and beef fat (108). The oxidative stability of lard was improved by the addition of up to 250-ppm tocopherol, whereas for BHA and BHT, the optimum effective dose was 200 ppm (66). Tocopherols also have the advantage of carry-through properties in baked and fried products prepared from lard, which will reduce oxidative instability in these products (109). In addition, the protective effects of major synthetic antioxidants on lard, and their carry-through effects in products made with lard, have been demonstrated (63). The best combinations of antioxidants and chelating agents for use in animal fats for particular applications has been reported (46). Natural antioxidants other than tocopherols also afford protection to animal fats (111, 112).

## 9. CHARACTERISTICS OF ANIMAL FAT-BASED SHORTENINGS AND FRYING FATS

Although their use in home baking and cooking in the United States has declined greatly in recent years, animal fat-based shortenings are to some extent used in industrial baking and in baking and deep fat frying elsewhere worldwide. Traditionally, the solids content, crystal structure, and working characteristics of lard made it the shortening of choice for pie crust. Vegetable shortenings can, however, be formulated to have characteristics similar to those of lard, and these shortenings have more favorable nutritional characteristics such as less saturated fat and no cholesterol. Deodorized and stabilized lard and tallow are examples of the lowest cost shortenings suitable for cookies (113).

The physical and textural characteristics of North American vegetable and animal fat shortenings have been compared (113). Selected data from this study are presented in Table 11. There was not much difference in the vegetable and animal fat shortenings as far as melting and crystallization temperatures were concerned, but the polymorphic forms were different. The animal fat shortenings were mostly in the  $\beta$  form, except for a tallow and a tallow-vegetable frying fat. The vegetable-based shortenings were mostly in the  $\beta'$  form. The texture of several of the meat-vegetable blend shortenings was comparable with that of the vegetable-palm shortenings, although the meat-vegetable samples had a higher solids content. The lards had high values for degree of deformation at breaking force, whereas the tallows

**TABLE 11. Selected Characteristics of Meat-Fat or Meat-Vegetable Fat Shortenings<sup>a</sup> (reprinted by permission of AOCS).**

Sample and Source	Dropping Point (°C)	Softening Point (°C)	Polymorphic Form	Hardness Index (g/min)	SFC (%) at 20°C (Tempered at 30°C)	Air Content
<i>Shortening</i>						
Lard, U.S.	45.2	44.4	$\beta$	4.8	26.9	2.3
Lard, Canada	40.6	41.9	$\beta \gg \beta'$	5.1	26.2	6.2
Lard, Canada	38.2	37.7	$\beta$	4.5	25.3	10
Tallow-lard, Canada	42.7	42.3	$\beta \gg \beta'$	11.8	27	8.8
Tallow-lard, Canada	42.3	43.7	$\beta$	14.6	28.7	9
Meat-vegetable, U.S.	44.6	45.8	$\beta' \gg \beta$	5.4	25	19.5
Meat-vegetable, U.S.	45.1	46	$\beta' = \beta$	5.9	26.9	21
Vegetable-tallow, Canada	50.6	50.7	$\beta$	8.1	26.6	4
<i>Frying Fat</i>						
Tallow, Canada	45.8	46.4	$\beta'$	8.9	—	—
Tallow-vegetable, Canada	44.8	44.7	$\beta'$	6.9	—	—

<sup>a</sup>See (113).

were not as pliable. Also, shortenings containing high levels of palm oil were able to withstand large deformations without breakage. The tallows and tallow-lard blends were very hard.

As in baking, animal fats were the item of choice for deep frying of foods in the United States until the 1980s. Meat fats exhibit good stability and have generally been economical to use. The flavors they impart have been considered desirable for some foods, as in the flavor added to french-fried potatoes by tallow and to pie crusts by lard. However, concerns regarding the relationship of dietary cholesterol and saturated fatty acid to coronary heart disease have caused the replacement of animal fats with unhydrogenated vegetable oils in U.S. deep fat frying. In other countries, animal fats are still used in frying, such as in the United Kingdom where the use of both tallow and lard is reported (114).

With the emphasis in some parts of the world on a reduction in the dietary consumption of animal fats, the possibility of using blends of animal and vegetable fat has been explored. It has been shown that such blends confer some beef-like flavor notes on fried foods, and that foods fried in straight vegetable oils lack the characteristic flavors imparted by beef tallow. It is presently unclear whether the use of such animal-vegetable fat blends in frying will be widely adopted.

Attempts have been made to extract and concentrate beef fat volatiles using supercritical carbon dioxide (115). Total volatiles were concentrated over controls



by 10–100 fold, with the lowest pressure extraction conditions yielding the highest concentration of volatiles. Similarly, it has been shown that the flavor volatiles of heated pork fat can be fractionated with supercritical carbon dioxide (116).

The cholesterol present in tallow used for frying undergoes oxidative changes (117, 118), and the generated products are found in fried foods. Thus, the presence of cholesterol oxides have been demonstrated in french-fried potatoes at concentrations approximately four times as high as those that existed in the heated tallow used for frying (119, 120), although some of the values may have included contributions from oxidized plant sterols (121).

## 10. RECENT DEVELOPMENTS

Since publication of the most recent prior edition of this chapter in 1996 (122), global events, scientific discoveries, and technological developments have impacted the real and potential use of animal fats in a number of areas. These should continue to affect lipid usage into the future. This section presents an introduction and overview of these developments.

### 10.1. Bovine Spongiform Encephalopathy (BSE)

In 1985, a new disease appeared in cattle, an unavoidably fatal neurological degeneration characterized by weight loss, a decline in milk yield, difficulty in walking, and a nervous appearance. Anatomically, it was shown to result in the formation of small holes throughout the brain, making it appear sponge-like, and leading to the name bovine spongiform encephalopathy, or BSE (123, 124). Because of its symptoms, the affliction is known as mad cow disease. The disease was first detected in the United Kingdom, and since the beginning of the outbreak, over 180,000 cattle have contracted and died from it there (125). This constitutes over 99% of known cases to date. The remaining occurrences have largely, but not exclusively, been limited to Europe. Pigs and poultry are not infected.

Although other theories for the cause of mad cow disease have been proposed, the most accepted one currently is that it is caused by a biochemically novel (and in many ways astounding) infectious agent termed a proteinaceous infectious particle, or prion (126, 127). Prions consist largely of protein, with minor amounts of sphingolipid and polysaccharide. They are not living organisms and in fact lack genetic material. Among other prion-mediated diseases are scrapie in sheep and Creutzfeldt–Jacob disease in humans. Prions are derivatives of natural body proteins synthesized and located in the brain of the host animal and normally serving their functions without incident. These proteins can undergo a change in their three-dimensional conformation, assuming the prion configuration, which renders them resistant to degradation and removal by normal body housekeeping machinery. They thus accumulate in the brain, causing the degeneration characteristic of the disease. This conformation change can occur spontaneously or as a result of genetic damage, and thus it occurs at a low rate in older animals.

Prions also have the ability to trigger conformational change in their normal counterpart proteins in the brain, causing these to also adopt the improper configuration. In this fashion, the prion can essentially replicate itself. Because the conformational structure assumed during prion formation is thermodynamically stable, prions are exceedingly resistant to inactivation (128, 129). In addition, they can be transmitted orally. The effect of these traits is that the disease is not solely dependent on spontaneous generation to infect an individual or population, but it also can be acquired by consumption of infected tissue.

These features, the fact that the disease typically takes 3 to 5 years to manifest itself, and the industry practice of capturing the nutritional and economic value of carcasses by feeding unused components, in the form of meat and bone meal, to succeeding generations of cattle combined to lead to widespread infection within the U.K. population when the disease did appear.

Before the development of full awareness of the BSE epidemic, infected animals continued to enter the human food supply. The degree of urgency of efforts to understand and control the epidemic was increased with the description, in 1996, of a similarly debilitating and fatal human disease, termed variant Creutzfeldt-Jacob disease (vCJD) that appears to be the result of transmission of BSE to humans (130–132). As of late 2002, 117 humans were known to have died from the disease in the United Kingdom (133). Because of the threat not only to the meat supply but also to human health, massive action was taken to halt the spread of BSE, including its designation as a reportable animal disease, the destruction of no less than 3 million head of cattle in the United Kingdom alone, and the adoption of regulations forbidding the feeding of cattle- and sheep- derived material to ruminants. In the 1990s, bans on the feeding of ruminant animal parts to ruminants were enacted in the United Kingdom and to some degree in the other European countries. In the United States and Canada, a ban restricting the feeding to ruminants of materials from any mammalian source (with some exceptions, including material of porcine and equine origin) was enacted in 1997 (134). In December 2000, EC Regulation 2000/766 came into force, banning the use of meat and bone meal in all feeds. This came about after BSE was found in Germany and Spain for the first time. As a result of such regulatory changes, and new surveillance policies, the incidence of BSE has fallen from a high of more than 37,000 reported cases in 1992, all located in the United Kingdom, to a world total of 2179 cases (1144 of these in the United Kingdom) in 2002 (135). Most incidents of BSE have been confined to Europe. Single or double cases detected in Israel, Greece, Canada, the United States, the Falklands, Oman, and Japan are in many cases attributable to the importation of U.K. beef or beef products. In mid-2003, Canada reported its first case of the disease in a reportedly native-born animal, an 8-year-old cow. Given the lengthy incubation period of the disease, it is possible that this animal was infected early in its life by contaminated imported meat and bone meal. In December of that same year, an infected animal was detected in the United States. Subsequent investigations determined that this individual had been imported from Canada.

To further combat the spread of the disease, European regulations categorize animal-based raw material into three classes and establish the appropriate uses for each

class. Only materials fit for human consumption are allowed for food, feed, and oleochemical uses. Nonedible material free of specific risk material (SRM: skull, eyes, brain, and spinal cord of cattle, sheep, and goats) can be used for industrial chemical technical uses. Finally, dead stock and SRM tissue may not be used, and must be destroyed. In addition, the feeding of animal proteins to animals of the same species is banned, and catering waste, which includes waste restaurant fats and oils, cannot be used as animal feed (136). Furthermore, only edible raw material can be used to produce tallow for food, animal feed, fertilizer, and cosmetic products (137).

In an attempt to prevent the further international spread of the disease, importation of animals from infected countries has been banned. Thus, for example, the United States in 1989 banned the importation of live ruminants and most ruminant products from the United Kingdom and other countries having BSE. The ban was extended to European products in 1997 after the discovery of the disease in some countries there.

The causes of the BSE epidemic have not been established with certainty. Scrapie is a similarly prion-mediated disease of sheep, known and present in U.K. sheep for centuries. Factors contributing to the BSE outbreak in cattle are suspected to be the inclusion of scrapie-infected sheep material in the rendering stream and industry changes in the United Kingdom during the 1970s and 1980s that reduced the rigor of heat treatment during rendering, thus (perhaps) allowing the BSE causative agent to persist in the resulting meat and bone meal and infect healthy cattle. In combating the disease, regulations were put in place in the United Kingdom that call for the processing of meat and bone meal at 133°C and 3-atm pressure for 20 minutes, which is believed to inactivate the BSE prion. It is unclear how such treatment will affect the quality of the resulting tallow.

Meat and bone meal has been identified as a vector for transmission in the BSE outbreak. Tallow is also an animal-derived product, is produced largely from cattle, and is a coproduct of MBM production. Thus, concerns existed regarding its health status. After an examination of existing data, the Scientific Steering Committee of the European Commission concluded that normal industrial tallow production processes result in a product that is free of detectable BSE infectivity, even if the source material was highly infective (138). In another study, the rates of prion inactivation during conventional oleochemical processing were determined and used to estimate that the risks of human infection caused by consumption of oleochemical products of bovine origin subjected to hydrogenation or high-temperature-high-pressure hydrolysis were less than the spontaneous rate of appearance of Creutzfeldt–Jakob disease (139). The European Commission considers tallow and its derivatives to be safe. The U.S. Food and Drug Administration has ruled that tallow and other rendered fats are safe, and it specifically omitted them from regulations prohibiting rendered products in feeds for cattle and other ruminants (140). The United Nations World Health Organization (WHO) has examined the issue and has concluded that because prions are proteinaceous, they would partition with the cellular residues of meat and bone during processing. The tallow fraction was therefore judged not a risk to human or animal health (141).

As animal fats are a potential feedstock for biodiesel production, Cummins et al. (142) assessed the danger of a human contracting CJD as a result of the use of tallow as a fuel in diesel engines. They concluded that the risk was several orders of magnitude less than the rate of spontaneous appearance of CJD. Thus, scientific analysis indicates that processed (i.e., rendered) animal fat is not an agent of transmission of BSE. Nonetheless, especially in the United Kingdom, the public remains skeptical. This has in some cases led to less use of animal fats in feed applications. Especially in the United Kingdom, the BSE epidemic has reduced the amount of domestically available tallow (because of condemnation) and increased the use of other lipids in place of animal fats.

The discovery of single infected animals in Canada and in the United States in 2003 reinvigorated the discussion of whether there should be a total ban on the use of mammalian products in animal feeds. Although the BSE situation seems to be under control at this time, new outbreaks could considerably impact the availability and the allowed uses of animal fats.

## 10.2. Use of Fats and Oils as Fuels

Sales restrictions by some petroleum exporting countries in the 1970s stimulated research in the oil-importing countries into the development of non-petroleum fuels for internal combustion engines. This led, in the case of compression ignition (diesel) engines, to a resurgence of interest in the use of vegetable oils and animal fats as fuels. Although Rudolph Diesel himself demonstrated more than a century ago that his engine would run on vegetable oils, the rise of the petroleum industry had largely extinguished research on this topic. Intact fats and oils are generally unsuitable as neat diesel engine fuels without fuel system modification, because they can lead to engine failure. However, conversion of the fatty acids in fats and oils to their simple alkyl esters, now known as biodiesel, produces a fuel that functions well in unmodified engines, while substantially reducing the undesirable emission of unburned hydrocarbons, particulates, carbon monoxide, and sulfur dioxide (143). In addition, biodiesel reduces net emissions of carbon dioxide (a "greenhouse gas" responsible for global warming), reduces dependence on imported energy sources, increases agricultural income, and increases fuel lubricity, thereby reducing wear in close-tolerance fuel injection systems. The removal of sulfur from petroleum diesel fuels will soon be implemented in some countries, including the United States. This process removes lubricity agents naturally present in the fuel. The enhanced lubricity properties of biodiesel may thus stimulate its greater use in the future.

Biodiesel is presently making the transition in many countries of the world from a research curiosity to an accepted alternative to petroleum-based fuel. Europe is the leading region for the production and use of biodiesel, with an estimated 2001 output of 757-million L. Biodiesel production in the United States in 2001 was estimated at 79.5-million L. Production and consumption are rapidly increasing worldwide, with estimates of combined US and European output in 2003 at around 1,628 million liters (144). Present and anticipated use constitutes but a fraction of

total fuel consumption, and it does not eliminate the use of petroleum-based fuels. However, anticipated vigorous future growth in this area has the potential to consume considerable volumes of fats and oils.

The predominant feedstocks for biodiesel production to date have been refined vegetable oils (rapeseed in Europe, soybean in the United States), although it appears that in Britain a subsidy program has had the effect of promoting the use of tallow and greases over vegetable oils (144). The production of biodiesel from animal fats has been reported (145–148), and the suitability of tallow-based biodiesel as an engine fuel has been demonstrated (149–151). Relative to biodiesel produced from vegetable oils, animal fat-based biodiesel offers the advantages of reduced raw material cost, increased cetane value (which improves engine performance), and greater oxidative stability. As raw material costs can contribute over 70% to the cost of biodiesel, the former advantage could be significant. On the other hand, the greater content of saturated fatty acids in animal fats raises the melting points of biodiesels made from them. For example, soy methyl ester has pour and cold points of  $-2^{\circ}\text{C}$  and  $0^{\circ}\text{C}$ , respectively, whereas the corresponding values for tallow methyl esters are  $15^{\circ}\text{C}$  and  $17^{\circ}\text{C}$  (147). In cold climates, this may lead to engine inoperability because of the plugging of fuel lines and filters. For heavy duty diesel engines, this is of concern only during nonoperational periods, because once the engine is running, the fuel recirculation loops keep the fuel fluid by circulating it through the warm engine. However, perhaps due to concerns regarding cold weather performance, the use of animal fats as a biodiesel feedstock has been negligible to date. As demand increases in the future, fat-based feedstocks may be more widely adopted, with such approaches as the use of low blend rates in petroleum diesel, the addition of freezing point depressants, the production of the lower melting esters of branched chain alcohols (147), or the blending of animal- and vegetable-biodiesels being taken to avoid low-temperature performance problems.

Waste greases, largely consisting of spent deep fat frying oils and fats, can also be used as feedstocks for biodiesel production (152–156), and the engine emissions of such fuels are comparable or superior with those of soy oil-based biodiesel (149, 153, 154). Acceptable engine wear and performance were obtained during extended use of grease-based biodiesel blends with petrodiesel in a heavy duty truck (157). As they are generally priced at about half the cost of refined edible oils, waste greases are an attractive feedstock. However, greases contain a higher content of free fatty acids than refined oils. These reduce the level of available alkaline catalyst in conventional transesterification reactions via the production of fatty acid soaps. Soaps can foster problematic emulsion formation during product washing. Thus, either the soaps must be isolated and discarded, representing a loss of potential feedstock and catalyst, or an acid-catalyzed esterification of free fatty acids must be adopted upstream of the conventional alkali-catalyzed acylglycerol transesterification reaction. Either way, a more complicated process is required to produce biodiesel from waste greases than from refined oils (158).

As lipases are able both to esterify free fatty acids and catalyze the alcoholysis of acylglycerols, they can in theory eliminate the need for multiple catalyst systems during biodiesel production from waste greases. Several groups have investigated

lipase-catalysis for the production of simple fatty acid esters for use as biodiesels from waste grease, as well as from animal fats (159–161). In some of these reports, esterification efficiencies in excess of 95% were described. Nonetheless, enzymatic catalysis has yet to be developed to the stage where it is efficient and economical for industrial use in biodiesel production.

As a consequence of increased biodiesel production, increasing amounts of byproduct glycerol will enter the market. There is some concern that this will depress glycerol prices. As glycerol is a valuable coproduct of the splitting of fats and oils to produce free fatty acids, this could negatively impact the economics of fat splitting. For this reason, glycerol utilization research is becoming a priority, although the industrial and market impact of such work has yet to be realized.

Another potential expansion in the use of animal fats (and vegetable oils) as fuels comes in their use to fire burners for steam and hot water production. In this situation, it is not necessary to convert the lipids to their simple alkyl esters, as must be done for use in internal combustion engines. Rather, the intact acylglycerols are used as fuels. They can be acceptable in this application because industrial boiler burners are both simpler and more robust than internal compression engines, and they are also subject to less emissions monitoring and fewer emissions regulations. During some winters near the recent turn of the century, agricultural lipid prices in the United States were in some cases competitive with that of fuel oil. This led to a number of unscientific, and largely unpublicized, tests of lipids as industrial boiler fuels. At least one scientific report of such an investigation has been released, demonstrating that tallow, white and yellow greases, and chicken fat were technically and economically viable fuels, either neat or in blends with U.S. No. 2 fuel oil, in an industrial boiler (162). The direct burning of lipids is more common in Europe, and to the extent that it is adopted more widely could represent a new lipid outlet, especially for low-value animal fats.

Increased consumption of agricultural lipids as fuels, either for boilers or diesel engines, could increase their prices and curtail their availability, impacting their use in traditional applications. All these changes are dependent on the future course of petroleum fuel prices, on research advances to reduce biofuel production costs, and/or on the establishment and continuation of government programs providing financial incentives that foster the use of renewable fuels.

### 10.3. *Trans*-Fatty Acids

The latter half of the twentieth century saw the use of hydrogenation technology to decrease the degree of unsaturation in vegetable oils. This converts the oils to fats (solids at room temperature) and increases their oxidative stability. Hydrogenated fats have been widely adopted in place of solid animal fats in such applications as table spreads, as is illustrated by the increasing consumption of vegetable-based lipids as margarines, as shortenings, and as frying fats for the production of deep fried pastries, potatoes, corn chips, chicken, and so on. In addition, as yellow grease is employed as a component of animal feeds, hydrogenated fatty acids may be incorporated intact into meat, tallow, mutton, lard, poultry fat, and so on that

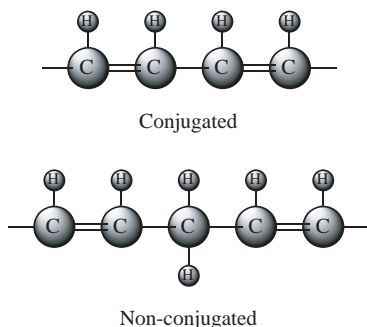
are subsequently consumed by humans. The degree of hydrogenation in these products is typically less than complete, because desired performance is achieved with partial saturation. One outcome of this partial saturation is the conversion of a proportion of the double bonds of the fatty acids from their original *cis*-form to the *trans*-configuration. It has become clear that *trans*-fatty acids are not metabolically equivalent to their *cis*-counterparts, but rather exhibit effects more similar to those of saturated fatty acids. *Trans*-fatty acids raise concentrations of total serum cholesterol, triacylglycerols, LDL cholesterol, and lipoprotein a, and they may lower HDL cholesterol (163–165). All of these effects increase the risk of coronary heart disease. Although ruminant fat also contains *trans*-fatty acids, the levels are substantially lower than are present in industrially hydrogenated lipids. It has been estimated that this increased consumption of *trans*-fatty acids results in 30,000 premature deaths annually in the United States alone (164).

In response to growing awareness in the scientific community and the populace at large of the negative health consequences of *trans*-fatty acid consumption, industry has refined hydrogenation catalysts and processes to reduce the production of *trans*-double bonds (166). Some major lipid-producing firms have voluntarily reduced or eliminated *trans*-fatty acids from their product lines. Following the conclusion by the Danish Nutrition Council that *trans*-fatty acids contribute to cardiovascular disease, to reduced fetal birthweight, and to the development of geriatric (type 2) diabetes, the Danish government as of June 2003 banned the sale of fats and oils with anthropogenically generated *trans*-fatty acid contents greater than 2%. This prohibition was extended to the oils and fats in processed foods in December 2003. In July 2002, the U.S. National Academy of Science issued a report concluding that the only safe intake of *trans*-fat is “zero”. Also in the United States, the *trans*-fatty acid contents of foods are due to be listed on the ingredients label as of January 2006. Actions such as these may renew interest and research in the development and use of animal fats, either neat or after mixing or interesterification with vegetable oils, in foods. This would trigger an increase in the consumption of animal fats.

#### 10.4. Conjugated Linoleic Acid

In general, multiple double bonds in the naturally occurring fatty acids are separated by one or more carbon atoms that are not involved in a double bond. Thus, for example, the two double bonds in the prevalent ( $\alpha$ -) isomer of linoleic acid occupy the 9, 10 and 12, 13 positions (numbering from the carboxyl terminus). The no. 11 carbon, separating these unsaturations, is a methylene carbon ( $\text{CH}_2$ ), being singly bonded to its neighboring carbons. It is also possible for two double bonds to be adjacent to one another, with no intervening methylene carbon, a condition known as “conjugation” (Figure 3). Such double bonds may be of the *cis*-or *trans*-configuration.

Fatty acids possessing conjugated double bonds occur in biological lipids, although their frequency is low. Conjugated linoleic acids (CLAs) are among the most common of the naturally occurring conjugated fatty acids (167, 168). CLA



**Figure 3.** Conjugated and nonconjugated carbon-carbon double bonds. (Used with the kind permission of the Institute of Shortening and Edible Oils, Inc. Washington, D.C.)

is rare to nonexistent in the lipids of plants and most monogastric animals, generally constituting 0.01–0.08% of the total lipid on a weight basis (169). In ruminant fats, however, CLA levels exceed these amounts by 10-fold (170). The most common of the ruminant CLAs is the *cis*-9, *trans*-11 isomer, known as rumenic acid, which constitutes upward from 85% of the total CLA content. In ruminants, two primary origins for CLA have been identified: as an escaped intermediate of the biohydrogenation of dietary linoleic and linolenic acids, and via delta-9 desaturation of vaccenic acid (*trans*-11 octadecenoic acid) generated in the rumen from dietary linoleic and linolenic acids (171). In dairy cows, the desaturase pathway is believed to be the predominant source of CLA.

Studies begun in the 1970s to identify carcinogenic chemicals in common foods found instead that fried hamburger actually contained an antimutagenic agent (172). This was identified as *cis*-9, *trans*-11 conjugated linoleic acid, or *c*-9, *t*-11 CLA, rumenic acid (173). Since then, extensive research has validated and extended these observations in animals and humans (174). Minor amounts of other CLAs are also found in ruminant lipids. The physiological effects of the *trans*-10, *cis*-12 isomer have been studied and have shown to include the reversal of obesity in test animals, and possibly in humans (175). Decreased atherosclerosis, improved hyperinsulinemia in prediabetic rats, and potentiation of the immune response have also been observed as a consequence of the consumption of CLAs.

Given the current widespread interest in reducing cancer, obesity, and other maladies, there is considerable interest in the use of the CLAs, either as a mixture or in the form of individual isomers, as beneficial dietary adjuncts. Cow's milk, beef tallow, and products made from them are natural sources of CLA. However, CLA is also readily synthesized in high yield in the laboratory from vegetable oils that are rich in linoleic acid, such as sunflower and safflower. The resulting synthetic product has CLA levels of about 80%, not the 0.3–0.5% (fat basis) found in beef tallow and dairy products (176). As a result, except for studies of the specific effects of foods containing CLA, vegetable oil is the typical source of CLA in contemporary studies and in commercial dietary supplements. This trend will probably continue.



Although it might trigger some increased consumption of dairy products and meats, it seems unlikely that the continued and increasing interest in CLA will translate to increased use of animal fats.

A continuously updated listing of publications relating to CLA can be found at [www.wisc.edu/fri/clarefs.htm](http://www.wisc.edu/fri/clarefs.htm).

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## REFERENCES

1. B. Rossell, ed., *Animal Carcass Fats*, vol. 2, Oils and Fats Series, Leatherhead Publishing, Leatherhead, U.K., 2001.
2. F. D. Gunstone, and F. B. Padley, *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997.
3. C. C. Akoh and D. B. Min, eds., *Food Lipids; Chemistry, Nutrition, and Biotechnology*, 2nd ed., Marcel Dekker, New York, 2002.
4. M. Bockisch, *Fats and Oils Handbook*, AOCS Press, Champaign, IL, 1998.
5. Darling International, Irving, TX. (2003) <http://www.darlingii.com>.
6. Darling International. (2003). Available: <http://www.darlingii.com/askross/Faq.htm> Fat8.
7. F. T. Orthoefer, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, 5th ed., Wiley, New York, 1996, pp. 19–43.
8. Institute of Shortening and Edible Oils. (1999). *Food Fats and Oils*, 8th ed. Available: <http://www.iseo.org>.
9. M. Enser, in J. B. Rossell and J. L. R. Pritchard, eds., *Analysis of Oilseeds, Fats and Fatty Foods*, Elsevier Applied Science, New York, 1991, pp. 329–394
10. F. J. Monahan, D. J. Buckley, P. A. Morrissey, P. B. Lynch, and J. I. Gray, *Meat Sci.* **31**, 229–241 (1992).
11. L. C. St. John, C. R. Young, D. A. Knabe, L. D. Thompson, G. T. Schelling, S. M. Grundy, and S. B. Smith, *J. Animal Sci.*, **64**, 1441–1447 (1987).
12. C. A. Morgan, R. C. Noble, M. Cocchi, and R. McCartnery, *J. Sci. Food Agric.*, **58**, 357–368 (1992).
13. D. K. Larick, B. E. Turner, W. P. Schoenherr, M. T. Coffey, and D. H. Pilkington, *J. Anim. Sci.*, **70**, 1397–1403 (1992).

14. I. W. McDonald and T. W. Scott, *World Rev. Nutr. Diet.*, **26**, 144–207 (1977).
15. D. C. Rule and D. C. Beitz, *J. Amer. Oil Chem. Soc.*, **63**, 1429–1436 (1986).
16. A. M. Pearson, J. D. Love, and F. B. Shoreland, in C. O. Chichester, E. M. Mrak, and G. F. Stewart, eds., *Advances in Food Research*, vol. 23, Academic Press, New York, 1977, pp. 2–74.
17. J. D. Wood, in J. Wiseman, ed., *Fats in Animal Nutrition*, Butterworth, London, 1984, pp. 407–435.
18. J. A. Marchello, D. A. Cramer, and L. J. Miller, *J. Anim. Sci.*, **26**, 294–297 (1967).
19. M. Enser, Production, Properties and Uses of Edible Oils and Fats in the Food Industry. Animal Fats: Chemical and Physical Properties and Applications/ Training Course Notes, Course 011E1, Leatherhead Food RA, Leatherhead, U.K., 1996.
20. W. E. Connor and S. L. Connor, *Current Atherosclerosis Rep.*, **4**, 425–432 (2002).
21. Anonymous, Nutritional Aspects of Cardiovascular Disease, Report on Health and Social Subjects, British Department of Health, London, U.K., 1994.
22. Anonymous, High Blood Cholesterol: Detection, Evaluation, Treatment, Third Report of the (U.S.) National Cholesterol Education Program (NCEP) on the Detection, Evaluation and Treatment of High Blood Cholesterol in Adults, National Cholesterol Education Program, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland. NIH Publication 02–5215. (2002). Available: <http://www.nhlbi.nih.gov/guidelines/cholesterol/atp3full.pdf>
23. R. Sieber, *Lebensmittel Wissenschaft und Technologie*, **26**, 375–387 (1993).
24. R. D. Kiley, C. T. Massie, A. L. Bachman III, and V. P. Wagher, *Lipid Technol.*, **8**, 5–10 (1996).
25. R. R. Chao, S. J. Mulvaney, and H. Huang, *J. Amer. Oil Chem. Soc.*, **70**, 139–143 (1993).
26. W. W. Christie, in R. J. Hamilton and J. B. Russell, eds., *Analysis of Fats and Oils*, Elsevier Science, New York, 1986.
27. H. Brockerhoff, R. J. Hoyle, and N. Wolmark, *Biochim. et Biophys. Acta*, **116**, 67–72 (1966).
28. W. C. Byrdwell, E. A. Emken, W. E. Neff, and R. O. Adlof, *Lipids*, **31**, 919–935 (1996).
29. H. R. Mottram, Z. M. Crossman, and R. P. Evershed, *Analyst*, **126**, 1018–1024 (2001).
30. H. Kallio, K. Yli-Jokipii, J.-P. Kurvinen, O. Sjoval, and R. Tahvonon, *J. Agric. Food Chem.*, **49**, 3363–3369 (2001).
31. W. E. Neff, W. C. Byrdwell, K. R. Steidley, G. R. List, and G. Snowder, *J. Liquid Chromatogr. Related Technol.*, **25**, 985–998 (2002).
32. A. Thomas, in W. Gerhartz, ed., *Ullman's Encyclopedia of Industrial Chemistry*, 5th ed., vol. A10, Ethanloamins to Fibers, VCH Publishers, New York, 1987, Table 22, p. 215.
33. J. Wang, R. Berthollet, P. Ducret, and M. Fleith, U.S. Patent 6,034,130, (1998).
34. K. Kennedy, M. S. Rewtrell, R. Morley, R. Abbott, and P. T. Gynlan, *Am. J. Clin. Nutr.*, **70**, 920–927 (1999).
35. T. Yang, X. Xu, C. He, and L. Li, *Food Chem.*, **80**, 473–481 (2003).
36. P. J. Lawler and P. S. Dimick, in C. C. Akoh and D. B. Min, eds., *Food Lipids; Chemistry, Nutrition, and Biotechnology*, 2nd ed., Marcel Dekker, New York, 2002.
37. N. Garti and K. Sato, eds., *Crystallization and Polymorphism of Fats and Fatty Acids*, Marcel Dekker, New York, 1988.

38. Anonymous, Color; *FAC Standard Color, AOCS Official Method Cc 13a-43, Official Methods and Recommended Practices of the AOCS*, 5th ed. and updates, American Oil Chemists' Society, Champaign, Illinois, 1997.
39. Anonymous, Color; *Wesson Method Using Color Glasses Calibrated in Accordance with the AOCS-Tintometer Color Scale, AOCS Official Method Cc13b-45*, American Oil Chemists' Society, Champaign, Illinois, 1997.
40. Anonymous, Color; *Lovibond Method Using Color Glasses Calibrated in Accordance with the Lovibond Tintometer Color Scale, AOCS Official Method Cc13e-92*, American Oil Chemists' Society, Champaign, Illinois, 1997.
41. Anonymous, Code of Federal Regulations, Part 319 — Definitions and Standards of Identity or Composition, Chapter III — Food Safety and Inspection Service, Department of Agriculture, Title 9 — Animals and Animal Products, U.S. Government Printing Office, Washington, D. C. (2003). Available: [http://www.access.gpo.gov/nara/cfr/waisidx\\_03/9cfr3319\\_03.html](http://www.access.gpo.gov/nara/cfr/waisidx_03/9cfr3319_03.html).
42. Anonymous, *Codex Standard for Named Animal Fats, Codex-Stan 211-1999*. Food and Agriculture Organization of the United Nations, World Health Organization, Rome, Italy (1999). Available: [http://www.codexalimentarius.net/standards\\_search.asp](http://www.codexalimentarius.net/standards_search.asp)
43. C. Paquot and A. Hautfenne, in *Determination of the Bömer Value (B.V.), Section 2.131, Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th ed. revised and enlarged, Blackwell Scientific Publications, Oxford, 1987, pp. 44–48.
44. R. S. Kirk and R. Sawyer, *Pearson's Composition and Analysis of Foods*, 9th ed., Langman Scientific and Technical, Essex, U.K., 1991, pp. 611–612.
45. M. L. Meara, in R. I. Hamilton and A. Bhati, eds., *Fats and Oils: Chemistry and Technology*, Applied Science, London, 1980, p. 196.
46. L. R. Dugan Jr., in J. F. Price and B. S. Schweigert, eds., *The Science of Meat and Meat Products*, 3rd ed., Food and Nutrition Press, Inc., Westport, Connecticut, 1987, pp. 507–529.
47. American Fats & Oils Association, Available: <http://www.foaonline.org>.
48. The Dupps Co. Available: <http://www.dupps.com>.
49. Anonymous, *BS3919:1987 Specification for technical tallow and animal grease*, BSI British Standards Headquarters, London, U.K. (1987). Available: <http://www.bsi-global.com>
50. U.S. Government Printing Office. (2002). *Agricultural Statistics*. Available: [http://www.usda.gov/nass/pubs/agroz/oz\\_ch3.pdf](http://www.usda.gov/nass/pubs/agroz/oz_ch3.pdf), and previous years 1970, 1980, 1990, 1994, 1997.
51. *INFORM*, **5**, 715 (1994).
52. R. B. Hu, M. J. Stampfer, J. E. Manson, A. Ascherio, G. A. Colditz, F. E. Speizer, C. H. Hennekens, and W. C. Willett, *Am. J. Clin. Nutr.*, **70**, 100–108 (1999).
53. S. Renaud and D. Lanzmann-Petithory, *Curr. Atheroscl. Res.*, **4**, 419–424 (2002).
54. W. E. Connor and S. L. Connor, *Curr. Atheroscl. Res.*, **4**, 425–432 (2002).
55. *Agricultural Statistics*, U.S. Government Printing Office, Washington, D. C., 1994.
56. See Schnell Publishing, *Chem. Mark. Rep.*, **257** (2000).
57. See Schnell Publishing, *Chem. Mark. Rep.*, **260** (2001).
58. See Schnell Publishing, *Chem. Mark. Rep.*, **263** (2003).
59. C. Watkins, *Inform*, **12**, 580–587 (2001).

60. B. Rossell, ed., *Animal Carcass Fats*, vol. 2, Oils and Fats Series, Leatherhead Publishing, Leatherhead, U.K., 2001, pp. 7–9.
61. J. Starkey, unpublished information, U.S. Poultry and Egg Association. Available: <http://www.poultryegg.org>
62. R. H. Rouse, in Multi-State Poultry Feeding and Nutrition Conference, Indianapolis, Indiana, 1998.
63. M. Bockisch, *Fats and Oils Handbook*, AOCS Press, Champaign, IL, 1998, pp. 131–138.
64. G. Pietroszek, *The National Provisioner*, June 27 p. 8–19 (1987).
65. N. O. V. Sonntag, in D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, vol. 2, 4th ed., Wiley & New York, 1979, pp. 289–477.
66. H. B. W. Patterson, *Bleaching and Purifying Fats and Oils: Theory and Practice*, AOCS Press, Champaign, Illinois, 1992.
67. *INFORM*, **1**, 638–644 (1990).
68. W. T. Koetsier, in F. D. Gunstone, and F. B. Padley, *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997.
69. A. Rozendaal and A. R. Macrae, in F. D. Gunstone, and F. B. Padley, *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997.
70. D. Rousseau and A. G. Marangoni, in C. C. Akoh and D. B. Min, eds., *Food Lipids; Chemistry, Nutrition, and Biotechnology*, 2nd ed., Marcel Dekker, New York, 2002.
71. W. M. Willis and A. G. Marangoni, in C. C. Akoh and D. B. Min, eds., *Food Lipids; Chemistry, Nutrition, and Biotechnology*, 2nd ed., Marcel Dekker, New York, 2002.
72. B. Sreenivasan, *J. Amer. Oil Chem. Soc.*, **55**, 796–805 (1978).
73. E. S. Lutton, M. F. Mallery, and J. Burgers, *J. Amer. Oil Chem. Soc.*, **39**, 233–235 (1962).
74. D. Chobanov and R. Chobanova, *J. Amer. Oil Chem. Soc.*, **54**, 47–50 (1977).
75. Y. C. Lo and A. P. Handel, *J. Amer. Oil Chem. Soc.*, **60**, 815–818 (1983).
76. U. Bornscheuer, ed., *Enzymes in Lipid Modification*, Wiley-VCH, Weinheim, 2000.
77. P. Quinlan and S. Moore, *INFORM*, **4**, 580–585 (1993).
78. N. Matsuo, *Lipid Technol.*, **13**, 129–133 (2001).
79. P. Forssell, R. Kervinen, M. Lappi, P. Linko, T. Suortti, and K. Poutanen, *J. Amer. Oil Chem. Soc.*, **69**, 126–129 (1992).
80. T. A. Foglia, K. Petruso, and S. H. Fearheller, *J. Amer. Oil Chem. Soc.*, **70**, 281–285 (1993).
81. A. Rodriguez, E. Castro, M. C. Salinas, R. Lopez, and M. Miranda, *J. Amer. Oil Chem. Soc.*, **78**, 431–436 (2001).
82. S. Bhattacharyya, D. K. Bhattacharyya, and B. K. De, *Eur. J. Lipid Sci. Technol.*, 323–328 (2000).
83. A. D. MacKenzie and D. E. Stevenson, *Enz. Microb. Technol.*, **27**, 302–311 (2000).
84. T. Yang, X. Xu, C. He, and L. Li, *Food Chem.*, **80**, 473–481 (2003).
85. F. D. Gunstone, ed., *Structured and Modified Lipids*, Marcel Dekker, New York, 2001.
86. K. T. Lee, T. A. Foglia, and M. J. Oh, *J. Food Sci.*, **67**, 1016–1020 (2002).
87. K.-T. Lee and T. A. Foglia, *J. Food Sci.*, **65**, 826–831 (2000).
88. K.-T. Lee and T. A. Foglia, *J. Amer. Oil Chem. Soc.*, **77**, 1027–1034 (2000).
89. H. T. Osborn and C. C. Akoh, *J. Food Sci.*, **67**, 2480–2485 (2002).

90. E. Deffense, in B. Rossell, ed., *Animal Carcass Fats*, vol. 2, Oils and Fats Series, Leatherhead Publishing, Leatherhead, U.K., 2001.
91. R. E. Timms, in F. D. Gunstone, and F. B. Padley, *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997.
92. W. Hamm, *Trends Food Sci. Technol.*, **6**, 121–126 (1995).
93. M. A. Grompone, *J. Amer. Oil Chem. Soc.*, **66**, 253–255 (1989).
94. M. A. Grompone, *J. Amer. Oil Chem. Soc.*, **67**, 980 (1990).
95. D. A. Glassner, E. A. Grulke, and J. I. Gray, *J. Amer. Oil Chem. Soc.*, **61**, 1919–1924 (1984).
96. J. P. Seuge, H. F. Vinconneau, *Oleagineux*, **30**, 25–30 (1975).
97. A. Tirtiaux, *J. Amer. Oil Chem. Soc.*, **60**, 473 (1983).
98. F. E. Luddy, J. W. Hampson, S. F. Herb, and H. L. Rothbart (to the U.S. Secretary of Agriculture), U.S. Patent 3,944,585, 1976.
99. H. H. Taylor, F. E. Luddy, J. W. Hampson, and H. L. Rothbart, *J. Amer. Oil Chem. Soc.*, **53**, 491–495 (1976).
100. B. F. Haumann, *J. Amer. Oil Chem. Soc.*, **64**, 789–795 (1987).
101. C. L. DeFouw, M. E. Zabik, and J. I. Gray, *J. Food Sci.*, **46**, 452–456 (1981).
102. N. O. V. Sonntag, in R. W. Johnson and E. Fritz, eds., *Fatty Acids in Industry; Processes, Properties, Derivatives, Applications*, Marcel Dekker, New York, 1989, pp. 23–72.
103. D. B. Min and J. M. Boff, in C. C. Akoh and D. B. Min, eds., *Food Lipids; Chemistry, Nutrition, and Biotechnology*, 2nd ed., Marcel Dekker, New York, 2002, pp. 335–363.
104. R. S. Parker, in J. E. Kinsella, ed., *Advances in Food Research*, vol. 33, Academic Press, New York, 1989, pp. 157–232.
105. R. J. Hamilton, in B. Rossell, ed., *Oils and Fats Handbook, vol. 1: Vegetable Oils and Fats*, Leatherhead Food RA Publishing, Leatherhead, Surrey, U.K., 1999, pp. st.1–8.
106. H. Kanematsu et al. *J. Jpn. Oil Chem. Soc.* (Yukaguba), **32**, 51–53 (1983).
107. J. Griewahn and B. F. Daubert, *J. Amer. Oil Chem. Soc.*, **25**, 26–27 (1948).
108. W. M. Cort, *J. Amer. Oil Chem. Soc.*, **51**, 321–325 (1974).
109. L. R. Dugan, Jr., and H. R. Kraybill, *J. Amer. Oil Chem. Soc.*, **33**, 527–528 (1956).
110. E. R. Sherwin and J. W. Thompson, *Food Technol.* **21**, 912–916 (1967).
111. C. M. Houlihan and C-T. Ho, in D. B. Min and T. H. Smouse, eds., *Flavor Chemistry of Fats and Oils*, AOCS Press, Champaign, Illinois, 1985, pp. 117–143.
112. C. Baniias, V. Oreopoulou, and C. D. Thanopoulos, *J. Amer. Oil Chem. Soc.*, **69**, 520–524 (1992).
113. L. deMan, J. M. deMan, and B. Blackman, *J. Amer. Oil Chem. Soc.*, **68**, 63–69 (1991).
114. B. Rossell, ed., *Animal Carcass Fats*, vol. 2, Oils and Fats Series, Leatherhead Publishing, Leatherhead, U.K., 2001, p. 185.
115. J. A. Merkle and D. K. Larick, *J. Food Sci.*, **59**, 478–483 (1994).
116. M. E. Bailey, R. R. Chao, A. D. Clarke, K.-W. Um, and K. O. Gerhardt, in A. M. Spanier, H. Okai, and M. Tamura, eds., *Food Flavor and Safety: Molecular Analysis and Design*, American Chemical Society, Washington, D. C., 1993, pp. 117–137.
117. S. W. Park and P. B. Addis, *J. Agric. Food Chem.*, **34**, 653–659 (1986).
118. S. W. Park and P. B. Addis, *J. Food Sci.*, **51**, 1380–1381 (1986).

119. T. C. Ryan and J. I. Gray, *J. Food Sci.*, **49**, 1390–1391, 1393 (1984).
120. S. W. Park and P. B. Addis, *J. Food Sci.*, **50**, 1437–1441, 1444 (1985).
121. K. Lee, A. M. Herion, and N. A. Higley, *J. Food Protect.*, **48**, 158–161 (1985).
122. J. A. Love, Animal Fats, in Y. H. Hui, ed., *Bailey's Industrial Oil & Fat Products, vol. 1, Edible Oil & Fat Products: General Applications*, Wiley, New York, 1996, pp. 1–18.
123. M. S. Brewer, *J Muscle Foods*, **10**, 97–117 (1999).
124. Recommended websites: [www.aphis.usda.gov](http://www.aphis.usda.gov); [www.bseinquiry.gov.uk](http://www.bseinquiry.gov.uk); <http://europa.eu.int/comm/food/fa/bse>.
125. Anonymous, U. K. Department for Environment, Food and Rural Affairs. Available: <http://www.defra.gov.uk/animal/bse/indec.html>.
126. S. B. Prusiner, *Science*, **216**, 136–144 (1982).
127. S. B. Prusiner, *Proc. Natl. Acad. Sci. USA*, **95**, 13363–13383 (1998).
128. A. Casolari, *Food Microbiol.*, **15**, 59–63 (1998).
129. K. Danner, *Pharm. Ind.*, **53**, 614–619 (1991).
130. R. G. Will, J. W. Ironside, M. Zeidler, S. N. Cousens, K. Estibeiro, A. Alperovitch, S. Poser, M. Pocchiarri, A. Hofman, and P. G. Smith, *Lancet*, **347**, 921–925 (1996).
131. P. Brown, *Brit. Med. J.*, **322**, 841–844 (2001).
132. J. Collinge, *Lancet*, **354**, 317–323 (1999).
133. Anonymous, *Render, the Bulletin of the National Renderers Association*, **832**, 2 (2002).
134. M. A. Friedman and D. E. Shalala, *Federal Register* **62(108)**, 30935–30978 (1997).
135. Anonymous, Office International des Epizooties. Available: <http://www.oie.int>.
136. Anonymous, Regulation (EC) 999/2001 of the European Parliament and of the Council of 22 May 2001, Laying down rules for the prevention, control, and eradication of certain transmissible spongiform encephalopathies. Available: [http://europa.eu.int/comm/food/fs/afs/marktlab/marktlab14\\_en.pdf](http://europa.eu.int/comm/food/fs/afs/marktlab/marktlab14_en.pdf). See especially Annex V.
137. Anonymous, Regulation (EC) 1772/2002 of the European Parliament and of the Council of 3 October 2002, Laying down health rules concerning animal by-products not intended for human consumption. Available: <http://www.eurits.org/pages/PDFs/Oct02healthrules.pdf>.
138. European Commission, 2000 Preliminary Report on Quantitative Risk Assessment on the Use of the Vertebral Column for the Production of Gelatine and Tallow. Submitted to the Scientific Steering Committee at its meeting of 13–14 April, 2000, Brussels, Belgium.
139. T. R. Appel, D. Riesner, F. Von Rheinbaben, and M. Heinzl, *Eur. J. Lipid Sci. Technol.*, **103**, 713–721 (2001).
140. Anonymous, U.S. Food and Drug Administration, Title 21 — Food and Drugs, Chapter 1 — Food and Drug Administration, Department of Health and Human Services, Part 589 — Substances Prohibited from Use in Animal Food or Feed — Table of Contents, Subpart B — Listing of Specific Substances Prohibited from Use in Animal Food or Feed, Sec. 589.2000 Animal Proteins Prohibited in Ruminant Feed. Available: [http://w3.aces.uiuc.edu/AnSci/BSE/BSE\\_FDA\\_Regul\\_Title\\_21\\_Part\\_589\\_2000.htm](http://w3.aces.uiuc.edu/AnSci/BSE/BSE_FDA_Regul_Title_21_Part_589_2000.htm).
141. World Health Organization, *INFORM*, **12**, 588 (2001).
142. E. J. Cummins, S. F. Colgan, P. M. Grace, D. J. Fry, K. P. McDonnell, and S. M. Ward, *Human Ecologi. Risk Assess.*, **8**, 1177–1192 (2002).

143. M. S. Graboski and R. L. McCormick, *Prog. Energy Combust. Sci.*, **24**, 125–164 (1998).
144. Anonymous, World Biodiesel Review, F. O. Lichts, *World Ethanol and Biofuels Report*, Vol. 1, No. 10, (2003). Available: <http://www.agra-net.com>.
145. C. L. Peterson, D. L. Reece, B. L. Hammond, J. Thompson, and S. M. Beck, *Appl. Eng. Agric.*, **13**, 71–79 (1997).
146. D. Zheng and M. A. Hanna, *Bioresource Technol.*, **57**, 137–142 (1996).
147. T. A. Foglia, L. A. Nelson, R. O. Dunn, and W. N. Marmer, *J. Amer. Oil Chem Soc.*, **74**, 951–955 (1997).
148. K. T. Lee, T. A. Foglia, and K. S. Chang, *J. Amer. Oil Chem. Soc.*, **79**, 191–195 (2002).
149. R. L. McCormick, M. Graboski, T. L. Alleman, and A. M. Herring, *Environ. Sci. Technol.*, **35**, 1742–1747 (2001).
150. Y. Ali and M. A. Hanna, *Trans. ASAE*, **39**, 793–797 (1996).
151. C. L. Peterson, D. L. Reece, B. L. Hammond, J. Thompson, and S. M. Beck, *Appl. Eng. Agric.*, **13**, 71–79 (1997).
152. M. J. Nye, T. W. Williamson, S. Deshpande, J. H. Schrader, and W. H. Snively, *J. Amer. Oil Chem. Soc.*, **60**, 1598–1601 (1983).
153. M. Mittelbach and P. Tritthart, *J. Amer. Oil Chem. Soc.*, **65**, 1185–1187 (1988).
154. W.-H. Wu, T. A. Foglia, W. N. Marmer, R. O. Dunn, C. E. Goering, and T. E. Briggs, *J. Amer. Oil Chem. Soc.*, **75**, 1173–1178 (1998).
155. M. Mittelbach and H. Enzelsberger, *J. Amer. Oil Chem. Soc.*, **76**, 545–550 (1999).
156. G. A. Lowe, C. L. Peterson, J. C. Thomopson, J. S. Taberski, P. T. Mann, and C. L. Chase, ASAE Paper No. 986081, Am. Soc. Automotive Engineers, St. Joseph, Michigan, 1998.
157. C. L. Chase, C. L. Peterson, G. A. Lowe, P. Mann, J. A. Smith and N. Y. Kado, SAE Technical Paper No. 2000–01-2647, Soc. Automotive Engineers, Warrendale, Pennsylvania, 2000.
158. Y. Zhang, M. A. Dube, D. D. McLean, and M. Kates, *Bioresource Technol.*, **89**, 1–16 (2003).
159. M. J. Haas, G. J. Piazza, and T. A. Foglia, in T. M. Kuo and H. W. Gardner, eds., *Lipid Biotechnology*, Marcel Dekker, New York, 2002, pp. 587–598.
160. Y. Watanabe, Y. Shimada, A. Sugihara, and Y. Tominaga, *J. Amer. Oil Chem. Soc.*, **78**, 703–707 (2001).
161. A. Hsu, K. Jones, T. A. Foglia, and W. N. Marmer, *Biotechnol. Appl. Biochem.*, **36**, 181–186 (2002).
162. T. T. Adams, J. Walsh, M. Brown, J. Goodrum, J. Sellers, and K. Das, A Demonstration of Fat and Grease as an Industrial Boiler Fuel, Engineering Outreach Service, University of Georgia, Athens, GA. (2002). Available: <http://www.engr.uga.edu/service/outreach/biofuel.htm>.
163. M. B. Katan, P. L. Zock, and R. P. Mensink, in D. B. McCormick, D. M. Bier, and A. G. Goodridge, eds, *Annu. Rev. Nutr.*, **15**, 473–493.
164. A. Ascherio and W. C. Willett, *Am. J. Clin. Nutr.*, **66**, 1006S–1010S (1997).
165. A. H. Lichtenstein, *Womens Health (Larchmt)*, **12**, 109–114 (2003).
166. J. M. Hasman, *INFORM*, **6**, 1206–1213 (1995).
167. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza and G. J. Nelson, eds., *Advances in Conjugated Linoleic Acid Research*, vol. 1, AOCS Press, Champaign, Illinois, 1999.

168. J.-L. Sebedio, W. W. Christie, and R. Adlof, eds., *Advances in Conjugated Linoleic Acid Research*, vol. 2, AOCS Press, Champaign, Illinois, 2003.
169. J. Fritsche, R. Rickert, and H. Steinhart, in M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza and G. J. Nelson, eds., *Advances in Conjugated Linoleic Acid Research*, vol. 1, AOCS Press, Champaign, Illinois, 1999.
170. J. Fritsche, R. Rickert, and H. Steinhart, in M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza and G. J. Nelson, eds., *Advances in Conjugated Linoleic Acid Research*, vol. 1, AOCS Press, Champaign, Illinois, 1999.
171. J. M. Griinari and D. E. Bauman, in M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza and G. J. Nelson, eds., *Advances in Conjugated Linoleic Acid Research*, vol. 1, AOCS Press, Champaign, Illinois, 1999.
172. M. W. Pariza, S. H. Ashoor, F. S. Chu, and D. B. Lund, *Cancer Lett.*, **7**, 63–69 (1979).
173. Y. L. Ha, N. K. Grimm, and M. W. Pariza, *Carcinogenesis*, **8**, 1881–1887 (1987).
174. M. W. Pariza, in M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza and G. J. Nelson, eds., *Advances in Conjugated Linoleic Acid Research*, vol. 1, AOCS Press, Champaign, Illinois, 1999.
175. U. Riserus, A. Smedman, S. Basu, and B. Vessby, *Lipids*, **38**, 133–137 (2003).
176. S. F. Chin, W. Liu, J. M. Storkson, YU. L. Ha, and M. W. Pariza, *J. Food Composit. Analy.*, **5**, 185–197 (1992).



# 6

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## *Vegetable Oils*

Frank D. Gunstone

### 1. INTRODUCTION

This chapter is concerned with the major and minor vegetable oils. It includes a brief account of the biosynthetic pathways for plant lipids and a description of minor, but important, components present in commercial vegetable oils. This is followed by a description of the major and minor vegetable oils. The major oils are discussed in more detail elsewhere in this work and in another recent book (1). The natural oils do not always meet human dietary requirements and may have to be modified. There is a discussion on what drives modification and of the various ways in which this can be achieved. Finally, some production and trade statistics are provided and discussed.

### 2. BIOSYNTHESIS

#### 2.1. Introduction

This section provides a brief account of the biosynthetic pathways to triacylglycerols in plants, but it requires a preliminary discussion of fatty acid biosynthesis.

The so-called acetate-malonate pathway leads to three different kinds of natural products depending on the detailed pathway followed. Fatty acids result from a reductive pathway to be described here, but acetate and malonate are also precursors for the isoprenoids (terpenes and sterols) produced via mevalonic acid (C<sub>6</sub>) and

for a wide range of phenolic compounds resulting from cyclization of polyacetate. This illustrates the observation that nature is economical in the range of both substrates and reactions employed in biosynthesis.

The major biosynthetic pathways to fatty in plants involve three stages (2–4):

- *de novo* synthesis of palmitic (or other alkanolic) acid from acetate (C<sub>2</sub>, a product of carbohydrate metabolism) by reaction with malonate (C<sub>3</sub>).
- Further chain-elongation of saturated or unsaturated acids by two-carbon units.
- Desaturation. Particularly of stearic acid, first to oleic acid and then to linoleic and linolenic acids.

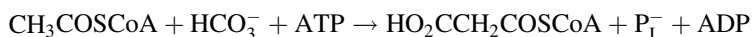
These changes take place in different parts of the cell, under the influence of specific enzymes or enzyme complexes, and they require the acids to be in appropriate substrate form.

## 2.2. *de novo* Synthesis of Saturated Acids

In plant systems, *de novo* synthesis occurs in the plastid and results mainly in the conversion of acetate to palmitate. All 16 carbon atoms in palmitic acid are derived from acetate—half from the methyl carbon and half from the acyl carbon. Two of the carbon atoms (C-15 and C-16) come directly from acetate, and the other 14 come from acetate via the more reactive malonate. Production of malonate requires the incorporation of an additional carbon atom into the acetyl group. This is supplied as bicarbonate, and this same carbon atom is subsequently lost as carbon dioxide. The acyl groups are attached to co-enzyme A (CoASH) during part of the cycle and to acyl carrier protein (ACPSH) during another part. The abbreviated symbols used for these co-enzymes emphasize the thiol groups (SH) to which the acyl chains are attached.

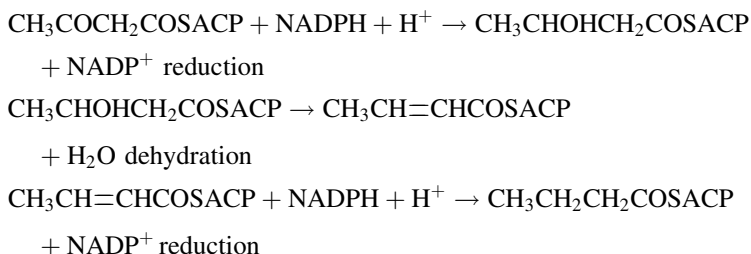
In the *de novo* pathway, acetate and malonate react through a series of steps converting acetate first to butanoate (C<sub>4</sub>), then to hexanoate (C<sub>6</sub>), and then sequentially, two carbon atoms at a time, to palmitate (C<sub>16</sub>). At this stage, a thioesterase liberates the acyl chain from ACP. The thioesterase is not completely specific, and acids of other chain lengths may be produced. This is obviously true in the lauric oils where the specificity of their thioesterases causes lauric acid (12:0) to be the major saturated acid, accompanied by lower levels of caprylic (8:0), capric (10:0), myristic (14:0), and palmitic acid (16:0).

### Conversion of acetyl-CoA to malonyl-CoA with a biotin enzyme (acetyl-CoA carboxylase)



### Conversion of acetyl-ACP to butanoyl-ACP (four-step cycle)





This four-step cycle includes condensation of acetate and malonate to give ketobutanoate with subsequent reduction to butanoate in three further steps. These are reduction to the 3*R* hydroxy acid, dehydration to the 2*t* acid, and reduction again. Reduction is affected by NADPH and a proton. The process is then repeated to add further two-carbon units until a thioesterase liberates the free acid. This sequence requires a fatty acid synthase, which contains the enzymes needed for each of the four steps viz.  $\beta$ -ketoacyl-ACP synthase,  $\beta$ -ketoacyl-ACP reductase,  $\beta$ -ketoacyl-ACP dehydrase, and enoyl-ACP reductase, respectively.

There are several minor modifications of the *de novo* process, but these are not important for the major fatty acids occurring in vegetable oils. They are detailed in more extensive accounts of this topic (2–4).

### 2.3. Desaturation to Monoene and Polyene Acids in Plant Systems

The first desaturation of a saturated acyl chain occurs in the plastid. The most common is the conversion of stearate to oleate and involves the removal of pro-(*R*) hydrogen atoms from C-9 and C-10 to give a *cis*-olefinic bond under the influence of a  $\Delta^9$  desaturase. The system is oxygen-dependent and involves the reduced form of ferredoxin.

Further desaturation, occurring in the cytoplasm, converts oleate, in the form of its phosphatidylcholine, to linoleate ( $\Delta^{12}$  desaturase) and linoleate, as the monogalactosyldiacylglycerol derivative, to linolenate ( $\Delta^{15}$  desaturase). The additional double bonds have *cis*-configuration and are in a methylene-interrupted relation to each other. This 1,4-diene unit is characteristic of polyunsaturated fatty acids and is to be distinguished from the 1,3 (conjugated) systems in carotenoids and the 1,5 system in terpenes. It is of interest that the enzymes converting oleate to the acetylenic acid crepenynic (9*c*12*a*–18:2) and to the epoxy acid vernolic (12,13-epoxyoleic) are very similar to  $\Delta^{12}$  desaturase, which converts oleate to linoleate. A small change in DNA sequence is sufficient to lead to these different fatty acids from the same substrate. The same holds for the conversion of oleate to ricinoleate where a small change alters a desaturase to a hydroxylase (5).

Linoleic acid and linolenic acid are essential fatty acids that cannot be made by animals and must be obtained by dietary intake from plant sources. When metabolized in animals, these two acids each give rise to a family of C<sub>18</sub>, C<sub>20</sub>, and C<sub>22</sub> n-6 and n-3 polyunsaturated fatty acids; thus:

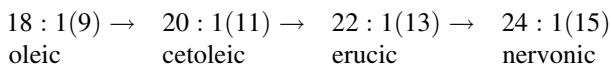
n-6 acids based on linoleic acid      18 : 2 → 18 : 3 → 20 : 3 → 20 : 4.

n-3 acids based on  $\alpha$ -linolenic acid   18 : 3 → 18 : 4 → 20 : 4 → 20 : 5 → 22 : 5  
 → 24 : 5 → 24 : 6 → 22 : 6

Although common in animal systems, the  $\Delta 6$  desaturase is less apparent in the plant world, although it must operate in the production of  $\gamma$ -linolenic acid (6, 9, 12–18:3) from linoleate and of stearidonic acid (6, 9, 12, 15–18:4) from  $\alpha$ -linolenate. The  $C_{20}$  and  $C_{22}$  polyenes that characterize animal systems and particularly fish lipids either do not exist in plant systems or are exceedingly rare. The production of important acids such as arachidonic (5, 8, 11, 14–20:4), eicosapentaenoic (5, 8, 11, 14, 17–20:5), and docosahexaenoic (4, 7, 10, 13, 16, 19–22:6) in plant systems is a challenge for plant geneticists (6).

## 2.4. Elongation

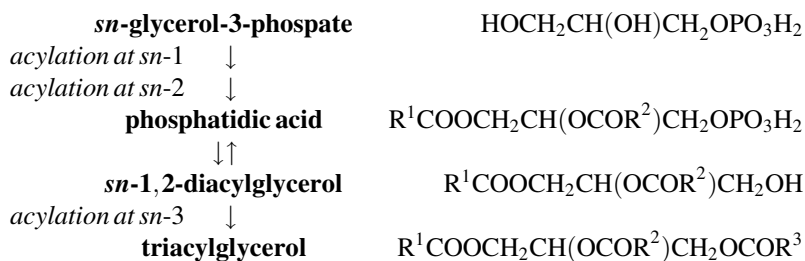
Elongation by two carbon atoms occurs commonly in fatty acid biosynthesis. It is a variant of *de novo* chain-lengthening and occurs with acetyl or malonyl CoA or ACP derivatives. The substrate is any preformed saturated or unsaturated acid. For example, erucic (22:1) in high-erucic acid rapeseed oil and nervonic acid (24:1) in seed oil are formed from oleic acid by two and three elongations, respectively:



## 2.5. Formation of Triacylglycerols

The glycerol present in glycerolipids is derived from glycerol (1,2,3-trihydroxypropane), glyceraldehyde (2,3-dihydroxypropanal), or dihydroxyacetone (1,3-dihydroxypropan-2-one), all of which are products of carbohydrate metabolism. The triacylglycerols are made from phosphatidic acids or 1,2-diacylglycerols, which are themselves interconvertible, and acylation is effected by fatty acids as their CoA thiol esters. The esterification reactions are enzyme-controlled with marked selectivity in the acylation of each hydroxyl group leading to a nonrandom distribution of fatty acids in plant lipids. Saturated acids are selectively bound at the *sn*-1 position and unsaturated acids at the *sn*-2 position. The acyl transferase for the *sn*-3 position is less selective, and fatty acids in this position are largely controlled by the composition of the acyl-CoA pool.

Although not shown in the following simplified scheme, the phosphatidic acid/1,2-diacylglycerol duo is also an intermediate in the pathway to the phospholipids (phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, phosphatidylinositols, and phosphatidylserines) and the mono- and digalactosyldiacylglycerols.



### 3. MINOR COMPONENTS

Crude vegetable oils are mainly triacylglycerols (around 95%) along with some free acids, monoacylglycerols, and diacylglycerols. They also contain variable amounts of other components such as phospholipids, free and esterified sterols, triterpene alcohols, tocopherols and tocotrienols, carotenes, chlorophylls and other coloring matters, and hydrocarbons as well as traces of metals, oxidation products, undesirable flavors, and so on. These are discussed in detail elsewhere. Refining procedures have been developed to convert the crude oil into a bland product that meets a defined specification. Some of the minor components are valuable in their own right and should be retained in the refined oil and/or trapped in a side stream for recovery and further utilization.

Crude oils generally contain phospholipids that are removed during the degumming stage of refining as a crude mixture (lecithin). This valuable product is the basis of the phospholipid industry, and phospholipids are used extensively in food products, in animal feeds, and in industrial processes. The major members are phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositols and are accompanied by smaller proportions of other phospholipids. Soybean oil (3.2%), rapeseed oil (2.5%), and sunflower seed oil (1.5%) contain the proportions of total phospholipids indicated in parentheses and are the main sources of commercial lecithins, especially soya lecithin. Palm oil contains little or no phospholipids (7–9).

Most vegetable oils contain 1000–5000 ppm (1–5 g/kg) of sterols, partly as free sterols and partly as esterified sterols. Higher levels are present in rapeseed oil (5–11 g/kg, mean ~7.5) and in corn oil (8–22 g/kg, mean ~14). Sitosterol is generally the major phytosterol (50–80% of total sterol) with campesterol, stigmasterol, and  $\Delta^5$ -avenasterol also frequently attaining significant levels. Brassicasterol is virtually absent from the major seed oils except for rapeseed oil where it comprises 10% of the total sterol. Cholesterol is generally considered to be a zoosterol and is not present in plant systems at any significant level. The normal value of 20–50 ppm in vegetable oils compares with the much higher levels reported for animal fats (up to 1000 ppm), fish oils (up to 7000 ppm), dairy fats (2000–3000 ppm), and egg yolks (12,500 ppm). Phytosterols (and other compounds) can be recovered from deodorizer distillate and are used to produce pharmaceutical steroids (10).

Tocol extracts are mixtures of up to eight compounds. There are four tocopherols with a saturated, branched C<sub>16</sub> side chain and four analogous tocotrienols with three double bonds in the side chain. The tocotrienols, although significant in palm oil, are generally less common than the tocopherols and much less is known about their biological properties. The four tocopherols differ in the number of methyl groups attached to the heterocyclic moiety. They are designated  $\alpha$  (5,7,8-trimethyl),  $\beta$  (5,7-dimethyl),  $\gamma$  (7,8-dimethyl), and  $\delta$  (8-methyl). The tocols have two valuable properties: They show vitamin E activity, and they are powerful antioxidants. These two properties are not identical. For vitamin E activity, the order is  $\alpha$  (1.0) >  $\beta$  (0.5) >  $\gamma$  (0.1) >  $\delta$  (0.03) with total activity usually expressed in  $\alpha$ -tocopherol equivalents. For antioxidants, this order is reversed.

Natural tocopherol mixtures are used as antioxidants, usually at levels up to 500 ppm, along with ascorbyl palmitate to extend the antioxidant activity. At higher levels (>1000 ppm),  $\alpha$ -tocopherol is considered to act as a pro-oxidant. As vegetable oils contain tocols at 200–800 ppm, further additions show only a limited effect. The tocols are very sensitive to oxidation and are more stable in esterified form where the all-important hydroxyl group is not free. However such compounds do not show antioxidant activity until they have been hydrolyzed *in vivo* to the free phenolic form (11).

Hydrocarbons are very minor components of oils and fats but are of dietary and legislative interest. They include alkanes, alkenes such as squalene and carotenes, and polycyclicaromatic hydrocarbons. Squalene (C<sub>30</sub>H<sub>50</sub>) is a highly unsaturated open-chain triterpene. It is used in the cosmetic industry after hydrogenation to squalane (C<sub>30</sub>H<sub>62</sub>). The most abundant source of squalene is the liver oil of the deep-sea dogfish (*Squalus acanthus*—hence the name squalene) and some other marine species. Vegetable sources of potential interest include olive oil and amaranthus (Section 6).

Carotenes are minor components in many vegetable oils and particularly in palm oil. They contain a long chain of conjugated unsaturation and are yellow/orange/red in color. Crude palm oil normally contains 500–700 ppm of carotenes. These are mainly  $\alpha$ -carotene (24–42% of total carotene) and  $\beta$ -carotene (50–60%) along with low levels of several other carotenes. Carotenes are also present in palm leaves and in pressed fiber remaining when oil has been expressed from palm fruits. Attempts have been made to retain these valuable materials in refined palm oil (red palm oil) or to recover them in concentrated form (12). Carotenes can be recovered from palm methyl esters. The esters are prepared by methanolysis of palm oil and are produced in large quantities for use as biodiesel, as a solvent, for conversion to alcohols, and so on. The carotenes can be recovered from the esters by chromatography in an open column or by molecular distillation. The latter gives a carotene concentrate (8%) which can be purified (>90%) by chromatography (13–15). The concentrates are used as food-dyes, as a vitamin additive, and by the pharmaceutical and cosmetic industries.

Polycyclic aromatic hydrocarbons are present at levels up to about 150  $\mu\text{g}/\text{kg}$  (ppb) in most crude vegetable oils, although slightly less after refining (<80 ppb). They are removed only to a small extent during bleaching and somewhat more

during deodorization. This holds more particularly for the more volatile tri- and tetracyclic compounds. The pentacyclic and other less volatile compounds are best removed with activated charcoal added to the earth during bleaching. These low values do not hold for crude coconut oil dried with combustion gases where values around 3000 ppb are routinely recorded. Normal values are obtained after charcoal treatment (16). Extracted oils may contain pesticides resulting from agricultural practices, but these are usually removed during deodorization.

## 4. CLASSIFICATION OF VEGETABLE OILS

### 4.1. Classification by Source Type

One market analyst provides regular information on the production and trade of 17 commodity oils and fats. In Oil World Publications (ISTA Mielke GmbH, Hamburg, Germany), these are listed in the order: soybean, cotton, groundnut, sunflower, rapeseed, sesame, corn, olive, palm, palm kernel, coconut, butter, lard, fish, linseed, castor, and tallow. The list includes 4 materials of animal origin and 13 of vegetable origin. This chapter will report on the 13 vegetable oils and some others of plant origin.

The above list does not include cocoa butter nor minor oils such as rice bran oil or safflower oil. Nor does it distinguish between oils from a common botanical source with a modified fatty acid composition, such as canola oil and high-erucic rape seed oil, linseed oil and linola, or the various types of sunflower oil.

The commodity vegetable oils can be classified in various ways. Some are byproducts so that decisions regarding their production are largely controlled by the nonoil component. Examples are corn oil and cottonseed oil. These are byproducts of cereal and fiber production, respectively. Also, rice bran oil is a byproduct of rice production. As a consequence, oil production is not the main economic factor that influences the areas cultivated by these crops. It is sometimes argued that soybean falls into this byproduct category and that the bean is grown as a source of protein with the oil as byproduct. It is true that the bean produces only 18% of oil against 79% of residual meal rich in protein, but the value of these two commodities is evenly balanced and there are times when the demand is oil-led and other times when it is meal-led.

Some commodity oils and fats such as palm, palm kernel, coconut, and olive are tree crops. Once the trees mature, they continue to produce fruit for many years and production levels cannot be greatly changed from season to season.

This leaves the annual crops of rape, sunflower, and linseed (often called soft oils) where planting decisions are made on a year-to-year basis depending on perceived benefit. Planting areas are decided on agricultural grounds (such as crop rotation) and on economic grounds. Low prices and/or high stocks in one season tend to lead to reduced plantings in the following season.

It is also possible to distinguish between oils from seeds, such as soybean and rapeseed, and those coming from the fleshy part of a fruit such as palm and olive. An important point here is that for oilseeds, exports and imports are as seeds as well

as extracted oil, whereas for the other group, trade is confined to extracted oil. When making comparisons, it is not sufficient to consider figures for traded oil without also including the oil-equivalent of traded seeds.

The annual production of soybean oil exceeds that of palm oil, but it is claimed that trade (imports/exports) in palm oil is larger. This view, based on the fact that trade in palm oil far exceeds that in soybean oil, does not take into account the very large exports of soybeans themselves. What are the numbers if exported beans are also considered in terms of their oil-equivalent? The following figures for year 2000/01 are taken from *Oil World Annual 2001*. For soybean, 7.4 million tons<sup>1</sup> were exported as oil and a further oil-equivalent of 7.8 million tons was exported in the form of beans. This latter figure is based on exports of 50.0 million tons of beans, of which 85.2% was crushed (world average) with an oil content of 18.3%. On this basis, the full level of exported soybean oil is about 15.2 million tons. This is still lower than the figure for palm oil (16.8 million tons), but the disparity is not so large (17).

The term *tropical oils* is correctly applied to oils and fats produced in the tropics and refers particularly to the (highly saturated) lauric oils (Sections 5.3 and 5.10) and to palm oil (Section 5.9). This term is frequently and unfairly used in a derogatory sense, partly through ignorance about the difference in fatty acid composition and use between lauric oils and palm oil and of the considerable nutritional value of the latter.

Castor oil is classed as an industrial oil because it is used only for nonfood purposes (Section 5.1). Linseed oil also is used almost entirely for industrial purposes. In its limited use as an edible oil, it is generally known by its alternative name of flaxseed oil (Section 5.7).

## 4.2. Classification by Fatty Acid Composition

The list of natural fatty acids exceeds 1000, but commercial interest is limited to a smaller number—perhaps around 20. Ignoring the lipid membrane, rich in  $\alpha$ -linolenic acid and present in all green tissue, the three dominant acids in the plant kingdom are palmitic, oleic, and linoleic, sometimes accompanied by stearic acid and by linolenic acid. Others, occurring in specialty oils, include myristic, lauric, erucic, hexadecenoic, petroselinic,  $\gamma$ -linolenic acid, eleostearic and isomers, ricinoleic, and vernolic (Table 1).

Although it is convenient to categorize oils by their fatty acid composition, it must be remembered that this is not the only index of their nutritional value or of their oxidative stability. Attention must also be given to the minor components in the crude oil and to those remaining after refining (see Section 3).

<sup>1</sup> The figures cited in tons in this chapter have been taken from publications using tonnes, and at the editor's request, the numbers have not been adjusted: 1 ton is equivalent to 0.984 tons, and the numbers are not significantly different in the present context.



**TABLE 1. Structures of the More Common Acids in Vegetable Oils.**

Trivial Name	Symbol	Unsaturation (if any)
<i>Saturated</i>		
Lauric	12:0	—
Myristic	14:0	—
Palmitic	16:0	—
Stearic	18:0	—
<i>Monounsaturated</i>		
Oleic	18:1	9c
Petroselinic	18:1	6c
Erucic	22:1	13c
<i>Polyunsaturated (non-conjugated)</i>		
Linoleic	18:2	9c12c
Linolenic ( $\alpha$ )	18:3	9c12c15c
Linolenic ( $\gamma$ )	18:3	6c9c12c
<i>Polyunsaturated (conjugated)</i>		
Eleostearic	18:3	9c11t13t
Calendic	18:3	8t10t12c
<i>Oxygenated</i>		
Ricinoleic	18:1	12-OH 9c
Vernolic	18:1	12,13-epoxy 9c

**TABLE 2. (a) Typical Fatty Acid Composition (%wt).**

Oil source	16:0	18:0	18:1	18:2	18:3
Cocoa butter	26	34	35	—	—
Corn	13	3	31	52	1
Cottonseed	27	2	18	51	Tr
Groundnut	13	3	38	41	Tr
Linseed	6	3	17	14	60
Olive	10	2	78	7	1
Palm	44	4	39	11	Tr
Palm olein	41	4	31	12	Tr
Palm stearin	47–74	4–6	16–37	3–10	—
Rape (high erucic)*	3	1	16	14	10
Rape (low erucic)	4	2	56	26	10
Rice bran oil	20	2	42	32	—
Safflower	7	3	14	75	—
Safflower (high oleic)	6	2	74	16	—
Sesame	9	6	41	43	—
Soybean	11	4	22	53	8
Sunflower	6	5	20	60	Tr
Sunflower (Sunola)	4	5	81	8	Tr
Sunflower (NuSun)	4	5	65	26	—

Adapted from Gunstone (11).

tr = trace (&lt;1%).

\* 20:1 6% and 22:1 5%.

**TABLE 2. (b) Typical Fatty Acid Composition (%wt) of Lauric Oils.**

Oil source	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2
Coconut	8	7	48	16	9	2	7	2
Palm kernel	3	4	45	18	9	3	15	2

Adapted from Gunstone (11).

**TABLE 3. Vegetable Oils by Fatty Acid Type.**

Acid(s)	Vegetable Oil
Lauric	coconut, palm kernel
Palmitic	palm, cottonseed
Oleic/linoleic	groundnut, safflower, sesame, sunflower, cottonseed, canola, soybean
High oleic	olive, safflower, sunflower, canola, groundnut, soybean
Linolenic	linseed, canola, soybean
Vegetable butters	cocoa butter
Erucic acid	HEAR*, crambe
Conjugated acid	tung, calendula
Oxygenated acids	castor, vernolic

\*HEAR high erucic acid rapeseed oil.

Most of these oils are described in more detail elsewhere in this work. Production and trading figures are discussed in Section 10 (see Tables 2 and 3).

**4.2.1. Lauric Oils** There are two major lauric oils—coconut oil and palm-kernel oil. Both are tropical oils, and both are tree crops. They differ from all other commodity oils in their higher level of medium chain acids, especially lauric, and slightly from one another as shown in Table 2(b). They find limited use in food products and are used extensively in the production of surface-active compounds. For more information, see Sections 5.3 and 5.10.

**4.2.2. Palmitic Acid Oils** The commodity oil richest in palmitic acid is palm oil (44%). This oil is also rich in oleic acid (37%), contains lower levels of linoleic acid (10%), and is a valuable source of minor components, especially carotenes, tocopherols, and tocotrienols (Section 3). Palm oil is an important world commodity in feeding the developing world. It is fractionated extensively to give a wider range of uses as palm olein and palm stearin. The only other commodity oil with a significant level of palmitic acid is cottonseed oil (27%).

To produce high-quality spreads, the solid portion should be in the  $\beta'$  form and this is most likely when the solid triacylglycerols contain acids of varying chain length—generally  $C_{16}$  and  $C_{18}$ . For this reason, palm oil or cottonseed oil are frequent components of the blend used to produce spreads.

**4.2.3. The Oleic/Linoleic Acid Group** This is the most common type of vegetable oil and includes peanut or groundnut (38% oleic and 41% linoleic acid), safflower (14% and 75%), sesame (38% and 45%), and sunflower (20% and 69%). The sum of these two acids is generally 80–90% so there can only be low levels of saturated or other acids. At the present time, there is a demand for high oleic oils, so variants of these oils enriched in oleic acid have been developed (Section 4.2.4). Cottonseed (18% and 51%) differs from the others cited here in its higher level of palmitic acid. Low-erucic rape/canola (56% and 26%) and soybean oil (22%

and 53%), while belonging to this oleic/linoleic group, also contain linolenic acid at levels of around 10% and 8%, respectively.

**4.2.4. High Oleic Oils** Olive oil is an important high-oleic oil (78%). It is generally consumed in an unrefined state and therefore retains all of the natural unsaponifiable material, including valuable antioxidants. Other high oleic oils have been developed by traditional breeding methods or by genetic engineering. These include variants of regular safflower (77% oleic acid), sunflower (80–90%), canola (78%), peanut (76%), and soybean (79–86%) (See appropriate entries in Section 5).

**4.2.5. Linolenic Acid Oils** The most familiar high-linolenic acid oil is linseed (50–60%), but rape/canola and soybean are important commodity oils containing linolenic acid at 10% and 8%, respectively. This triene acid has both positive and negative connotations. It is very easily oxidized, and its oxidation products have strong undesirable flavors. It therefore contributes to a shortening of shelf life and is not favored by food processors for this reason. Hence, these last two oils are frequently subjected to brush hydrogenation (at least) to reduce the level of linolenic acid. Also, seed breeders are striving to produce low-linolenic forms of rape/canola and soybean. However, nutritional scientists tell a different tale. There is a growing awareness that the present ratio of dietary n-6 to n-3 acids at between 5 and 10 to 1 is too high and should be changed to the lower end of this range or below. This is particularly a problem for those countries whose diet contains high levels of n-6 PUFA oils as in the United States (18, 19).

**4.2.6. Vegetable Butters** Most fats/oils derived from vegetable sources are liquid, reflecting the unsaturated nature of most of their component acids. The few that are solid (i.e., have melting points above ambient temperature) are known as butters. The best-known and most important member of this class is cocoa butter (Section 5.2), which is the major or only, fat component in chocolate. Others discussed in Section 6 include illipe butter (Borneo tallow), kokum butter, mango kernel fat, sal fat, and shea butter. These along with palm oil are, in some countries, permitted replacements, in part, for cocoa butter in chocolate (20, 21).

**4.2.7. Erucic Acid Oils** Traditional rapeseed oil (colza), which was used as an illuminant as well as for food, was rich in erucic acid (22:1). Certain findings with animals suggested that this was not a healthy oil, and although the matter was never proved for humans, new varieties of rape (canola) were developed, principally in Canada, with virtually no erucic acid. However, erucic acid and its oils have a number of oleochemical uses and there is a continuing if limited demand for erucic oils. Crambe oil (*Crambe abyssinica* and *C. hispanica*) is being developed as an alternative erucic-rich oil. A major use of erucic acid involves the formation of erucamide (RCONH<sub>2</sub>), which acts as a nonslip agent in polythene and polypropylene. Some 30,000 tons of this is used each year in “clingfilm” and related materials. Oleamide can be used as an inferior alternative (22–25).

**4.2.8. Conjugated Acid Group** Most of the important polyunsaturated fatty acids such as linoleic and linolenic are 1,4-dienes with methylene-interrupted unsaturation, and this structural unit relates to many of the characteristic properties of these acids. However, a smaller number of vegetable oils have acids with conjugated unsaturation. Most of these are C<sub>18</sub> acids with three double bonds, although some have four. The best known acid in this group is  $\alpha$ -eleostearic acid (9c11t13t-18:3). This is a major component of tung oil (china wood oil, *Aleurites fordii* and *A. montana*), which contains ~70% of eleostearic acid. The oil polymerizes readily. Calendula oil (*Calendula officinalis*) is being developed as a source of calendic acid (8t10t12c-18:3) (Section 6).

**4.2.9. Hydroxy and Epoxy Acid Group** Although a number of oils contain acids with hydroxy, epoxy, or oxo (keto) functions, only one is readily available. Castor oil contains over 90% of ricinoleic acid (12-hydroxyoleic acid) and about 1% of 9,10-dihydroxystearic acid (section 5.1).

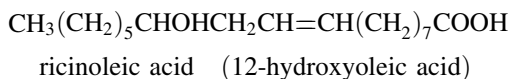
Some contain vernolic acid (12,13-epoxyoleic acid). This has several potentially useful properties, and attempts are being made to produce an economically viable crop.

## 5. THE MAJOR VEGETABLE OILS AND FATS

There follows a brief account of the commodity oils. This includes the nature of the oil and any special features, its production levels, and major areas of production. Most of these oils are discussed in greater detail elsewhere in this series and in Gunstone (1). Triacylglycerol composition is indicated by three-letter symbols that include all of the isomeric triacylglycerols containing the acids designated where P = palmitic, St = stearic, S = saturated, O = oleic, L = linoleic, and Ln = linolenic.

### 5.1. Castor Oil

Castor oil is derived from the plant *Ricinus communis* grown mainly in India, Brazil, and China at a world production level of about 0.5 million tons of oil. This oil differs from all other commercial oils in being rich in ricinoleic acid (~90%, 12-hydroxyoleic). Compared with the common vegetable oils, castor oil is more viscous, less soluble in hexane, and more soluble in ethanol, all as a consequence of the presence of the hydroxy acid. This hydroxy acid has several interesting properties by which it can be converted to useful products.



- Sulfation converts the hydroxyl group to a sulfate ( $-\text{OSO}_2\text{OH}$ ) with improved surfactant properties. Apart from soap, it is the earliest anionic surfactant (dating back to 1874) and is still used in textile processing, leather treatment, and as an additive for cutting oils and hydraulic fluids. The sulfated hydrogenated oil has the consistency of an ointment and gives adjustable viscosity to water-based formulations with excellent skin compatibility.
- Dehydration of castor oil and of castor acids gives products enriched in diene acids, some with conjugated unsaturation. These products are valuable alternatives to drying oils such as tung oil.
- Hydrogenated castor oil and hydrogenated castor acids, with higher melting points than the nonhydrogenated material, are used in cosmetics, coatings, and greases. Greases prepared from tallow are much improved when salts of 12-hydroxystearic acid are added.
- Castor oil reacts with isocyanates to give polyurethanes that are much used for wood preservation and have been developed as encapsulating materials.
- Splitting ricinoleic acid with caustic soda gives  $\text{C}_8$  and  $\text{C}_{10}$  products. At 180–200°C with a 1:1 caustic/castor ratio, the major products are 2-octanone and 10-hydroxydecanoic acid. At 250–275°C and a 2:1 ratio, the products are 2-octanol and sebacic (decanedioic) acid. The dibasic acid, when reacted appropriately, gives a nylon (polyamide) and efficient lubricants (esters).
- Splitting ricinoleic acid with steam gives  $\text{C}_7$  and  $\text{C}_{11}$  products. This splitting process has been much improved by the development of a continuous steam cracking process. Heptanal is used in perfumes, and 10-undecenoic acid can be converted to a polyamide (Rilsan) while its salts show antifungal properties. Several new uses developed for these  $\text{C}_7$  and  $\text{C}_{11}$  compounds have been described by Caupin (26).

## 5.2. Cocoa Butter

The cocoa bean (*Theobroma cacao*) is the source of two important ingredients of chocolate: cocoa powder and a solid fat called cocoa butter. The usefulness of cocoa butter for this purpose is related to its fatty acid and triacylglycerol composition. The major triacylglycerols are symmetrical disaturated oleic glycerol esters of the type SOS and include POP (18–23%), POST (36–41%), and StOST (23–31%). Cocoa butter generally commands a premium price, and cheaper alternatives have been developed. These are known as cocoa butter alternatives, cocoa butter equivalents, cocoa butter improvers, cocoa butter replacers, and cocoa butter substitutes. They may or may not have similar chemical composition to cocoa butter, but they must display similar melting behavior. The annual production of cocoa beans is about 2.7 million tons containing 45–48% cocoa butter, i.e., 1.2–1.3 million tons of fat. Most fats/oils derived from vegetable sources are liquid, reflecting the unsaturated nature of most of their component acids. Only a few are solid (i.e., have melting points above ambient temperature). The best-known and most important member of this class is cocoa butter. This is the major fat component in chocolate.

To be a satisfactory ingredient in chocolate, the fat must have certain defined melting behavior. It must be hard and brittle at ambient temperature, have a steep melting curve, and be completely melted at mouth temperature. These properties give a cooling sensation on the tongue. In addition, the chocolate should break with a snap. This behavior is associated with triacylglycerols with the structure SOS. A few less well-known fats have similar composition and properties. Other fats with similar properties can be produced more economically. These can be used in confectionery fats, but the composition of chocolate is now defined by law, although not identically in all countries. The degree of substitution of cocoa butter by other hard fats is limited both in the fats that can be used and in the level at which they may be added. This varies between zero and a maximum of 5% of the product. The permitted list is palm oil, illipe butter (Borneo tallow), kokum butter, mango kernel fat, sal fat, and shea butter, which are detailed in Section 6 (20, 21, 27).

### 5.3. Coconut Oil

Coconut oil from the coconut palm (*Cocos nucifera*) is one of two important lauric oils (see also palm-kernel oil, Section 5.10). Annual production exceeds 3 million tons and comes mainly from Indonesia and the Philippines (Section 10). It is characterized by its high level of lauric acid (12:0) accompanied by the 8:0–14:0 acids. A detailed fatty acid composition of this oil is given in Table 2(b). The oil is used in the food industry and in the nonfood industry. In the latter case, it is used mainly as derivatives of the corresponding alcohols (dodecanol or coco alcohol).

Attempts are being made to develop oils of various cuphea species rich in one or the other of the  $C_8$  to  $C_{14}$  acids. (Section 6). Genetically modified rapeseed oil with lauric acid is also available but has not yet proved to be economically viable (28–30).

### 5.4. Corn Oil

Corn oil is a major vegetable oil with an annual production of around 2 million tons obtained from corn or maize (*Zea mays*) by wet milling, particularly in the United States. The major acids are palmitic (9–17%), oleic (20–42%), and linoleic (39–63%), and the major triacylglycerols are typically LLL (15%), LLO (21%), LLS (17%), LOO% (14%), LOS (17%), LSS (5%), OOO (6%), and OOS (4%). Despite its high unsaturation, the oil has good oxidative stability. The refined oil is used as a frying oil, a salad oil, and in the production of spreads after partial hydrogenation (31).

### 5.5. Cottonseed Oil

The cotton plant is grown for its fiber with the oil being a byproduct and representing only about 11–12% of the gross value of the product. Cottonseed oil was once the major vegetable oil in competition with the more widely used animal fats.

Today it occupies ninth place in production tables after the four major vegetable oils (soybean, palm, rape/canola, and sunflower), peanut oil, and three land animal fats (tallow, lard, and butter). With an annual production of about 3.9 million tons, it is grown mainly in China (1.1 million tons) and at lower levels of 0.4–0.2 million tons in India, the United States, the ex-USSR, Pakistan, Brazil, and Turkey. The oil is consumed mainly in the country of origin with only limited exports/imports. Cottonseed oil is unusual among commodity vegetable oils in that it contains a relatively high level of palmitic acid (27%) along with oleic (18%) and linoleic acids (51%). Linolenic acid is virtually absent. Low levels of malvalic and sterculic acids (cyclopropene acids) are removed during refining. Gossypol present in the crude oil gives it a strong yellow color (32).

### 5.6. Linseed (Flaxseed, Linola)

Different varieties of flax (*Linum usitatissimum*) are grown for fiber and for oil. Linseed oil is well known as one of the most unsaturated vegetable oils, resulting from its high level of linolenic acid (50–60%, Table 4). As a consequence of this, it oxidizes and polymerizes very readily and is used in paints, varnishes, and inks, in the production of linoleum, and as a sealant for concrete. These uses diminished with the appearance of alternative petroleum-based products, but the natural oil is coming back into favor on environmental grounds (33).

With recognition of the importance of n-3 acids in the diet, the oil and seed—under the name of flaxseed—are being used increasingly in food products both for humans (cereals and breads) and for animals. This is independent of the growing use of linola oil (solin) discussed below.

Using chemical mutation, plant breeders in Australia developed a variety of linseed with a low level of linolenic acid (~2%) and a high level of linoleic acid. This is called linola and is a linoleic-rich oil like sunflower. Solin is the generic name given to a similar Canadian flaxseed oil with <5% of linolenic acid. To distinguish these from traditional linseed oil, they must have yellow seed coats. They can be grown in the same temperate zones as rapeseed (canola), and the oil is used as an alternative to sunflower seed oil in the production of spreads rich in EFA. It is being grown in Australia, Canada, and Europe (34–38) (Table 4).

### 5.7. Olive Oil

Olive oil is a major vegetable oil obtained from the mesocarp of the fruits of the olive tree (*Oleo europaea*). Annual production is about 2.5 million tons, and commercial growth of the tree is confined almost entirely to the Mediterranean

TABLE 4. Fatty Acid Composition of Linseed and Linola Oils.

Oil source	Saturated	18:1	18:2	18:3
Linseed	10	16	24	50
Linola	10	16	72	2

countries of Italy, Greece, Spain, Turkey, and Tunisia. Virgin olive oil is produced from the first pressing, and other grades of lower quality are produced subsequently. The oil is characterized by a high level of oleic acid with *Codex* ranges of 8–20% for palmitic acid, 55–83% for oleic acid, and 4–21% for linoleic acid. The major triacylglycerols are typically OOO (43%), LOO (11%), and POO (22%), and the oil is characterized by a range of unsaponifiable constituents, that confer high oxidative stability (60). The oil contains squalene at a higher level (150–170 mg/100 ml) than in other vegetable oils (5–50 mg/100 ml), and this can be recovered from deodorizer distillate (Section 3.5) (39).

## 5.8. Palm Oil

The oil palm (*Elaies guinensis*) produces two distinct oils—palm oil from the fleshy endosperm and palm-kernel oil (Section 5.10) from the kernels. It grows in the tropical regions of Asia, Africa, and America and predominantly in Malaysia and Indonesia. At an average of about 4 tons per hectare of the two oils combined on well-managed plantations, the oil palm outcrops all other oil crops. Fruit bunches of 4–20 kg contain 200–2000 individual fruits that furnish palm oil (20–24%) and palm-kernel oil (2–4%). Through seed breeding, palm trees are being developed with lower height, higher oil yields, more unsaturated oil, and a higher proportion of kernel (40, 41).

The supply of palm oil has risen considerably since around 1980. It was almost 24 million tons per annum in 2001–2002 and is predicted to exceed the production of soybean oil during the period 2011–2015 at around 37 million tons. The oil contains almost equal proportions of saturated (palmitic ~48% and stearic ~4%) and unsaturated acids (oleic ~37% and linoleic ~10%). The major triacylglycerols are POP (30–40%) and POO (20–30%). The oil is used mainly for food purposes but finds some nonfood uses. It is a source of valuable byproducts such as carotene and tocopherols and tocotrienols (vitamin E). Red palm oil is a carefully prepared oil that retains about 80% (500–700 ppm) of the carotenes present in the crude oil and is a valuable dietary source of these important compounds (42).

Palm oil, melting in the range 21–27°C, can be fractionated to give solid (palm stearin, 30–35%, mp 48–50°C) and liquid fractions (palm olein, 65–70%, mp 18–20°C), thereby extending the range of usefulness of this oil. With improved filtration procedures, the yield of olein has been increased to 71–78%. This olein has a cloud point of 7–10°C and can be fractionated further to give even more unsaturated oleins and palm mid fraction (Table 5). Palm olein is a high-quality, highly stable

**TABLE 5. Fatty Acid Composition of Palm Oil and its Fractions.**

Oil source	16:0	18:0	18:1	18:2
Palm oil	44	4	39	11
Palm olein	41	4	41	12
Palm stearin	47–74	4–6	16–37	3–10

Source: Lin (42).



frying oil, and the major export of palm oil from Malaysia is now in the form of palm olein. Palm stearin is the less valuable commodity, but it can be used as a hard fat in the production of spreads and as a vegetable alternative to tallow in the oleochemical industry.

## 5.9. Palm-Kernel Oil

Palm-kernel oil is produced from the kernels of the oil palm, usually by solvent extraction and is an important lauric oil (see also coconut oil, Section 5.3). Its fatty acid composition is detailed in Table 2(b). Annual production is about 2.3 million tons. The kernels originate mainly in the oil palm growing areas of Malaysia and Indonesia and are crushed almost entirely in the country of origin (28, 29).

### 5.10. Peanut Oil (Groundnut Oil)

Peanut oil (groundnut), obtained from the legume *Arachis hypogea*, is also known as monkeynut oil and arachis oil. The plant is grown widely and especially in India, China, and the United States. A lot of the nuts are consumed as snacks, but crushing still yields about 4.6 million tons of oil each year. Its major acids are palmitic (8–14%), oleic (36–67%), and linoleic (14–44%) along with 5–8% (total) of C<sub>20</sub>, C<sub>22</sub>, and C<sub>24</sub> saturated and monoene acids. The major triacylglycerols in one sample of oil were reported to be LLL (6%), LLO (26%), LLS (8%), LOO (21%), LOS (13%), OOO (5%), OOS (16%), and other (5%). The oil shows high oxidative stability and is considered to have a desirable nutty flavor. There is also a high-oleic variety with 76% of oleic acid (Section 4.2.4) (43).

### 5.11. Rapeseed Oil

The seed oil of *Brassica napus* or *B. campestris* was typically rich in erucic acid (22:1), and the seed meal had an undesirably high level of glucosinolates. These components reduced the value of the oil and the protein meal but both have been bred out of modern rapeseed, known as double zero or canola. Rapeseed (of all kinds) is now the third largest source of oil at about 14.1 million tons a year (2000/01) after soybean oil and palm oil. It is grown mainly in Western Europe, China, India, and Canada (where the canola varieties were developed). Typically it contains palmitic (4%), stearic (3%), oleic (56%), linoleic (26%), and linolenic (10%) acids with less saturated acids than any other commodity oil. In one example, its major triacylglycerols were LLL (5%), LLO and LnOO (19%), LOO (27%), and OOO (41%). Rapeseed oil lends itself to genetic modification, and several rapeseed varieties giving oils with modified fatty acid composition have been developed. It is still not clear how many of these will be economically viable. Rapeseed oils containing less linolenic acid, or enhanced levels of lauric acid, stearic acid, oleic acid, or containing unusual acids such as  $\gamma$ -linolenic acid, ricinoleic acid, or vernolic acid have all been developed with a view to commercial exploitation (see Section 6 borage oil). An oleic-rich variety developed in Australia, called

Monola, contains about 78% oleic acid (Section 4.2.4). Efforts to modify rapeseed oil by conventional breeding and by genetic engineering are detailed in Sections 9.3 and 9.4 (44).

### 5.12. Rice Bran Oil

Rice (*Oryza sativa*) is an important cereal with an annual production of over 500–800 million tons. To produce white rice, the hull is removed and the bran layer is abraded giving 8–10% of the rice grain. The bran contains the testa, cross cells, aleurone cells, part of the aleurone layer, and the germ and includes almost all of the oil of the rice coreopsis. Gopala Krishna (45) considers that there is a potential for over 5 million tons of rice bran oil per annum, but present production is only about 0.7 million tons and not all of this is of food grade. India (0.50 million tons), China (0.12 million tons), and Japan (0.08 million tons) are the major countries producing rice bran oil.

Lipases liberated from the testa and the cross cells promote rapid hydrolysis of the oil, and therefore, it should be extracted within hours of milling. Attempts have been made to upgrade oil with 30% free acid by reaction with glycerol and the enzyme Lipozyme (*Mucor miehei* lipase) followed by neutralization. The major acids in rice bran oil are palmitic (12–18%, typically 16%) oleic (40–50%, typically 42%), and linoleic acid (29–42%, typically 37%). The oil contains phospholipids (~5%), a wax that may be removed and finds industrial use, and unsaponifiable matter including sterols, 4-methylsterols, triterpene alcohols, tocopherols, and squalene among others.

Refined rice bran oil is an excellent salad oil and frying oil with high oxidative stability resulting from its high level of tocopherols and from the presence of the oryzanols (ferulic acid esters of sterols and triterpene alcohols). The oxidative stability of this oil is exploited in “Good Fry Oil.” This is a frying oil based on oleic-rich sunflower oil to which is added up to 6% of rice bran and/or sesame oil to confer high oxidative stability. Rice bran oil also finds several nonfood uses (45).

Rice bran oil is reported to lower serum cholesterol by reducing LDL and VLDL without changing the level of HDL. This effect seems not to be related to fatty acid or triacylglycerol composition but to the unsaponifiable fraction and probably to the oryzanols (1.5–2.0% of the oil). These can be isolated in concentrated form from rice bran oil soapstock but have not yet been accepted for food use (46–51).

### 5.13. Safflower Oil

Safflower seed oil is a minor oil obtained from the seed of *Carthamus tinctorius*, grown particularly in India as a source of a valuable red-yellow or orange dye. Annual production of seed varies between 600,000 and 800,000. Normally it is a linoleic-rich oil (~75% linoleic acid) with LLL (47%), LLO (19%), and LLS (18%) as the major triacylglycerols. An oleic-rich variety (~74% oleic acid) has been developed and designated saffola (52).

### 5.14. Sesame Oil

Sesame oil comes from the plant *Sesamum indicum*. This is grown mainly in India and China and in Myanmar (Burma), Sudan, Mexico, and Egypt with a total annual production of oil of ~0.8 million tons. The seed has 40–60% of oil with almost equal levels of oleic (range 35–54, average 46%) and linoleic acids (range 39–59, average 46%). The oil contains sesamin (0.5–1.1%) and sesamol (0.3–0.6%), which give the oil high oxidative stability (51, 53). It may be added to other oils to enhance oxidative stability as in the preparation of “Good-Fry Oil” (Section 5.11) (50).

### 5.15. Soybean Oil

The seed of *Glycine max* is grown as a source of high-grade protein (79%) used in many human foods and the most dominant protein in animal feed. It is also the source of a high-quality oil (~18%). The oil contains palmitic acid (typically 11%, range 7–14%), oleic acid (typically 23%, range 19–30%), linoleic acid (typically 53%, range 44–62%), and linolenic acid (typically 7%, range 4–11%). Triacylglycerols exceeding 5% include LeLL (7%), LeLO (5%), LLL (15%), LLO (16%), LLS (13%), LOO (8%), LOS (8%), OOS (5%), and other (19%) at the approximate levels shown.

Soybean oil is produced in a larger amount than any other vegetable oil and is grown primarily in the United States, Brazil, Argentina, and China (see Section 10). It is a very significant part of the diet in the first three of these countries (perhaps over 80% of dietary fat intake in the United States). Because it contains linolenic acid, it is usually subjected to a light hydrogenation before being used as a frying oil or as a salad oil and is more extensively hydrogenated for the production of spreads and cooking fats (54). Nonfood applications are based mainly on the high unsaturation of this oil and include surface coatings, dimer acids, and epoxidized oil.

Soybean oil is richly endowed with several minor components that can be recovered during the refining process. These include phospholipids recovered as lecithin, mixed sterols that serve as a starting material for production of valuable pharmaceutical products, and tocopherols (vitamin E) (10).

Attempts are being made to modify the fatty acid composition of soybean oil to enhance its usefulness. Oils with less or more saturated acid, with less linolenic acid, and with high levels of oleic acid are in various stages of development (55).

### 5.16. Sunflower Oil

Sunflower seed oil is obtained from *Helianthus annuus* grown mainly in the USSR, Argentina, Western and Eastern Europe, China, and the United States. The oil normally contains 60–75% of linoleic acid, >90% of oleic and linoleic acids combined, and virtually no linolenic acid. Its major triacylglycerols are typically LLL (14%), LLO (39%), LLS (14%), LOO (19%), LOS (11%), and other (3%).

**TABLE 6. Fatty Acid Composition of Tall Oil.**

Source	Sat (a)	18:1	18:2	(b)	(c)	(d)
American	2.5	46	36	2	9	1-5
Scandinavian	2.5	30	45	9	5	1-5

(a) 16:0 + 18:0; (b) pinolenic acid, 5c9c12c-18:3; (c) conjugated diene acids; (d) rosin acids and unsaponifiable.

It is widely used as a cooking oil and is valued as an important component of soft spreads. High oleic varieties have been developed. Sunola or Highsun has about 85% oleic acid (some samples reach 90%). These are used to meet the growing demand for high oleic oils. NuSun with ~ 60% oleic acid has been developed in the United States [Table 2(a)]. It is hoped that it will replace regular sunflower oil in the United States, and by 2001, it represented 32% of all sunflower grown there (56).

### 5.17. Tall Oil

The term tall oil comes from the Swedish word for pine oil (*tallolja*). It is a mixture of fatty acids and some neutral material. Tall oil fatty acids are a byproduct of the wood pulp industry and result when pine wood chips are digested, under pressure, with an alkaline solution of sodium sulfate or an acidic solution of sodium sulfite. Tall oil is produced mainly in North America (~250,000 tons) and Scandinavia (~90,000 tons), but the products from these two sources differ in composition because of the differences in wood species being pulped. The crude extract is distilled to separate fatty acids (with less than 2% of resin acids) from resin acids (with less than 2% of fatty acids). The former is a good and cheap source of an oleic–linoleic acid mixture (75–80%). However, tall oil fatty acids contain sulfur compounds that interfere with catalytic processes, so the acids are not usually converted to alcohols or to nitrogen-containing compounds. They are used instead to prepare dimer acids, alkyds and coatings, detergents, and lubricants and are being examined for use as solvent, in inks, and for biodiesel production (57). Tall oil pitch is a valuable source of sterols. These are hydrogenated and acylated for use in cholesterol-lowering spreads (58) (see Table 6).

## 6. SPECIALITY AND MINOR OILS

The term *speciality oil* is a vague term used to describe oils that usually have been carefully refined to retain the special qualities of color and flavor normally associated with the oil. For the most part, they are used as food ingredients and in cosmetic and pharmaceutical products.

These oils are generally available in only limited quantities, and it is essential to ensure that the sources located will provide a reliable and adequate supply of good

quality material. As the oils are to be used as dietary supplements, health foods, or gourmet oils, it is important that the seeds be handled, transported, and stored under conditions that will maintain quality. It may be necessary to consider growing crops under conditions that minimize the level of pesticides.

Many fruits are now being processed in large amounts at centralized facilities. This means that larger quantities of “waste products” are available at one center and can be more easily treated to recover oil and other valuable byproducts. This is particularly relevant in the fruit industry where pips, stones, and kernels are available in large supply.

Extraction can be carried out in several ways, including cold-pressing at temperatures not exceeding 45°C, pressing at higher temperatures, and/or solvent extraction. Solvent extraction is not favored for high-quality gourmet oils. Supercritical fluid extraction with carbon dioxide is an acceptable possibility, but there is no evidence that this technique is widely used for this purpose. A further possibility is to use enzymes to break down cell walls followed by extraction under the mildest possible conditions.

Some speciality oils such as walnut, virgin olive, hazelnut, pistachio, and sesame can be used as expressed, merely after filtering, but for others, some refining is generally necessary. On the other hand, if the oil has a characteristic flavor of its own, it may be desirable to retain this and high-temperature deodorization must then be excluded or reduced to a minimum. Once obtained in its final form, the oil must be protected from deterioration—particularly by oxidation. This requirement necessitates the use of stainless steel equipment, blanketing with nitrogen, and avoiding unnecessary exposure to heat and light. At the request of the customer, natural and/or synthetic antioxidant can be added to provide further protection (59).

There follows a description of many minor oils and for convenience these are presented in alphabetical order (60, 61). Useful fatty acid data for many oils are given in an AOCS publication (62) and in a book by Ucciani (63).

**Aceituno oil** (*Simarouba glauca*). This tree grows in Central and South America. Its seeds produce oil (about 30%), which is rich in oleic acid (~58%), and contains significant levels of stearic (~28%) and palmitic (12%) acids. (64).

**Almond** (*Prunus dulcis*, *P. amygdalis*, *Amygdalis communis*). Almond oil is generally considered as an oleic-rich oil (65–70%), but its fatty acid composition can vary widely. The triacylglycerol composition of the oil has also been reported. Low-saturated, high-monounsaturated oils show high oxidative and cold weather stability (i.e., they are slow to deposit crystals) (65, 66).

**Amaranthus** (*Amaranthus cruentus*). Amaranthus or amaranth is a grain containing low levels (6–9%) of oil. A study of 21 accessions gave the following results: oil content 5–8% (mean 6.5), palmitic 8–22% (mean 19), stearic 1–4% (mean 3), oleic 16–25% (mean 22), linoleic 41–61% (mean 45), and tocopherols 2.8–7.8 mg/100 g (mean 4.9). Amaranthus oil is unusual in that it has a relatively high level (6–8%) of squalene, and this concentration can be raised 10-fold by short-path high-vacuum distillation. There is no other convenient vegetable source of squalene apart from olive oil, which has a squalene level of 0.3–0.7% rising to 10–30% in deodorizer distillate (67–70).

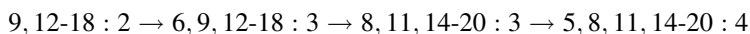
**Apricot** (*Prunus armeniaca*). Apricot seed oil is used in cosmetics and is available as a speciality oil for food use. It contains oleic (58–74%) and linoleic acids (20–34%). One study gives values of palmitic 5%, stearic 1%, oleic 66%, and linoleic acid 29%. With its low content of saturated acids, it shows excellent cold weather stability (71, 72). The fatty acid composition of the phospholipids has been reported (73).

**Avocado** (*Persea americana*). The avocado grows in tropical and subtropical countries between 40°N and 40°S and is available particularly from California, Florida, Israel, New Zealand, and South Africa. Like the palm and the olive, lipid is concentrated in the fruit pulp (4–25%) from which it can be pressed. There is very little oil in the seed (2%). The oil is used widely in cosmetic products as it is easily absorbed by the skin, and its unsaponifiable material is reported to provide some protection from the sun. It is also available as a high-oleic speciality oil for food use. It is rich in chlorophyll, making it green before processing. It contains 16:0 (10–20%), 18:1 (60–70%), and 18:2 (10–15%) as its major fatty acids. Its unsaponifiable matter, total sterol, and tocopherol levels have been reported (74–78).

**Babassu** (*Orbignya martiana* and *O. oleifera*). This palm, grown in South and Central America, contains a lauric oil in its kernel. Annual production is small and uncertain (100–300 kt), but Codex values have been established. In line with other lauric oils, it contains 8:0 (6%), 10:0 (4%), 12:0 (45%), 14:0 (17%), 16:0 (9%), 18:0 (3%), 18:1 (13%), and 18:2 (3%) acid (79).

**Blackcurrant** (*Ribes niger*) see Borage.

**Borage** (*Borago officinalis*).  $\gamma$ -Linolenic acid (6,9,12–18:3, GLA) is now recognized as an interesting material with beneficial health properties. Claims have been made for its use in the treatment of multiple sclerosis, arthritis, eczema, premenstrual syndrome, and other diseases. It is a biological intermediate in the conversion of freely available linoleic acid to the important but less readily available arachidonic acid. This change is a three-step process involving 6-desaturation, elongation, and 5-desaturation, of which the first step is rate-determining.



GLA is commercially available in three seed oils: blackcurrant, borage, and evening primrose. The production and use of these oils has been reviewed by Clough (80, 81). See also references 82–85 and Section 2.3 (Table 7).

**TABLE 7. Component Acids of Oils Containing  $\gamma$ -Linolenic Acid (Typical Results,% wt).**

Oil source	16:0	18:0	18:1	18:2	$\gamma$ -18:3	other
Evening primrose	6	2	9	72	10	1
Borage	10	4	16	38	23	9 (a)
Blackcurrant	7	2	11	47	17	16 (b)

(a) 20:1 (4.5), 22:1 (2.5), and 24:1 (1.5).

(b)  $\alpha$ -18:3 (13) and n-3 18:4 (3).

**Borneo tallow** (*Shorea stenoptera*). This solid fat, also known as illipe butter, contains palmitic (18%), stearic (46%), and oleic acid (35%). It is one of six permitted fats (palm oil, illipe butter, kokum butter, sal fat, shea butter, and mango kernel fat), which, in some countries at least, can partially replace cocoa butter in chocolate (86, 87).

**Buffalo gourd** (*Cucurbita foetidissima*). The buffalo gourd is a vine-like plant that grows in semiarid regions of the United States, Mexico, Lebanon, and India. The seed contains good quality oil (32–39%) and protein. The oil is very variable in fatty acid composition, thus lending itself to seed breeding. A typical sample contains 16:0 (9%), 18:0 (2%), 18:1 (25%), and 18:2 (62%) (88).

**Calendula** see Marigold.

**Camelina** see Gold of Pleasure.

**Candlenut** (lumbang, kemiri, kukui, *Aleurites moluccana*). This is a tropical tree whose nuts contain a very unsaturated oil: 16:0 (6–8%), 18:0 (2–3%), 18:1 (17–25%), 18:2 (38–45%), and 18:3 (25–30%). Its iodine value, however, is not as high as that of linseed oil. It is used for cosmetic purposes and has been recommended for the treatment of burns (89).

**Caraway** (*Carum carvii*). This is one of a group of plants whose seed oils contain petroselinic acid (6–18:1). This acid reaches levels of 35–43% in caraway, 66–73% in carrot, 31–75% in coriander, and ~80% in parsley. This isomer of oleic acid has some potential use as a source of lauric and adipic acids, produced by oxidative cleavage. The latter, an important component of many polyamides (nylons), is usually made from cyclohexane by a reaction that is reported to be environmentally unfriendly (90).

**Carrot** (*Daucus carta*). See caraway.

**Cashew** (*Anacardium occidentale*). Toschi et al. (91) have given details of the fatty acids, triacylglycerols, sterols, and tocopherols in cashew nut oil. The major fatty acids are palmitic (9–14%), stearic (6–12%), oleic (57–65%), and linoleic (16–18%), and the major triacylglycerols are OOO, POO, OOST, OOL, and POL.

**Cherry** (*Prunus cerasus*). Obtained by cold pressing and filtering, this oil is sold in the unrefined state for use as a speciality oil for salad dressings, baking, and shallow frying and in the production of skin-care products. Its fatty acid composition is unusual in that in addition to oleic (30–40%) and linoleic acids (40–50%), it also contains  $\alpha$ -eleostearic acid (6–12%, 9c11t13t-18:3). Some of these potential uses are perhaps surprising for an oil containing a conjugated triene acid (92–95). The fatty acid composition of the phospholipids has been reported (96).

**Chia** (*Salvia hispanica*). Chia seeds contain 32–38% of a highly unsaturated oil (97). The fatty acid composition for five samples from Argentina have saturated acids (9–11%), oleic (7–8%), linoleic (20–21%), and linolenic acid (52–63%).

**Chinese vegetable tallow and stillingia oil** (*Sapium sebiferum*, *Stillingia sebifera*). This seed is unusual in that it yields lipid from its outer seed coating (Chinese vegetable tallow, 20–30%) and from its kernel (stillingia oil, 10–17%), which are very different (96). The former, with ~75% palmitic acid and 20–25% oleic acid, is mainly a mixture of PPP (~70%) and POP (20–25%) triacylglycerols and is a potential confectionery fat. However, it is difficult to obtain the fat free of stillingia

oil (the kernel oil), which is considered to be nutritionally unacceptable. *Stillingia* oil is different, with oleic (13%), linoleic (23%), and linolenic acids (47%) and novel C<sub>8</sub> (hydroxy allenic) and C<sub>10</sub> (conjugated dienoic) acids that occur together as a C<sub>18</sub> estolide attached to glycerol at the *sn*-3 position, thus,

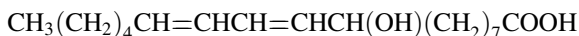


**Coriander** (*Coriandrum sativum*). See caraway. Attempts are being made both to develop coriander as an agricultural crop and to transfer the necessary  $\Delta$ -6 desaturase to the rape plant (98).

**Crambe** (*Crambe abyssinica*, *C. hispanica*). Present interest in this oil, particularly in North Dakota and in Holland, depends on the fact that it is a potential source of erucic acid (50–55%) that finds several industrial uses. This was once the major acid in rapeseed oil, but modern varieties of this seed produce a low-erucic oil (such as canola) suitable for food use. High-erucic rapeseed oil is still grown for industrial purposes, and attempts are being made to increase the level of this C<sub>22</sub> acid from around 50% to over 65% and even to 90% by genetic engineering (22–23, 44, 99–102).

**Cuphea**. *Cuphea* plants furnish seeds with oils that may be rich in C<sub>8</sub>, C<sub>10</sub>, C<sub>12</sub>, or C<sub>14</sub> acids. They generally contain >30% of oil and are expected to produce a commercial crop in the period 2005–2010. Problems of seed dormancy and seed shattering have already been solved. As markets for lauric oils already exist, there should be no difficulty in substituting cuphea oils. More recently, it has been reported that cuphea will be used as a commercial source of lauric acid from 2003 onward (30, 102, 103). Pandey et al. (104) have described the oil (17–29%) from *Cupea procumbens* containing 89–95% of decanoic acid. See also Section 9.2.

**Dimorphotheca** The seed of *Dimorphotheca pluvialis* is not very rich in oil (13–28%, typically about 20%), but it contains an unusual C<sub>18</sub> hydroxy fatty acid (~60%) with hydroxyl group adjacent (allylic) to a conjugated diene system. This is very unstable and easily dehydrates to a mixture of conjugated 18:3 acids (105).



Dimorphecolic acid (9-OH10t12c-18 : 2)

**Evening Primrose** (*Oenothera biennis*, *O. lamarckiana*, and *O. parviflora*). see Borage.

**Gold of Pleasure** (*Camelina sativa*, also called false flax). In addition to its interesting fatty acid composition, this plant attracts attention because it grows well with lower inputs of fertilizers and pesticides than traditional crops like rapeseed and linseed. The plant can also be grown on poorer soils and shows better gross margins than the other two plants after allowing for direct costs and (EU) subsidy payments. The seed yield is in the range 1.5–3.0 t/ha, and the oil content is between 36% and 47%. The oil has an unusual fatty acid composition. It contains



significant levels of oleic acid (10–20%), linoleic acid (16–24%), linolenic acid (30–40%), and C<sub>20</sub> and C<sub>22</sub> acids, especially 20:1 (15–23%). Another publication reports 30–38% oil containing oleic (14–20%), linoleic (19–24%), linolenic (27–35%), eicosenoic (12–15%), and other acids (12–20%) along with a range of tocols (5–22, mean 17 mg/100 g). Despite its high level of unsaturation, the oil shows reasonable oxidative stability. Attempts are being made to optimize the agronomy. Its use in paints, varnishes, inks, cosmetics, and even as a food oil is being examined and developed. Permission for food use has been granted in France and Britain (106–110).

**Grapeseed** (*Vitis vinifera*). These seeds produce variable levels of oil (6–20%), now available as a gourmet oil and for which Codex values have been reported. The oil is rich in linoleic acid (60–76%) and contains palmitic (6–8%), stearic (3–6%), and oleic acids (12–25%). In common with other oils rich in linoleic, it is reported to have a beneficial effect on the skin (79). Moret et al. (111) have described the effect of processing on the content of polycyclic aromatic hydrocarbons in this oil.

**Hazelnut** (*Corylus avellana*, also called filberts). The oil is rich in oleic acid (65–75% or even higher) and contains linoleic acid (16–22%). Its levels of saturated acids are low. Grown in Turkey and New Zealand, the nuts produced 55–63% of oil with saturated acids (6–8%), monoene acids (74–80%), and linoleic acid (6–8%). A recent study indicates the presence of several monoene acids in the C<sub>16</sub>–C<sub>22</sub> range, although this may refer to a different species (79, 112–115).

**Hemp** (Marijuana, *Cannabis sativa*). Hemp seed oil has an interesting fatty acid composition. One report gives the following values: palmitic (4–9%), stearic (2–4%), oleic (8–15%), linoleic (53–60%),  $\alpha$ -linolenic (15–25%),  $\gamma$ -linolenic (0–5%), and stearidonic acid (0–3%). The oil is being used in cosmetic formulations (116). Evidence from a study in Finland indicates that dietary consumption of hemp seed oil leads to increased levels of  $\gamma$ -linolenic acid in blood serum (117). The growing of hemp is banned in the United States, and therefore, hemp seed oil must be imported into that country (118–119).

**Honesty** (*Lunaria annua*). This seed oil contains significant levels of erucic (22:1, 41%) and nervonic acids (24:1, 22%) and is being studied as a new crop because it is a good source of the latter acid, which may be useful in the treatment of demyelinating disease (120).

**Illipe** (*Shorea stenoptera*, also called Borneo tallow). This is one of a group of tropical fats that are often confused with one another. They generally resemble cocoa butter in their proportions of palmitic, stearic, and oleic acids and therefore have similar triacylglycerol composition and display similar melting behavior. Values of 18%, 46%, and 35% have been reported for palmitic, stearic, and oleic acids and POP (7%), POST (34%), and StOST (47%) for the major triacylglycerols (121–125). It is one of six permitted fats (palm oil, illipe butter, kokum butter, sal fat, shea butter, and mango kernel fat), which, in some countries at least, can partially replace cocoa butter in chocolate (86–87).

**Kapok** (*Bombax malabaricum*, *Ceiba pentandra*). This name is applied to a number of tropical trees of the bombax family. The oil is a byproduct of kapok fiber production. Its major component acids are palmitic (22%), oleic (21%), and linoleic

TABLE 8. Fatty Acids and triacylglycerols of Kokum and Madhua Fats.

Oil source	Fatty Acids				Triacylglycerols		
	16:0	18:0	18:1	18:2	StOSt	POSt	POP
Kokum	2.0	49.0	49.0	0	72.3	7.4	0.5
Madhua	23.5	20.0	39.0	16.7	10.6	22.2	18.9
Stearin*	15.7	37.8	35.5	11.1	46.2	15.0	9.7

\*Obtained by dry fractionation of a 1:1 mixture of the two oils.

(37%), but it also contains about 13% of cyclopropene acids (malvalic and sterculic), which make it unsuitable for food use.

**Kokum** (*Garcinia indica*). Both kokum and mahua fats are rich in saturated and oleic acid and contain high levels of SOS triacylglycerols. They can be fractionated separately or as blends of the two oils to produce stearins that can be used as cocoa butter extenders (Table 8) (125). Kokum butter is one of six permitted fats (palm oil, illipe butter, kokum butter, sal fat, shea butter, and mango kernel fat), which, in some countries at least, can partially replace cocoa butter in chocolate (90).

**Lesquerella**. The only oil of significance with a hydroxy acid is castor oil (Section 5.1) but among the new crops being seriously developed are two containing hydroxy acids. Lesquerella oils have some resemblance to castor oil, but *Dimorphotheca pluvialis* seed oil contains a different kind of hydroxy acid.

Plants of the *Lesquerella* species are characterized by the presence of the C<sub>20</sub> bis-homologue of ricinoleic acid—lesquerolic acid—sometimes accompanied by other acids of the same type at lower levels:

Ricinoleic acid	12-OH 9-18 : 1
Densipolic acid	12-OH 9, 15-18 : 2
Lesquerolic acid	14-OH 11-20 : 1
Auricollic acid	14-OH 11, 17-20 : 2

A typical analysis of *L. fendleri* seed oil showed the presence of 16:0 (1%), 18:0 (2%), 18:1 (15%), 18:2 (7%), 18:3 (14%), lesquerolic (54%), and auricollic (4%) acids. As lesquerolic acid is the C<sub>20</sub> homologue of ricinoleic with the same  $\beta$ -hydroxy alkene unit, it undergoes similar chemical reactions but produces (some) different products. For example, pyrolysis should give heptanal and 13-tridecenoic acid (in place of 11-undecenoic acid). This could be converted to 13-aminotridecanoic acid, the monomer required to make nylon-13. Similarly, alkali-fusion will give 2-octanol and dodecanedioic acid in place of decanedioic (sebacic) acid. This C<sub>12</sub> dibasic acid is already available from petrochemical products and has a number of applications. A recent account of the status of this oil is available (126).

**Macadamia** (*Macadonia integrifolia*, *M. tetraphylla*). The nuts are used as a snack food. They are rich in oil (60–70%), which is used in cosmetics and is available as a gourmet oil. It is characterized by its high level of monoene acids [total ~80%, 16:1 16–23%, 18:1 55–65%, 20:1 1–3%] and is a convenient source of the

relatively uncommon palmitoleic acid. Its high level of monoene acids makes it good for skin care, but low levels of tocopherols limit its oxidative stability (127–128).

**Mahua** (*Madhuca latifolia*). see Kokum fat and Mango kernel fat.

**Mango** (*Mangifer indica*). Mango is consumed in large quantities as fruit. The kernel contains 7–12% of lipid with palmitic (3–18%), stearic (24–57%), oleic (34–56%), and linoleic acid (1–13%). In a typical case, these values were 10.3%, 35.4%, 49.3%, and 4.9%, respectively. It is fractionated to give an olein that is lower melting than mango fat and has excellent emollient properties and a stearin. The stearin can serve as a cocoa butter equivalent (POP 1%, POST 12%, StOST 56%) (124, 129, 130) and as a component with fractionated mahua fat of a *trans*-free bakery shortening. It is one of six permitted fats (palm oil, illipe butter, kokum butter, sal fat, shea butter, and mango kernel fat), which, in some countries at least, can partially replace cocoa butter in chocolate (86).

**Marigold** (*Calendula officinalis*). Interest in this seed oil is based on the fact that it contains significant levels (53–62%) of calendic acid along with linoleic acid (28–34%). Calendic acid (8*t*,10*t*,12*c*-18:3) is a conjugated trienoic acid, and this makes the oil an effective drying agent. Its alkyl esters can be used as a reactive diluent in alkyd paints replacing volatile organic compounds. The crop is being studied particularly in Europe (131–133).

**Meadowfoam** (*Limnanthes alba*). This oil is unusual in that over 95% of its component acids are C<sub>20</sub> or C<sub>22</sub> compounds and include 5–20:1 (63–67%), 5–22:1 (2–4%), 13–22:1 (16–18%), and 5,13–22:2 (5–9%). It is being grown in the United States, and its potential uses are being thoroughly examined. Winter cultivars now being developed are expected to improve the suitability of the crop to conditions in Northern Europe. Potential uses of this oil include cosmetic applications, production of dimer acid, as a lubricant, and via a wide range of novel derivatives based on reaction at the  $\Delta 5$  double bond (134–138).

**Melon** (*Citrullus colocythis* and *C. vulgaris*). This seed oil has been examined in terms of its fatty acids and phospholipids by Akoh and Nwosu (139). The major fatty acids in the total lipids are palmitic (11% and 12%), stearic (7% and 11%), oleic (10% and 14%), and linoleic acid (71% and 63%) for two samples.

**Mowrah** (*Madhuca latifolia*, *M. longifolia*, *M. indica*). This is mainly an Indian product where the fat is used for edible and industrial purposes. The nuts contain 46% of oil with variable levels of palmitic (15–32%), stearic (16–26%), oleic (32–45%), and linoleic acid (14–18%) (140).

**Mustard** (*Brassica alba*, *B. hirta*, *B. nigra*, *B. juncea*, *B. carinata*). The seeds contain 24–40% of oil characterized by the presence of erucic acid. Typical values are oleic 23%, linoleic 9%, linolenic 10%, eicosenoic 8%, and erucic acid 43% (141, 142). The plant is grown extensively in India (59, 79).

Canadian investigators have bred *Brassica juncea* (oriental mustard) from an Australian line with low erucic acid and low glucosinolate so that it has a fatty acid composition (palmitic 3%, stearic 2%, oleic 64%, linoleic 17%, and linolenic acid 10%) similar to that of canola oil from *B. napus* and *B. rapa*. This makes it possible to expand the canola growing area of Western Canada (143).

**Neem** (*Azadirachta indica*). This interesting seed oil contains chemicals used to control 200 species of insects. The oil prevents some insect species from maturing past the larval stage (144).

**Nigella** (*Nigella sativa*, black cumin). Typically, nigella oil contains palmitic (10%), oleic (35%), and linoleic acid (45%). Related species (*N. arvensis* and *N. damascena*) give similar oils with less oleic and more linoleic acid. The presence of low levels of 20:1 (11c, 0.5–1.0%) and higher levels of 20:2 (11c14c, 3.6–4.7%) in all of these oils may be of taxonomic significance. In one analysis, the oil contained the following major triacylglycerols: LLL 25%, LLO 20%, LLP 17%, LOP 13%, and LOO 10% reflecting the high level of linoleic acid. The seeds appear to contain an active lipase, and the oil quickly develops high levels of free acid. The oil is reported to be a good source of thymoquinone and to assist in the treatment of prostate problems (145–148).

**Niger** (*Guizotia abyssinica*). This oil comes mainly from Ethiopia. The seeds contain 29–39% of oil rich in linoleic acid (71–79%) along with palmitic, stearic, and oleic acids, each at levels of 6–11%. It is used for both edible and industrial purposes. It is rich in  $\alpha$ -tocopherol and is therefore a good source of vitamin E (149).

**Nutmeg** (*Myristica malabarica* and other *M.* species). Not surprisingly, considering its botanical name, seeds of the *Myristica* species are rich in myristic acid (~40%). Higher levels (60–72%) were quoted in earlier work (150).

**Oats** (*Avena sativa*). This grain seed contains 4–8% of lipid, although somewhat more in certain strains. The major component acids are palmitic (13–28%), oleic (19–53%), linoleic (24–53%), and linolenic acid (1–5%) The oil contains triacylglycerols (51%), di- and monoacylglycerols (7%), free acids (7%), sterols and sterol esters (each 3%), glycolipids (8%), and phospholipids (20%). The special features of this oil are used in various ways. It is reported to show cholesterolemic and antithrombotic activity, it is present in Olibra used as an appetite-suppressant, it is used in cosmetics by virtue of its glycolipids (151–153), and it can be used in baking at levels as low as 0.5% to increase loaf volume. Oat lipids are the subject of recent reviews (154, 155).

**Oiticica** (*Licania rigida*). The kernel oil obtained from this Brazilian tree is characterized by its high level (~78%) of licanic acid (4-oxo-9c11t13t-octadecatrienoic acid)—a keto derivative of the more familiar eleostearic acid. The oil shows drying properties but does not dry as quickly as tung oil (156).

**Parsley** (*Petroselinium sativum*). See carrot.

**Passionfruit** (*Passiflora edulis*). This popular fruit contains about 20% of oil in its seed and is available as a gourmet oil for use in speciality foods and salad dressings. It is a linoleic-rich (65–75%) but also contains palmitic (8–12%) and oleic acids (13–20%). Its high level of linoleic acid makes the oil good for skin care (157).

**Perilla** (*Perilla frutescens*). Perilla is a linolenic-rich oil (57–64%) used as a drying oil. It also contains oleic (13–15%) and linoleic acids (14–18%) and comes mainly from Korea or India. Recent descriptions of this oil come from these two countries (158–160).

**Pistachio** (*Pistachio vera*). Pistachio nuts, produced mainly in Iran, are widely consumed as shelled nuts. They contain about 60% of an oil that may be used for cooking and frying. Mean fatty acid values for five varieties are given as palmitic (10%), stearic (3%), oleic (69%), and linoleic (17%). Triacylglycerol composition has been suggested as a method of determining the country of origin of pistachio nuts (161–163).

**Poppy** (*Papaver somniferum*). Opium is obtained from unripe capsules and from the straw of the poppy plant. The narcotic is not present in the seed, which is much used for birdseed. It contains 40–70% of a semi-drying oil used by artists and as an edible oil. Rich in linoleic acid (72%), it also contains palmitic (10%), oleic (11%), and linolenic acids (5%) (79, 164).

**Purslane** (*Portulaca oleracea*). The plant (leaves, stem, and whole plant) is reported to be the richest vegetable source of n-3 acids, including low levels of the 20:5, 22:5, and 22:6 members. This is such a surprising result that it should be confirmed. These acids have not been identified in the seed oil, which contains palmitic (15%), stearic (4%), oleic (18%), linoleic (33%), and linolenic acids (26%) (165).

**Sal fat** (*Shorea robusta*). This tree, which grows in Northern India, is felled for timber. Its seed oil is rich in stearic acid, and it can be used as a cocoa butter equivalent (CBE). The major acids are palmitic (2–8%), stearic (35–48%), oleic (35–42%), linoleic (2–3%), and arachidic acid (6–11%). Its major triacylglycerols are of the SUS type required of a cocoa butter equivalent. Sal olein is an excellent emollient, and sal stearin, with POP 1%, POST 13%, and StOSt 60%, is a superior cocoa butter equivalent (122–124). It is one of the six permitted fats (palm oil, illipe butter, kokum butter, sal fat, shea butter, and mango kernel fat), which, in some countries at least, can partially replace cocoa butter in chocolate (86).

**Sea buckthorn** (*Hippophae rhamnoides*). This is a hardy bush growing wild in several parts of Asia and Europe and now cultivated in Europe, North America, and Japan. It is resistant to cold, drought, salt, and alkali. Different oils are available from the seeds and from the pulp/peel, but these are not always kept separate. Several health benefits are claimed for this oil, which is now available in encapsulated form and is being incorporated into functional foods. The oil is rich in sterols, carotenoids, and tocopherols. The seed oil is rich in 18:1, 18:2, and 18:3, but the berry oil contains significant levels of 16:1 (16–22%) (166–169).

**Shea** (*Butyrospermum parkii*, shea butter, karite butter). This fat comes from trees grown mainly in West Africa and contains an unusually high level of unsaponifiable material (~11%), including polyisoprene hydrocarbons. It is rich in stearic acid, but its fatty acid composition varies with its geographical source. It contains palmitic (4–8%), stearic (23–58%), oleic (33–68%), and linoleic acid (4–8%). It can be fractionated to give a stearin (POP 1%, POST 8%, and StOSt 68%), which can be used as a cocoa butter equivalent (79, 122–124). It is one of the six permitted fats (palm oil, illipe butter, kokum butter, sal fat, shea butter, and mango kernel fat), which, in some countries at least, can partially replace cocoa butter in chocolate (86).

**Tobacco**. Tobacco seeds contain an oil rich in linoleic acid (>70%) but with virtually no linolenic acid. After refining, it can be used for edible purposes or as a

**TABLE 9. Minor Oils Rich in Particular Fatty Acids.**

Fatty Acid	Sources
Lauric and myristic	Babassu, nutmeg
Stearic	Aceituno, illipe, mango, mowrah, sal, shea
Petroselinic	Caraway, carrot, coriander, parsley
Oleic	Aceituno, almond, avocado, apricot, cashew, hazelnut, pistachio
Linoleic	Amarantus, buffalo gourd, grape seed, hemp, melon, nigella, niger, passionflower, poppy, tobacco, walnut, wheatgerm
Linolenic	Candlenut, flax, gold of pleasure, hemp, mustard, perilla
Conjugated triene acids	Cherry, marigold, oiticica, tung,
Long-chain monoene acids C <sub>20</sub> –C <sub>24</sub>	Crambe, gold of pleasure, honesty, meadowfoam, mustard

nonyellowing drying oil. In one sample of the oil that was analyzed, the major triacylglycerols were LLL (38%), LLO (24%), and LLS (20%) (79).

**Tung oil** (*Aleurites fordii*). This oil comes mainly from China, which explains its alternative name of China wood oil. It is characterized by the presence of a conjugated triene acid ( $\alpha$ -eleostearic, 9c11t13t-18:3, ~69%). The oil dries more quickly than linseed with its nonconjugated triene acid, but oxidized tung oil contains less oxygen (5%) than does oxidized linseed oil (12%). Put another way, tung oil hardens at a lower level of oxygen-uptake than linseed oil. This oil is exported mainly from China (30–40,000 tons) and is imported mainly by Japan, South Korea, Taiwan, and the United States (each 6000–7000 tons). Starting in 1993, attempts have been made to develop this crop in Mississippi. It is planned to have 15,000 acres planted by 2006 producing 30,000 tons of oil (79, 170).

**Walnut** (*Juglans regia*). Walnut oil is an unsaturated oil containing both linoleic (50–60%) and linolenic acids (13–15%) and rich in tocopherols (~1500 mg/kg of oil). It is used as a gourmet oil in Japan, France, and other countries. A recent paper gives the detailed composition (fatty acids, triacylglycerols sterols, and tocopherols) of oil extracted with hexane and with supercritical carbon dioxide (171).

**Wheatgerm** (*Triticum aestivum*). This oil is highly unsaturated with linoleic (~60%) and some linolenic acid (~5%). It is valued for its high tocopherol levels (~2500 mg/kg of oil) (172–173).

Oils rich in a particular fatty acid are listed in Table 9.

## 7. MODIFICATION OF OILS AND FATS

The oils and fats provided by nature are not always ideal for their ultimate use, whether for food or nonfood purposes, and scientists and technologists have devised procedures for changing the natural oils. The major reasons for modification are nutritional, physical, and economic.

## 7.1. Nutritional Reasons for Modifying a Fat

Although ideas about the nutritional values of oils and fats change over time and will probably continue to do so, present consensus is based on the lipid hypothesis that is conveniently expressed in the following four statements:

- Diets with high contents of fat, of saturated fatty acids (SFA), and of cholesterol lead to high concentrations of cholesterol in blood and especially in low-density lipoprotein (LDL).
- This leads to high morbidity and mortality from coronary heart disease (CHD).
- Reducing the amount of fat/SFA/cholesterol in the diet reduces blood cholesterol and especially LDL cholesterol.
- This reduction leads to a lower risk of CHD and eventually to lower morbidity and mortality from the disease.

Perhaps this is oversimplified. The following points certainly need to be considered:

- All saturated acids do not behave identically. Short- and medium-chain acids up to 10:0 are rapidly metabolized by a different pathway from the longer chain acids and have no effect on cholesterol level. Stearic acid appears to lead only to a marginal cholesterol rise. This leaves lauric acid (12:0), myristic acid (14:0), and palmitic acid (16:0), among which myristic acid has the greatest cholesterol-raising effect. Some authorities have argued that even this is only a problem if there is an inadequate intake of linoleic acid.
- Monounsaturated acids (mainly oleic acid) are in great favor at the present time, but this relates only to the natural *cis*-isomers, and there is concern that acids with *trans*-unsaturation behave like the saturated acids in their effect on blood cholesterol levels. Dietary acids with *trans*-unsaturation come mainly from three sources: (1) Partially hydrogenated vegetable oils resulting mainly from heterogeneous catalytic reduction (nickel catalyst) of linoleic acyl groups. The product is a complex mixture of *cis*- and *trans*-18:1 esters. The major *trans*-acids in this mixture are  $\Delta 8$ – $\Delta 12$ , but others are also present. (2) Animal fats in ruminant meat and in dairy products contain *trans*-acids formed by enzymatically controlled biohydrogenation of linoleic acid. These are mainly 18:1 acids with the 11*t* isomer (vaccenic) dominant, but they also contain some diene acids referred to as conjugated linoleic acid (CLA) and mainly rumenic acid (9*c*11*t*-18:2). CLA is considered to have some positive health benefits. (3) *Trans*-isomers of PUFA result from high-temperature isomerization during deodorization ( $\sim 250^\circ\text{C}$ ). The detailed composition of dietary *trans*-18:1 depends mainly on the ratio of ruminant fats to partially hydrogenated vegetable oils. The difference between French diets (rich in dairy fats) and U.S. diets (rich in partially hydrogenated soybean oil) accounts for the differing dietary intake of *trans*-acids in these two countries (174).

- So far as polyunsaturated fatty acids are concerned, it is now considered that n-6 acids (linoleic and its metabolites) should not exceed present levels of around 6% of energy and that the n-6/n-3 ratio should be 5:1 or lower. There have even been serious claims that linoleic acid should not exceed 3% of energy (175). Some countries, including the United States, have a very low intake of n-3 acids and possibly an excessive intake of n-6 acids leading to a high n-6/n-3 ratio (18, 19).
- Unsaturated fat intake should always be accompanied by an adequate supply of antioxidants from fruit and vegetables.

## 7.2. Physical Reasons for Modifying a Fat

The important physical properties are most commonly associated with crystallization, crystal form, and melting behavior. For example, frying oils and lubricants ideally do not contain crystals and should therefore be free of those triacylglycerols that crystallize readily and promote crystallization. For spreads, where solids are needed, it is desirable that these be in the  $\beta'$ -crystal form and remain in this form.  $\beta'$ -Crystals are relatively small and can incorporate large volumes of oil. They give the product a glossy surface and a smooth luster.  $\beta$ -Crystals, on the other hand, although initially small, grow into needle-like agglomerates that produce a grainy texture and are less able to incorporate liquid. Oils with mixed chain-lengths (usually  $C_{16}$  and  $C_{18}$ ) are more likely to exist in the  $\beta'$  crystalline form, whereas those comprising almost entirely of  $C_{18}$  acids are known to be  $\beta$ -tending (176).

## 7.3. Economic Reasons for Selecting a Fat

Through changes that can be produced in natural oils and fats by application of appropriate technologies (Section 8), there is a high measure of interchangeability among these materials. Food processors in different countries use different recipes to produce very similar properties. This flexibility means that economic factors can also be part of the basis of selection. See Section 8.1.

# 8. TECHNOLOGICAL PROCEDURES USED FOR LIPID MODIFICATION

Although it may be necessary to modify natural oils to achieve desired functionality and properties, there is an economic cost for all of these processes and they will not be undertaken unnecessarily. Most of these procedures are discussed in detail elsewhere in this series so that only a brief outline will be given here. The objective is to give an overall view of the range of procedures (177) (see Table 10).

## 8.1. Blending

The mixing of oils and fats to produce blends with improved nutritional or physical properties has a long history. This method continues to find favor and is illustrated



**TABLE 10. Methods Employed to Extend the Usefulness and Improve the Properties of Oils.**

Blending	mixing of two or more oils
Fractionation	separating oils into two or more fractions
Partial hydrogenation	saturation of some double bonds accompanied by double-bond isomerization
Interesterification by chemical or enzymatic catalyst	reorganization of fatty acids among triacylglycerol molecules
Domestication of wild crops	conversion of wild crops to crops that can be cultivated commercially
Seed-breeding by traditional methods	interspecies crossing using irradiation or mutagenesis if necessary
Seed-breeding by genetic modification	crossing between species

in the production of Good-Fry oil (see below). Most spreads contain blends of two or more oils to combine desirable nutritional and essential physical properties. Interesterification is usually carried out on oil blends. Oils are also blended to obtain the desired mixture at minimum cost and computer programs to give the best solution have been developed (178).

Good-Fry is a blend of high-oleic vegetable oil such as sunflower mixed with up to 6% of sesame and/or rice bran oil, both of which show high oxidative stability by virtue of the antioxidants among their minor components. It is of interest that some of these antioxidants are particularly active at frying temperatures. The nature of the bulk oil (with its low levels of linoleic acid) and of the minor oils (with their high oxidative stability) combine to produce a very stable frying oil. Good-Fry can therefore be used longer than other frying oils. This makes it safer because of its reduced levels of oxidized and polymerized products and more economical because it does not have to be replaced so frequently (50).

## 8.2. Fractionation

Fractionation is a procedure for separating oils and fats into two or more components depending on their solubility and melting point. This topic has been reviewed by Timms (179) and Gibon and Tirtiaux (180). The less-soluble, higher melting fractions are called "stearins," and the more-soluble, lower melting fractions are called "oleins." The two products extend the range of use of the original oil or fat. Sometimes both fractions have added value, but on other occasions, only one fraction is of enhanced value and efforts have to be made to find a use for the less valuable fraction. Fractionation can be repeated to give even more refined fractions, but this is only commercially practicable when high-value products are obtained, such as cocoa butter replacers. Although other procedures have been employed in the past, this process is now usually carried out through dry fractionation (179–182).

Dry fractionation is a two-step operation involving crystallization that should be allowed to proceed slowly to the equilibrium state, followed by filtration of the solid from the liquid phase. Crystallization occurs over several hours and requires good temperature control. The temperature must be lowered at a fixed rate to the selected value, and this must be combined with efficient but slow agitation. Good filtration—aiming at complete separation of solid and liquid—is important and may be carried out under reduced pressure using a Florentine filter or under pressures up to 5 Mpa (50 bar) with a membrane filter. Fractionation is applied mainly to palm oil but also to lauric oils (coconut and palmkernel), butter oil, beef tallow, hardened soybean, and cottonseed oil.

Palm oil is fractionated more than any other oil. A single fractionation converts palm oil (IV 51–53) to palm olein (IV 56–59) and to hard stearin (IV 32–36). Each of these can be fractionated a second or a third time to give a range of products, including superolein (IV 64–66), topolein (IV 70–72), soft stearin (IV 40–42), super stearin (IV 17–21), soft palm mid-fraction (IV 42–48), and hard palm mid-fraction (IV 32–36). These materials have a wide range of food and nonfood uses and extend considerably the use of palm oil.

Palm-kernel oil (IV 18) is fractionated to give a stearin of IV  $\sim 7$ , which can be used as a cocoa butter substitute and as an olein of IV  $\sim 25$ . These fractions are also useful after complete hydrogenation.

### 8.3. Hydrogenation

Just over 100 years ago (1897), Sabatier and Senderens demonstrated that olefinic compounds could be reduced with hydrogen in the presence of nickel or other metallic catalyst. Shortly after, the German chemist Normann applied the process to unsaturated fatty materials. Partial hydrogenation has since developed into a much-used process for modifying liquid oils from oilseeds or from fish (178).

In 1990, it was claimed that among all edible fats, one-third was hydrogenated and only one-tenth was fractionated or interesterified. These proportions are now probably different because of the increasing volumes of palm oil available for fractionation and of concern about *trans*-acids formed during partial hydrogenation. Hydrogenation is appropriate for highly unsaturated oils such as soybean, rapeseed, and cottonseed, and for fish oils, whereas fractionation is better applied to palm oil and other more saturated oils. The following changes take place when an oil is partially hydrogenated:

- There is a change in the melting behavior of the oil as a consequence of the increased proportion of saturated and/or *trans*-monoene acids and this affects spreadability, oral response, and baking performance.
- There is an improvement in stability toward atmospheric oxidation resulting from reduced levels of the methylene-interrupted polyene acids that are so easily oxidized.
- There is a reduction in the nutritional value of the product, related to lower levels of essential fatty acids ( $\alpha$ -linolenic and linoleic acids) and enhanced

levels of both *trans*-monoene and saturated acids. It is possible to add back essential fatty acids into the final product by blending with appropriate oils.

- Through reaction with hydrogen in the presence of a heterogeneous catalyst, the unsaturated centers in the oil being hydrogenated may suffer one of three fates. (1) The double bond can react with hydrogen and become saturated; as a consequence of this, diene acids are reduced to monoenes and monoene acids become saturated. (2) The double bond may change configuration and the natural *cis*-isomers may become largely *trans*: Such acids have a higher melting point than the *cis*-isomers, so stereomutation leads to a rise in melting point without any uptake in hydrogen or change in iodine value. (3) Interaction among double bond, catalyst, and hydrogen can lead to double bond migration.

When partially hydrogenated, linoleic acid might be expected to give only  $\Delta 9$  and  $\Delta 12$   $C_{18}$  monoenes. Each of these may then react further, but under conditions of extended selective hydrogenation, the product is more complex and the  $C_{18}$  monoene esters may include the *cis*- and *trans*-isomers from  $\Delta 5$  through to  $\Delta 15$  (i.e., 22 isomers).

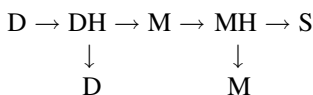
Partial hydrogenation is a flexible process and can produce different products depending on the:

- nature of the starting material
- extent of hydrogenation
- selectivity of the catalyst, which influences the proportion of *cis*- and *trans*-monoenes and of saturated acyl chains.

The nature of the hydrogenation products is controlled by the process conditions. Important factors are catalyst properties (pore diameter, pore length, activity level, and amount), reaction temperature, hydrogen pressure, and the degree of agitation (which affects the transfer of hydrogen and oil to and from the catalyst surface). Hydrogenation (reduction) is favored by a high concentration of hydrogen on the catalyst through increased pressure or increased stirring. Isomerization is favored by factors such as increased temperature, more catalyst, a more active catalyst, or a more highly unsaturated oil, all of which lead to an increased hydrogen demand that cannot be completely satisfied.

Dijkstra (177, 184) has proposed a modification of the Horiuti–Polanyi mechanism to explain the changes that occur during partial hydrogenation of fatty acids and their esters. In the sequence given below, the horizontal line shows the conversion of diene (D) to monoene (M) and of monoene to saturated acid/ester via the half hydrogenated states DH and MH. The steps shown vertically are the reverse processes whereby DH reverts to diene and MH reverts to monoene. It is during these reverse processes that *trans*- and positional isomers are formed. There are six stages altogether, and it is important to know the relative rates of these.

- In the conversion of D to M, the first step is rate-determining and the second step is fast. The conversion of DH back to D is slow and is only important in the unusual situation that hydrogen is present in very low concentration.
- In the conversion of M to S, the final stage is slow and rate-determining, thus making it more likely that there will be considerable recycling of M and MH leading to formation stereochemical and positional isomers.



The only useful commercial catalyst now used is nickel, available at a 17–25% level on a support and suspended in hardened edible oil or tallow. This preserves the activity of the nickel in a form in which it can be safely and easily handled. Catalyst can be recovered and reused but will be less active. Reaction is usually effected at temperatures between 180°C and 200°C and at a pressure of about 0.3 MPa (3 bar). The catalyst is quickly poisoned by fatty acids, soaps, phospholipids, oxidized acids, sulfur compounds, halogen compounds, carbon monoxide, oxygen, and water. As a consequence, both the oil and the hydrogen should be as pure as possible.

Catalysts are continually being improved. Hastert reports (185) that nickel loading has fallen continuously from 0.25% (prior to 1960) to 0.1% (by 1970) and 0.05–0.1% (by 1990), and that 0.025–0.05% is now normal. This is partly a consequence of improved plant design, but catalyst surface area increased from 70 m<sup>2</sup>/g in 1970 to 180 m<sup>2</sup>/g in 1993, and there is increasing recognition of the importance of using pure hydrogen and highly refined oil. Interesting developments now taking place involve the use of precious metals (platinum and palladium), which although more expensive, offer higher reaction rates at lower temperatures with formation of less *trans*-isomer. In these ways, hydrogenation will probably continue as a useful processing technique for many years to come.

#### 8.4. Interesterification with a Chemical Catalyst

The production of fat spreads as an alternative to butter led to an increased demand for solid fats. For the most part, this demand has been met by the use of partially hydrogenated vegetable oils (Section 8.3), but concern about the health effects of *trans*-unsaturated acids has raised interest in an alternative way of producing fats with the required melting behavior. This can be achieved by interesterification of blends of natural or fractionated fats. Products obtained in this way will probably contain more saturated acids than their partially hydrogenated equivalents, but they will have no *trans*-acids. This section is devoted to interesterification carried out under the influence of a chemical catalyst (177, 186, 187). Similar reactions with enzymes are discussed in the following section.

Interesterification is generally effected in 10–15 ton batches at 80–90° over 30–60 minutes at a cost not very different from that for partial hydrogenation. It does not require expensive equipment nor use explosive gases. To get a product with the desired properties, a soft oil is interesterified with a hard stock, which may be a fractionated stearin, a lauric oil, or a fully hydrogenated seed oil. This last is a scientifically acceptable choice but has the disadvantage that the word “hydrogenated” will have to appear on the label. The average customer does not appreciate the difference between partially hydrogenated (with *trans*-acids) and fully hydrogenated (without unsaturated acids).

The catalyst normally employed is an alkali metal at a level of 0.1–0.2% or a sodium alcoholate (usually sodium methoxide) at a 0.2–0.3% level. The true catalyst is believed to be a diacylglycerol anion resulting from interaction of alkali and triacylglycerol. The catalyst is easily destroyed by acid, water, or peroxides, so the feedstock oil should be free of these impurities (188).

Natural oils and fractionated oils usually have their acyl chains organized in a nonrandom manner, but they become randomized after interesterification with a chemical catalyst. There is no change in fatty acid composition, only in triacylglycerol composition, but this leads to a modification of the physical properties. More selective interesterification can be achieved with enzymic catalysts (Section 8.5).

The following are typical applications of interesterification:

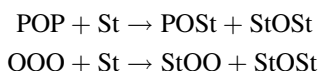
- Lard, with an unusually high level of palmitic acid in the  $\beta$ -position, crystallizes naturally in the  $\beta$  form. When randomized, the content of 2-palmitoglycerol esters is reduced from around 64% to 24% and the interesterified product crystallizes in the  $\beta'$  form with consequent improvement in shortening properties.
- The crystal structures of margarines based on sunflower or canola oil (rape-seed) along with hydrogenated oil are stabilized in the  $\beta'$  form by interesterification leading to randomization of the glycerol esters.
- Solid fats with about 60% of essential fatty acids can be obtained by (reduced temperature) interesterification of sunflower oil and about 5% of hard fat.
- Margarine made, for example, by interesterification of palm stearin and sunflower oil (1:1), contains no hydrogenated fat and therefore no *trans*-acids.
- Chemical interesterification is used in the production of caprenin, salatrim, and olestra.

### 8.5. Interesterification with an Enzymic Catalyst

Intesterification can also be catalysed by enzymes, many of which show useful specificities. The 1,3-specific lipases, such as those derived from *Aspergillus niger*, *Mucor javanicus*, *M. miehei*, *Rhizopus arrhizus*, *R. delemar*, and *R. niveus*, are particularly useful for interesterification. They are used to effect acyl exchange at the *sn*-1 and 3 positions while leaving acyl groups at the *sn*-2 position unchanged.

Many interesting changes of this type have been affected on a bench scale, but as yet only a few have been commercialized and then only for products of high value (186–190).

Unilever developed a method for upgrading palm mid-fraction (PMF) as a cocoa butter equivalent. The PMF is too rich in palmitic acid and has too little stearic acid, but this deficiency can be repaired by enzyme-catalysed acidolysis with stearic acid. Reaction is confined to the exchange of palmitic acid by stearic acid at the *sn*-1 and 3 positions with no movement of oleic acid from the *sn*-2 position. A similar product is produced enzymatically by acidolysis of high-oleic sunflower oil (rich in triolein) and stearic acid.



Chemical interesterification would lead to randomization of all of the acyl chains, and the products would have different melting behavior from that required by a cocoa butter equivalent (188).

Another product manufactured by Loders-Croklaan (Unilever) and named Betapol consists mainly of triacylglycerols of the type UPU. This is used as a constituent of infant formulas (191). Human milkfat is unusual in that it contains a significant proportion of its palmitic acid in the *sn*-2 position. Although this is true also for lard (pig fat), it is not a feature of vegetable oils. Betapol is made from tripalmitin and oleic acid using the lipase from *Mucor miehei* to promote 1,3-acyl exchange. These materials are expensive, and in practice, fractionated palm oil, rich in tripalmitin, is reacted with acids from high-oleic sunflower or safflower or from olive oil.

Bohenin (BOB) is the name given to glycerol 1,3-behenate 2-oleate, which inhibits fat bloom when added to chocolate. It is produced in Japan by enzymic interesterification of triolein and behenic (22:0) acid or ester in the presence of a 1,3 stereospecific lipase.

Diacylglycerols are being produced and used in growing quantities because of their beneficial effects in the management of obesity. Cooking oil containing at least 80% of diacylglycerols (mainly the 1,3- isomer) has been marketed in Japan since 1999 by the Kao company and thereafter in other countries, including the United States by ADM.

There are many reports showing how, with an appropriate enzyme (*Mucor miehei* and *Candida antarctica* are frequently used), long-chain polyunsaturated fatty acids such as eicosapentaenoic acid and/or docosahexaenoic acid can be introduced into vegetable oils or synthetic glycerides to give products with enhanced nutritional value. In a similar way, C<sub>8</sub> and C<sub>10</sub> acyl chains can be introduced into vegetable oils or fish oils with a consequent change in nutritional properties and energy values. The products are triacylglycerols with either one long and two short chains (LS<sub>2</sub>) or two long and one short chain (L<sub>2</sub>S). This reaction can be used to produce triacylglycerols with easily metabolizable short- and medium-chain acids at the *sn*-1 and 3 positions and an essential fatty acid at the crucial *sn*-2 position. This topic is the subject of some recent reviews (190, 192, 193).

## 9. BIOLOGICAL METHODS OF LIPID MODIFICATION

### 9.1. Introduction

New sources of oils and fats develop from plants in three different ways. One possibility is to take a wild plant that produces oil with an interesting fatty acid and/or triacylglycerol profile and to make it suitable for commercial growing and harvesting. This is generally a slow process requiring many years. Traits developed over an evolutionary time scale to maintain the plant in the wild are not always appropriate in domesticated plants and have to be bred out. This approach is being pursued, particularly in North America and in Europe, for a number of species identified as promising, and they are at differing stages of development (see Section 9.2).

A second approach, when a diverse gene pool is available, is to interbreed species with appropriate traits by standard seed-breeding processes. This has been done very effectively with species of brassica to yield the modern oilseed rape (canola). If necessary, the gene pool can be extended by mutation resulting from chemical treatment or from irradiation. This may produce novel varieties with interesting traits and is the basis of the low-linolenic lines from linseed, and other examples are described in Section 9.3.

Finally, genes required for particular aspects of fatty acid and triacylglycerol biosynthesis can be identified in appropriate sources, cloned, and transferred to other plants. Rapeseed has proved to be particularly flexible in this respect, and its fatty acid composition has been modified in several ways, some of which have now reached or are very close to commercial application (Section 9.4). Genetic modification procedures are also applied to soybean and other oilseed crops.

The commercial introduction of a new lipid source is not a trivial matter. Unless the oil has some specific and novel property (like oils containing  $\gamma$ -linolenic acid, for example), it will have to compete with existing oils available in bulk at commodity prices. This exerts a number of constraints.

- The new crop should be easily cultivated, harvested, processed, and marketed. Additional costs may result from the need to have separate and distinct harvesting, storage, processing, and marketing facilities.
- The new crop must quickly become available in good and reliable quantities at acceptable prices.
- The demand for and interest in some new crops may come more from the oleochemical industry than from the food industry, but traditionally the oleochemical industry has used lower grade and cheaper oils than the food industry.
- Because the supply of new oil must start small and grow with demand, it is useful to find some low-volume, high-value products that will support the crop through its early years of development until the supply is adequate to be used for high-volume, low-value products.
- The demand must be market-led (at least after the first few years). At present, there is an interest in new crops that produce oils with high levels of a single

acid such as lauric, oleic, petroselinic, erucic, or acids with hydroxy or epoxy groups.

- Although agronomists must help to produce oils meeting these requirements, chemists and technologists must assist in the substitution of existing oils by new oils and in the development of new uses for new oils.
- In addition, regulatory requirements will have to be met when the seed and/or its products are novel. This topic has been reviewed (194, 195).

## 9.2. Domestication of Wild Crops

Serious attempts are being made in Europe and North America to develop a range of wild oilseed crops, generally with a high content of one particular acid. The following account shows the variety of species being examined. Reference has already been made to some of these in Sections 4 and 6.

*Cuphea* oils are interesting because they come from annual plants producing glycerol esters based mainly on capric (10:0) or lauric acid (12:0) or occasionally on caprylic (8:0) or myristic acid (14:0). *Cuphea* plants exhibit several wild plant characteristics that need to be bred out. These include dormancy, nonuniform germination, indeterminate flowering, seed maturation over a broad time period (six weeks), extreme dehiscence (pod shattering), and the presence of viscid hairs on stem, leaves, flowers, and fruit. Several species are being studied in attempts to make them commercial (30).

*Oleic oils.* Oleic acid is an important source material for the oleochemical industry, and as the most common monoene acid, it has a good rating on dietary grounds. For many oleochemical purposes, the presence of some saturated acid (palmitic, stearic) is not significant but levels of linoleic and linolenic acid should be as low as possible because they promote undesirable oxidation. All oils contain oleic acid, and frequently, it is the dominant component. For example, the oils of rapeseed (56%), macadamia (56% along with a further 22% of 16:1), almond (61%), high-oleic safflower (74%), olive (78%), and high-oleic sunflower (82%) contain the levels of oleic acid indicated. Of these, almond and rape also contain 25–30% of linoleic acid. *Euphorbia lathyris* (caper spurge) is a Mediterranean annual containing about 50% of oil in its seed and 80–90% of oleic acid in the oil. This would make it an excellent source of oleic acid. At present, it suffers from a number of deficiencies, especially seed shattering and the presence of a cocarcinogenic milky sap. But it is hoped that these problems will be overcome through plant breeding. Other good sources of oleic acid—both existing and potential—are discussed in Sections 9.3 and 9.4.

*Petroselinic acid oils.* Petroselinic acid is the  $\Delta^6$  isomer of the more common oleic acid ( $\Delta^9$ ). Although they share many properties, these acids display an interesting difference in melting point. Petroselinic acid and its glycerol ester melt at 33°C and 28°C, respectively. The corresponding figures for oleic acid and its glycerol ester are lower at 12° and 16° (two forms) and 5°. Attempts are being made to develop a better source of petroselinic acid by improved cultivation of coriander (~80%



petroselinic acid) or by transferring the appropriate genes from this plant to rape (Section 9.4) (196).

*Oils containing C<sub>18</sub> polyene acids: Calendula officinalis* seed oil. *Calendula* oil (from marigold) is of interest because it contains about 58% of calendic acid (8t10t12c-18:3). This unusual acid is an isomer of  $\alpha$ -eleostearic (9c11t13t-18:3) present in tung oil, and calendula oil should also be a good drying oil. The presence of linoleic acid (30%) will add to the unsaturated nature of this oil (131–133).

*Camelina sativa* seed oil. This plant is also known as gold of pleasure or false flax. In addition to its interesting fatty acid composition, it attracts attention because it grows well with lower inputs of fertilizers and pesticides than more traditional crops like rape and linseed. The plant can also be grown on poorer soils and shows better gross margins than the other two plants after allowing for direct costs and subsidy payments. The seed yield is in the range 1.5–3.0 t/ha and the oil content between 36% and 47%. The oil has an unusual fatty acid composition. It contains significant levels of linolenic acid (31–41%) and of C<sub>20</sub> and C<sub>22</sub> acids, especially 20:1 (15–23%). Despite its high level of unsaturation, it shows reasonable oxidative stability. Attempts are being made to optimize the agronomy. Its use in paints, varnishes, and inks, in cosmetics, and even as a food oil is being examined and developed (108–110).

*Oils containing erucic and other long-chain monounsaturated acids.* In 1994, it was reported (22) that the demand for erucic acid-based oleochemicals was about 20 kt of compounds derived from 55–60 kt of high-erucic oil. These oleochemicals include materials obtained from erucic acid (22:1) or from behenic acid (22:0) and brassylic acid (the C<sub>13</sub> dibasic acid resulting on ozonolysis). The demand is mainly for erucamide (7 kt), other erucic acid nitrogen compounds (2.7 kt), erucic esters (1.8–2.3 kt), erucyl alcohol (4.5 kt), behenyl alcohol (2.7 kt), and glycerol tribehenate (1.1–1.4 kt). High-erucic oils are reported to have a growth rate of about 6%.

The traditional source of erucic acid was rapeseed oil before this acid was bred out of that oil because of its reported adverse health effects. Most rapeseed oil now contains less than 2% of erucic acid. The two major sources of erucic acid are high-erucic rapeseed oil (HEAR) containing about 50% of erucic acid and crambe oil with 55–60% of erucic acid. As will be reported later (Section 9.4), attempts to produce a still higher erucic rapeseed oil are being made by genetic engineering. Crambe oil (from *Crambe abyssinica*) is grown most extensively in North Dakota and to a lesser extent in Holland.

*Meadowfoam oil* from *Limnanthes alba* seed oil is unusual in that over 95% of its component acids are C<sub>20</sub> or C<sub>22</sub> and include 5–20:1 (63–67%), 5–22:1 (2–4%), 13–22:1 (16–18%), and 5,13–22:2 (5–9%). It is being grown in the United States, and its potential uses thoroughly examined. The crop yields 1000–1500 kg of seed per hectare and contains 25% oil. Potential uses of this oil include cosmetic applications, production of dimer acid, as a lubricant, and via a wide range of novel derivatives based on reaction at the  $\Delta^5$  double bond (134–138).

*Simmondsia chinensis* seed oil. Jojoba oil is another source of C<sub>20</sub> and C<sub>22</sub> compounds that has already been developed as a marketable product but in limited supply (195). It is produced by a drought-resistant plant that withstands desert heat.

It takes 5–7 years to first harvest, 10–17 years to full yield, and has a life span of around 100 years. It is being grown in the Southwestern United States and Mexico mainly, but also in Latin America, Israel, South Africa, and Australia. Yields are reported to be about 2.5 ton of oil/hectare.

Joboba oil is not a triacylglycerol but a mixture of wax esters based mainly on 20:1 and 22:1 acids and alcohols. It contains C<sub>40</sub>, C<sub>42</sub>, and C<sub>44</sub> esters with two isolated double bonds (one in the acyl chain and one in the alkyl chain). The oil serves as replacement for sperm whale oil, which is proscribed in most countries because the sperm whale is an endangered species. At present, jojoba oil is a high-priced oil used mainly in cosmetics, but it has excellent lubricating properties and could be used extensively for this purpose if available in sufficient quantity at an appropriate price.

The oil is fairly pure as extracted, has a light color, and because the double bonds are well separated, it is resistant to oxidation. The oil can be chemically modified by reaction of the double bonds (hydrogenation, stereomutation, epoxidation, sulfochlorination) (197).

Honesty seed oil (*Lunaria biennis*) is characterized by its high levels of monoene acids, including 18:1 (23%), 22:1 (46%), and 24:1 (23%). It is being developed as a commercial crop for nutritional research based on its significant level of nervonic acid (24:1) (120).

*Oils containing hydroxy acids:* The only oil of significance containing a hydroxy acid is castor oil, but among the new crops being seriously developed are two that contain hydroxy acids. Lesquerella oils have some resemblance to castor oil, but *Dimorphotheca pluvialis* seed oil contains a different kind of hydroxy acid (see Section 6).

*Oils containing epoxy acids:* Several natural epoxy acids are known, but vernolic acid (12,13-epoxyoleic) is the most common and occurs at high levels in several seed oils. Of these, serious attempts are now being made to develop *Vernonia galamensis* (73–78% vernolic acid) and *Euphorbia lagascae* (57–62% vernolic acid) as commercial crops (198). Several potential uses of this acid and the seed oils in which it occurs are being explored.

### 9.3. Oils Modified by Conventional Seed Breeding

Seed breeding of industrial crops is a continuous activity. Much of this is concerned with agronomical factors and is not of concern here. More significant for the present purpose are those developments leading to new or improved seed oils. Many of the changes are small and incremental and only become apparent after many years. Others are more dramatic. Some examples that are already well established will be discussed.

*Rapeseed oil:* Low-erucic rapeseed oil is now the third largest source of oil after soybean and palm. The seed contains over 40% of oil and this represents about 80% of the seed's commercial value. Seed breeders have developed seeds which produce oil low in erucic acid (<2%) and meal low in the undesirable sulfur-containing glucosinolates (i.e. double low varieties). This low-erucic oil (LEAR)

finds many food uses and also some non-food uses (biodiesel, lubricants). The crude oil is rich in phospholipids (~3.5%) though these are reduced to 10–300 ppm (phosphorus) after refining and are themselves a useful by-product (Section 3). The plant grows in cooler agricultural regions including China, Northern Europe, and Canada as well as in the Indian sub-continent. In common with other Brassica species rapeseed oil contains brassicasterol at much higher levels (~600 ppm) than is observed in other seed oils. Low-erucic rapeseed oil has a very low level of saturated acids and a high level of oleic acid: palmitic 4%, stearic 2%, oleic 56%, linoleic 26%, linolenic 10%, and others 2% (194, 199).

*Linseed oil:* Linseed oil is well known as one of the most unsaturated vegetable oils with a high level of linolenic acid (~50%). As a consequence of this it oxidizes and polymerizes very readily and is used in paints, varnishes, inks, linoleum, and as a sealant for concrete. Using chemical mutation, plant breeders in Australia (33) developed a variety of linseed with a low level of linolenic acid (~2%) and a high level of linoleic acid. This is called linola and is a linoleic-rich oil like sunflower (Table 4). The oil has GRAS status in USA.

*High-oleic sunflower and safflower oils:* By taking advantage of the wide range of natural sunflower and safflower varieties seed breeders have developed lines which, in place of the normal high levels of linoleic acid, have high levels of oleic acid (Table 2a). These are commercially available as Sunola (~85% oleic acid) and Saffola (~75% oleic acid) (49). They are used in Good-Fry (Section 2) and as an alternative to triolein in some enzymic processes (Section 8.5). A third type of sunflower oil (Nu-Sun) with an intermediate level of oleic acid (65%) and reduced levels of saturated acids is now available.

*The oil palm* is already the most productive source of vegetable oil at an average level of over 3t/ha/yr. Seed breeding through the last 25 years has led to palms which in the best environments can produce 10 tons per hectare. According to Jalani et al (38, 39) further objectives being pursued include the following.

- High palm plants present harvesting problems. With shorter plants, there is easier harvesting and a longer planting cycle because the trees do not need to be replanted so often. Plants that grow only 15–25 cm/yr are now available in place of the usual 45–75 cm/yr.
- Oils with higher iodine value (normally 53, raised to 63) contain less palmitic acid and more oleic acid. When fractionated, they produce more of the valuable olein fraction (Section 5).
- Kernels are normally about 6% of the fruit, but palms with 12% kernel have now been developed. This is advantageous because palm-kernel oil commands a higher price than palm oil.

#### 9.4. Oils Produced Through Genetic Engineering

*Genetically modified seed oils:* Recent years have witnessed great strides in the understanding and application of genetic engineering. This has been applied to

oilseed plants to produce mainly agronomic benefits such as resistance to herbicides and to pests, shorter times between sowing and harvest, increased yield, and so on. But these techniques have also been applied to changing fatty acid composition and hence triacylglycerol composition. A few products are commercially available at the present time, others are at the stage of field trials, and yet more have been obtained in the laboratory. The number of available products should increase rapidly in the next few years (199–201). The number of known fatty acids exceeds 1000, although only a small number of these—perhaps around 25—are of common concern. Most vegetable oils contain only palmitic, oleic, and linoleic acids as major components in varying proportions. These are accompanied by stearic and linolenic acid in some seed oils. Others that become major components in selected seed oils include 8:0, 10:0, 12:0, 14:0, 18:1(6c), 20:1, 22:1, 24:1, ricinoleic, vernolic, and GLA. Some of these are related biosynthetically as already discussed (Section 2).

This range shows that plant lipids as a whole can produce a wide variety of fatty acids, sometimes at very high levels. It follows that the enzymes necessary to produce these less common acids are available somewhere within the plant kingdom. It is then possible to identify these, clone them, and introduce them into species that are already cultivated on a large scale such as rapeseed, soybean, maize (corn), sunflower, and linseed among others (143, 202–204).

The rape plant seems to lend itself to genetic manipulation, and the first genetically modified oilseed with changed fatty acid profile was canola oil containing lauric acid. This was developed by Calgene, and the crop is grown in the United States, although successful field trials have been conducted elsewhere. To obtain this new oil, Calgene scientists isolated the thioesterase, which produces lauric acid in the Californian Bay tree and transferred it to the rape plant. With this, *de novo* synthesis stops mainly at the C<sub>12</sub> level rather than the more usual C<sub>16</sub> acid. When introduced into rapeseed, the oil contained more than 50% of lauric acid, although this was somewhat reduced in the commercial crop. To go beyond this

**TABLE 11. Fatty Acid Composition of Commodity Canola Oil and Some Genetically Modified Oils Based on it.**

Oil source	12:0	14:0	16:0	18:0	18:1	18:2	18:3	Other
Rape	—	—	2	2	13	12	9	62*
Canola	—	—	4	2	62	20	9	3
High in								
16:0	—	—	10	1	51	19	13	6
16:0/18:0	—	—	9	10	57	14	4	6
16:0	—	—	29	2	31	22	13	3
18:0	—	—	4	34	22	18	18	4
12:0	40	4	3	1	29	12	8	3
14:0	—	40	3	1	29	10	7	10
18:1	—	—	4	1	84	5	3	3
18:2	—	—	4	2	33	49	7	5

\*20:1 (7%) and 22:1 (54%).

Based on Huang and Ziboh (84).

**TABLE 12. Fatty Acid Composition of Commodity Soybean Oil and Some Genetically Modified Oils Based on it.**

Soybean oil	16:0	18:0	18:1	18:2	18:3	other
Commodity	10	4	23	52	8	2
Saturated (L)	4	3	23	60	10	—
Linoleic (H)	11	6	28	52	3	—
Palmitic (H)	24	4	15	44	11	2
Stearic (H)	8	25	17	39	8	3
Saturated (H)	22	18	9	38	11	2
Sat/len (L)	4	3	28	61	3	1
P/len (H)	19	4	23	48	3	3

H = high, L = low.

Based on Huang and Ziboh (84).

level, it is necessary to introduce a further gene (lysophosphatidic acid acyl transferase, LPAT), which will promote the acylation of the *sn*-2 position with lauric acid. There is some concern about the economic viability of this project.

Other oils at various stages of development include the following (200):

- Rapeseed oils still higher in lauric acid, high in erucic, palmitic, oleic, or linoleic acid, or containing C<sub>8</sub> and C<sub>10</sub> acids, myristic, stearic, petroselinic, ricinoleic, vernolic, or  $\gamma$ -linolenic acid, and wax esters in place of the normal triacylglycerols (Table 11).
- Soybean oils with lower saturated acids, lower linolenic acid, and higher stearic acid as well as seeds producing meal of enhanced nutritional value (Table 12).
- Sunflower oil with high palmitic, stearic, oleic, or linoleic acid (Table 13).
- Corn oil with high oleic acid.

The level of linolenic acid is being reduced because its oxidation leads to undesirable flavors. Saturated acids are being increased to produce oils that can be used to make spreads without partial hydrogenation (see Section 6).

**TABLE 13. Fatty Acid Composition of Commodity Sunflower Oil and Some Genetically Modified Oils Based on it.**

Sunflower oil	16:0	16:1	18:0	18:1	18:2	Other
Commodity	7	—	5	28	59	1
High oleic	3	—	2	92	2	1
High linoleic	8	—	2	13	76	1
High stearic/oleic	5	—	11	79	2	3
High palmitic/oleic	25	6	3	60	4	2
High palmitic/linoleic	27	4	3	17	47	2

Based on Huang and Ziboh (84).

## 10. PRODUCTION AND TRADE STATISTICS

Table 14 contains production and export data for 13 vegetable oils. The figures are given as annual average values for the four 5-year periods from 1991–1995 to 2005–2010 and thus cover a 20-year period. Readers who prefer information for individual years can take the four columns of figures to be close to values reported or expected for the midyear in each quinquennium. viz. 1993, 1998, 2003, and 2008. These figures are taken from “*The Revised Oil World 2020—Supply, Demand and Prices*” produced by ISTA Mielke GmbH of Hamburg in 2002 (205). This company has been producing and interpreting data for oilseeds, oils and fats, and seed meals since 1958. The 2002 publication contains much more relevant information. Attention is drawn to the following points.

- During the 15 years (1993–2008) covered in Table 14, production of oils and fats is expected to rise 69% from 86.8 to 146.7 million tons and exports are expected to double, rising from 25.3 to 50.8 million tons. Comparisons between production and exports of oils and fats are sometimes complicated by the fact that for oilseeds, there is trade in oilseeds as well as in the extracted oils. This does not apply to palm oil traded only as oil.
- Three oilseeds and four oils dominate production and export and have become more dominant with the passage of time. These are soybean oil (produced mainly in the United States, Brazil, Argentina, and China), palm oil (Malaysia and Indonesia), rape/canola oil (China, EU-15, India, and Canada), and

**TABLE 14. Annual Average Production (million tons) of Oils and Fats for the 5-year Periods 1991–1995 to 2006–2010.**

	Production				Exports			
	91–95	96–00	01–05	06–10	91–95	96–00	01–05	06–10
Oil								
World total	86.82	105.06	126.47	146.73	25.29	32.93	41.88	50.82
Soybean	17.90	23.14	29.56	33.60	4.12	6.81	8.88	10.63
Cotton	3.94	4.00	4.60	5.35	0.25	0.22	0.26	0.32
Peanut	4.09	4.56	5.45	5.72	0.27	0.25	0.29	0.31
Sunflower	7.96	9.11	9.83	12.43	2.16	2.93	2.83	4.17
Rape/canola	9.66	12.64	15.34	17.72	1.59	1.90	1.85	2.48
Sesame	0.62	0.70	0.75	0.85	0.02	0.02	0.03	0.03
Corn	1.67	1.91	2.18	2.49	0.49	0.71	0.86	1.04
Olive	1.96	2.47	2.58	2.75	0.38	0.47	0.57	0.62
Palm	13.34	18.72	25.24	31.43	9.55	12.76	18.55	22.66
Palmkernel	1.72	2.34	3.11	3.84	0.84	1.11	1.49	1.81
Coconut	3.03	3.01	3.33	3.70	1.50	1.64	1.91	2.00
Linseed	0.64	0.70	0.69	0.81	0.14	0.13	0.12	0.16
Castor	0.46	0.46	0.54	0.62	0.20	0.25	0.28	0.32
Animal fats*	19.80	21.30	23.26	25.42	3.77	3.69	3.94	4.26

\* Butter, lard, tallow, fish oil.

Adapted from Mielke (205).

**TABLE 15. Production and Export of Three Major Oilseeds and Four Major Oils. Figures are Percent of Total Production and Exports Based on Average Annual Levels (Million Tons) for the 5 year Period 2001–2005.**

	Oilseeds		Oils and Fats	
	Production%	Exports%	Production%	Exports%
Soybean	55.2	79.5	23.4	21.2
Rape/canola	12.6	10.4	12.1	4.4
Sunflower	8.4	3.8	7.8	6.8
Palm	—	—	19.9	44.3

Adapted from Mielke (205).

sunflower oil (ex-USSR, EU-15, and Argentina). These are produced mainly in the countries listed (Table 14 and 15).

- Cottonseed and groundnut (peanut) oils and some of the minor oils are used almost entirely in the country of origin and exports amount to very little.

## 11. CONCLUSION

The vegetable oils are important materials, significant for agriculture, the refining and processing industries, and for the food industry. Their production and use is based on a wide range of supporting sciences (physics, chemistry, biochemistry, agriculture, seed breeding, molecular biology, engineering, food science, nutrition, and medicine among others). At present, we are particularly dependent on five oils from four sources: soybean, the oil palm producing two different oils, rapeseed (canola), and sunflower. These, and the minor vegetable oils, are produced and used at increasing levels each year and are essential components of the human diet. Although some individuals consume too much, others have too little and demand is expected to grow for many years yet. As the land available to grow these materials is limited, it is essential to increase yields. The vegetable oils do not always have ideal physical and nutritional properties, so methods of modifying the oils have been developed. For the most part, these have been technological in the past, but increasingly in the future, they are expected to be biological. In the second half of the twentieth century, soybean oil and palm oil have risen to prominence. Will there be new major oils developed in the next half century? This seems unlikely, but we may yet be surprised.

## REFERENCES

1. F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, U.K., 2002.
2. J. L. Harwood, *Biochim. Biophys. Acta*, **1301**, 7–56 (1996).

3. J. L. Harwood, in F. D. Gunstone, ed., *Lipid Synthesis and Manufacture*, Sheffield Academic Press, Sheffield, U.K., 1999, pp. 422–466.
4. K. L. Parkin, in C. C. Akoh and D. B. Min, eds., *Food Lipids—Chemistry, Nutrition, and Biochemistry*, 2nd ed., Marcel Dekker, New York, 2002, pp. 909–965.
5. P. Broun, J. Shanklin, E. Whittle, and C. Somerville, *Science*, **282**, 1315–1317 (1998).
6. E. Heinz, *Eur. J. Lipid Sci. Technol.*, **104**, 1–2 (2002).
7. B. F. Szuhaj, *Lecithins: Sources, Manufacture, and Uses*, AOCS Press, Champaign, Illinois, 1989.
8. M. Schneider, in F. D. Gunstone and F. B. Padley eds., *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997, pp. 51–78.
9. F. D. Gunstone, in F. D. Gunstone, ed., *Structured and Modified Lipids*, Dekker, New York, 2001, pp. 241–250.
10. J. P. Clark, *Lipid Technol.*, **8**, 111–114 (1996).
11. F. D. Gunstone, in W. Hamm and R. J. Hamilton, eds., *Edible Oil Processing*, Sheffield Academic Press, Sheffield, U.K., 2000, pp. 1–33.
12. Y. B. Che Man and C. P. Tan, in F. D. Gunstone, ed., *Lipids for Functional Foods and Nutraceuticals*, Oily Press, Bridgwater, U.K., 25–52 (2003).
13. C. K. Ooi, Y. M. Choo, S. C. Yap, Y. Basiron, and A. S. H. Ong, *J. Amer. Oil Chem. Soc.*, **71**, 423–426 (1994).
14. B. S. Baharin, K. A. Rahman, M. I. A. Karim, T. Oyaizu, K. Tanaka, Y. Tanaka, and S. Takagi, *J. Amer. Oil Chem. Soc.*, **75**, 399–404 (1998).
15. N. V. Yaniishlieva, K. Aitzetmuller, and V. G. Raneva, *Fett/Lipid*, **100**, 444–462 (1998).
16. B. K. Larsson, A. T. Eriksson, and M. Cervenka, *J. Amer. Oil Chem. Soc.*, **64**, 365–370 (1987).
17. F. D. Gunstone, *Inform*, **13**, 65–68 (2002).
18. H. Okuyama, T. Kobayashi, and S. Watanabe, *Prog. Lipid Res.*, **35**, 409–457 (1997).
19. H. Okuyama, *Lipid Technol. Newslett.*, **6**, 128–132 (2000).
20. I. M. Stewart and R. E. Timms, in K. K. Rajah, ed., *Fats in Food Technology*, Sheffield Academic Press, Sheffield, U.K., 2002, pp. 159–191.
21. R. E. Timms, *Confectionery Fats Handbook*, The Oily Press, Bridgwater, U.K., 2003.
22. C. Leonard, *Lipid Technol.*, **6**, 79–83 (1994).
23. C. E. Temple-Heald, *Lipid Technol. Newslett.*, **6**, 59–63 (2000).
24. C. E. Temple-Heald, in F. D. Gunstone, ed. *Rapeseed and Canola Oil—Production, Processing, Properties and Uses*, Blackwell Publishing, Oxford, U.K., 2004, pp. 111–130.
25. R. Przybylski and T. Mag, in F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, U.K., 2002, pp. 98–127.
26. H-J. Caupin, in F. D. Gunstone and F. B. Padley, eds, *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997, pp. 787–795.
27. C. Crews, in M. Jee, ed., *Oils and Fats Authentication*, Blackwell Publishing, Oxford, U.K., 2002, pp. 66–94.
28. Anon., *Inform*, **3**, 1080–1090 (1992).
29. T. P. Pantzaris and Y. Basiron, in F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, U.K., 2002, pp. 157–202.



30. T. A. Isbell, *Lipid Technol.*, **14**, 77–80 (2002).
31. R. A. Moreau, in F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, U.K., 2002, pp. 278–296.
32. R. D. O'Brien, in F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, U.K., 2002, pp. 203–230.
33. B. E. Prentice and M. D. Hildebrand, *Lipid Technol.*, **3**, 83–89 (1991).
34. S. C. Cunnane and L. U. Thompson, eds., *Flaxseed in Human Nutrition*, AOCS Press, Champaign, Illinois, 1995.
35. A. G. Green, and J. C. P. Dribnenki, *Lipid Technol.*, **6**, 29–33 (1994).
36. Anon., *Lipid Technol. Newslett.*, **1**, 80–81 (1995).
37. Advisory Committee on Novel Foods and Processes (UK), Annual Report, 1997, p. 8.
38. Anon. *Lipid Technol. Newslett.*, **4**, 13–15 (1998).
39. D. Boskou, in F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, U.K., 2002, pp. 244–277.
40. B. S. Jalani and N. Rajanaidu, *Lipid Technol.*, **12**, 5–8 (2000).
41. B. S. Jalani, S. C. Cheah, N. Rajanaidu, and A. Darus, *J. Amer. Oil Chem. Soc.*, **74**, 1451–1455 (1997).
42. S. W. Lin, in F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, U.K., 2002, pp. 59–97.
43. Sanders, in F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, U.K., 2002, pp. 231–243.
44. F. D. Gunstone, ed., *Rapeseed and Canola Oil—Production, Processing, Properties and Uses*, Blackwell Publishing, Oxford, U.K., 2004.
45. A. G. Gopala Krishna, *Lipid Technol.*, **14**, 80–84 (2002).
46. B. Sayre and R. Saunders. *Lipid Technol.*, **2**, 72–76 (1990).
47. Anon., *J. Amer. Oil Chem. Soc.*, **66**, 615–618 (1989).
48. H. P. Gupta, *J. Amer. Oil Chem. Soc.*, **66**, 620–623 (1989).
49. S. Bhattacharyya and D. K. Bhattacharyya, *J. Amer. Oil Chem. Soc.*, **66**, 1469–1471 (1989).
50. Anon., *Inform*, **8**, 968 (1997).
51. S. P. Kochar, in F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, U.K., 2002, pp. 297–326.
52. J. R. Smith, *Safflower*, AOCS Press, Champaign, Illinois, 1996.
53. S. P. Kochar, *Lipid Technol. Newslett.*, **6**, 35–39 (2000).
54. T. Wang, in F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, U.K., 2002, pp. 18–58.
55. F. D. Gunstone, *J. Oleo Sci.*, **50**, 269–279 (2001).
56. M. K. Gupta, in F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, U.K., 2002, pp. 128–156.
57. A. Hase and S. Pajakkala, *Lipid Technol.*, **6**, 110–114 (1994).
58. Anon., *Lipid Technol.*, **10**, 98 (1998).
59. G. Webster, *Lipid Technol. Newslett.*, **4**, 38–43 (1998).

60. F. D. Gunstone, *Fett/Lipid*, **101**, 124–131 (1999).
61. F. D. Gunstone, in F. D. Gunstone, ed., *Structured and Modified Lipids*, Dekker, New York, 2001, pp. 185–208.
62. D. Firestone, *Official Methods and Recommended Practices of the AOCS, Physical and Chemical Characteristics of Oils, Fats and Waxes*, Champaign, Illinois, 1997.
63. E. Ucciani, *Nouveau Dictionnaire des Huiles Vegetales*, Paris, France, 1995.
64. F. B. Padley, in F. D. Gunstone, J. L. Harwood, and F. B. Padley, eds., *The Lipid Handbook*, 2nd ed., Chapman and Hall, London, 1994, p. 53.
65. K. Aitzetmuller and M. Ihrig, *Fat Sci. Technol.*, **90**, 464–470 (1988).
66. M. S. Prats-Moya, N Grane-Teruel, V. Berenguer-Navarro, and M. I. Martin-Carattalla, *J. Amer. Oil Chem. Soc.*, **76**, 267–272 (1999).
67. C. K. Lyon and R Becker, *J. Amer. Oil Chem. Soc.*, **64**, 233–236 (1987).
68. J. T. Budin, W. M. Breene, and D. H. Putnam, *J. Amer. Oil Chem. Soc.*, **73**, 475–481 (1996).
69. H. Sun, D. Wiesenborn, K. Tostenson, J. Gillespie, and P. Rayas-Duarte, *J. Amer. Oil Chem. Soc.*, **74**, 413–418 (1997).
70. F. Jahaniavali, Y. Kakuda, and M. F. Marcone, *J. Amer. Oil Chem. Soc.*, **77**, 847–852 (2000).
71. B. S. Kamel and Y. Kakuda, *J. Amer. Oil Chem. Soc.*, **69**, 492–494 (1992).
72. Advisory Committee on Novel Foods and Processes (UK), Annual Report, 1993.
73. M. Zlatanov and I. Janakieva, *Fett/Lipid*, **100**, 312–315 (1998).
74. M. J. Weerman and L. Neeman, *J. Amer. Oil Chem. Soc.*, **64**, 229–232 (1987).
75. H. E. Swisher, *J. Amer. Oil Chem. Soc.*, **65**, 1704–1706 (1988).
76. Y. F. Lozano, C. D. Mayer, C. Bannon, and E. M. Gaydou, *J. Amer. Oil Chem. Soc.*, **70**, 561–565 (1993).
77. L. Eyres, N. Sherpa, and G. Hendricks, *Lipid Technol.*, **13**, 84–88 (2001).
78. J. Birkbeck, *Food New Zealand*, April/May, 40–42 (2002).
79. J. L. R. Pritchard in J. B. Rossell and J. L. R. Pritchard, eds., *Analysis of Oilseeds, Fats, and Fatty Foods*, Elsevier, London, 1991, pp. 77–97.
80. P. M. Clough, in F. D. Gunstone, ed., *Structured and Modified Lipids*, Marcel Dekker, New York, 2001, pp. 75–117.
81. P. M. Clough, *Lipid Technol.*, **13**, 9–12 (2001).
82. Y-S. Huang and D. E. Mills,  *$\gamma$ -Linolenic Acid*, AOCS Press, Champaign, Illinois, 1996.
83. S. P. J. N. Senanayake and F. Shahidi, *J. Amer. Oil Chem. Soc.*, **77**, 55–61 (2000).
84. Y-S. Huang and V. A. Ziboh,  *$\gamma$ -Linolenic Acid: Recent Advances in Biotechnology and Clinical Applications*, AOCS Press, Champaign, Illinois, 2001.
85. Y-S. Ju and T-C. Chen, *J. Amer. Oil Chem. Soc.*, **79**, 29–32 (2002).
86. I. M. Stewart and R. E. Timms, in K. K. Rajah, ed., *Fats in Food Technology*, Sheffield Academic Press, Sheffield, U.K., 2002, pp. 159–191.
87. I. Campbell (2002). Available: <http://www.britanniafood.com/invite>.
88. J. A. Vasconcellos, W. P. Bemis, J. W. Berry, and C. W. Weber, in E. H. Pryde, L. H. Princen, and K. D. Mukherjee, eds., *New Sources of Fats and Oils*, AOCS Press, Champaign, Illinois, 1981, pp. 55–68.

89. F. B. Padley, in F. D. Gunstone, J. L. Harwood, and F. B. Padley, eds., *The Lipid Handbook*, 2nd ed., Chapman and Hall, London, 1994, pp. 54–55.
90. F. D. Gunstone, in F. D. Gunstone, ed., *Structured and Modified Lipids*, Marcel Dekker, New York, 2001, p. 191.
91. T. G. Toschi, M. F. Caboni, G. Pennazzi, G. Lercker, and P. Capella, *J. Amer. Oil Chem. Soc.*, **70**, 1017–1020, 1993.
92. B. S. Kamel and Y. Kakuda, *J. Amer. Oil Chem. Soc.*, **69**, 492–494 (1992).
93. F. Comes, M. Farines, A. Aumelas, and J. Soulier, *J. Amer. Oil Chem. Soc.*, **69**, 1224–1227 (1992).
94. Advisory Committee on Novel Foods and Processes (UK), Annual Report (1993).
95. Anon, *Lipid Technol.*, **5**, 53 (1993).
96. B. S. J. Jeffrey and F. B. Padley, *J. Amer. Oil Chem. Soc.*, **68**, 123–127 (1991).
97. R. Ayerza, *J. Amer. Oil Chem. Soc.*, **72**, 1079–1081 (1995).
98. F. D. Gunstone, in F. D. Gunstone, ed., *Structured and Modified Lipids*, Marcel Dekker, New York, 2001, p. 192.
99. K. J. Lessman and W. P. Andersom, in E. H. Pryde, L. H. Princen, and K. D. Mukherjee, eds., *New Sources of Fats and Oils*, AOCS Press, Champaign, Illinois, 1981, pp. 223–246.
100. M. A. Reuber, L. A. Johnson, and L. R. Watkins, *J. Amer. Oil Chem. Soc.*, **78**, 661–664 (2001).
101. G. Steinke and K. D. Mukherjee, *J. Amer. Oil Chem. Soc.*, **77**, 361–366 and 367–371 (2000).
102. Anon, *Inform*, **12**, 921 (2001).
103. D. P. Geller and J. W. Goodrum, *J. Amer. Oil Chem. Soc.*, **77**, 111–114 (2000).
104. V. Pandey, R. Banerji, B. S. Dixit, M. Singh, S. Shukla, and S. P. Singh, *Eur. J. Lipid Sci. Technol.*, **102**, 463–466 (2000).
105. F. D. Gunstone, in F. D. Gunstone, J. L. Harwood, and F. B. Padley, eds., *The Lipid Handbook*, 2nd ed., Chapman and Hall, London, 1994, pp. 15 and 109.
106. J. T. Budin, W. M. Breene, and D. H. Putnam, *J. Amer. Oil Chem. Soc.*, **72**, 309–315 (1995).
107. A. Bonjean, B. Monteuis, and A. Messean, *Oleagineux Corps gras Lipides*, **2**, 97–100 (1995).
108. J. Zubr, *Ind. Crops Products*, **6**, 113–119 (1997).
109. A. Hebard, *Lipid Technol.*, **10**, 81–83 (1998).
110. E. C. Leonard, *Inform*, **9**, 830–838 (1998).
111. S. Moret, A. Dudine, and L. S. Conte, *J. Amer. Oil Chem. Soc.*, **77**, 1289–1292 (2000).
112. G. P. Savage, D. L. McNeil, and P. C. Dutta, *J. Amer. Oil Chem. Soc.*, **74**, 755–759 (1997).
113. C. Bertoli, M. Stancanelli, D. Gummy, and P. Lambelet, *J. Amer. Oil Chem. Soc.*, **75**, 1037–1040 (1998).
114. M. H. Gordon, C. Covell, and N. Kirsch, *J. Amer. Oil Chem. Soc.*, **78**, 621–624 (2001).

115. M. L. Ruiz del Castillo, M. Herraiz, M. D. Molero, and A Herrera, *J. Amer. Oil Chem. Soc.*, **78**, 1261–1265 (2001).
116. Anon., *Lipid Technol. Newslett.*, **4**, 25–26 and 48 (1998).
117. Anon., *Inform*, **12**, 1213 (2001).
118. Anon., *Inform*, **11**, 404–405 (2000).
119. R. Przybylski and K. C. Fitzpatrick, *Inform*, **13**, 489–491 (2002).
120. J. Sargent and K. Coupland, *Lipid Technol.*, **6**, 10–14 (1994).
121. V. K. S. Shukla and U. Blicher-Mathiesen, *Fat Sci. Technol.*, **95**, 367–369 (1993).
122. V. K. S. Shukla, in R. J. Hamilton, ed., *Developments in Oils and Fats*. Blackie, London, 1995, pp. 66–94.
123. M. Lipp. and E. Anklam, *Food Chem.*, **62**, 73–97 (1998).
124. J. Birkett, in B. Rossell, ed., *Oils and Fats Handbook, Vol 1, Vegetable Oils and Fats*, Leatherhead Food RA, Leatherhead, U.K., 1999, pp. Ch. 1–22.
125. Y. Jeyarani and S. Yella Reddy, *J. Amer. Oil Chem. Soc.*, **76**, 1431–1436 (1999).
126. T. Abbott, *Inform*, **8**, 1169–1175 (1997).
127. N. Macfarlane and R. V. Harris, in E. H. Pryde, L. H. Princen, and K. D. Mukherjee, eds., *New Sources of Fats and Oils*, AOCS Press, Champaign, Illinois, 1981, pp. 103–108.
128. C. Croy, *Inform*, **5**, 970–971 (1994).
129. S. M. Osman, in E. H. Pryde, L. H. Princen, and K. D. Mukherjee, eds., *New Sources of Fats and Oils*, AOCS Press, Champaign, Illinois, 1981, pp. 129–140.
130. S. Yella Reddy and T. Jeyarani, *J. Amer. Oil Chem. Soc.*, **78**, 635–640 (2001).
131. R. J. Janssens, *Lipid Technol. Newslett.*, **7**, 133–137 (1999).
132. R. J. Janssens and W. P. Vernooij, *Inform*, **12**, 468–477 (2000).
133. R. J. Janssens, *Lipid Technol.*, **12**, 53–58 (2000).
134. G. D. Jolliff in E. H. Pryde, L. H. Princen, and K. D. Mukherjee, eds., *New Sources of Fats and Oils*, AOCS Press, Champaign, Illinois, 1981, pp. 269–285.
135. T. A. Isbell, *Lipid Technol.*, **9**, 140–144 (1997).
136. T. A. Isbell, in F. D. Gunstone, ed., *Lipid Synthesis and Manufacture*, Sheffield Academic Press, Sheffield, U.K., 1998, pp. 401–421.
137. H. B. Frykman, T. Isbell, and S. C. Cermak, *J. Surfactants Detergents*, **3**, 179–183 (2000).
138. T. A. Isbell and S. C. Cermak, *J. Amer. Oil Chem. Soc.*, **78**, 527–531 (2001).
139. C. C. Akoh and C. V. Nwosu, *J. Amer. Oil Chem. Soc.*, **69**, 314–316 (1992).
140. F. B. Padley, in F. D. Gunstone, J. L. Harwood, and F. B. Padley, eds., *The Lipid Handbook*, 2nd ed., Chapman and Hall, London, 1994, pp. 77–78.
141. J-W. Kao, E. G. Hammond, and P. J. White, *J. Amer. Oil Chem. Soc.*, **75**, 1103–1107 (1998).
142. F. Bondioli, C. Mariani, A. Lanzani, E. Fideli, and A. Muller, *J. Amer. Oil Chem. Soc.*, **70**, 763–766 (1993).
143. F. D. Gunstone, *J. Oleo Science*, **50**, 269–279 (2000).
144. Anon., *Inform*, **5**, 713 (1994).
145. G. Ustun, L. Kent, N. Cekin, and H. Civelekoglu, *J. Amer. Oil Chem. Soc.*, **67**, 958–960 (1990).

146. M. A. M. Zeitou and W. E. Neff, *Oleagineux Corps gras Lipides*, **2**, 245–248 (1995).
147. K. Aitzetmuller, G. Werner, and S. A. Ivanov, *Oleagineux Corps gras Lipides*, **4**, 385–388 (1997).
148. H. R. H. Takruri and M. A. F. Dameh, *J. Sci. Food Agric.*, **76**, 404–410 (1998).
149. P. C. Dutta, S. Helmersson, E. Kebedu, G. Alemaw, and L. A. Appelqvist, *J. Amer. Oil Chem. Soc.*, **71**, 839–845 (1994).
150. T. P. Hilditch and P. N. Williams, *The Chemical Constitution of Natural Fats*, 4th ed., Chapman and Hall, London, 1964, p. 336.
151. Anon., *Lipid Technol. Newslett.*, **4**, 105–106 (1998).
152. J. M. Holmbach, A. A. Karlsson, and K. C. Arnoldsson, *Lipids*, **36**, 153–165 (2001).
153. B. G. Herslof, *Lipid Technol.*, **12**, 125–128 (2000).
154. M. Zhou, K. Robards, M. Glennie-Holmes, and S. Helliwell, *J. Amer. Oil Chem. Soc.*, **76**, 159–169 (1999).
155. D. M. Peterson, *Lipid Technol.*, **14**, 56–59 (2002).
156. F. B. Padley, in F. D. Gunstone, J. L. Harwood, and F. B. Padley, eds., *The Lipid Handbook*, 2nd ed., Chapman and Hall, London, 1994, p. 79.
157. Advisory Committee in Novel Foods and Procedures (UK), Annual Report, 1991.
158. T. Longvah and Y. G. Deosthale, *J. Amer. Oil Chem. Soc.*, **68**, 781–784 (1991).
159. H-S Shin and S-W Kim, *J. Amer. Oil Chem. Soc.*, **71**, 619–622 (1994).
160. I-H. Kim, H. Kim, K-T. Lee, S-H. Chung, and S-M. Ko, *J. Amer. Oil Chem. Soc.*, **79**, 363–367 (2002).
161. S. M. Dyszel and B. C. Pettit, *J. Amer. Oil Chem. Soc.*, **67**, 947–951 (1990).
162. M. Maskan and S. Karatas, *J. Sci Food Agric.*, **77**, 334–340 (1998).
163. M. Yildiz, S. T. Gurcan, and M. Ozdemir, *Fett/Lipid*, **100**, 84–86 (1998).
164. S. P. Singh, S. Shukla, K. R. Khanna, B. S. Dixit, and R. O. Banerji, *J. Sci. Food Agric.*, **76**, 168–172 (1998).
165. T. R. Omara-Alwala, T. Mebrahtu, D. E. Prior, and M. O. Ezekwe, *J. Amer. Oil Chem. Soc.*, **68**, 198–199 (1991).
166. B. Yang and H. Kallio, *J. Agric. Food Chem.*, **49**, 1939–1947 (2001).
167. H. Kallio, B. Yang, P. Peippo, R. Tahvonen, and R. Pan, *J. Agric. Food Chem.*, **50**, 3004–3009 (2002).
168. B. Yang, R. Karlsson, P. Oksman, and H. Kallio, *J. Agric. Food Chem.*, **49**, 5620–5629 (2001).
169. Yang Baoru, F. D. Gunstone, and H. Kallio, in F. D. Gunstone, ed., *Lipids for Functional Foods and Nutraceuticals*, Oily Press, Bridgwater, U.K., 2003, pp. 263–290.
170. Anon., *Lipid Technol. Newslett.*, **2**, 100 (1996).
171. R. Oliveira, M. F. Rodrigues, and M. G. Bernardo-Gil, *J. Amer. Oil Chem. Soc.*, **79**, 225–230 (2002).
172. P. J. Barnes and P. W. Taylor, *J. Sci. Food Chem.*, **31**, 997–1006 (1980).
173. P. J. Barnes, *Fat Sci. Technol.*, **84**, 256–269 (1982).
174. R. L. Wolff, N. A. Combe, F. Destailats, C. Boue, D. Precht, J. Molкетин, and B. Enstressangles, *Lipids*, **35**, 825–825 (2000).
175. E. Vos, *Abstracts, AOCS Meeting*, Montreal, QE, Canada, 2002.

176. T. Koyano and K. Sato, in K. K. Rajah, ed., *Fats in Food Technology*, Sheffield Academic Press, Sheffield, U.K., 2002, pp. 1–29.
177. A. J. Dijkstra, in K. K. Rajah, ed., *Fats in Food Technology*, Sheffield Academic Press, Sheffield, U.K., 2002, pp. 123–158.
178. J. M. Block, D. Barrera-Arellano, M. F. Figueiredo, and F. A. C. Gomide, *J. Amer. Oil Chem. Soc.*, **74**, 1537–1541 (1997).
179. R. Timms, in F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997, pp. 199–222.
180. V. Gibon and A. Tirtiaux, *Lipid Technol.*, **14**, 33–36 (2002).
181. T. Willner and K. Weber, *Lipid Technol.*, **6**, 57–60 (1994).
182. E. Deffense, *Lipid Technol.*, **7**, 34–38 (1995).
183. T. Koetsier, in F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997, pp. 285–303.
184. A. J. Dijkstra, *Inform*, **8**, 1150–1158 (1997).
185. R. C. Hastert, *Lipid Technol.*, **10**, 101–105 (1998).
186. D. Allen, *Lipid Technol.*, **10**, 29–33 and 53–57 (1998).
187. D. Rousseau and A. G. Maringoni, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry Nutrition, and Biotechnology*, 2nd ed., Marcel Dekker, New York, 2002, pp. 301–333.
188. A. Rosendahl and A. R. Macrae, in F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997, pp. 245–259.
189. K. D. Mukherjee, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry Nutrition, and Biotechnology*, 2nd ed., Marcel Dekker, New York, 2002, pp. 751–812.
190. W. M. Willis and A. G. Maringoni, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry Nutrition, and Biotechnology*, 2nd ed., Marcel Dekker, New York, 2002, pp. 839–876.
191. A. R. Kavanagh, *Oleagineux Corps gras Lipides*, **4**, 165–168 (1997).
192. C. C. Akoh, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry Nutrition, and Biotechnology*, 2nd ed., Marcel Dekker, New York, 2002, pp. 877–908.
193. U. T. Bornscheuer, M. Adamczak, and M. M. Soumanou, in F. D. Gunstone, ed., *Lipids for Functional Foods and Nutraceuticals*, Oily Press, Bridgwater, U.K., 2003, 149–182.
194. Anon., *Inform*, **2**, 678–692 (1991).
195. L. J. M. van Soest and F. Mulder, *Lipid Technol.*, **5**, 60–65 (1993).
196. F. D. Gunstone, in F. D. Gunstone, ed., *Structured and Modified Lipids*, Marcel Dekker, New York, 2001, p. 192.
197. J. Wisniak, ed., *The Chemistry and Technology of Jojoba Oil*, AOCS Press, Champaign, Illinois, 1987.
198. L. G. Angelini, E. Moschemi, G. Collonna, B. Polloni, and E. Bonari. *Ind. Crops Products*, **6**, 313–323 (1997).
199. R. K. Downey and D. C. Taylor, *Oleagineux Corps gras Lipides*, **3**, 9–13 (1996).
200. A. J. Kinney, *Lipid Technol. Newslett.*, **5**, 36–39 (1999).
201. W. Friedt and W. Luhs, *Fett/Lipid*, **100**, 219–226 (1998).
202. J. Reske, J. Siebrecht, and J. Hazebroek, *J. Amer. Oil Chem. Soc.*, **74**, 989–998 (1997).

203. F. D. Gunstone and R. M. Pollard, in F. D. Gunstone ed., *Structured and Modified Lipids*, Dekker, New York, 2001, pp. 155–184.
204. V. C. Knauf and A. J. DelVecchio, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry Nutrition, and Biotechnology*, 2nd ed., Marcel Dekker, New York, 2002, pp. 967–996.
205. S. Mielke, *The Revised Oil World 2020—Supply, Demand and Prices*, ISTA Mielke GmbH, Hamburg, Germany, 2002.

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## *Lipid Oxidation: Theoretical Aspects*

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### **1. INTRODUCTION**

Many excellent chapters and books have been written on lipid oxidation (1–11). Studies of lipid oxidation are provided differently by different authors: each scientist studying lipid oxidation focuses on a different single aspect, such as following early kinetics by oxygen uptake or LOOH production, determining volatile products by gas chromatography (GC) or nonvolatile products by high-performance liquid chromatography (HPLC), or analyzing specific catalyst or antioxidant effects on oxidation; oxidation mechanisms are then interpreted in that context. There have been few attempts to integrate multiple stages or approaches to lipid oxidation, and as a result, descriptions of lipid oxidation have been disparate and totally dependent on the individual aspect being studied. This can be quite confusing to anyone not deeply immersed in the field. That is not to say that any of the published information is incorrect. Much of it, however, has been presented in too narrow of a context to provide an accurate overall picture of complex lipid oxidation reactions.

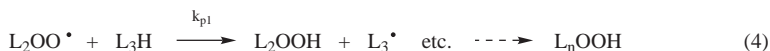
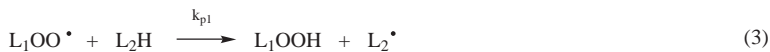
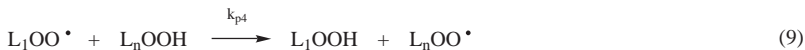
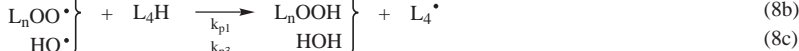
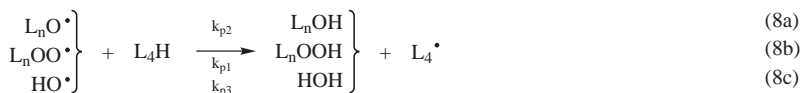
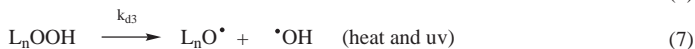
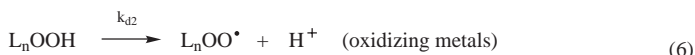
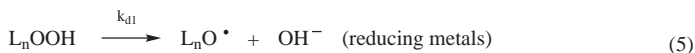
Part of the problem stems from considering lipid oxidation as precisely following classic free radical chain reactions. To be sure, lipids do oxidize by a radical chain mechanism, and they show initiation, propagation, and termination stages



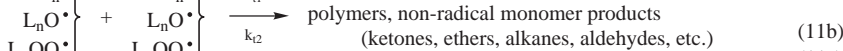
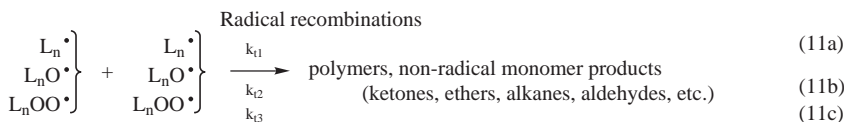
## CLASSIC FREE RADICAL CHAIN REACTION MECHANISM OF LIPID OXIDATION

Initiation (formation of *ab initio* lipid free radical)

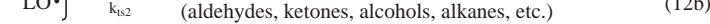
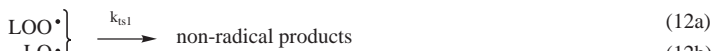
Propagation

*Free radical chain reaction established**Free radical chain branching (initiation of new chains)*

Termination (formation of non-radical products)



Radical scissions



i - initiation; o - oxygenation;  $\beta$  -  $O_2$  scission; p - propagation; d - dissociation; t - termination;  
ts - termination/scission

**Figure 1.** Classic free radical chain reaction mechanism of lipid oxidation with propagation by a series of hydrogen abstractions.

as is normally depicted (Figure 1). However, the generalized reactions of the classic free radical chain reaction scheme are very much oversimplified and, because they do not portray the wide range of competing side reactions that contribute to the great complexities of lipid oxidation, they are often inconsistent with observed oxidation kinetics and product mixes.

Thus, this chapter presents lipid oxidation from a broad systems perspective to make the overall process logical, reconcile some common inconsistencies in proposed mechanisms, address some of the complexities that are important in directing downstream pathways and ultimate product mix, and develop an integrated view of lipid oxidation. In doing so, attempts are made to bridge basic chemistry to applied lipid and food chemistry. "Old" literature is cited liberally, despite current trends to ignore anything outside the previous two to five years, because the fundamental chemistry is still relevant, the early researchers in the field deserve recognition for their ground-breaking observations, and the information needs to be revisited to remind us of what already has been done to prevent "rediscovering the wheel." Furthermore, consideration of fundamentals too often gets lost in the sophistication of applications, particularly in biological systems. Lipid oxidation processes in foods or biological tissues may be more complicated, but will still follow fundamental mechanisms identified in simpler chemical reactions. Greater consideration of details learned from fundamental chemistry should help clarify and elucidate mechanisms and kinetics in complex media.

In particular, this chapter will stress the need to look beyond the classic radical chain reaction. Lipid oxidation mechanisms have been proposed based on kinetics, usually of oxygen consumption or appearance of specific products (e.g., LOOH) or carbonyls (e.g., malonaldehyde), assuming standard radical chain reaction sequences. However, when side reactions are ignored or reactions proceed by a pathway different from that being measured, erroneous conclusions can easily be drawn. The same argument holds for catalytic mechanisms, as will be shown in the discussion about metals. In the past, separation and analysis of products was laborious, but contemporary methods allow much more sensitive detection and identification of a broad mix of products. Thus, multiple pathways and reaction tracks need to be evaluated simultaneously to develop an accurate picture of lipid oxidation in model systems, foods, and biological tissues.

*In vivo* lipid oxidation will not be covered, although the fundamental chemistry presented certainly applies wherever lipid oxidation occurs. Also, in light of the product and reaction pathway complexities presented in this chapter, kinetics of lipid oxidation will not be covered. That is not to say that kinetics are not important. However, kinetic analyses are always based on assumptions, and kinetic equations derived in different studies are often difficult to reconcile even in simple systems. The broader consideration being urged in this chapter poses even greater challenges. A citation from the past remains cogently relevant today: "in view of the numerous possible routes that might be followed in the initiation, propagation, and termination stages of the decomposition process, kinetic analysis of the results has proved to be difficult" [(12) citing (13)].

## 1.1. Classic Radical Chain Reaction Scheme

Lipid oxidation has long been recognized as a free radical chain reaction (14–18), and the classic chain reaction scheme with three phases has been repeated in many forms. Figure 1 is one version. Sometimes secondary abstraction reactions of lipid alkoxyl radicals ( $\text{LO}^\bullet$ ) and peroxy radicals ( $\text{LOO}^\bullet$ ) are presented as initiation reactions because they form  $\text{L}^\bullet$  radicals. That is true when lipid oxyl radicals are from outside sources, e.g., lipoxygenase reactions followed by  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  reactions with  $\text{LOOH}$ . However, in the following discussion,  $\text{LO}^\bullet$  and  $\text{LOO}^\bullet$  deriving from the initial  $\text{L}^\bullet$  or its subsequent reactions are considered to mediate propagation or chain branching (initiation of secondary chains) rather than *ab initio* initiation.

The driving force in the chain reaction is the repeated abstraction of hydrogens by  $\text{LOO}^\bullet$  to form hydroperoxides plus free radicals on a new fatty acid. The process continues indefinitely until no hydrogen source is available or the chain is intercepted. The radical chain reaction imparts several unique characteristics to lipid oxidation:

1. Lipid oxidation is autocatalytic—once started, the reaction is self-propagating and self-accelerating.
2.  $G$  (product yield)  $\gg 1$ , i.e., many more than one  $\text{LOOH}$  are formed and more than one lipid molecule are oxidized per initiating event. Chain lengths as long as 200 to 300 lipid molecules have been measured (19, 20) showing how effective a single initiating event can be. However, this also points out one reason why it has been so difficult to study initiation processes—initiators become the proverbial needle in a haystack once oxidation chains become established.
3. Very small amounts of pro- or antioxidants cause large rate changes.
4. The reaction produces multiple intermediates and products that change with reaction conditions and time.

These features present distinct challenges in measuring and controlling lipid oxidation, and are part of the reason why lipid oxidation is a major problem *in vivo* and in storage stability of foods.

Citation of the classic chain reaction for lipid oxidation persists even though, as product analysis and studies of mechanisms have become more sophisticated, there is now considerable evidence that only Reactions 1, 2, and 5 (and perhaps also 6) of Figure 1 are always present. Research has shown that, although hydrogen abstraction ultimately occurs, it is not always the major fate of the initial peroxy or alkoxyl radicals. Indeed, lipid alcohols from H abstraction are relatively minor products of lipid oxidation. There are many competing alternative reactions for  $\text{LOO}^\bullet$  and  $\text{LO}^\bullet$  that propagate the radical chain but lead to different kinetics and different products than expected from the classic reaction sequence (5, 6, 21). A more detailed consideration of each stage shows how this basic radical chain sequence portrays only a small part of the lipid oxidation process and products, and a new overall reaction scheme for lipid oxidation is needed.

## 2. INITIATION (LH $\rightarrow$ L $\cdot$ )

Initiation of lipid oxidation produces the *ab initio* lipid free radicals, L $\cdot$ . The initiation process is not well understood, so it is usually represented in reaction schemes merely as an “X” or “?” over the reaction arrow. Lipid oxidation is a very facile reaction that is nearly ubiquitous in foods and biological systems, so it is often treated as an instantaneous reaction that just happens, and has been referred to as “spontaneous” (22). Nevertheless, lipid oxidation is not a spontaneous reaction! Thermodynamically, oxygen cannot react directly with double bonds because the spin states are different (Reaction 1). Ground state oxygen is in a triplet state (two free electrons in separate orbitals have same spin direction, net positive angular momentum), whereas the double bond is in a singlet state (no unpaired electrons, paired electrons are in the same orbital and have opposite spin, no net angular momentum). Quantum mechanics requires that spin angular momentum be conserved in reactions, so triplets cannot invert (flip spins) to singlet states. Reaction then demands that the double bond be excited into a triplet state, which requires prohibitive amounts of energy ( $E_a = 35\text{--}65$  kcal/mole). Thus, no direct reaction occurs.



To overcome this spin barrier, initiators or catalysts are *required* to start the lipid oxidation process by removing an electron from either the lipid or oxygen or by changing the electron spin of the oxygen. As only trace amounts of catalysts are needed, many situations that appear to be spontaneous or uncatalyzed are actually driven by contaminants or conditions that have gone undetected or unconsidered. Indeed, in most foods, biological systems, and laboratory experiments, it is fair to say that multiple catalysts and initiators are always operative.

The most common initiators are described below. Somewhat more detail than in most reviews of lipid oxidation is presented because control of lipid oxidation ultimately demands control of initiation. Antioxidants that scavenge lipid free radicals after they are formed are always playing “catch up,” and may be totally or partially ineffective if the total radical load from initiation (whether from known or unknown sources) is excessive. To achieve full protection against lipid oxidation and attain long-term stability of any material, control strategies must include elimination, or at least inhibition, of initial alkyl radical production in lipids.

### 2.1. Catalysts

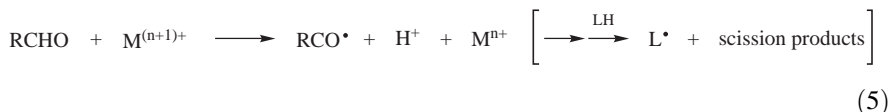
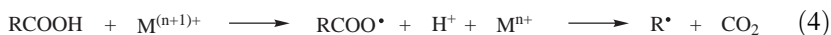
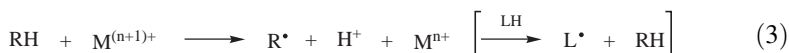
#### 2.1.1. Metals

Redox-active metals are the initiators of perhaps greatest importance for lipid oxidation in oils, foods, and biological systems because they are ubiquitous and active in many forms, and trace quantities ( $\ll$  micromolar) are sufficient for effective catalysis (23–26). Only metals undergoing one-electron transfers appear to be active catalysts; these include cobalt, iron, copper, manganese, magnesium, and

vanadium. Metals that oxidize by two-electron transfers, e.g.,  $\text{Sn}^{2+}$  and  $\text{Tl}^+$ , are not active (23).

The mechanisms and rates of metal-catalyzed initiation operative in individual reaction systems are determined by a complex mixture of factors: the metal and type of complexes it forms (inner sphere or outer sphere), the chelator or complexing agent, redox potential of the metal and its complexes, solvents, phase localization of the metal, and availability of oxygen or preformed hydroperoxides. The reactions outlined below show the multiplicity of mechanisms possible.

**Direct initiation through higher valence metals** involves direct electron transfer from the metal to a bond in the lipids and is the simplest mechanism for metal catalysis. Electron transfer to methyl linoleate is exothermic ( $\Delta H = -62.8 \text{ kJ}, -15 \text{ kcal}$ ), so is probably the dominant initiation mechanism with lipids (23, 27). *Ab initio* lipid radicals are formed directly by removing an electron from a double bond (Reaction 2) (28, 29) or, more generally, from the C-H bond of any labile H in lipid molecules (e.g., allylic hydrogens) (Reaction 3), or via subsequent secondary hydrogen abstraction reactions, as designated in the bracketed reactions.



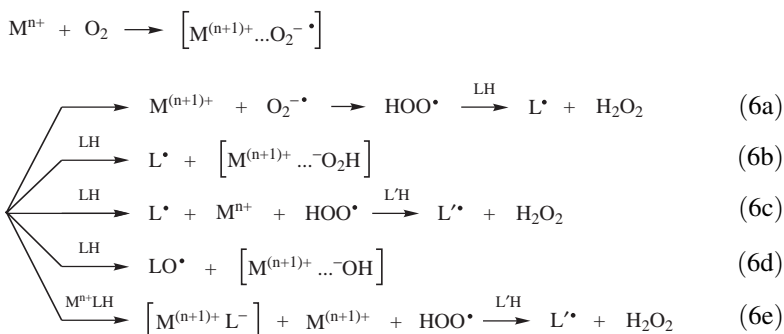
Reactions 2 and 3 have been proposed as the primary mode of catalysis for Co (30), Mn (31), and Cr (32). However, it must be pointed out that metal reactivity can change tremendously with complexing agent, which shifts redox potentials, and with solvent, which alters acid/base properties and electron transfer efficiency. Electron transfer oxidations to generate  $\text{L}\cdot$  are extremely rapid in nonpolar media (33, 34), including neat oils, and are less efficient in aqueous or polar protic solvents.

Analogous electron transfers involving the carboxylic acid group of fatty acids (Reaction 4) or lipid oxidation products such as aldehydes (Reaction 5) (35) can also occur to form radicals that are potential initiators. Reaction 4 with free carboxylic acids has been demonstrated with cobalt and short-chain organic acids (29, 36, 37), so the potential exists for its occurrence with fatty acids. The aldehyde reaction (Reaction 5) is strongly catalyzed by  $\text{Cu}^{2+}$ ,  $\text{Co}^{3+}$ , and  $\text{Mn}^{2+}$  (38-40) and, being inhibited by water competition for ligand sites, occurs primarily in organic solvents or neat lipids. However, the reaction is relatively slow and not competitive with the first three reactions under most food conditions.

The rate and selectivity of the direct electron transfers of Reactions 2-5 are influenced by the type of metal complex formed. In outer sphere complexes, electrons flow directly between the valence shell of the metal and the target group;

electron transfer is fast and selective. Inner sphere complexes involve ligand binding to the metal and electron flow is through the ligands; electron flow is slow and less discriminating (41). Iron forms mostly outer sphere complexes. Copper forms mostly inner sphere complexes with organic substrates, especially in nonpolar solvents, but most inorganic copper salts catalyze direct electron transfer through outer sphere complexes. Cobalt forms inner sphere ligand complexes in nonpolar solvents such as oils (42); but in polar solvents and with polar ligands, cobalt catalyzes electron transfer by an outer sphere mechanism (29, 43, 44). The difference may seem academic, but it partially explains differences in reactivity, kinetics, and products for different metals and in some cases for different complexing agents, and it points out the need to understand mechanisms when determining which products to analyze to most accurately evaluate extent of oxidation.

**Direct initiation by lower valence states ( $M^{n+}$ ) of metals** proceeds through formation of activated complexes with  $O_2$  (23, 45)—mostly via inner sphere complexes. As free reduced metals react rapidly with oxygen (Reaction 6a), this mechanism is active primarily when chelators specifically stabilize the reduced metals. These reactions also proceed mostly facily in nonpolar solvent (46), e.g., in hydrophobic lipid phases of membranes or in oils.



Direct initiation by either mechanism is characterized by a lack of induction period (47) and is most efficient by metals that are strongly oxidizing (Co and Fe) or can form metal-oxygen complexes (Co and Cu).

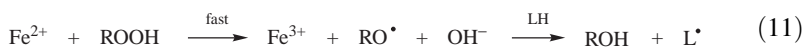
Indirect initiation of lipid oxidation by reduced metals ( $Co^{2+}$ ,  $Fe^{2+}$ ,  $V^{2+}$ ,  $Cr^{2+}$ ,  $Cu^+$ ,  $Ce^{3+}$ ,  $Mn^{2+}$ ) occurs by two different mechanisms, depending on the  $pO_2$  of the system and levels of preformed or nonlipid hydroperoxides:

- a. autoxidation of reduced metals to generate oxygen radicals that then react with lipids (27) occurs at moderate to high  $pO_2$ , e.g., for iron:

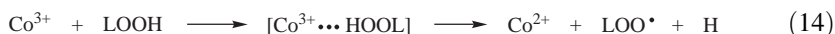


Evidence for this process has been obtained in systems of charged micelles prepared from linolenic acid (48) and by chemiluminescence in very early stages of lipid oxidation in oils and a variety of foods (49).

- b. reduction or oxidation of hydroperoxides (either from other sources or from preformed lipid hydroperoxides) to  $\text{RO}^\bullet$  or  $\text{ROO}^\bullet$ , respectively (Reactions 11 and 12), which then react with lipids; dominates under conditions of low metal, substrate, and oxygen concentration (27, 35). Lipid hydroperoxide reduction is an extremely facile reaction. The activation energy is considerably lower than that of  $\text{H}_2\text{O}_2$  ( $E_{a_{\text{LOOH}}} = 12.5 \text{ kcal}$ ;  $E_{a_{\text{HOOH}}} \sim 35 \text{ kcal}$ ) and the rate of reduction is correspondingly several orders of magnitude faster:  $k_{\text{LOOH}} = 5 \times 10^9 \text{ L} \cdot \text{mol}^{-1} \text{sec}^{-1}$  (50, 51) and  $k_{\text{HOOH}} \sim 10^4 \text{ M}^{-1} \text{sec}^{-1}$  (52).

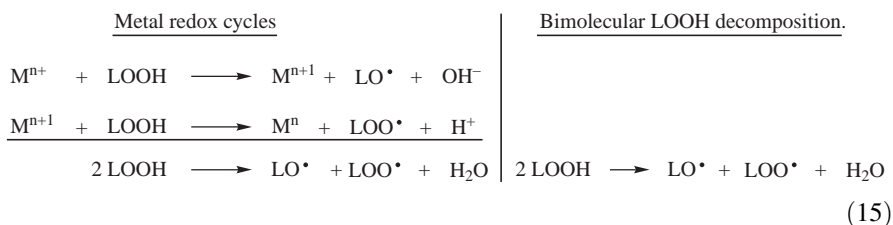


Metals that form complexes with oxygen also form intermediate complexes with hydroperoxides during oxidation and reduction, particularly at low hydroperoxide concentrations and in nonpolar solvents, as shown in Reactions 13 and 14 for cobalt (53–57). However, in polar solvents, cobalt acts by direct electron transfer, as in Reactions 11 and 12 (58). Copper forms similar complexes with hydroperoxides (59).



Metal autoxidation and hydroperoxide decomposition are both very active processes in foods, oils, and biological tissues where metals are always present. Considering the constant presence of peroxides from various sources in all natural materials, it could reasonably be argued that peroxide decomposition is the major practical source of initiators for lipid oxidation. However, these reactions are perhaps even more important in accelerating chain branching in later stages of oxidation when higher concentrations of LOOH accumulate.

Whatever the operative mechanism for a given system, the effect of metals is tremendously amplified when redox cycling occurs. Coordination of redox pairs of metals has the same effect in early stages of lipid oxidation that bimolecular decomposition has in later stages (60):

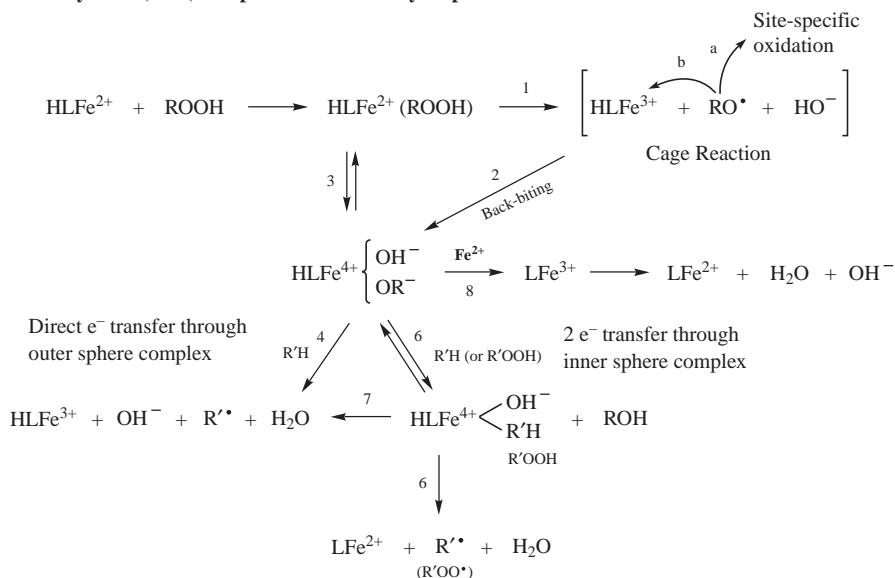
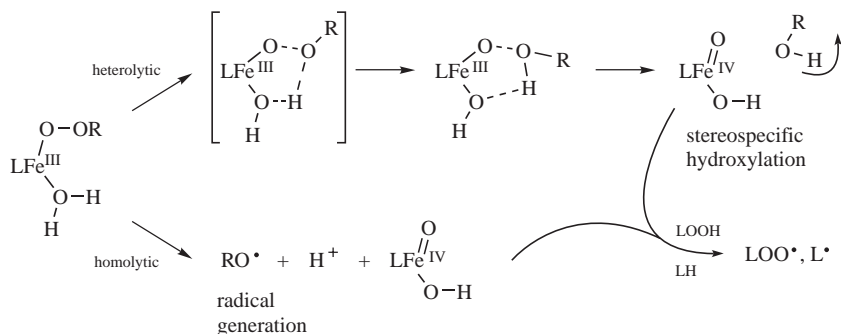


**Initiation by hypervalent metal-oxygen complexes, e.g.,  $\text{Fe}^{4+}=\text{O}$ .** The question of oxidation catalysis by hypervalent iron also needs to be raised because new evidence is suggesting that some of the mechanisms of metal catalysis described above may actually be driven by hyperoxidized iron. Ferryl iron complexes [ $\text{Fe}(\text{IV})=\text{O}$ ;  $\text{FeO}^{2+}$ ] (61) and perferryl iron [ $\text{Fe}(\text{V})$ ] catalyze oxygen insertion into C–H to yield epoxides, ketones, and alcohols. However, the mechanisms for both formation and reactions of  $\text{Fe}(\text{IV})$  complexes are still unclear, and their involvement in initiation of free radical autoxidations is hotly debated. Walling (62), highly respected for his research on Fenton chemistry, disputes the  $\text{Fe}^{4+}$  pathway and argues that one-electron oxidation to  $\text{Fe}^{3+}$  is the major pathway for most iron compounds. Nevertheless, it is well-known that hypervalent iron complexes are transient intermediates in many heme enzyme mechanisms, as will be discussed later, and there is now unequivocal spectroscopic and EPR evidence for  $\text{Fe}(\text{IV})$  participation in nonheme iron enzymes as well (63–65). Still, hypervalent iron complexes were considered too difficult to form and too unstable to be relevant in solution chemistry without porphyrins or proteins as electron sinks until observations that iron reacted with hydrogen peroxide in acid to give the same nonselective products as  $\text{HO}^\bullet$  in pulse radiolysis, whereas in neutral and alkaline solutions, products were more stereospecific and selective (66). This led to the proposal that hypervalent iron does form transiently in some solution reactions and may be the catalytic species involved rather than hydroxyl radicals. The two-electron oxidation of ferrous iron yields an equivalent ferryl peroxy complex,  $2 \text{Fe}^{\text{II}} + \text{O}_2 = [\text{FeOOFe}]^{\text{IV}}$ . There are ten total unpaired spins on each side of the equation, the thermodynamics are favorable ( $\Delta H^\circ = 17 \text{ kCal}$ ,  $\Delta F^\circ = 11$ ), and the reaction can occur without a net spin change (67). Pulse radiolysis studies show that  $\text{FeIV}$  and  $\text{FeV}$  have significant lifetimes when complexed with simple ligands like hydroxide and pyrophosphate and, as such, are plausible intermediates in iron-catalyzed oxidations of organic compounds (68). Thus, participation of  $\text{Fe}(\text{IV})=\text{O}$  may explain aspects of kinetics and product distributions that have not fit traditional  $\text{Fe}^{3+}/\text{Fe}^{2+}$  mechanisms (Reactions 6–14).

There is now substantial evidence that the metal-oxygen complexes described above do indeed form hypervalent intermediates that catalyze both radical and nonradical oxidations (63, 69–78). Most is known about  $\text{Fe}(\text{IV})$  and  $\text{Fe}(\text{V})$  complexes, providing support for the idea that hypervalent iron is at least one catalyst in Fenton reactions (79); analogous complexes have been identified for  $\text{Cu}^{2+}$  (73, 80) and  $\text{Co}^{2+}$  (73). Both  $\text{Fe}^{2+}$  (74) and  $\text{Fe}^{3+}$  (63, 75) complexes participate, although through different routes:  $\text{Fe}^{2+}-\text{HOOH}$  yields  $\text{Fe}^{4+}=\text{O}$ ;  $\text{Fe}^{3+}-\text{HOOH}$  yields the  $\text{Fe}^{3+}-\bullet\text{OH}$  complex, which is functionally equivalent to  $\text{Fe}^{4+}$  (79).

Figure 2 presents overall reaction schemes for the  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  reactions. The schemes include radical and nonradical pathways and represent reactions for both  $\text{H}_2\text{O}_2$  and  $\text{ROOH}$ . In the figure,  $\text{ROOH}$  is used to indicate lipid hydroperoxides to avoid confusion with metal ligands, L, and for simplicity, only the lipid species are carried completely through reaction sequences. These reactions have been determined using  $\text{H}_2\text{O}_2$ , but have not yet been demonstrated specifically with lipids. Nevertheless lipid hydroperoxides are expected to follow the same general



A. Ferryl iron ( $\text{Fe}^{4+}$ ) complexes from  $\text{Fe}^{2+}$ -hydroperoxide reactions:B. Ferryl ( $\text{Fe}^{4+}$ ) and perferryl ( $\text{Fe}^{5+}$ ) iron complexes from  $\text{Fe}^{3+}$ -hydroperoxide reactions:

**Figure 2.** Formation of ferryl iron in initiation and catalysis of lipid oxidation: Reaction schemes for formation of hypervalent iron states by  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  complexes and subsequent reactions leading to radicals that can initiate lipid oxidation. L, metal ligand; R, alkyl or acyl group.  $\text{Fe}^{2+}$  sequence (71, 73);  $\text{Fe}^{3+}$  sequence (81), adapted.

pathways as  $\text{H}_2\text{O}_2$ , although perhaps even more readily because the O—O bond energy is lower in lipids ( $\text{HOOH} = 51 \text{ kcal mol}^{-1}$  vs.  $\text{LOOH} = 25\text{--}35 \text{ kcal mol}^{-1}$ ).

In  $\text{Fe}^{2+}$  reactions (Figure 2A), the initial Fe-hydroperoxide complex formed with  $\text{H}_2\text{O}_2$  or LOOH can undergo a traditional one-electron oxidation (Reaction 1), yielding  $\text{Fe}^{3+}$  and hydroxyl or alkoxy radicals, respectively, in a

cage reaction. In systems where the radicals can diffuse out readily, they escape to react and initiate new lipid oxidation chains (A), or while still in the reaction cage, the oxyl radical can backbite on the  $\text{Fe}^{3+}$  (B) and oxidize it to  $\text{Fe}^{4+}$  (Reaction 2). Alternatively, the Fe-hydroperoxide complex can generate the ferryl iron complex directly by two-electron oxidation to the  $\text{Fe}^{4+}$  complex (Reaction 3).  $\text{Fe}^{4+}$  reactions are responsible for the catalytic power and greatly increased radical production.  $\text{Fe(IV)=O}$  abstracts hydrogens even more rapidly than  $\text{HO}^\bullet$  ( $k > 10^9 \text{ L M}^{-1}\text{s}^{-1}$ ). It can abstract allylic hydrogens from unsaturated fatty acids to form the *ab initio*  $\text{L}^\bullet$  radical or it can abstract H from lipid hydroperoxides to give  $\text{LOO}^\bullet$  that will propagate radical chains. Thus, through either a one-electron process involving outer sphere complexes (Reaction 4) or a two-electron process with inner sphere complexes (Reaction 5–6), radicals are produced in any unsaturated fatty acid or lipid hydroperoxide that comes in contact with the  $\text{Fe}^{4+}$  complex.

It should be stressed that the radicals evolving from Reaction 3 are not from the initial complexed hydroperoxide, but rather are in new lipid molecules. The initial hydroperoxide serves only to activate the iron to  $\text{Fe}^{4+}$  in contrast to Reaction 1 in which the hydroperoxide was the direct reactant and source of propagating radicals. In ferryl iron reactions, oxygen groups from the initial hydroperoxides are inserted or transferred directly to a substrate without radical intermediates, yielding alcohols, ketones, epoxides, or water. This finally explains earlier observations of “crypto  $\text{HO}^\bullet$ ,” hidden  $\text{HO}^\bullet$  that hydroxylated target compounds but could not be detected free in solution (82). In terms of kinetics, oxidation rates much greater than would be predicted for trace levels of hydroperoxides and iron can thus be achieved by  $\text{Fe}^{4+}$  because Reactions 3–7 in Figure 2A are much faster than Reaction 1, the selectivity of  $\text{Fe}^{4+}$  in hydrogen abstractions is greater than either  $\text{HO}^\bullet$  or  $\text{RO}^\bullet$ , and  $\text{Fe}^{4+}$  both initiates and propagates radical chains. Reaction 8 depicts the reduction of  $\text{Fe}^{4+}$  complexes in the presence of excess  $\text{Fe}^{2+}$  to yield two  $\text{Fe}^{3+}$  complexes with concurrent release of water and hydroxylated products. This is one explanation for the loss of catalytic effectiveness at high concentrations of metals.

In the  $\text{Fe}^{3+}$  reactions (Figure 2B), hydroperoxides bind to the iron atom and subsequent formation of the  $\text{Fe}^{4+}$  complex is accompanied by either heterolytic scission of the O–O bond to form hydroxylated products or homolytic scission to release hydroxyl or lipid alkoxy radicals. Current evidence suggests that  $\text{Fe}^{3+}-\text{H}_2\text{O}_2$  and  $\text{Fe}^{3+}-\text{LOOH}$  form different  $\text{Fe}^{4+}$  complexes, so  $\text{H}_2\text{O}_2$  undergoes preferential heterolytic scission, whereas homolytic scission is the almost exclusive route for organic hydroperoxides (81). For  $\text{LOOH}$ , increased conversion to initiating  $\text{LO}^\bullet$  and rapid H abstractions by  $\text{Fe}^{4+}$  to produce  $\text{L}^\bullet$  or  $\text{LOO}^\bullet$  combine to tremendously accelerate generation of new chains of radical reactions, and it accounts, at least in part, for the great catalytic effectiveness of even traces of lipid hydroperoxide.

Both  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  complexes undergo two-electron oxidations to yield  $\text{Fe}^{4+}$  and  $\text{Fe}^{5+}$  states, respectively. The  $\text{Fe}^{5+}$  state, in particular, is achievable with inorganic and small organic ligands because both electrons needed for oxidation come from the Fe. This doesn't happen with hemes, where one electron comes from the iron and the other is taken from the porphyrin or apoprotein (81).

There is much still to be learned about conditions required for formation of fer-ryl or other hypervalent iron complexes, the actual structure of the complexes under different circumstances, the kinetics and mechanisms by which they react, and the overall consequences to lipid oxidation. The factors that appear to be most important include the following:

1. Ligand structure. Highly electrophilic ligands are most effective in producing  $\text{Fe}^{4+}$  (73). Changing the ligands alters the lifetime of  $\text{FeIV}=\text{O}$  complexes. Longer lifetime translates as lower reactivity; shorter lifetime results from higher reactivity, but makes the state more difficult to detect and study (65).
2. Redox potential of the complex (73).
3. Spatial arrangement of ligand components relative to the iron atom (64, 71, 74, 78).
4. Acid-base properties of the ligands (64, 77). The presence of a Lewis base in the ligand exerts a tremendous push effect on the  $-\text{OH}$  group in the hydroperoxide, enhancing both formation of  $\text{Fe}^{\text{IV}}=\text{O}$  and homolysis of  $\text{O}-\text{O}$  in  $\text{Fe}^{3+}-\text{OOH}$  complex (increased release of  $\text{HO}^\bullet$ ) (77).
5. Relative proportions of iron and hydroperoxide. High iron favors oxygen insertion and formation of ketones, whereas 1 : 1  $\text{Fe}$  : hydroperoxide shifts products to epoxides (73); excess  $\text{ROOH}$  yields large amounts of free radicals caused by a shift of the iron to high spin [ $\text{FeL}(\eta^1\text{-OOH})^{2+}$ ] states and rapid reaction of iron with  $\text{ROOH}$  instead of substrates (83).
6. Solvent and presence of water. 1–5% water decreases the redox potential of iron complexes and increases homolytic scission to  $\text{HO}^\bullet$  radicals; in aprotic solvents, heterolytic scission and oxygen insertion products predominate (69).
7. Chemical structure of hydroperoxide forming the initial complex. This alters the structure and spin state of the  $\text{Fe}^{4+}$  complex and, consequently, affects dominant product pathways (73).  $\text{H}_2\text{O}_2$  forms low spin complexes that undergo heterolytic scission, whereas alkyl hydroperoxides form high spin complexes that release alkoxy radicals in homolytic scissions (81).

## 2.1.2. Light

**2.1.2.1. Ultraviolet Light—Direct Effects** Direct initiation of lipid oxidation by ultraviolet light,



requires either direct deposition to sufficient energy to break covalent bonds or transformation of light energy to chemical energy that can catalyze the reaction. The  $E_a$ 's for  $\text{L}-\text{H}$  and  $\text{L}-\text{L}$  scission reactions are higher than the corresponding bond energies ( $\sim 98.4$  kcal/mol and  $83.1$  kcal/mol, respectively), and this photon energy is available only at wavelengths  $< \sim 254$  nm (Table 1). In fact, however, most ultraviolet light damage to lipids occurs at wavelengths less than 200 nm.

**TABLE 1. Energies of Light at Various Wavelengths vs. Typical Energies of Bonds in Lipids.**

	eV <sup>a</sup> (Physicists)	kJ <sup>b</sup> (Chemists)	kCal <sup>b</sup> (Biologists)	Bond Dissociation Energy $\Delta E$		
				Bond	kJ/mol <sup>c</sup>	kCal/mol <sup>d</sup>
200	6.2	596	143	C=C	612	146
230	5.4	518	124	O—H	463	111
260	4.8	458	110	C—H	412	99
290	4.3	411	98	C—O	360	86
320	3.9	372	89	C—C	348	83
350	3.5	341	82	C—N	305	73
380	3.3	314	75	O—O	157	35
410	3.0	291	70			
440	2.8	271	65			
470	2.6	254	61			
510	2.4	234	56			
540	2.3	221	53			
570	2.2	209	50			
600	2.1	199	48			
630	2.0	189	45			
660	1.9	181	43			
700	1.8	170	41			

<sup>a</sup>See (84).<sup>b</sup>Calculated from  $E = Nhc/\lambda$ , where  $N = \text{Avogadro's no.}(6.02 \cdot 10^{23} \text{ photons/mol})$ ,  $h = \text{Planck's constant}(1.58 \cdot 10^{-34} \text{ cal/s or } 6.6 \cdot 10^{-34} \text{ J/s or } 4.36 \cdot 10^{-15} \text{ ev/Hz})$ ,  $c = \text{speed of light}(3 \cdot 10^{17} \text{ nm/s})$ ,  $\lambda = \text{wavelength}(84)$ .<sup>c</sup>See (85).<sup>d</sup>See (86).

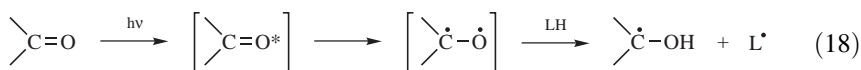
Although ultraviolet light is thermodynamically capable of producing  $L^\bullet$  radicals directly in lipids, the process is not a competitive reaction. In solution, ionization generally requires energies of about 5–6 eV (87), available only at wavelengths < 230 nm, so direct  $L^\bullet$  production is not easily achieved by ultraviolet irradiation. When ionization does occur, there usually is not enough energy to push molecular segments apart, except when the sample is heated, so radicals recombine in cage reactions and do not initiate chains. Also, UV initiation is kinetically slow and very selective because the absorbed energy must match  $\Delta E$  between energy states of elements and bonds.

The principal light-absorbing groups of lipids are double bonds, peroxide O—O bonds, and carbonyls; the last two are most important. The primary mechanism by which ultraviolet radiation initiates lipid oxidation is actually indirect, mediated through homolytic scission of any preformed hydroperoxides to generate the true initiators— $LO^\bullet$ ,  $HO^\bullet$ , and  $RO^\bullet$ —that abstract hydrogens from lipid molecules and form the *ab initio*  $L^\bullet$ .



When the reaction involves LOOH, UV light is also a potent catalyzer of propagation and, from a practical standpoint, exerts its main effects in that stage. In fact, it is often difficult to maintain LOOH on the lab bench for reaction or analysis, especially under fluorescent lights, because the decomposition is quite rapid. Handling samples for analysis of LOOH and separation of hydroperoxides by column chromatography are best done under red light or at least with the vessel or column wrapped in aluminum foil or other light-impermeable material—and also in the cold, as will be shown later.

A second source of UV-induced radicals to initiate lipid oxidation is excitation of carbonyl compounds (88). The carbonyl  $n \rightarrow \pi^*$  transition (340 kJ/mol) occurs when light is absorbed at 350 nm and lower wavelengths (87).



The production of  $\text{H}_2\text{O}_2$  during UV-irradiation in aqueous solutions should not be overlooked as another source of initiating radicals from ultraviolet light.  $\text{H}_2\text{O}_2$  yields of 3.7 and 1.3 mmol per mole L and Ln, respectively, have been measured in solutions exposed to UV light (89), more than enough for very active initiation of lipid autoxidation.

Contrary to what might be expected from their reactivity, double bonds are not effective targets for UV light. The energy of the  $\pi \rightarrow \pi^*$  excitation transition in conjugated dienes is 560 kJ/mol and in isolated double bonds is 680 kJ/mol, which is only achievable at the lower limit of the ultraviolet ranges (215 nm and <180 nm, respectively) (87). Long periods of irradiation are required because absorption of light must produce excited states and ionization before bond scission can occur. Free fatty acids, even saturated ones, are more susceptible to UV radiation than esters because the C—C and C—H bonds  $\alpha$  to the —COOH are activated 5–8 kcal/mol by mesomerism and thus are more susceptible to rupture by light energy. Chain reactions are not involved, and decarboxylation products result (12).

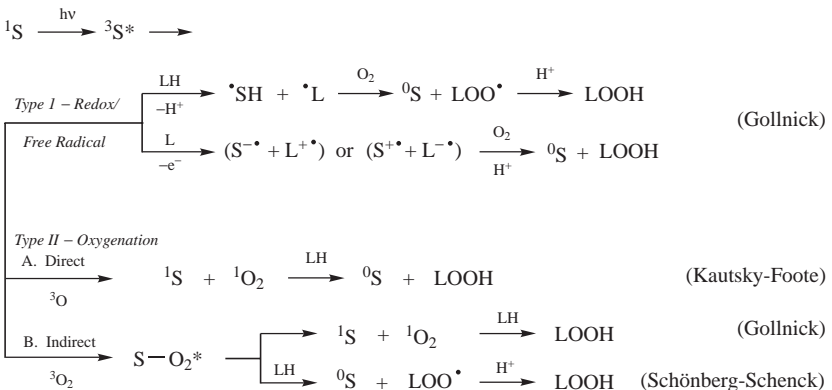
Electron paramagnetic resonance (EPR) studies of lipid free radical production during UV radiation have found it exceedingly difficult to detect  $\text{L}^{\bullet}$  or subsequent  $\text{LOO}^{\bullet}$  in highly purified systems (90). Using nitrosodurene as a spin trap to detect free radicals too short-lived for direct observation by EPR, a mixture of free radical adducts were observed, consistent with H abstractions at allylic carbons for unsaturated fatty acids and carbons  $\alpha$  and  $\beta$  to COOH in saturated fatty acids (91, 92). However, since UV irradiation produces radicals in both benzene and nitrosodurene (both of which were trapped), the lipid radicals detected are more likely to have been produced by secondary H abstractions than light-induced bond scissions. Similarly, radicals were only detected in light-irradiated unsaturated fatty acids at 77 K when photosensitizers were included. Thus, UV-induced direct bond scission that could start radical chain reactions in lipids does not seem likely.

**2.1.2.2. Photosensitization of lipid oxidation by visible light** Visible light (>400 nm) lacks the energy to produce radicals directly. However, when the low level quantum energy of visible light is collected by specifically absorbing molecules, it is transformed to chemical energy that can drive reactions. This process, called photosensitization, involves excitation of the sensitizer, then transfer of the excitation energy to bonds to form free radicals directly (Type 1) or to oxygen to form singlet oxygen, which then adds to double bonds of unsaturated fatty acids without generating radicals (Type 2):

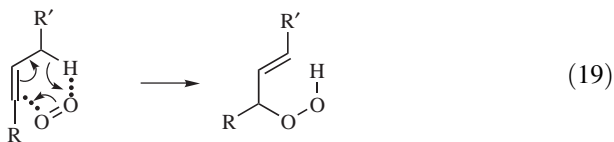
Type 1 sensitization (free radical)  $\longrightarrow L^{\bullet}$  ( $e^-$ -transfer reaction)

Type 2 sensitization ( $^1O_2$ , singlet oxygen)  $\longrightarrow LOOH$  (no free radicals produced)

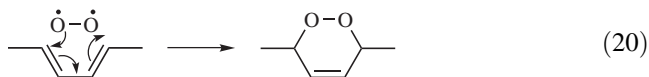
By these two reactions, photosensitization provides the spin state requirements cited above for reaction of oxygen with double bonds, namely a change in oxygen spin state from triplet to singlet or loss of a bonding electron from the target molecule. Type 1 reactions are oxidations, whereas Type 2 reactions are oxygenations (oxygen insertions) that are 1500 times faster than with normal triplet oxygen (93). Photosensitizers in foods and biological materials are usually, but not exclusively, pigments. Chlorophyll, in particular, acts as Nature's light gatherer, collecting low-energy visible light and converting it to chemical energy in plants. Other photosensitizers include flavins (especially riboflavin), porphyrins, aromatic amino acids, and any molecules with carbonyls or an extended conjugated double bond system (94). Some photosensitizers, including chlorophyll, catalyze by both free radical and singlet oxygen mechanisms, with the dominant reactions depending on substrate and reaction conditions (95–97). Other photosensitizers are very specific in their reactions (98). With nearly all sensitizers, regardless of final mechanism or product, the initial steps involve excitation of the sensitizer to its lowest triplet level,  $^3S^*$  (requires the least amount of energy). The triplet sensitizer then directs subsequent reaction, transferring the excitation either to the lipid substrate (Type I) or to oxygen (Type II) (97, 99).



$^1\text{O}_2$  itself is not a free radical generator, but rather generates hydroperoxides that are precursors for initiating radicals. In a concerted “ene” reaction,  $^1\text{O}_2$  attaches to either carbon of a double bond and abstracts an allylic proton to form a hydroperoxide directly (Reaction 19); no free radical is involved. There is little preference for carbon position, so approximately equal amounts of LOOH are produced at both ends of the original double bond. At the same time, a new double bond in *trans*-configuration is formed between the other double bond carbon and the allylic position. When the hydroperoxide is decomposed to radicals by metals, light, or heat, subsequent hydrogen abstractions initiate autoxidation chain reactions.

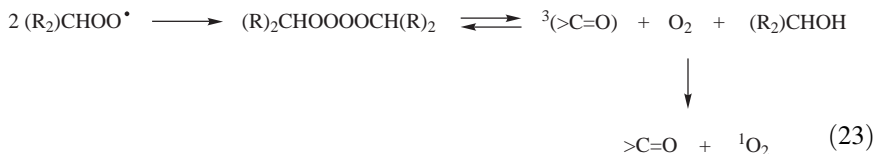
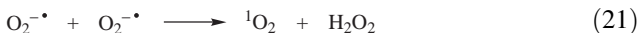


In polyunsaturated fatty acids with nonconjugated double bonds,  $^1\text{O}_2$  reacts with each C=C as if it were isolated, so it yields roughly equivalent amounts of hydroperoxides at both internal and external positions. However, if the double bonds are conjugated (e.g., natural conjugated linoleic acid or oxidized linoleic acid), cyclic endoperoxides are formed.



Dioxetane formation by  $^1\text{O}_2$  does not occur in lipids because it requires an electron-donating atom such as N or S next to the double bond (100).

It has been proposed that  $^1\text{O}_2$  can be generated in chemical reactions (so-called dark biochemistry) by unstable oxygen adducts, endoperoxides, metal complexes (101, 102), and peroxy radical recombinations (103–105), and that the low levels of internal hydroperoxides produced thereby initiate lipid autoxidation chains (102, 106, 107, respectively).



However, the production of  $^1\text{O}_2$  in the dark remains highly controversial. Bielski and Allen, Matsoura et al., and Nilssa and Kearns have shown that this reaction is highly unlikely both on thermodynamic grounds (108) and because  $^1\text{O}_2$  is converted to  $\text{HOO}^\bullet$  so rapidly by phenols (Reaction 24) (109) that it cannot be detected (110).



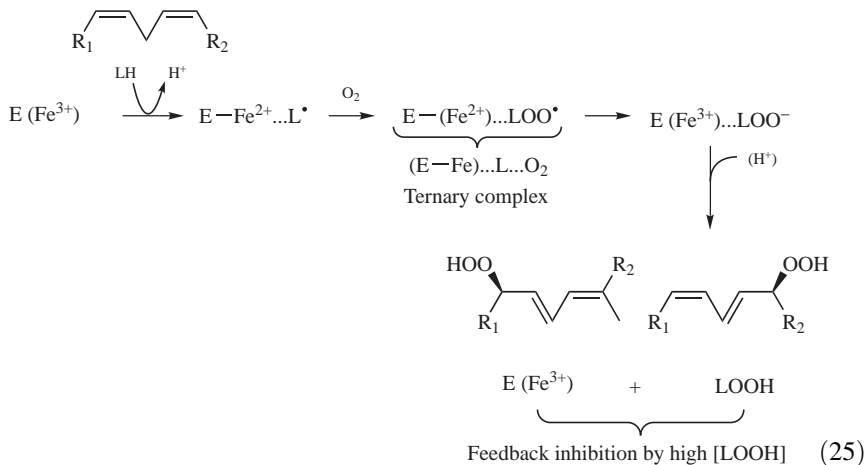
The general consensus remains that if  $^1\text{O}_2$  production in dark biochemistry occurs, it is not competitive with other modes of catalysis and cannot be considered an important initiator of lipid oxidation.

**2.1.3. Heat** High temperatures (e.g., frying temperatures) have sufficient energy to break covalent C–C or C–H bonds in the acyl backbone to form a variety of lipid alkyl radicals (111, 112), which then start the radical chains of oxidation. Moderate temperatures have lower energy, so act primarily by breaking O–O bonds in traces of ROOH or LOOH preformed by other reactions, particularly metals, lipoxygenase, or photosensitizers. The  $\text{RO}^\bullet$ ,  $\text{LO}^\bullet$ , and  $\bullet\text{OH}$  thus generated abstract hydrogens from neighboring lipids to form  $\text{L}^\bullet$  and initiate radical chains. As shown by the activation energies for the individual stages of lipid oxidation, LOOH decomposition and its subsequent contribution to propagation is the major catalytic effect of heat (113, 114). Effects of increased LOOH decomposition are amplified by increased rates of subsequent H abstractions by  $\text{LO}^\bullet$  and  $\text{LOO}^\bullet$ , which is reflected in the doubling of oxidation rate for every  $10^\circ\text{C}$  rise in temperature (115).

Reaction	Activation Energies ( $E_a$ ) kCal/mole
$(\text{L}^\bullet + \text{O}_2)$	0
$k_p (\text{LOO}^\bullet + \text{LH})$	$\sim 5\text{--}15$
$k_t (2 \text{ROO}^\bullet)$	$\sim 4$
$k_t (2 \text{R}^\bullet)$	5
$k_t (\text{R}^\bullet + \text{ROO}^\bullet)$	1
* $k_i$ (monomolecular)	31
* $k_{ii}$ (bimolecular)	50 uncatalyzed system

**2.1.4. Lipoxygenase** Lipoxygenases catalyze the aerobic oxidation of fatty acids with *cis*-nonconjugated pentadiene structures to generate optically active conjugated LOOH without releasing a lipid free radical. Hydroperoxides are synthesized in a cage reaction involving electron transfer to the lipid from the ferrous iron atom in the enzyme's active site (116) and removal of the bisallylic hydrogen as the rate determining step (117–119). Oxygen bound to a separate site on the enzyme is activated to react with the free radical, then  $\text{H}^+$  donation from the enzyme completes the LOOH before it is released. As the oxygen always adds anti to the hydrogen removal, the resulting conjugated dienes are always *trans*-, *cis*-relative to the hydroperoxide (117).





Radical oxidation chains are initiated when LOOH is decomposed to initiating  $\text{LO}\cdot$  and  $\cdot\text{OH}$  radicals by light and heat, to  $\text{LO}\cdot / \text{LOO}\cdot$  by metals, or to  $\text{LO}\cdot$  by the enzyme itself (120). Very low levels produced in plant or animal tissues may provide the “invisible” initiators that make lipid oxidation sometimes appear spontaneous. Perhaps just as important, LOOH produced by lipoxygenase can accumulate to relatively high levels under appropriate conditions (e.g., cold and dark, as in frozen unblanched materials), then lead to a cascade of rapid oxidation when LOOH decomposes.

It should be noted that although oxygen is not required for formation of the bisallylic radical, it is necessary for formation of high yields of hydroperoxides. Hence, when lipoxygenase is being used to synthesize lipid hydroperoxides, full oxygenation must be ensured, and conversely, when lipoxygenase action needs to be inhibited without thermal inactivation, reduced oxygen pressures offer an excellent means of control.

**2.1.5. Heme Proteins and Porphyrins** Heme catalysis of lipid oxidation was first reported in 1924 (121), but it was another 30 years before research to determine mechanisms and effects began in earnest. In pioneering studies, Watts and Chang (148) observed that ferric heme forms were the most active catalysts and proposed a fundamental electron transfer mechanism (122–125). A few years later, Tappel’s work in model systems suggested that hemes form complexes with preformed hydroperoxides, and radicals are generated in subsequent decomposition of the complex (126–132). Love and Pearson (133) then proposed that free inorganic iron released from hemes, rather than the hemes themselves, catalyzed lipid oxidation in meats. However, this theory was inconsistent with earlier observations that hemes were more effective catalysts than free iron, and questions were further raised when  $\text{Fe}^{3+}$ -heme complexes were more active in model emulsions than  $\text{FeSO}_4$  and  $\text{FeCl}_3$  (134). Although all these theories address some behaviors of

heme systems, none of them completely accounts for the kinetics, product mixes, and solvent effects of heme catalysis (99).

That heme compounds catalyze lipid oxidation in food and biological systems has been extensively documented (128, 135–140), but how this occurs is still not clear. The greatest obstacle for unraveling heme catalysis in foods is the complicated composition and structure of the reaction system. The kind of compartmentalization that isolates heme proteins in living tissues may or may not be retained after food processing, the cellular chemistry maintaining redox balance begins to decline immediately after slaughter or harvest, and previously protected sites become exposed. Under these conditions, overall measures of increased lipid oxidation can be obtained, but it is exceedingly difficult to determine details of reaction mechanisms.

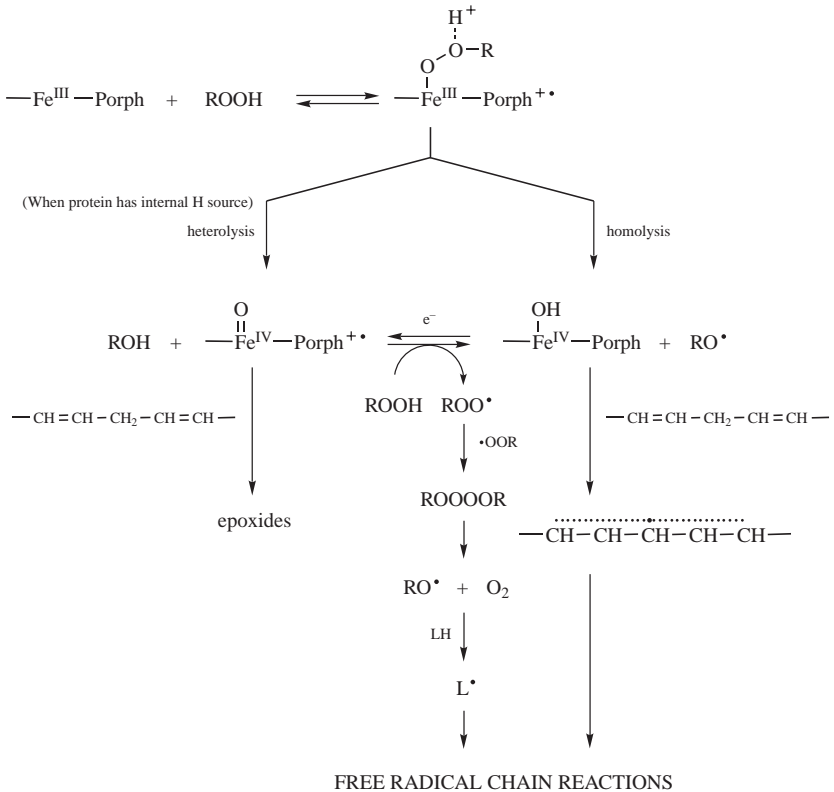
Application of data obtained from simple “clean” reaction systems in biological or chemical studies of heme catalysis also has its problems. Chemical model systems use chelators, model hemes, and substrate structures that are quite different from those existing in foods. Reaction sequences change with heme, substrate, solvent, and reaction conditions. Intermediates are often difficult to detect (141), and derivations of mechanisms by measuring products and product distributions downstream can lead to erroneous or incomplete conclusions. It is no surprise, then, that there remains considerable controversy over heme catalysis mechanisms. Furthermore, mechanisms determined in these defined model systems with reaction times of seconds to minutes may or may not be relevant to lipid oxidation being measured in the complex matrices of foods stored for days or weeks under conditions where phospholipids, fatty acid composition, heme state, and postmortem chemistry complicate the oxidation once it is started (142). Hence, the mechanisms outlined below should be viewed as guides rather than absolutes. More research should be focused on determining, by kinetic and product analyses, which reactions actually occur and are of practical importance in specific food systems.

Current evidence indicates that hypervalent iron complexes—ferryl iron (FeIV,  $\text{FeO}_2^{2+}$ ,  $\text{Fe(IV)=O}$ ) or perferryl iron (FeV)—are involved in the catalytic mechanism, but there is still controversy over the details of reaction mechanisms and what proportion of heme catalysis it accounts for. Very recently, some very elegant chemistry has elucidated binding and O—O bond scission mechanisms and identified heme structural elements critical for oxidation catalysis (143, 144). Paradoxically, although the early theories of heme catalysis have been largely dismissed, they nevertheless are consistent with aspects of hypervalent iron behavior. Ferryl iron chemistry encompasses and explains the most important features noted in early studies (99):

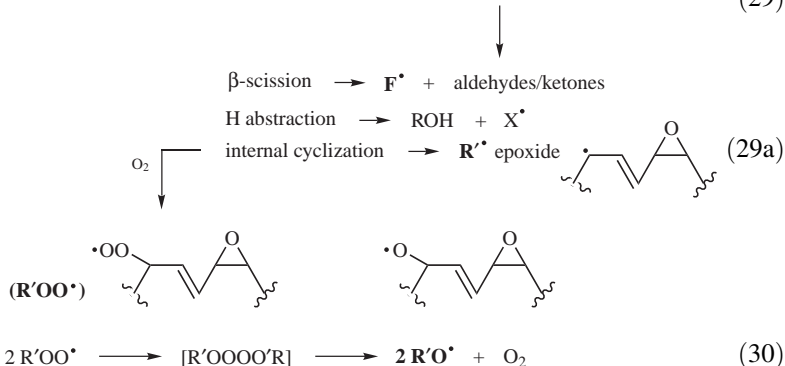
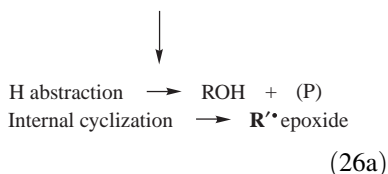
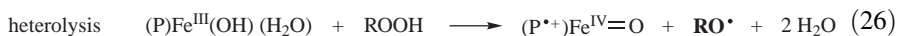
1. The porphyrin-Fe structure is an absolute requirement for catalysis.
2. Catalytic activity varies tremendously among heme proteins, partly due to exposure/or accessibility of the heme structure and partly due to other unidentified factors.

3.  $Fe^{3+}$ -hemes are most active even without oxygen;  $Fe^{2+}$ -hemes require oxygen for catalysis.
4. No change of heme iron valence seems to be involved.
5. There is a solvent-related pH dependence that varies with the specific reaction components and conditions (e.g., liposomes vs. membranes vs. emulsions, fatty acids vs. phospholipids, buffer type, heme compound).
6. Catalysis reverses to inhibition at high heme levels.

The simplified reaction scheme given in Reactions 26–30 is a synthesis from several authors (139, 141, 143–158) and together with Figure 3 provide a general representation of current understanding of heme-mediated formation and reaction of  $Fe^{4+}$ . All iron is complexed to a porphyrin, P, and has hydration and hydroxyl ligands; F is a scission fragment; and radicals capable of initiating lipid oxidation are noted in bold type. An extensive discussion of heme catalysis with major emphasis on foods is available in a recent review by Baron and Anderson (159).



**Figure 3.** Heme-catalyzed formation of species that can initiate lipid oxidation: generation and reaction of ferryl iron complexes [ $Fe^{IV} = O$ ,  $Fe^{IV}(OH)$ ]. Adapted (143, 160); used with permission.



For hemes to be more effective initiators than  $\text{Fe}^{3+}$  and  $\text{Fe}^{2+}$ , either removal of an electron from the double bond, reduction of preformed hydroperoxides to generate  $\text{L}^{\bullet}$  or  $\text{LO}^{\bullet}$ , or both of these reactions must be activated, or another mechanism entirely must be operative. Model system studies have now shown that the basic activating reaction involves binding of preformed hydroperoxides, either  $\text{H}_2\text{O}_2$  or  $\text{LOOH}$ , to ferric hemes to generate hypervalent Fe in a very fast reaction ( $k \sim 10^9$ ). In the concerted process, the negatively charged porphyrin ligand releases  $\text{H}_2\text{O}$  and weakens the O—O bond, the hydroperoxide is decomposed heterolytically (Reaction 26; left reaction series, Figure 3) to produce an alcohol, or homolytically (Reaction 27; right reaction series, Figure 3) to produce alkoxy radicals, respectively, and an O is transferred to the iron to form the ferryl complex,  $\text{Fe}^{4+}=\text{O}$ . This reaction is very sensitive to environment, particularly solvent and proton availability; and the O—O scission mode and products vary with the heme, hydroperoxide structure, solvent, and reaction environment. Heterolytic scission results in one of the oxidizing equivalents being transferred to the porphyrin apoprotein, forming a free radical that localizes on tyrosine (161–163) or tryptophan (164). This radical can be quenched by H abstraction from hydroperoxides, producing peroxy radicals (30, 31).

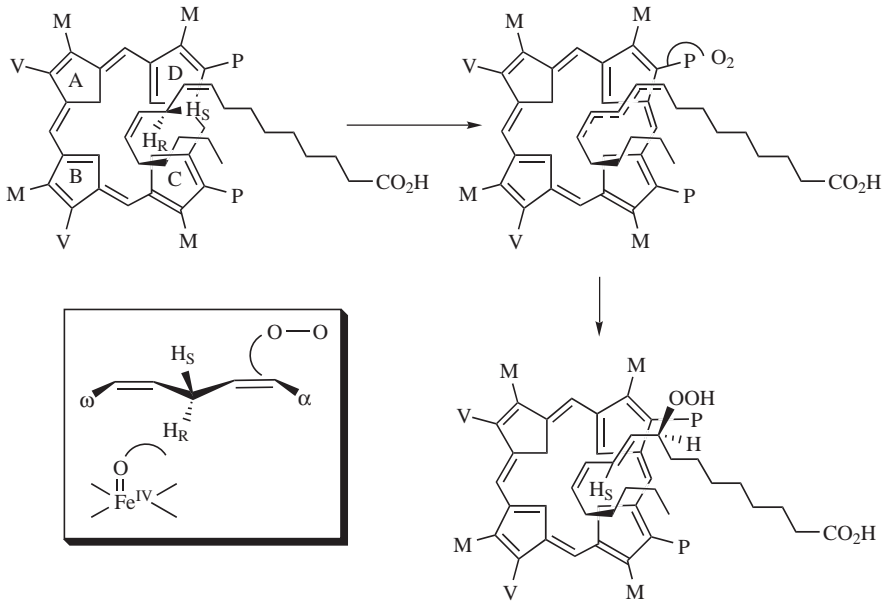
In protective heme enzymes such as catalase and peroxidases, the dominant process is heterolytic, and amino acids such as histidine in the apoprotein are in close proximity in the active site to transfer protons to the  $\text{RO}^{\bullet}$  in situ. For

ROOH = LOOH, a lipid alcohol is released and no initiation or branching can occur. In aprotic solvents or acid environments, however, H abstraction is delayed and the radicals remain active. When the heme is myoglobin, hemoglobin, or a heme protein where an internal proton source is not available, the reaction mechanism is more likely to be homolytic, yielding alkoxy radicals with no radical on the porphyrin.

There is disagreement about the fate of the RO•, R'•, and F• radicals, and it is even less clear which species initiate new lipid oxidation chains. Most obviously, any of these would appear to be potential initiating radicals for lipid oxidation, but perhaps not directly. Unless a proton source is immediately available, there is a strong driving force for the bound LO• to cyclize internally to epoxides, at the same time generating epoxyallylic radicals (Reactions 26a and 29a) that are more stable than alkoxy radicals (see Section 3.2.2). Indeed, most model system studies of heme catalysis have found that cyclization dominates overwhelmingly with fatty acids (147, 148, 156). The peroxy radicals formed by oxygen addition to epoxyallylic sites (Reaction 29a) are slow and specific in reaction, giving them a much better chance of escaping the heme complex reaction cage to react elsewhere. Even so, the slowness of peroxy radical reactions also argues against their initiating lipid reactions much faster than normal autoxidation, so it is much more likely that the peroxy radicals recombine outside the reaction cage (but still inside the heme crevice) and dismutate to LO• radicals, which react much more rapidly.

Although the distinctions between heterolytic and homolytic pathways may be important for enzymes *in vivo* and may also provide some support for Tappel's theory of lipid hydroperoxide decomposition, what happens to the activating ROOH (HOOH or LOOH) is inconsequential to lipid oxidation in foods. HO• from H<sub>2</sub>O<sub>2</sub> may be diffusible and highly reactive, but it does not initiate lipid oxidation (144). Subsequent abstraction reactions of LO• dominate with Hb (165) and Myb (166), giving 9-LOOH as in autoxidation. Increased decomposition of H<sub>2</sub>O<sub>2</sub> or lipid hydroperoxides alone cannot account for the explosive oxidation that can occur in the presence of hemes because (1) catalysis rate would then be directly proportional to the hydroperoxide concentration, and (2) final rates would approximate those controlled by LO• in secondary stages of autoxidation. Neither condition seems to fit existing data. Something more is needed to connect these reactions to active, accelerated lipid oxidation.

One missing link was provided in observations that myoglobin-H<sub>2</sub>O<sub>2</sub> catalysis of linoleic acid oxidation gives highly regio- and stereospecific hydroperoxides, almost exclusively 9S-OOH (144, 167), which indicated some type of specific fatty acid binding to myoglobin. Comparative studies with Myb mutants revealed that fatty acids bind at the entrance to the heme pocket (Figure 4). The hydrocarbon terminus of a fatty acid penetrates into the crevice in the geometry required to form a *trans*-10,11-double bond. Abstraction of the pro-*R* hydrogen generates a planar 1,4-dienyl radical directed toward the heme ring and protected from oxygen. Oxygen then adds from the opposite direction (from outside the crevice), and the 9-OOH forms preferentially because it is exposed, while C-13 is inside the crevice.



**Figure 4.** Model proposed for the binding and oxidation of polyunsaturated fatty acids in the myoglobin heme crevice. From (144), used with permission.

A second missing link is that the critical driver responsible for the dramatically increased lipid oxidation rate is the  $\text{Fe}^{4+}$  itself, not radicals from the decomposition of contaminating hydroperoxides. Ferryl iron is a strong oxidant, kinetically equivalent to  $\text{HO}^\bullet$  in reactivity (154) but more selective due to its lower redox potential (168). Ferryl iron rapidly abstracts H from the doubly allylic C-11 of linoleate (now conveniently oriented toward the heme iron core) (144) and it abstracts hydrogens from hydroperoxides even more rapidly (154), in contrast to the very slow oxidation with nonheme  $\text{Fe}^{3+}$ :



This has two consequences: (1) most importantly, direct initiation of radicals in lipids bound to the heme, and (2) assurance of lipid release as  $\text{LOO}^\bullet$  rather than  $\text{LOOH}$ . Chain propagation may proceed through  $\text{LOO}^\bullet$  directly or through epoxyallylic peroxy radicals from  $\text{LOO}^\bullet$  cyclization.

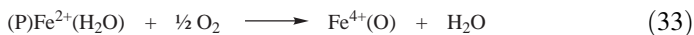
A third missing link important for rapid catalysis was recognition that once formed,  $\text{Fe}^{4+}$  states could be maintained by electron transfers to the apoprotein without involving the iron center. This is shown as the reversible reaction



in Figure 3. Thus, electrons can be shuttled facily between two reactive states without the loss of oxidizing power and reduction of  $\text{Fe}^{4+}$  to less reactive  $\text{Fe}^{3+}$

(160). Together, these three factors provide a powerful system for extremely effective catalysis of lipid oxidation.

Fe<sup>2+</sup>-hemes also generate ferryl complexes, albeit more slowly, and this oxidant source may be important over longer reaction times or during storage. With H<sub>2</sub>O<sub>2</sub> as the oxygen source, Fe<sup>2+</sup>-myoglobin catalysis of lipid oxidation is initially slower but eventually reaches the same rate as Fe<sup>3+</sup>-myoglobin (169). However, peroxides are not always absolute requirements. Direct (slow) reaction of heme-Fe<sup>2+</sup> with oxygen,



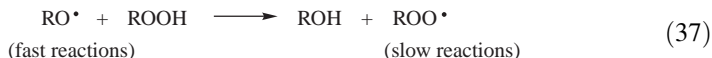
may not be detected as activity in short-term assays designed to mimic enzyme active sites, but nevertheless may provide a nonhydroperoxide source replenishing Fe<sup>4+</sup>(O) in longer reactions. Fe<sup>2+</sup> hemes may also contribute to delayed catalysis by regenerating Fe<sup>3+</sup> hemes after some peroxy radicals have been formed (2, 149, 152),



Variable catalytic activity between different heme proteins (137, 170, 171) and between the same hemes from different species (172) has long been recognized. The recent elucidation of the fatty acid binding (144) and clarification of O—O bond cleavage mechanisms by ferryl complexes (143, 173, 174) provide insights into why this happens. The composition and arrangements of amino acids in the heme crevice, as well as heme pocket size and orientation, affect lipid binding and proton transfer, while the heme structure and ligands influence electron transfer processes and stabilization of the ferryl complex. Attainment and stabilization of Fe<sup>4+</sup>(O) long enough for reaction requires both appropriate adjustment of the heme redox potential and steric shielding of the bound oxygen at a fixed coordination position on the iron. Small perturbations in the active site deactivate oxygen and lead to its release as O<sub>2</sub><sup>-</sup>/HO<sub>2</sub><sup>•</sup> (149), which are not very reactive with lipids. All of these factors and the reaction environment influence whether O—O bond cleavage is homolytic or heterolytic, pro-oxidant or antioxidant, under given conditions (143). Considering this new information on lipid binding and mechanisms of ferryl iron formation, it should now be straightforward to interpret, model, and even predict catalytic activity based on individual heme protein and ligand structures.

Similarly, this new information provides explanations for the shift from pro-oxidant to antioxidant at high heme concentrations that has long been recognized (123, 175–177). High heme concentrations increase heme association and limit fatty acid access to the heme pocket (177). Under low oxygen conditions or when oxygen has been depleted by reaction, excess ferrous hemes oxidize instead by combination with reactive ferryl complexes, reducing them to ferric complexes (Reaction 35) with lower reactivity. High heme concentrations oxidize the radicals generated during formation of Fe<sup>4+</sup>(O) (Reaction 36), or reduce them if the hemes are ferrous, so

no subsequent reactions can occur. Any alkoxy radicals produced in ferryl formation, although kinetically inconsequential at low concentrations, become competitive at high heme concentrations and can convert the hydroperoxides being generated to alcohols and peroxy radicals (Reaction 40)—a net reduction in propagation capacity.



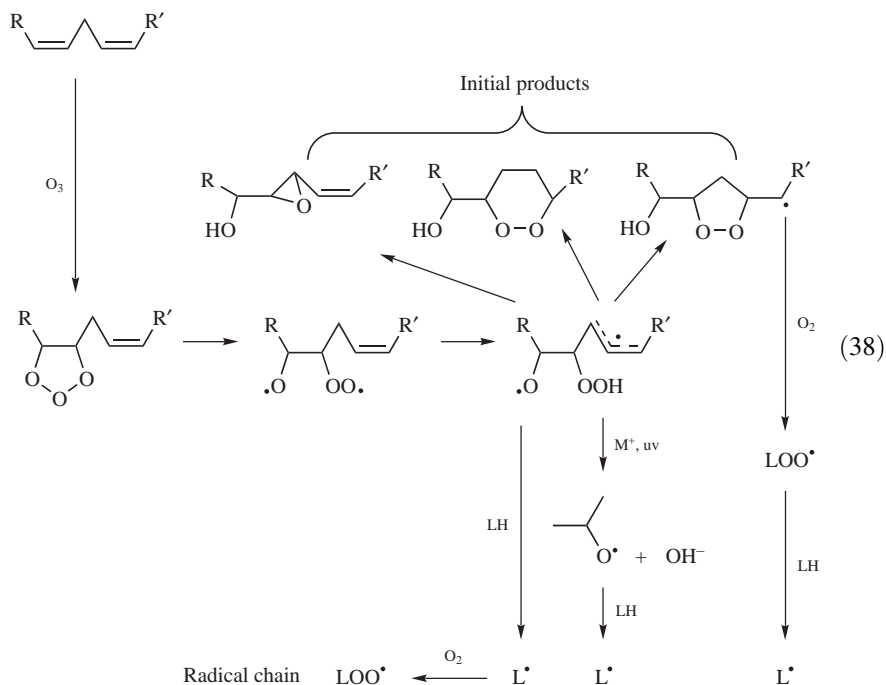
Whether the porphyrin apoprotein radical shown in reactions above has a role in catalyzing lipid oxidation is still being debated. Current evidence suggests that the heme protein radical is required for electron transfer in the ferryl iron-heme complexes (157) and that it may co-oxidize proteins or other molecules (163, 178), but is probably not involved in direct catalysis of lipid oxidation (144).

For food applications, another mechanism must also be considered as a possible minor contributor. Considering the photosensitization capabilities of porphyrin rings in chlorophyll, Schaich (99) questioned whether analogous reactions could be catalyzed by hemes in foods in which normal molecular and cell environments are disrupted and porphyrin rings can become exposed. This possibility has now been verified by EPR spin trapping evidence that hematin, but not intact heme proteins, produce  $^1O_2$  (179), and in observations that protoporphyrin IX catalyzed oxidation of rat liver microsomes only in the light, whereas in the dark it inhibits lipid oxidation (180). Photosensitization, which can only occur at the surface, would not be expected to compete with ferryl iron produced by intact hemes in the interior of muscle foods before cooking, but it may indeed contribute to oxidation in processed foods in which some disintegration of the heme complexes occurs.

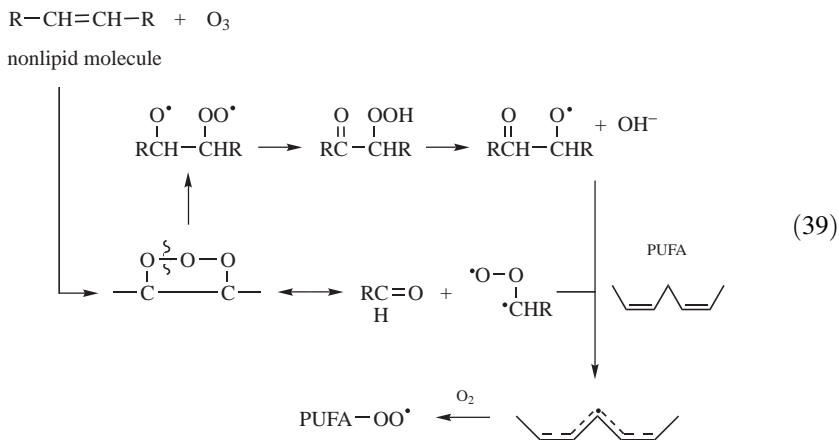
**2.1.6. Ozone** The reactivity of ozone with unsaturated fatty acids has long been recognized, and indeed, the reaction has practical applications in localization of double bonds (181). As a damage reaction, atmospheric ozone ( $O_3$ ) [e.g., from pollution or sterilization processes (182)] rapidly adds across double bonds in nearly all organic molecules to form ozonides (trioxides), which then undergo a number of different subsequent reactions, not all of which produce free radicals. However, there remains some controversy over whether direct or indirect mechanisms dominate.

Ozone adds directly to double bonds in fatty acids to form ozonides (183–185). These decompose to lipid alkoxy and peroxy radicals that abstract hydrogens to initiate radical chains (186). In the process, internal rearrangements within the original lipid molecule(s) yield hydroxy epoxides and hydroxy epidioxides with 1,3- and 1,4-cyclic hydroperoxides:





Indirect initiation of lipid oxidation by ozone is similar except that it occurs via decomposition of ozonides in non-lipid molecules to form alkoxy and peroxy radicals that subsequently abstract hydrogens from fatty acids. Two mechanisms have been proposed, both of which yield the same final lipid products (186):



Ozone preferentially reacts with the most unsaturated fatty acids present (187); arachidonic acid and higher PUFAs are particularly sensitive. *Trans*-double bonds and fatty acids have been reported to react with ozone much more slowly than *cis*-double bonds (21), but this observation may be an artifact of measuring only initial ozonides. In fact, *trans*-fatty acids do react with ozone, but the initial ozonides decompose and rearrange more rapidly to generate peroxy-epoxide or peroxy-ozonide complexes and free acids (188). This is another example of how, as in lipid oxidation itself, downstream as well as initial products must be measured to obtain a full and accurate picture of reaction.

Ozone reactions are not very fast ( $k \sim 10^5$ ) and do not change the rate or product mix of lipid autoxidation once established (189). Nevertheless, ozone markedly shortens induction periods by contributing to early accumulation of the critical concentration of lipid radicals and hydroperoxides necessary to trigger the onset of rapid oxidation. Ozone also reacts with LOOH to produce radicals that propagate the oxidation chain:



Whichever initial reaction occurs with ozone, once active oxidation equilibrium is established,  $\text{LOO}^\bullet$  and  $\text{LO}^\bullet$  propagation reactions dominate and effects of ozone on oxidation rates and product mixes becomes insignificant (190).

**2.1.7. Free Radicals** In the discussion above, all the initiating processes generate some form of radical that ultimately reacts with lipids to produce the *ab initio* lipid radical that starts the autoxidation chain. The kinetics of the initiation, however, are governed by the speed of individual radical reactions with lipids, which can vary tremendously. Table 2 lists rate constants for a number of reactions important in initiation of lipid oxidation. For the most part, the rate constants speak for themselves. Nevertheless, a few comments need to be added.

Not surprisingly, hydroxyl radicals have the fastest reaction rates with lipids. However,  $\text{HO}^\bullet$  are so strongly oxidizing that their reactions are also very nonspecific, and they attack lipids indiscriminantly at all sites along acyl chains (195, 207). These radicals then “migrate” (by intramolecular abstraction) to the doubly allylic H’s in dilute monomer solutions, or abstract H’s from doubly allylic sites of neighboring lipids in concentrated solutions, yielding the dienyl radicals that, when oxygenated to  $\text{LOO}^\bullet$ , become the main chain carriers.

It is important to note that saturated fatty acids are not immune to effects of oxidation. The strongly oxidizing radicals  $\text{HO}^\bullet$  and  $\text{RO}^\bullet$  abstract hydrogens at reasonable rates even from saturated fatty acids ( $10^6$  for  $\text{RO}^\bullet$  and  $10^9$  for  $\text{HO}^\bullet$ ). The subsequent  $\text{L}_{\text{sat}}\text{OO}^\bullet$  radicals then abstract hydrogens from neighboring unsaturated fatty acids and thus can be sources of external radicals initiating radical chains in PUFA’s (9, 208).

Values for  $\text{ROO}^\bullet$  are average rates for all organic peroxy radicals; peroxy radical rate constants vary little with R structure unless there is a halogen atom  $\alpha$  to the radical peroxy group (9). Although  $\text{O}_2^\bullet$  has been invoked as an initiator of lipid

**TABLE 2. Lifetimes and Hydrogen Abstraction Rates of Various Radicals that Initiate Lipid Oxidation.**

Radical	Half-life with Typical Substrate, 10 <sup>-3</sup> M, 37°C		Ave. rx Rate, k (L mol <sup>-1</sup> sec <sup>-1</sup> )		Reference
HO•	10 <sup>-9</sup> sec		10 <sup>9</sup> -10 <sup>10</sup>		191
RO•	10 <sup>-6</sup> sec		10 <sup>6</sup> -10 <sup>8</sup>		191
ROO•	10 sec		10 <sup>1</sup> -10 <sup>3</sup>		191
L•	10 <sup>-8</sup> sec		10 <sup>4</sup> -10 <sup>8</sup>		191
AnOO•	10 <sup>-5</sup> sec				192 <sup>a</sup>
O <sub>2</sub> <sup>-•</sup>			~1		193 <sup>b</sup>
HOO•			10 <sup>0</sup> -10 <sup>3</sup>		194 <sup>b</sup>
	18:1	18:2	18:3	20:4	
HO•	~10 <sup>9</sup>	9.0 × 10 <sup>9</sup>	7.3 × 10 <sup>9</sup>	~10 <sup>10</sup>	9,195
Monomer		8.0 × 10 <sup>9</sup>	8.0 × 10 <sup>9</sup>		196
Micellar		1.3 × 10 <sup>9</sup>	2.5 × 10 <sup>9</sup>		195
Non-allylic H	4 × 10 <sup>2</sup>	3.4 × 10 <sup>3</sup>	7.0 × 10 <sup>3</sup>	1.0 × 10 <sup>4</sup>	196
RO•	3.3 × 10 <sup>6</sup>	8.8 × 10 <sup>6</sup>	1.3 × 10 <sup>7</sup>	2.0 × 10 <sup>7</sup>	9
t-BuO•	3.8 × 10 <sup>6</sup>	9.1 × 10 <sup>6</sup>	1.3 × 10 <sup>7</sup>	2.1 × 10 <sup>7</sup>	197
( <i>trans</i> ) aqueous	3.3 × 10 <sup>6</sup> 6.8 × 10 <sup>7</sup>	( <i>trans</i> ) 8.8 × 10 <sup>6</sup> 1.3 × 10 <sup>8</sup>	1.6 × 10 <sup>8</sup>	1.8 × 10 <sup>8</sup>	197 198
ROO•	1.1	6 × 10 <sup>1</sup>	1.2 × 10 <sup>2</sup>	1.8 × 10 <sup>2</sup>	199-201
O <sub>2</sub> <sup>-•</sup>	no rx	no rx	<1	<1	196, 200
(MLOOH)	7.4 × 10 <sup>3</sup>				202
HOO•	no rx.	1.1 × 10 <sup>3</sup>	1.7 × 10 <sup>3</sup>	3.1 × 10 <sup>3</sup>	193
		<3 × 10 <sup>2</sup>			200
O <sub>3</sub> -CCl <sub>4</sub>	6.4 × 10 <sup>5</sup>	6.9 × 10 <sup>5</sup>			203
-aq SDS	9.5 × 10 <sup>5</sup>	1.1 × 10 <sup>6</sup>			203
SO <sub>3</sub> <sup>-•</sup>		1.8 × 10 <sup>6</sup>	2.8 × 10 <sup>6</sup>	3.9 × 10 <sup>6</sup>	194
GS•	<2 × 10 <sup>6</sup>	8 × 10 <sup>6</sup>	1.9 × 10 <sup>7</sup>	3.1 × 10 <sup>7</sup>	204
<sup>1</sup> O <sub>2</sub>	0.74 × 10 <sup>5</sup>	1.3 × 10 <sup>5</sup>	1.9 × 10 <sup>5</sup>	2.4 × 10 <sup>5</sup>	205
O <sup>-•</sup>	7.5 × 10 <sup>2</sup>	9.7 × 10 <sup>3</sup>	1.2 × 10 <sup>4</sup>	1.9 × 10 <sup>4</sup>	196
NO <sub>2</sub> <sup>•</sup>	1.2 × 10 <sup>6</sup>	6.2 × 10 <sup>6</sup>	6.6 × 10 <sup>6</sup>		206

<sup>a</sup>Aqueous solution.<sup>b</sup>H abstraction from unsaturated alkenes.

oxidation, the rate constants in Table 2 show clearly that O<sub>2</sub><sup>-•</sup> does not react with unsaturated fatty acids or their hydroperoxides. O<sub>2</sub><sup>-•</sup> is a weak reactant, both as a reducing and oxidizing agent (E° = -0.33 V for O<sub>2</sub>/O<sub>2</sub><sup>-•</sup> and +0.94 V for O<sub>2</sub><sup>-•</sup>/H<sub>2</sub>O<sub>2</sub>) (209). In lipid oxidation, O<sub>2</sub><sup>-•</sup> is probably most active in recycling traces of contaminating metals, particularly iron, or as a source of highly reactive HO•, which very rapidly take over reactions, obscuring initial effects of O<sub>2</sub><sup>-•</sup>. This has been demonstrated in reactions of Fe-EDTA complexes with linolenic acid (210). However, the conjugate acid, HO<sub>2</sub><sup>•</sup>, abstracts doubly allylic H atoms of linoleic, linolenic, and arachidonic acids (211). At pH 7.0, only about 1% of O<sub>2</sub><sup>-•</sup> solutions is present as HO<sub>2</sub><sup>•</sup>, but the latter drives any radical abstractions. In acid solution, only HO<sub>2</sub><sup>•</sup> is active.

**2.1.7.1. Radicals from Secondary Reactions** One area of initiation that has gone totally unnoticed is reaction of radicals produced in solvents or other system

components, which then react with lipids. Whether the primary initiator is heat, radiation, or metals, many of the initial oxygen radicals produced react more rapidly with solvent components than with lipids. For example,  $\text{HO}^\bullet$  react with alcohols (e.g., used as solvents in model systems) at rates as high as  $10^{12} \text{ L mol}^{-1} \text{ s}^{-1}$  (212, 213), and the alcohol radicals then react with lipids. Decomposition of MLOOH in 80% ethanol, for example, yields >7% ethoxylated products (214), and more than 60% of products from photolysis of MLOOH in methanol were methoxylated (215). Radicals induced in cyclohexane by photolysis also react with MLOOH (216). Similar co-oxidation occurs with Triton-X as an emulsifier (217). Tris, phosphate, and other buffer components form radicals that can be detected by EPR, and EDTA forms several radicals that are strongly reducing in nature (218–220). The role these “system” radicals may play in overall lipid oxidation is not yet known, but their possible involvement should be considered in designing test systems and calculating and interpreting oxidation kinetics.

## 2.2. Sites of Radical Initiation by Hydrogen Abstraction and Formation of Peroxyl Radicals

Hydrogen abstraction by free radicals is generally quite specific, occurring preferentially at allylic hydrogens where the C–H bond energies are lowest (Table 3). The order of reactivity is doubly allylic H's between two double bonds > singly allylic H's next to double bonds  $\gg$  H's  $\alpha$  to the  $-\text{COOH}$  group > H's on methylene groups farther down the acyl chains. The one exception to this “rule” is the hydroxyl radical,  $\text{HO}^\bullet$ , which is so electrophilic and reactive that it abstracts H's indiscriminantly from all positions along the acyl chain (195). The radicals formed either migrate to the acyl carbon with the weakest bonding, i.e., the allylic H's, or abstract allylic hydrogens from a neighboring lipid molecule.

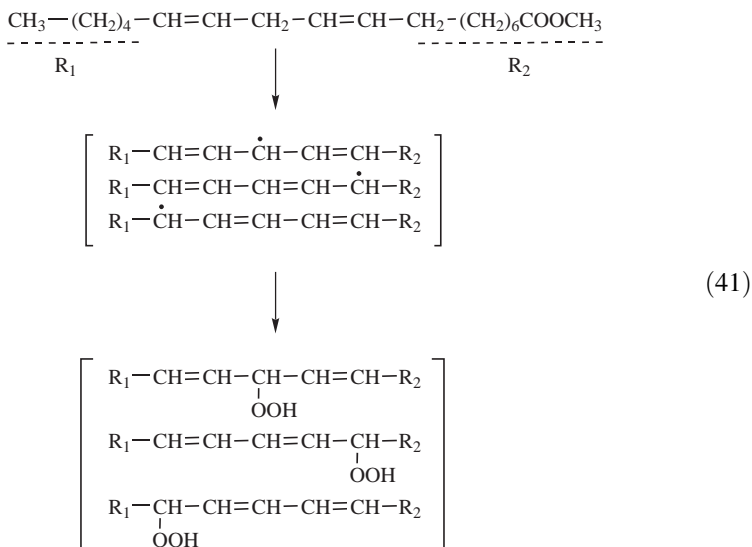
Older literature always presents the initial radicals in equivalent resonant positions with equal probability of forming hydroperoxides. The three resonant positions for linoleic acid or its ester and the three hydroperoxides resulting from these are shown in Reaction 41 (224). Comparable resonant structures have been published for oleate, linolenate, and arachidonate (222, 225).

**TABLE 3. Bond Energies of Hydrogens at Various Positions in Acyl Chains (bold font): Sites of Preferential Hydrogen Abstraction.**

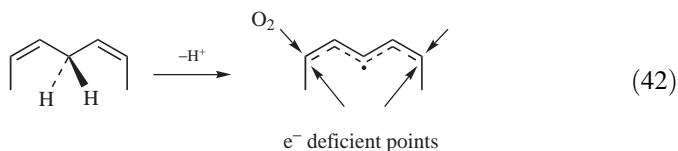
	E (kJ/mol)	E (kcal/mol) <sup>a</sup>	Relative Ease of H Abstraction <sup>b</sup>
<b>H–CH=CH<sub>2</sub></b>	431	105	
<b>H–CH<sub>2</sub>–CH<sub>2</sub>–CH<sub>3</sub></b>	419	99	
<b>H–CH<sub>2</sub>–CH=CH<sub>2</sub></b>	356	85	
<b>R–HCH–CH=CH–CH<sub>2</sub>–CH<sub>3</sub></b>	322	77	
R(CH <sub>2</sub> =CH)– <b>HCH–CH<sub>2</sub>–</b>	310	74	1
R– <b>CH=CH–HCH–CH=CH–</b>	272	65	62
<b>ROOH</b>	377	90	

<sup>a</sup>See (221, 222).

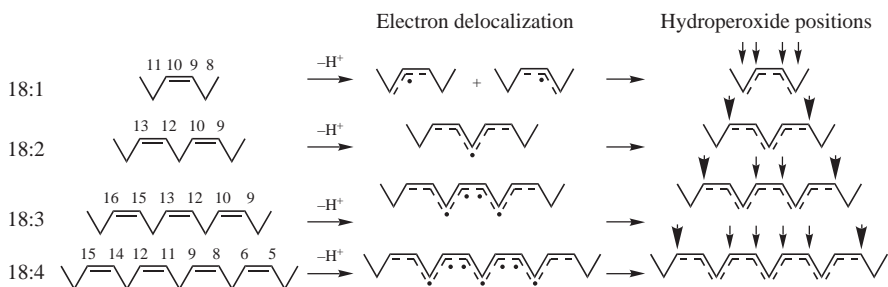
<sup>b</sup>See (223).



Product distributions show the inaccuracy of this notion. It is now recognized that after hydrogen abstraction from the allylic hydrogens, the free electron becomes distributed across a resonance stabilized double bond system (Reaction 42). The highest electron density is in the center, and the outside positions are relatively electron deficient. Thus, oxygen preferentially adds at the outermost points. When oxygen pressures are greater than 100 mm Hg, the addition occurs at diffusion-controlled rates ( $k > 10^9 \text{ L M}^{-1}\text{sec}^{-1}$ ) (223), so is essentially instantaneous as long as oxygen is available—one reason  $\text{L}^\bullet$  radicals are so difficult to detect, even by electron paramagnetic resonance.



Translating this into observed behavior, isolated double bonds behave as if there were two separate resonant systems of equal probability, so oleic acid yields (C9 + C11) and (C8 + C10) hydroperoxides from the two resonance systems, respectively, in approximately equivalent amounts (18:1, Figure 5). In 1,4-diene systems, H abstraction occurs preferentially at the doubly allylic hydrogen between the two double bonds, and the resonance system with the unpaired electron extends across both double bonds with electron density focused at the central carbon (11) and electron deficient positions at external carbons 9 and 13 (18:2, Figure 5). In higher polyunsaturated fatty acids with multiple 1,4-diene structures (18:3 and 20:4, Figure 5), the resonant systems from multiple doubly allylic radicals



**Figure 5.** Doubly allylic H abstraction sites, electron resonance distributions, and corresponding locations of hydroperoxide formation in unsaturated fatty acids. Heavy arrows denote dominant positions for hydroperoxide formation.

overlap. In theory, then, hydroperoxides should form at internal positions in equal proportion to the external positions. Nevertheless, only minor amounts of internal hydroperoxides are observed, and then only with three or more double bonds, because they lack the C—OO• bond stabilization by conjugation and undergo rapid  $\beta$ -elimination of the oxygen to regenerate the original 1,4-diene radical (226). In addition, internal peroxides have a very strong tendency toward cyclization (227–230). Consequently, the dominant hydroperoxides of autoxidizing fatty acids are always found at the external positions, regardless of the number of double bonds, except under two circumstances; (1) in autoxidation, equal distribution of LOOH at all positions without any cyclic products is found only in media of high H donating power—e.g., when 3–5% tocopherol is added (6), and (2) internal hydroperoxides are characteristic of singlet oxygen photosensitized oxidation, as was discussed in Section 2.1.2.

Hydroperoxide positional distributions in unsaturated fatty acids undergoing autoxidation and photosensitized oxidation are presented in Table 4.

Hydroperoxides have geometric as well as positional isomers on lipid chains. When the hydrogen is abstracted at an allylic carbon, the double bond shifts one carbon to a position  $\beta$  to the abstraction site, and it reforms in the *trans* rather than *cis* configuration. The *trans,cis*-conjugated diene structure is retained whether oxygen adds or not, and provides the first detectable intermediate in lipids during autoxidation (238).



For a long time, it was thought that the *trans,cis*-conjugated double bonds isomerize to *trans,trans* as oxidation progresses, so both *trans,cis* and *trans,trans* forms are typically isolated for each hydroperoxide position. Linoleic acid, for example, forms 9*tc*, 9*tt*, 13*tc*, and 13*tt* hydroperoxides. It is now known that this

**TABLE 4. Hydroperoxide Positional Distributions in Oxidizing Fatty Acids.**

	5-OOH	6-OOH	8-OOH	9-OOH	10-OOH	11-OOH	12-OOH	13-OOH	14-OOH	15-OOH	16-OOH	Ref.
<b>18:1Δ9</b>												
Autoxidation			26.4	24.2	22.8	26.6						231
Photo-ox $^1\text{O}_2$				47.5	52.3							232
Photo-ox Chl <sup>†</sup>				49.1	50.8							233
Thermal oxidation			25.1	25.1	24.9	24.9						231
<b>18:2Δ9,12</b>												
Autoxidation			1	51	tr		tr	49	1			234
Photo-ox $^1\text{O}_2$				31.9	16.7		17	34.5				232
Photo-ox Chl <sup>*</sup>				30.2	19.8		19.8	30.1				233
<b>18:3Δ9,12,15</b>												
Autoxidation				33.4			10.1	12.5			43.9	235
Photo-ox $^1\text{O}_2$				22.7	12.7		12.0	14.0		13.4	25.3	232
Photo-ox Chl <sup>†</sup>				21.6	14.3		15.3	15.7		12.0	21.1	233
<b>20:4Δ5,8,11,14</b>												
Autoxidation	27		7	9		11	6			40		9
Photo-ox $^1\text{O}_2$	14.4	4.8	12.9	13.2		14.4	13.3		6.9	20.3		236
<b>22:6Δ4,7,10,13,16,19</b>												
Autoxidation	% of -OOH at C20 (27.1), C17 (7.9), C16 (9.2), C14 (10.8), C13 (8.9), C11 (7.3), C10 (7.3), C8(7.9), C7(7.0), C4(6.5)											237

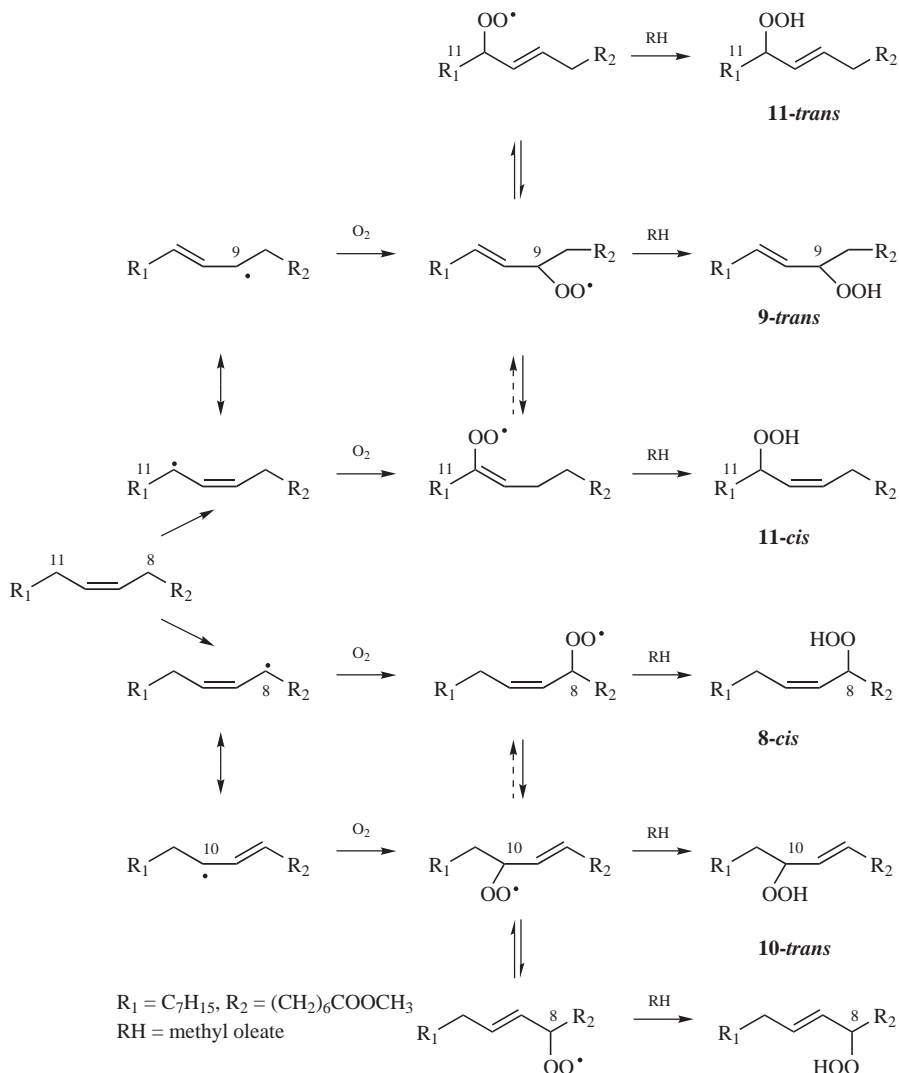
explanation is inaccurate. The fundamental mechanisms underlying *cis-trans* isomerization and distributions in lipid hydroperoxides were recently elucidated by Porter and his colleagues (7, 226, 239). Two critical factors control the process: reversible  $\beta$ -elimination of oxygen from peroxy radicals, and availability of strong hydrogen donors. Reversible addition of oxygen to the pentadienyl system was first proposed as the major action during the induction period about thirty years ago, based on kinetic (240), EPR (241), and  $^{17}\text{O}$  evidence (242, 243). Since then, Porter has contributed much new documentation of the phenomenon, but the concept still does not seem to be recognized widely and incorporated into general schemes of lipid oxidation.

Data of Porter and colleagues (5, 7, 11, 226, 244–257) shows quite conclusively that both positional and geometric isomerism proceed through the delocalized allyl radical for oleate (Figure 6) or dienyl radical for linoleate and higher PUFAs (Figure 7) via alternating removal of the peroxy oxygen by  $\beta$ -scission, migration of the free radical, and readdition of the oxygen at a new carbon position or orientation (5, 7, 11, 226–257). There can be interconversion of peroxy position and orientation indefinitely as long as the radical is in the manifold. Once the peroxy radical is protonated, it becomes fixed as the hydroperoxide, but can return to the manifold if the LOOH hydrogen is abstracted.

To explain different proportions of *trans,cis* and *trans,trans* isomers, Porter distinguishes thermodynamic and kinetic processes (5, 226, 253). Kinetically, hydroperoxides will form whenever an abstractable hydrogen atom is available, but thermodynamically, the system equilibrium moves toward *trans,trans* isomers in the absence of good H donors, as in organic solvents (248). The observed isomer mix reflects the balance and competition between these two processes in a given system. When good H donors are present, the *trans,cis* isomers kinetically form first. The H atoms can come from a protic solvent, an antioxidant, a cosubstrate, or the allylic hydrogens of the fatty acid chains themselves. For oleic and linoleic acids with only slightly bent chains, *trans,cis* formation is favored in oriented systems or at high concentrations that increase interchain contact. *Trans,trans* isomers are favored in dilute solutions, aprotic solvents, and at elevated temperatures in which there is less interchain contact and decreased H availability. With linolenic, arachidonic, and higher polyunsaturated fatty acids, the fatty acid chains bend back on each other, bringing double bonds and allylic hydrogens from opposite ends of the chain into proximity with the peroxy radicals. When oxidized neat, higher PUFAs thus have an immediate internal H source and characteristically yield high proportions of *trans,cis* peroxides (kinetic products). However, when an H donor is lacking (e.g., low concentrations, aprotic solvent, elevated temperature), *trans,trans* cyclic hydroperoxides become dominant (251).

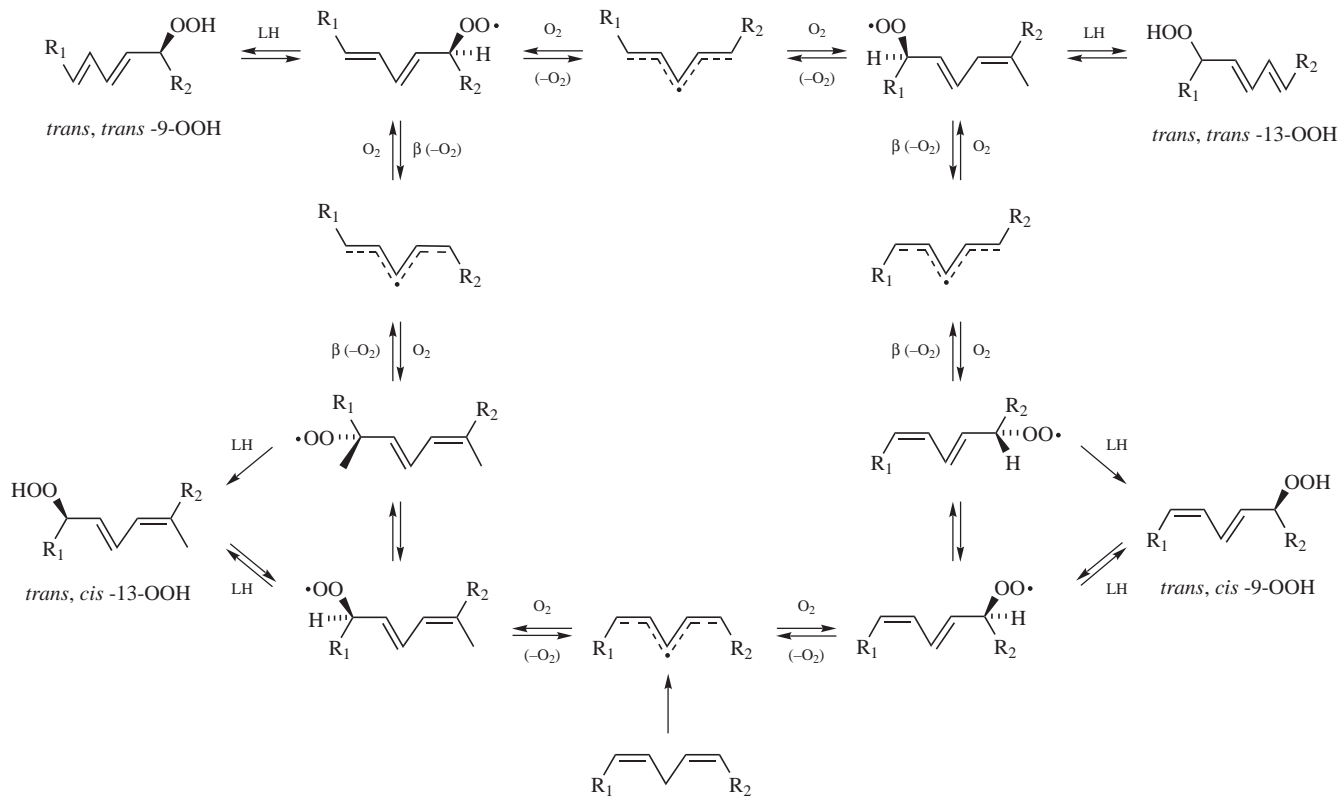
The tendency for higher *cis* isomers at external hydroperoxides and positions closer to the  $-\text{COOH}$  terminus reflects the greater H abstracting power of those positions. Conversely, the increase in *trans* isomers with internal hydroperoxides and as the hydroperoxide position moves toward the distal end of the fatty acid chain reflects depressed activity at those sites. The *cis/trans* ratio changes with





**Figure 6.** Radical sites and  $\beta$ -elimination manifold leading to isomerization of hydroperoxides in oleic acid. Adapted from (11).

reaction system and with temperature. *Cis* isomers are enhanced by the presence of antioxidants such as tocopherol and by high concentrations of lipids, whereas *trans* isomers are enhanced by even mild heating which reduces contact between lipid and potential H donors. Contrary to earlier reports, the *cis/trans* ratio does not vary with extent of oxidation unless reaction conditions are changing or H abstraction from LOOH is occurring, allowing LOO $\bullet$  to undergo  $\beta$ -scission.



**Figure 7.** Reaction scheme for positional isomerization of double bonds and formation of *trans*-, *trans*-hydroperoxides during oxidation of linoleic acid via reversible  $\beta$ -scission of oxygen. Adapted from (246, 248, 250).

### 3. PROPAGATION

The classic free radical chain depicts propagation as proceeding directly and entirely by hydrogen abstraction. In reality, however, H abstraction by  $\text{LOO}^\bullet$  is very slow ( $k = 36\text{--}62 \text{ L mol}^{-1} \text{ sec}^{-1}$ ) (200, 258) and selective, abstracting only hydrogens with low bond energy (e.g., doubly allylic  $-\text{CH}_2-$ , thiols, phenols) (259). Consequently, there is plenty of time for alternative reaction pathways to compete and change the direction of oxidation (260) yielding distinctly different products at different rates and having significant consequences to the ultimate mixture of products. Addition, cyclization, and scission reactions compete with H abstraction to reroute  $\text{LO}^\bullet$  and generate products and additional radical species. Ultimately, radicals are always transferred between molecules by hydrogen abstractions, but the original  $\text{LOO}^\bullet$  may not be the propagating radical, and the product mix is much more complicated than implied by the simple free radical chain. At least one of the reactions ( $e^-$  transfer) stops rather than propagates the radical chains.

Multiple mechanisms are well established in radical chemistry and have been applied to peroxy and alkoxy radical reactions in lipid oxidation (6, 7, 261), although not all rate constants and reaction details are available. Consideration of the multiple competing pathways discussed below can explain complicated oxidation kinetics, account for complex product mixes, enable more accurate evaluation of the extent of oxidation, and facilitate design of more effective antioxidant strategies.

#### 3.1. Chain Propagation by $\text{LOO}^\bullet$

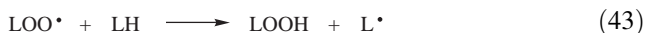
$\text{LOO}^\bullet$  are the chain carriers in early stages of lipid oxidation. Competing reactions of  $\text{LOO}^\bullet$  include:

- a. atom or group transfer (H-abstraction)
- b. rearrangement/cyclization
- c. addition to double bonds ( $\rightarrow$  crosslinks)
- d. disproportionation
- e.  $\beta$ -scission
- f. recombination
- g.  $e^-$  transfer ( $\text{LOO}^\bullet + e^- \rightarrow \text{LOO}^-$ )

The first four reactions all contribute to chain propagation, although under different conditions. Disproportionation leads to branching and a shift in kinetics, and  $\beta$ -scission mediates isomerization, as was described in the previous section. Recombination (f) and electron transfer (g) terminate radical chains. Electron transfer is an active antioxidant mechanism that occurs particularly in the presence

of active redox agents such as metals. The mechanism(s) occurring in any given system are determined by ease of H abstraction and double bond structure in the target molecule, solvent, and reaction conditions, particularly temperature.

**3.1.1. Atom Transfer (hydrogen abstraction) by  $\text{LOO}^\bullet$   $\longrightarrow$  Free Radical Chain Reactions** Hydrogen abstraction is the heart of the classic free radical chain reaction schemes (Figure 1). Peroxyl radicals initially formed at any site on a fatty acid pass the unpaired electron to adjacent lipid molecules by abstracting hydrogens from an allylic position or a hydroperoxide, and the process repeats itself indefinitely until the chain is intercepted.



H abstraction from dienes by peroxyl radicals (Reaction 43) is slow ( $k = 62 \text{ L M}^{-1}\text{s}^{-1}$ ) (258) and highly selective for doubly allylic hydrogens (261, 262). H abstraction from hydroperoxides (Reaction 44) is ten times faster (223). Two factors govern H abstraction by  $\text{LOO}^\bullet$ : (1) relative availability of H sources in solvent and lipids, and (2) viscosity of medium (263). H abstraction from other lipids (i.e., chain propagation) is facilitated in neat lipids and aprotic solvents in which the lipid allyls are the only source of hydrogens, at high lipid concentrations where fatty acid chains come in closer contact, and in higher polyunsaturated fatty acids with multiple bisallylic hydrogens. Hydrogen abstraction is also facilitated in low viscosity media, whereas chain lengths are greatly shortened in viscous solvents (11, 264).

On the other hand, when the solvent or other components in the system have H sources, competitive abstraction from nonlipid sites occurs and the net result is to quench the radical and interrupt the chain rather than propagate it. Abstraction from multiple H sources in a system is common, and subsequent oxidation at nonlipid sites may account for oxygen consumption that exceeds LOOH formation in many systems.

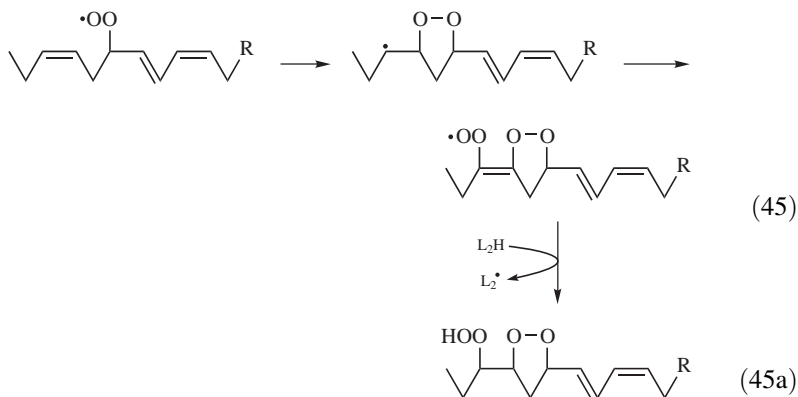
Hydrogen abstraction also increases at elevated temperature as thermal energy decreases bond dissociation energy. Typical H abstraction rates for  $\text{ROO}^\bullet$  at room temperature are  $\leq 1 \text{ M}^{-1}\text{s}^{-1}$ , but this increases to  $10^3\text{--}10^4 \text{ L M}^{-1}\text{s}^{-1}$  at  $65^\circ\text{C}$  (223). For example, in linolenic acid autoxidized neat at room temperature to PV 1113, products were not quantified, but estimates from intensities of HPLC peaks gave about 40%  $\text{LnOOH}$ , 12% dihydroperoxides, 12% hydroperoxy epoxidides, and 4% epoxidides (228). At  $40^\circ\text{C}$ , H abstraction occurred more as a secondary process. Hydroperoxides *per se* were still the main products, but fewer were present as mono- and dihydroperoxides (36% total) and more had formed after cyclization or addition (31%). Data are not available to distinguish whether this

occurred because rates of  $\text{LOO}^\bullet$  cyclization increase more than H abstraction with temperature or, alternatively, the increased rates of H abstraction by Reaction 44 [ $12.75 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$  for Reaction 43 vs.  $5.6 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$  for Reaction 44 (261)] forces a shift in the equilibrium balance and cyclization products accumulate at the expense of hydroperoxides in secondary processes. However, at  $80^\circ\text{C}$ , H abstraction clearly dominated, yielding 84% LOOH and 16% cyclic products.

Hydroperoxides were the first lipid oxidation products discovered (115), and eventually a hydroperoxide should result from each alternative pathway, as will be described below. Thus, there is logic to the common practice of measuring hydroperoxides to detect early stages of lipid oxidation. Nevertheless, hydroperoxides alone do not give an accurate quantitative or qualitative picture of the extent of lipid oxidation because there is no way to account for either LOOH decomposition or alternative reactions. Oxidation can be seriously underestimated and system effects can be misinterpreted when monohydroperoxides are considered to be the only product and determinations of oxidation extent and kinetics are based on LOOH concentrations alone. Although many studies have focused on identifying structures of products, few have actually calculated total product yields and distributions. The limited data available show clearly that LOOH is not the only product, even in early stages of lipid oxidation. In some cases, hydroperoxides may ultimately form only after addition, cyclization, or other rearrangement; in some systems, conventional monohydroperoxides may not form at all.

**3.1.2. Rearrangement/Cyclization of  $\text{LOO}^\bullet$**  When abstractable hydrogens are not immediately available, peroxy radicals find pairing electrons by adding to down-chain double bonds, forming cyclic products. The most important internal rearrangement or cyclization of  $\text{LOO}^\bullet$  proceeds by 1,3-addition of the peroxy radical to the neighboring *cis*-double bond, attaching to the  $\beta$  carbon to form a 5-exo ring and leaving a radical on the  $\gamma$  carbon of the double bond (Reaction 45). Addition of oxygen to the radical generates a second peroxy radical (new position), which abstracts a hydrogen from a neighboring lipid molecule to propagate the chain and form a hydroperoxy epoxide product (Reaction 45a) (265). 5-exo cyclization by  $\text{LOO}^\bullet$  ( $k \sim 10^3 \text{ s}^{-1}$ ) (7) is faster than  $\beta$ -scission of oxygen ( $27\text{--}430 \text{ s}^{-1}$ ) (11) and H abstraction ( $<1\text{--}400 \text{ M}^{-1}\text{s}^{-1}$ ) (88, 223, 247), so it should be able to compete as an initial process, especially in fatty acids with three or more double bonds.

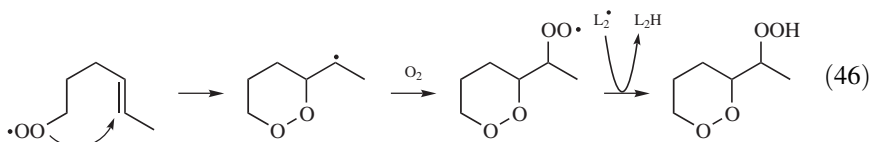
Reaction sequence 45 shows this process at C-13 of linolenic acid for simplicity, but comparable cyclization also occurs at C-10 in linolenic acid and at C-8, C-9, C-11, and C-12 in arachidonic acid (252). The cyclic product mixes of oxidized Ln and An typically show multiple positional and geometric isomers (227, 266). In the interest of space, the isomerization and racemization that accompanies cyclization will not be discussed here. The reader is referred to papers by Gardner (6, 267) and Porter (7, 11, 252, 268) for more details.



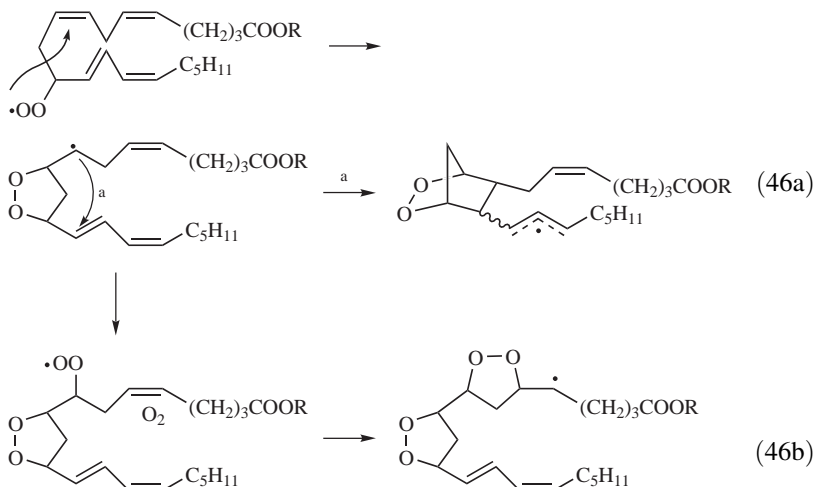
Cyclization requires the presence of a *cis*-double bond homoallylic to a hydroperoxide (230, 269), as shown in Reaction 45. In addition, cyclization of peroxy radicals at internal positions is considerably faster than secondary oxidations of hydroperoxides at either external position. About 25% of peroxy radicals in linolenic acid and 33% of peroxy radicals in arachidonic acid are internal (Table 4). Thus, linolenic and arachidonic acids are particularly prone to formation of cyclic peroxides. These factors together make intramolecular cyclization 4–6 fold faster than  $\beta$ -scission in higher polyunsaturated fatty acids (247).

Initial cyclization of  $\text{LOO}^\bullet$  via 1,4-addition to the  $\gamma$ -carbon of the neighboring double bonds forces endo cyclization to a 6-oxo ring and is kinetically unfavorable ( $k \sim 10 \text{ s}^{-1}$ ) (11, 230). However, both 6-oxo exocyclic peroxides (Reaction 46) and endoperoxides (Reaction 46a) have been observed as secondary oxidation or rearrangement products in arachidonic acid oxidation (252, 270). The acyl chains of fatty acids with four or more double bonds (An, EPA<sup>1</sup>, and DHA<sup>1</sup>) have hairpin-like configurations, bringing double bonds from opposite ends into close proximity. Although the 1,3-cyclic peroxides are all *exo*, with these fatty acids there is increased tendency towards cross-chain addition to form endo peroxides (270) and toward multiple internal  $\text{LOO}^\bullet$  additions to form bicycloperoxides (Reaction 46a) and polyperoxides (Reaction 46b) in prostaglandin-like structures (11, 230, 261, 271, 272). Note that each cyclization produces another radical capable of initiating new oxidation chains!

Without enzymatic catalysis, the endo and bicyclic peroxides (Reaction 46a) usually account for less than a few percent of arachidonic acid oxidation products, and the dominant pathways are formation of *exo* peroxides (Reaction 45) and polyperoxides (Reaction 46b).

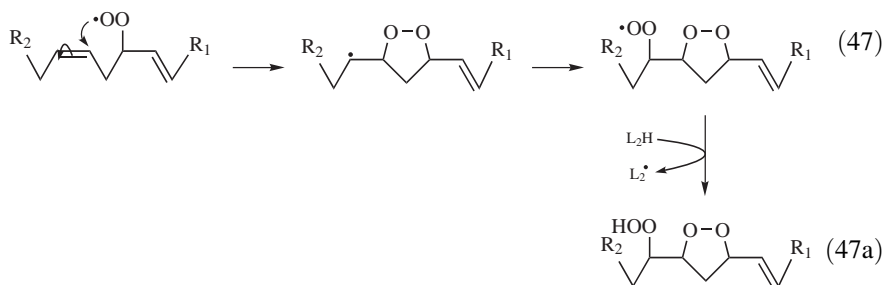


<sup>1</sup>EPA: eicosapentaenoic acid, 20:5 $\omega$ 3; DHA: docosahexaenoic acid, 22:6 $\omega$ 3.



As mentioned earlier, in linolenic acid and higher PUFAs, even with cyclization, some peroxy radical may eventually abstract a hydrogen externally to propagate the oxidation chain. The net result of internal cyclization, however, is a reduction of the number of molecules oxidized: Two or more moles of oxygen are absorbed per fatty acid, but only one radical transfer occurs and the chain length is extended by only one. Hence, although the most highly unsaturated fatty acids are innately the most oxidizable, paradoxically their oxidation chains may be shorter and their propagation rates may be lower than for linoleic acid.

Peroxy radicals of linoleic acid do not undergo cyclization to epidioxides during autoxidation because the requisite *cis*-double bond-hydroperoxide structure is not present. However,  $^1\text{O}_2$  photosensitized oxidations produce hydroperoxides at the internal 10- and 12- positions in quantities almost as high as at the external 9- and 13- positions, and internal hydroperoxides do have the required  $\beta$ -*cis*-double bond. Hydrogen abstraction from the internal hydroperoxides yields  $\text{LOO}\cdot$  that undergo cyclization and produce propagating hydroperoxy epidioxide radicals (Reaction 47) and the corresponding epidioxy-hydroperoxide products (Reaction 47a) in high proportions (232, 273, 274).



**TABLE 5. Product Distributions in Methyl Linolenate (MLn) Autoxidized Neat at 40°C. Data from (228).**

			PV	904	1286		
9-OOH	c/t 27.8%	~1:1 ratio	} MLn (unreacted)	87.9	74.8%		
	t/t 24.5			0.2	0.3		
13-OOH	c/t 27.8			Mono-OOH <sup>a</sup>	3.5	8.4	
	t/t 20.7			HOO-epidioxide <sup>b</sup>	3.8	7.7	
				Epoxy-HO dienes	tr	tr	
				Di-OOH	0.9	2.9	
			Polars	3.7	5.9		

<sup>a</sup>Mixture of 9-OOH and 13-OOH.<sup>b</sup>All at internal positions.

Availability of hydrogens drives abstraction reaction. Thus, solvent, lipid concentration, extent of oxidation, and temperature all play critical roles in shifting the balance between external hydrogen abstraction and internal addition, i.e., direct chain propagation vs. internal cyclization. Low oxygen pressures particularly favor cyclization (275). In aprotic solvents and dilute solutions at room temperature, in which external abstractable hydrogens are absent or limited, LOO• cyclization at various positions accounts for all the products (266). However, in neat lipids, a situation that provides both availability and proximity of abstractable hydrogens, abstraction competes with cyclization to generate mixed products. The apparent proportions vary with temperature and extent of oxidation. At least two studies have found about 30% cyclic products at 40°C (228, 265).

Epidioxide-OO• radicals are very reactive and are particularly prone to dimerization with even moderate heat (276). This makes detection of their involvement in oxidations sometimes difficult. Using the example cited above (228), with continued oxidation to PV = 1286 meq oxygen/kg oil, epidioxides remained a major product, but mono- and dihydroperoxides, as well as the polar products, increased more (Table 5). Dimerization of the hydroperoxy epidioxides accounted for at least part of the decrease in proportional percentage of epidioxides as well as the increase in polar dimers. Increased H abstraction rates at elevated temperatures contributed to the higher hydroperoxides, but increased decomposition of hydroperoxides to alkoxy radicals at the elevated temperature also introduces more competing reactions. Faster H abstractions by LO• at external positions lead to increased propagation rates to form new hydroperoxides, pointing out how even moderate heat can introduce considerable complication in propagation mechanisms.

**3.1.3. Addition of LOO• to Double Bonds** Peroxyl radicals are quite specific in their addition preferences, and competition with hydrogen abstraction is generally unfavorable except under select conditions. The ROO• addition becomes competitive when abstractable hydrogens are limited (aprotic solvents, low temperature) and when there is a double bond that is conjugated, terminal, or 1,1-disubstituted



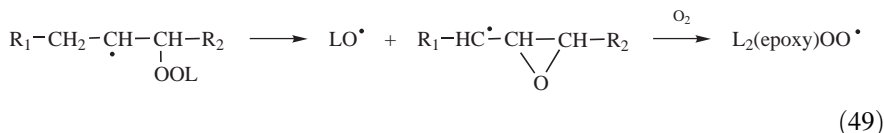
(277). Hence, simple short-chain alkenes with allylic hydrogens react 80–100% by hydrogen abstraction, whereas alkenes that are conjugated or have radicals at a terminal double bond with no allylic hydrogens (as in some scission products) react 80–100% by addition (278, 279). Longer chain alkenes such as fatty acids give mixed products.

Factors controlling addition are strength of the bond formed, steric hindrance, polar effects, and stability of the resulting radical adduct (280). Although addition reactions of small molecular  $\text{ROO}^\bullet$  and  $\text{RO}^\bullet$  can be very fast, steric factors and the large number of reactive  $\text{CH}_2$  groups on unsaturated fatty acids decrease the ease of addition reactions in lipids. Conjugation and *trans*-double bonds in oxidized lipids counterbalance the steric impediments and enhance peroxy radical additions (281). Copper salts catalyze addition reactions of peroxy radicals (282), which has some interesting implications in food systems.

Propagation via addition of  $\text{LOO}^\bullet$  to double bonds forms both monomer products (epoxides and epidioxides) and dimers or polymers; the propagating species are peroxy radicals formed at new positions and alkoxy radicals released by  $\beta$ -elimination. In early stages of oxidation,  $\text{LOO}^\bullet$  adds to double bonds to form an initial dimer complex (Reaction 48), which then reacts further to generate new radicals. The ultimate product depends on the nature of the target double bond.



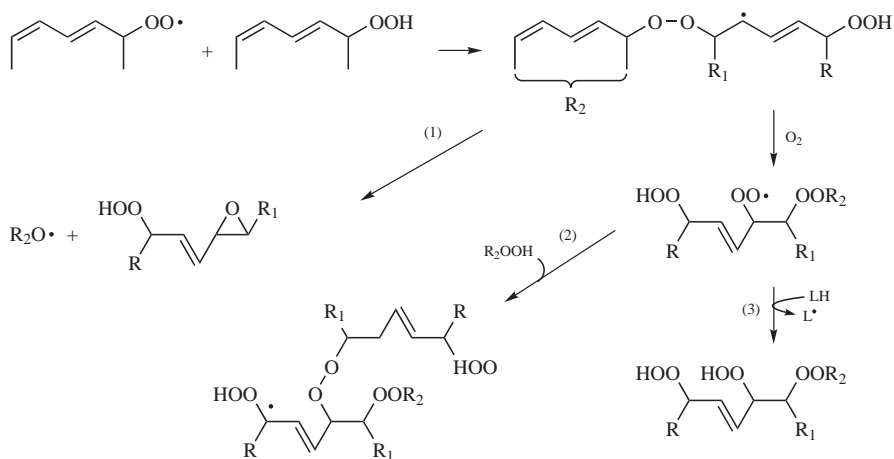
$\text{LOO}^\bullet$  adds to isolated or nonconjugated double bonds, then undergoes 1,3-cyclization to form an epoxide and an allylic radical, eliminating  $\text{LO}^\bullet$  in the process. The allylic radical then adds oxygen to form a new peroxy radical (Reaction 49). This is a true branching reaction as two new propagating radicals ( $\text{LO}^\bullet$  and epoxy $\text{OO}^\bullet$ ) with increased reactivities are generated from the initial  $\text{LOO}^\bullet$ .



This presents an interesting analytical quandary. Epoxides are major products of lipid oxidation and derive from  $\text{LO}^\bullet$  cyclization as well as  $\text{LOO}^\bullet$  additions (see Section 3.2.2). Consequently, it may be difficult to determine the mechanism that is operative in a given reaction system, and indeed, both may contribute. For example, Hendry (283) reacted a series of  $\text{ROO}^\bullet$  with their parent compounds at 60°C and found 40% of the products were epoxides. Rate constants of  $k = 20$  to  $1130 \text{ M}^{-1}\text{sec}^{-1}$  were calculated assuming the reactions were all additions, but at the elevated temperature of the study, hydrogen abstraction to form the hydroperoxides, followed by homolytic scission to alkoxy radicals, could also have contributed to the yields.

In later stages of oxidation, the likelihood of  $\text{LOO}^\bullet$  addition increases because doubly allylic hydrogens have been removed during initial reactions and the double bond system has been shifted to conjugated. This limits competition by hydrogen abstraction and greatly facilitates  $\text{LOO}^\bullet$  addition. Thus, addition reactions of  $\text{LOO}^\bullet$  to conjugated oxidation products (Reaction 50) increase during later stages of oxidation and produce the characteristic polymers and increased viscosity of very oxidized oils (284–286). Note that even in advanced oxidation, the polymer product still retains a propagating free radical, distinguishing this from peroxy radical recombinations that yield peroxy dimers without radicals (see Termination reactions). Gardner (6) hypothesized that  $\text{LOO}^\bullet$  could add to *cis*-bonds of unoxidized linoleic acid as well as conjugated double bonds of products to form the same type of polymer as in Reaction 48. Whether LH or  $\text{LOOH}$  is the  $\text{LOO}^\bullet$  target for addition, chain propagation could then continue by three pathways: (1) eliminate an alkoxy radical and form epoxides via Reaction 49, or add oxygen to regenerate peroxy radicals and then (2) add to another  $\text{LOOH}$  to continue the polymerization process, or (3) abstract a hydrogen to propagate the radical chain and form a stable dihydroperoxide dimer. Elevated temperatures favor polymerization via pathway 2 (287). Pathway 3 could account for the low levels of dimers that have been detected in early stages of oxidation (288).

$\text{LOO}^\bullet$  additions increase with heat (289), extent of oxidation (290), and solvent polarity (266). Dimer levels of methyl linolenate autoxidized neat at room temperature varied from 0.1% to 10.1%, proportional to peroxide values (290).  $\text{ML}_n$  autoxidized at  $40^\circ\text{C}$  to PV 1062 gave 6.8% dimers; 80% of these were from  $\text{LOO}^\bullet$  and 20% were from epidioxide- $\text{OO}^\bullet$  additions. The dimer linkages were mostly  $\text{C}-\text{O}-\text{O}-\text{C}$  at lower temperatures, but shifted to  $\text{C}-\text{C}$  and  $\text{C}-\text{O}-\text{C}$  as the temperature increased (276). At PV = 4002,  $\text{LOO}^\bullet$  additions increased to 55% of the products. Epidioxide peroxy radicals, in particular, showed a very strong tendency to add to double bonds, with greater than 90% dimerization at  $40^\circ\text{C}$ .





**TABLE 6. Isomerization of 13-OOH to 9-OOH and Corresponding Shift in Products During Heating of Linoleic Acid. Data from (297).**

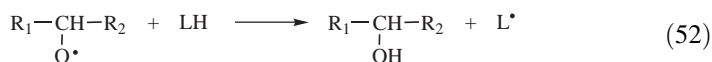
Product	Scission Point	% yield			
		9-OOH	13-OOH	Pure 9-OOH	Pure 13-OOH
Hexanal	13-OOH $\alpha$	1.7	8.3	1	28
Me octanoate	9-OOH $\alpha$	5.0	4.3	37	24
2,4-decadienal	9-OOH $\alpha$	20.0	12.5	51	33
Me oxononanoate	9-OOH $\beta$	10.5	15.2	12	16

**3.2. Propagation by Alkoxy Radicals,  $LO^\bullet$**  Alkoxy ( $LO^\bullet$ ) radicals are responsible for propagation of the radical chain during the very rapid oxidation that ensues after the induction period ends. In the earliest stages of oxidation,  $LOO^\bullet$  cyclization and addition reactions can proceed before  $LOOH$  formation via H abstraction, but  $LO^\bullet$  can only be generated via  $LOOH$  decomposition, so their reactions become important as secondary events in oxidation. Nevertheless, because  $LO^\bullet$  react faster than  $LOO^\bullet$  by several orders of magnitude,  $LO^\bullet$  becomes dominant almost as soon as  $LOOH$  breaks down.

There are four major mechanisms for radical chain propagation by alkoxy radicals. The mechanism dominating in a given system is determined largely by double bond structure, solvent conditions, and steric factors (21):

- hydrogen abstraction
- rearrangements/cyclization
- addition
- $\alpha$ - and  $\beta$ -scission (fragmentation)

**3.2.1. Hydrogen Abstraction by  $LO^\bullet$**   $LO^\bullet$  abstractions are very fast ( $k \sim 10^7$ – $10^8$  L M<sup>-1</sup>s<sup>-1</sup>), but less selective than  $LOO^\bullet$  (198); they abstract both allylic and bis-allylic hydrogens, whereas  $LOO^\bullet$  abstracts only the latter (261). Allylic hydrogens are particularly susceptible to abstraction by sec alkoxy radicals (21), so the H abstractions by lipid alkoxy radicals, as written in the classic free radical chain (Reaction 52), should be a preferred reaction in lipid oxidation:



However, cyclization and scission reactions of  $LO^\bullet$  compete with H abstraction and can often limit the effectiveness of this reaction in chain propagation.

Factors influencing the rates of H abstraction by alkoxy radicals are H abstractability on target molecules > structure of the alkoxy radical > solvent system (298). Hydrogen availability and solvent have critical effects in lipid oxidation;

**TABLE 7. Rate Constants for H Abstraction from PUFA by *t*-BuO<sup>•</sup> in Various Solvents (197, 198). Reactivity of LO<sup>•</sup> is Comparable (299).**

Fatty Acid	$k \times 10^{-6} \text{ L mol}^{-1} \text{ s}^{-1}$	
	Nonpolar Solvent	Aqueous Solution
Oleic	3.8	68
Linoleic	8.8	130
Linolenic	13.0	160
Arachidonic	20.5	180

the structure is essentially constant as sec alkoxy radicals. Hydrogen abstraction from other lipid chains by LO<sup>•</sup> is most effective in neat lipids in which the lipid allylic groups are the only source of hydrogens. The relative rates of abstraction from different fatty acids are approximately proportional to the number of allylic or doubly allylic hydrogens, as is shown in the reaction rate hierarchy of O < L < Ln < An in Tables 2 and 7, but beyond that there seems to be little preference for one bis-allylic position over another (197, 300). Interestingly, the high susceptibility of allylic hydrogens to abstraction, along with the bent chain configurations of polyunsaturated fatty acids, also enhances preferential internal H abstraction by lipid alkoxy radicals, leading to competing cyclization and epoxide formation (301–303). This will be discussed in more detail in Section 3.2.3.

Hydrogen abstraction by LO<sup>•</sup> to propagate free radical chains is facile also in nonpolar aprotic solvents when lipids are at high concentrations. However, at moderate lipid concentrations, H abstraction must compete with internal rearrangements and scission (304), and at low concentrations it may become insignificant (305).

Surprisingly, the most important effect of LO<sup>•</sup> on propagation may well be via abstraction of hydrogens from the lipid hydroperoxides as they form (Reaction 53), thus regenerating LOO<sup>•</sup> and eliminating the need for other catalysts to decompose the hydroperoxides and begin chain branching.



The bond dissociation energy of the hydroperoxide hydrogen is higher than the allylic hydrogens (90 vs. 65–85 kCal mol<sup>-1</sup>, respectively), but hydrogen bonding between the LO<sup>•</sup> and LOOH greatly decreases the E<sub>a</sub> for the abstraction (306). In mixtures of fatty acids and their hydroperoxides, *t*-butoxy radicals abstract hydrogens almost exclusively from the hydroperoxides (230). The rate constant for (*t*-BuO<sup>•</sup> + ROOH) is 2.5 × 10<sup>8</sup> M<sup>-1</sup>s<sup>-1</sup>, nearly diffusion controlled (307). Similarly, cumylalkoxy radicals abstract H from hydroperoxides faster than reported for alkyl substrates (306).

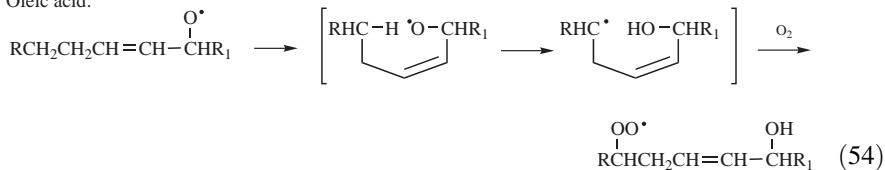
In aqueous and protic solvents where H sources are plentiful, hydrogen abstractions by LO<sup>•</sup> are faster kinetically, but less effective in chain propagation (Table 7). Production of LOH can be detected in protic solvents (308), but the yields of hydroxylated products remain low because selectivity of H abstraction decreases and H

abstraction must compete with increasing rates of  $\beta$ -scission (309) (see Section 3.2.4). The availability of hydrogens from water and other dissolved solutes increases the likelihood of H abstraction from molecules other than fatty acids (310), in which case the chain reaction is not propagated (2, 9). H abstraction as a termination reaction will be discussed further in Section 4.

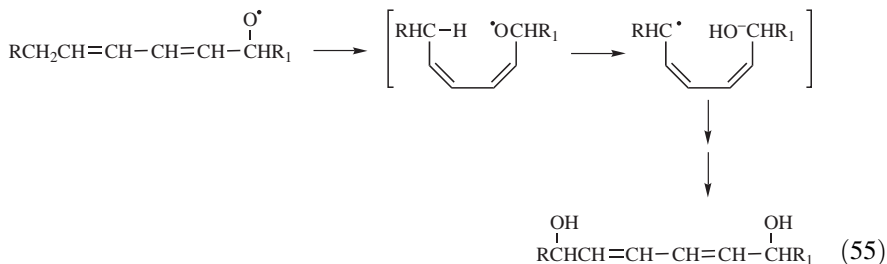
The rate of H abstraction by  $\text{RO}^\bullet$  increases with temperature in all solvents. This leads to marked acceleration of oxidation in neat lipids and in nonpolar solvents where the only H sources are fatty acids, and it also favors LOOH formation over cyclization. This is evident in the marked increase of mono-, di-, and trihydroperoxides over epoxides as oxidation temperature increases from room temperature to about  $80^\circ\text{C}$  (228, 276, 311, 312). However, heat has less effect in polar and aqueous solvents (310). The activation energy for H abstraction is lower than for  $\beta$ -scission, so there is less thermal enhancement of abstraction rate and also less selectivity of abstraction sites in polar solvents. More importantly, higher temperatures enhance scission more than abstractions so, particularly at  $T > 100^\circ\text{C}$ , the relative importance of H abstraction by  $\text{LO}^\bullet$  and  $\text{LOO}^\bullet$  in propagation is diminished (278, 313) and secondary processes begin to dominate.

One additional H abstraction reaction must be mentioned. Internal 1,5 (Reaction 54) or 1,6 (Reaction 55) hydrogen abstraction generates an alcohol and a radical (21) in a position that may or may not be normal for autoxidation. Intramolecular H abstraction involving a six-membered transition state (Reaction 55) has been identified in saturated alkyls with long side chains (304). Occurrence of the corresponding reaction in unsaturated fatty acids would produce oxidation at sites previously attributed to  $\text{HO}^\bullet$  attack (314).

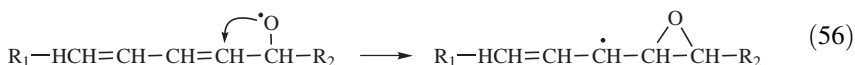
Oleic acid:



Linoleic acid:



**3.2.2. Rearrangement/Cyclization of LO<sup>•</sup>** Cyclization of LO<sup>•</sup> involves 1,2 addition to an adjacent double bond to form epoxides and epoxyallylic radicals (Reaction 56).



This is a very fast reaction that, under some conditions, can even exceed rates of H abstraction (309). Cyclization of LO<sup>•</sup> to epoxides is the dominant reaction in aprotic solvents (including neat lipids), when lipids are at low concentration (275) or highly dispersed on a surface (315, 316), at room temperature (147, 308, 317), and at low oxygen pressures (275, 278); and the reaction accelerates with increasing polarity of the aprotic solvent (308–310). However, the stability of LO<sup>•</sup> is reduced considerably in polar solvents (309, 310). Although epoxyallylic radicals from cyclization have been observed in pulse radiolysis studies of LO<sup>•</sup> in aqueous solutions (308), H abstraction and scission reactions are much faster. This pattern can be seen in the change of cyclic products yields when oxidation was conducted in different solvents (Table 8). The change in competition over time is also apparent.

Cyclization of LO<sup>•</sup> is stereospecific. The configuration of epoxides is fixed by the conformation of the fatty acid alkoxy radical at the point of cyclization rather than postcyclization isomerization (319, 320). As with LOO<sup>•</sup>, there is a stronger tendency for LO<sup>•</sup> to cyclize from internal positions, probably due to the orientation of the -O<sup>•</sup> relative to the bis-allylic hydrogens, and consequently, photosensitized oxidations yield high concentrations of cyclic products (321). The levels and positional distribution of these products are characteristic markers distinguishing autoxidation from photosensitized oxidation.

Temperature has relatively little effect on cyclization because the activation energy for the rearrangement is low. Cyclization thus dominates in neat lipids at

**TABLE 8. Variation in Dominant Propagation Mechanism and Product Distribution for Linoleic Acid Oxidized in Different Solvents.**

Solvent and System	Product Distribution (%)					Reference
	LOH/LOOH <sup>a</sup>	Cyclic <sup>b</sup>	Scission	Other	Unknown	
CHCl <sub>2</sub> , FeCl <sub>3</sub> , early		100				266
Anhydrous MeOH	3–8	75–80	13–15			318
Cyclohexane, 7.5 mM	15	68		18 <sup>c</sup>		216
80% ethanol	30	11	—	7 <sup>d</sup>	7 <sup>e</sup>	214
FeCl <sub>3</sub> /cysteine						

<sup>a</sup>Total of all H abstraction products, all isomers.

<sup>b</sup>Total of all products that had any cyclic component.

<sup>c</sup>Oxo dienes.

<sup>d</sup>Hydroxyl ethoxylated products from rx with solvent radicals.

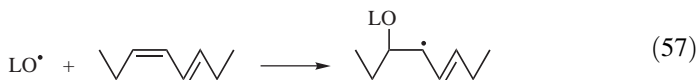
<sup>e</sup>Unidentified soluble products and volatile scission products.

**TABLE 9. Effect of Alkene Structure on Preference for Addition vs. Abstraction by *t*-BuO<sup>•</sup> Radicals at 40°C. Data from (21).**

Alkene	Abstraction (%)	Addition (%)
R-CH=CH-R (trans)	95	3-4
R-CH=CH-R (cis)	83	17
R-CH=CH <sub>2</sub>	97	3
R <sub>2</sub> -C=CH <sub>2</sub>	83	17

room temperature, but as the temperature increases, H abstraction and scission become more important directors of propagation. At high temperatures (>100°C), rearrangement is a relatively minor process (278, 317). Metals, particularly Fe and Cu, activate cyclization and direct internal rearrangements to dihydroxy and hydroxyene multiple positional isomers (317). Iron catalyzes isomerization and conversion of HO-epoxides to ketols (322).

**3.2.3. Addition of LO<sup>•</sup> to Double Bonds** Addition of LO<sup>•</sup> to double bonds does not occur with the ease of LOO<sup>•</sup> additions. Alkoxy radicals have unusually strong preference for allylic attack, so intermolecular H abstraction or internal cyclization will dominate as long as allylic hydrogens are present. Addition is favored by absence of allylic hydrogens and by conjugation (Table 9), conditions that only hold after oxidation has started. Hence, propagation by LO<sup>•</sup> addition is most active in catalyzing chain branching in secondary stages of oxidation. In contradistinction to LOO<sup>•</sup> additions, LO<sup>•</sup> addition increases with *cis* configuration and asymmetrical substitution on double bonds (323), so when LO<sup>•</sup> does add to lipid hydroperoxides, it adds to the *cis*-rather than *trans*-double bonds (324).



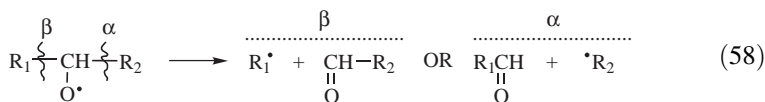
Propagation by LO<sup>•</sup> addition is most important in neat lipids and organic solvents (308). Although LO<sup>•</sup> additions do occur in aqueous solvents, they are generally not competitive with scission and rearrangement reactions. Heat catalyzes the addition. Addition of LOOH to methyl linoleate at 210°C results in complete conversion of the LOOH to dimers containing both reactants (289). Although the exact structure was not determined, the dimers were presumably LO-ML adducts forming after thermal decomposition of LOOH to LO<sup>•</sup>.

**3.2.4. β-Scission of LO<sup>•</sup>** Beta-scission of alkoxy radicals leads to scission of the C-C bond on either side of the LO<sup>•</sup> group to yield a mixture of carbonyl products and free radicals, typically aldehydes, alkanes, and oxo-esters, from the initial alkoxy radicals (297). Scission produces the volatile products so characteristically

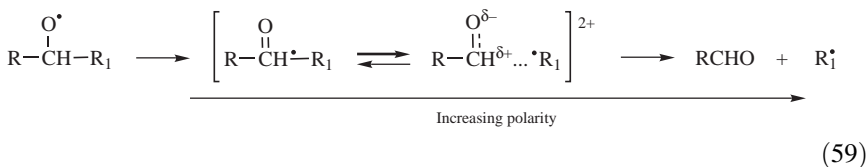


associated with rancidity, and the mix can become quite complex in secondary stages of oxidation.

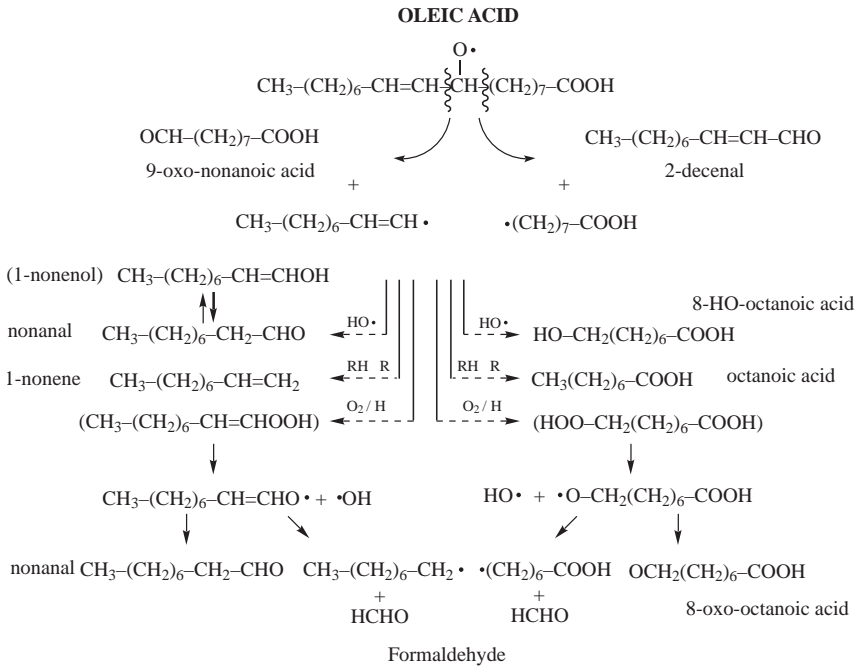
A simplified scission is shown in Reaction 58. The  $\alpha$  and  $\beta$  fragmentation in this case refer to the position of chain scission relative to the  $-\text{COOH}$  on the fatty acid. More complete scission maps for oleic, linoleic, and linolenic acids are presented in Figures 8–10. Some of the radicals deriving from the scissions rearrange to nonradical products internally, but most of them abstract hydrogens to propagate the radical chain. Unsaturated fragments, particularly those containing conjugated dienes, are still susceptible to oxidation and their subsequent reactions also contribute to chain branching.



Scission of alkoxy radicals is a solvent-dependent, solvent-driven process (325). Fragmentation of the carbon chain proceeds through formation of a transition state, which mediates the transformation from nonpolar alkoxy radical to polar cleavage products. Water and polar protic solvents stabilize both the increasingly polar transition state and the carbonyl products by providing solvation and hydrogen bonding to support the transition state and reduce the activation energy for bond rupture (263, 305, 323, 326, 327);  $\text{H}^+$  from the solvent then adds immediately to the scission radicals to provide the driving force for the reaction (224). This process is shown in Reaction 59 for  $\alpha$ -scission (21). Scission is also favored when lipids are in dilute solution in nonpolar organic solvents where there is reduced competition from hydrogen abstraction.



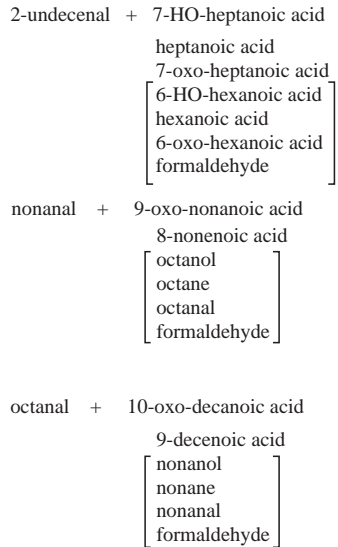
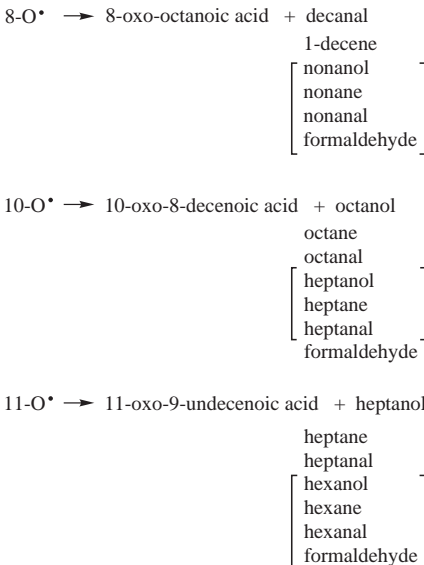
Scission is rapid in polar solvents. The  $k_s$  for alkoxy radicals in aqueous solution is  $10^6$ – $10^7 \text{ s}^{-1}$  (328–330), 10–100 times faster than rates in nonpolar organic solvents (331–333) that have dielectric constants comparable with fatty acid methyl esters. Even though this is somewhat slower than H-abstraction (Table 10), scission usually competes effectively, and under appropriate conditions, scission can dominate. In polar media, scission accounts for at least half of the  $\text{LO}^\bullet$  reactions even in early oxidation. For example, Bors (308) found  $\sim 48\%$  fragmentation,  $\sim 48\%$  H abstraction, and 4% unreacted  $\text{t-BuO}^\bullet$  in aqueous solution on a pulse radiolysis time scale (ms to s). n-6 Fatty acids oxidized in  $\text{Tris-KCl} + \text{FeSO}_4/\text{ascorbic acid}$  for up to 24 hrs gave the scission fragment 2-hydroxyheptanal as the sole product (334). Scission accounted for 7–10% of the oxidation products in neat triolein, but



Following the same fragmentation pattern -

$\beta$ -scission

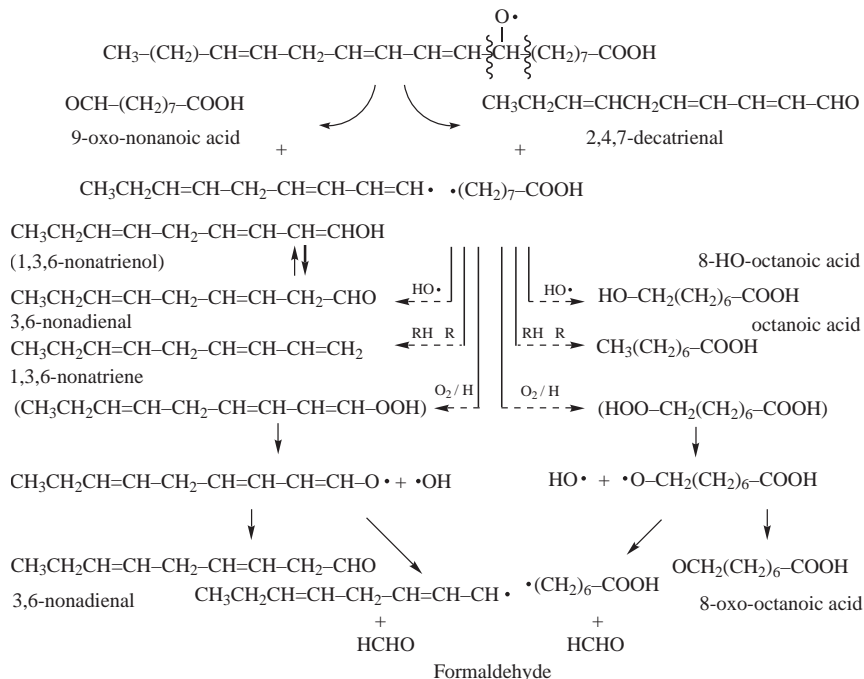
$\alpha$ -scission



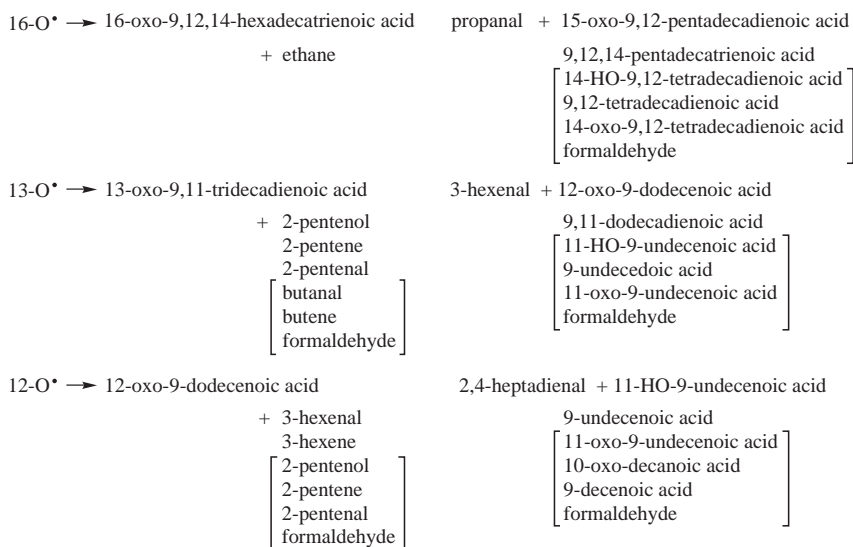
**Figure 8.** Typical initial scission patterns of oxidizing oleic acid. Data from (340, 341). Parentheses indicate unstable intermediates; brackets denote products from secondary scissions.



## LINOLENIC ACID



Following the same fragmentation pattern -

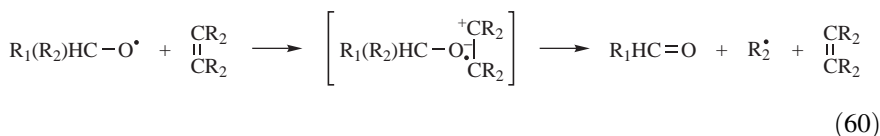
 $\beta$ -scission $\alpha$ -scission

**Figure 10.** Typical initial scission patterns of oxidizing linolenic acid. Data from (340, 341). Parentheses indicate unstable intermediates; brackets denote products from secondary scissions.

**TABLE 10. Solvent Effects on Rates of H Abstraction ( $k_a$ ) and  $\beta$ -Scission ( $k_\beta$ ) of Cumyloxy Radicals (CumO $\cdot$ ). Data from (327).**

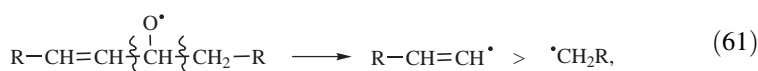
	H Abstraction	$\beta$ -Scission	$k_a/k_\beta \text{ M}^{-1}$
	$k_a \times 10^{-6} \text{ M}^{-1}\text{s}^{-1}$	$k_\beta \times 10^{-5} \text{ s}^{-1}$	
$\text{CCl}_4$	1.1	2.6	4.5
$\text{C}_6\text{H}_6$	1.2	3.7	3.2
$\text{C}_6\text{H}_5\text{Cl}$	1.1	5.5	2.0
$(\text{CH}_3)_3\text{COH}$	1.3	5.8	2.3
$\text{CH}_3\text{CN}$	1.2	6.3	1.9
$\text{CH}_3\text{COOH}$	1.3	19	0.7

on a total weight basis. The fact that it is observed at all under these conditions is probably due to acceleration of scission in the presence of double bonds through the increase in polarity. Dipole-dipole interaction between alkoxy radicals on one fatty acid with double bonds on an adjacent unsaturated fatty acid forms a charge transfer transition state that induces electron and charge redistributions, thus facilitating scission (263).



Alkoxy radical ( $\text{LO}\cdot$ ) scission makes its greatest contribution to propagation at elevated temperatures (323) that overcome the large  $E_a$  and  $\log A$  (Arrhenius factor) for scission (338). Heat accelerates alkoxy radical scissions in all solvents, although the pattern of cleavage may change as temperature increases because primary scission of the alkoxy radical (Reaction 49) gradually increases at the expense of alternative reactions (339), and at high enough temperatures, secondary scissions also occur. Cleavage of polyperoxides, for example, is unimportant at  $60^\circ\text{C}$ , but becomes a major contributor to propagation at  $T > 100^\circ\text{C}$  (278). Similarly, fragmentation of sulfenyl alkoxy radicals varies from 21% at  $10^\circ\text{C}$  to 40% at  $50^\circ\text{C}$  (338). Shifts in scission products at different temperatures have been reviewed in detail by Grosch (340).

What determines whether a scission will be  $\alpha$  or  $\beta$  relative to the  $-\text{COOH}$  is an age-old question that still has not been completely answered. There are suggestions in the chemical literature that scissions should occur between the alkoxy radical and the double bond (314, 341),



**TABLE 11. Distribution of  $\alpha$  and  $\beta$  Scissions as a Function of Alkoxy Radical Distance from  $-\text{COOH}$  in Oleic Acid. Data from (312).**

LO Position	% $\alpha$ -Scission	% $\beta$ -Scission
8	3.2	7.4
9	10.4	8.0
10	22.0	6.5
11	23.0	10.6

but this has been questioned on energetic grounds, i.e., the dissociation energy for vinyl bonds is 109 kcal but for allylic bonds is 60 (111). An alternative explanation is that scission will occur preferentially at the site fulfilling the thermodynamic requirement to form the most stable product, e.g., saturated aldehydes are more stable than unsaturated aldehydes. However, there are disagreements over whether the stability of the radical (333, 342–344) or the carbonyl product (327, 341) is the determining force. Inductive effects of the  $-\text{COOH}$  group increase the tendency toward  $\alpha$  scission, but this has less overall influence than the aforementioned factors. The presence of an acid group favors selective cleavage between  $-\text{OOH}$  and double bond ( $\alpha$  or  $\beta$  depending on position of  $-\text{OOH}$ ). Heat and metals induce one-electron redox reactions, which generate  $\alpha$ -monocarbonyls (scission on  $-\text{COOH}$  side of alkoxy radicals) and an ejected radical that can initiate new chains (335). Thus, the scission pattern for oxidizing fatty acids is mixed and varies with the product structure and reaction conditions.

Evaluation of products from oleate oxidation provides a simple example of how these factors interact in directing scission (312). The tendency for  $\alpha$ -cleavage increases as the alkoxy radical position moves away from the carboxylic acid; there is relatively little positional preference for  $\beta$ -cleavage (Table 11). This pattern is consistent with preferential scission between the alkoxy radical and the double bond as well as formation of saturated aldehydes.

The dominant products do indeed derive from scission between the alkoxy radical and the double bond, but a variety of scissions that are less favorable thermodynamically occur at the same time, generating the complex mixture of products shown in Figures 8–10 and Table 12. For monohydroperoxides, scission varies with the position of the alkoxy radical, with the longest saturated product receiving preference. For alkoxy radicals from dihydroperoxides, dominant cleavages are still between the  $-\text{CO}^\bullet-$  and double bond, but  $\sim 40\%$  occur at the alkoxy nearest the  $-\text{COOH}$ , and half that occur on the  $\text{CH}_3$  terminal alkoxy radical (345).

It is important to recognize that scission does not necessarily stop after reaction of initial alkoxy radicals. Scissions of secondary products generated during lipid oxidation also contribute to propagation and to the ultimate product mix (346). Malonaldehyde is perhaps the best known example of this, as will be discussed further in Section 4.2.

**TABLE 12. Scission Products from Unsaturated Fatty Acids Oxidized at Room Temperature. (Data from 3, 8, 273, 290, 314, 340, 341, 345, 347–349.)**

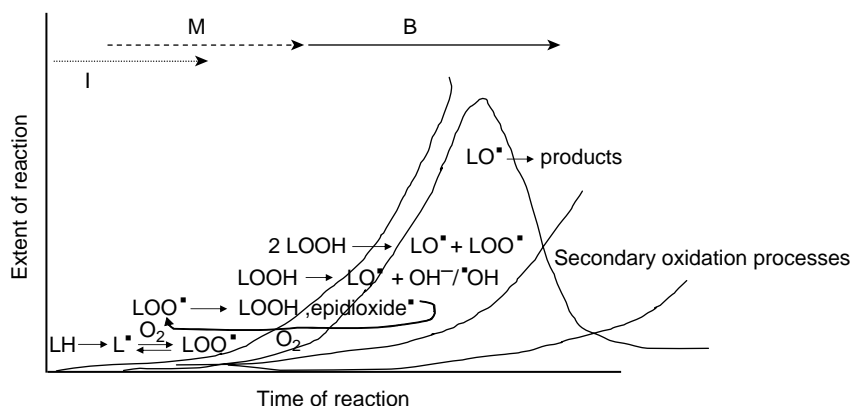
OLEIC ACID		
Major Products	Product Classes and Carbon Chain Length	
Nonanal	Hydrocarbons	6–8
Octanal	Alkanals	2–4, 5, 6, 7, 8, 9, 10, 11
Undec-2-enal	2-Alkenals	6–9, 10, 11
Undecanal	Acids	1, 6–9
	Alkanols	5–8
2-decenal	Alkylformates	2–8
LINOLEIC ACID		
Major Products	Product Classes and Carbon Chain Length	
Hexanal	Hydrocarbons	3–5
2,4-decadienal	Alkanals	3, 4, 5, 6, 7, 8
2-octenal	Alkenals	7, 8, 9, 10
2-heptenal	Dienals	9, 10
	Oxo-alkanals	7, 8, 9
	Ketones	7, 8
	Alcohols	3, 4, 5, 6, 7, 8
	Acids	1, 5, 6, 7, 9
	Esters	1, 6, 7, 8
LINOLENIC ACID		
Major Products	Product Classes and Carbon Chain Length	
2,4-heptadienal	Hydrocarbons	1–3
3-hexenal	Alkanals	1–3, 6
Propanal	Alkenals	4, 5, 6, 7
2,4,7-decatrienal	Dienals	7, 8, 9
2-pentenal	Trienals	10
Octadienal	Ketones	5, 18
Pentene-3-one	Alcohols	3, 4, 5, 6, 7, 8
Octadiene-2-one	Acids	1, 5, 6, 7, 9
	Esters	1, 6, 7, 8
	Oxo-alkanals	1, 6, 7, 8
ARACHIDONIC ACID		
Major Products	Product Classes and Carbon Chain Length	
Hexanal	Alkanal	2, 6, 7
2,4-decadienal	Alkenal	7, 8, 9, 11
2,4,7-tridecatrienal	Dienal	9, 10, 11, 12
2-heptenal	Ketones	
2-octenal	Alkanes	5, 6
Pentanal	Aldehyde esters	4, 5
1-octen-3-one		
4-decenal		
3,5-undecadien-2-one		
2,6-dodecadienal		
5-oxo-pentaoate		

### 3.3. Propagation Reactions of LOOH; Mono- vs. Bimolecular LOOH Decomposition and Chain Branching

It should be obvious from the discussion above that hydroperoxides are the key intermediates controlling the progress of lipid autoxidation. As long as peroxy radicals remain in the  $\beta$ -scission manifold, with  $O_2$  continuously being added to or eliminated from lipid alkyl radicals, perceptible oxidation does not progress. This is the well-known induction period (Figure 11) during which oxygen absorption and traditional chemical changes in the lipids are difficult to detect for several reasons:

1. Until H abstraction occurs, no net change occurs.
2. Standard analytical techniques are not sensitive enough or fast enough to detect the low levels of initial products.
3. Only traditional products such as conjugated dienes and hydroperoxides have been analyzed in most cases. If cyclization occurs before H abstraction to form hydroperoxides, the oxidation may be missed by standard peroxide value analyses.
4. Hydroperoxides are often breaking down as fast as (or faster than) they are formed.

Ultimately, production of lipid hydroperoxides, even by circuitous routes, becomes the major process driving the oxidation reaction forward. LOOH are the first stable products of lipid oxidation, accumulating in the absence of pro-oxidant heat, metals, hemes, ultraviolet light, peroxy radicals, or antioxidant acids or nucleophiles. However, from a practical standpoint, one or more of these or other decomposing factors are nearly always present, so the low energy O—O and O—H bonds undergo a variety of scission reactions. Indeed, a large proportion of the

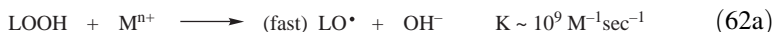


**Figure 11.** Diagrammatic representation of changes in dominant reactions and products over the course of lipid oxidation. Three separate rate periods are usually designated: Induction period (I), monomolecular rate period (M), and bimolecular rate period (B).



LOO• and all of the LO• involved in propagation are not *ab initio* radicals, but derive from some form of LOOH decomposition (350).

To briefly recap what has already been covered in Section 2, redox-active metals break the O-O bond by electron transfer, hence LOOH decomposes heterolytically to generate radicals and ions. Reducing metals such as Fe<sup>2+</sup> and Cu<sup>+</sup> generate alkoxy radicals (LO•) and hydroxide ions (OH<sup>-</sup>), whereas oxidizing metals such as Fe<sup>3+</sup> and Cu<sup>2+</sup> give peroxy radicals (LOO•) and hydrogen ions (H<sup>+</sup>):



Hydroperoxides are also “recycled” by reaction with peroxy radicals:

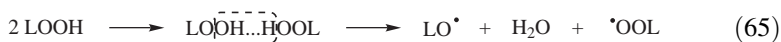


These reactions must be distinguished from homolytic decomposition by heat and UV light that break the O—O bond by energy deposition, yielding alkoxy and hydroxyl radicals (•OH). The O—O in organic hydroperoxides (BDE = 25–38 kCal/mole) begins decomposing at about 50°C and is completely decomposed at 160°C (297).

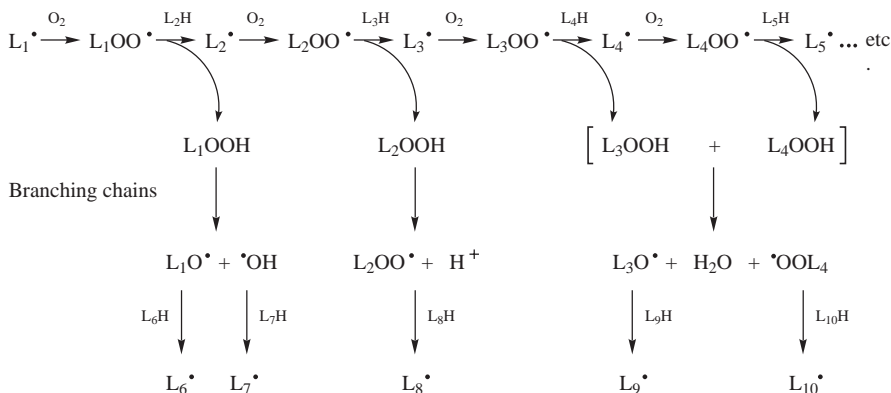


Homolytic scission is much more catastrophic in terms of lipid oxidation because two propagating radicals are released per hydroperoxide, LO• is more reactive and more selective than LOO•, and HO• is extremely reactive. HO• is rather unselective, abstracting hydrogen atoms all along the acyl chain, and it can also readily add to double bonds (still generating a radical). Hence, the net effect of LOOH decomposition is a transition in mechanism and kinetics. Lipid oxidation essentially gathers steam, increasing in rate and extent as LO• becomes the dominant chain carrier and secondary chains are initiated. This process, often referred to as chain branching, greatly amplifies and broadcasts the effects of initiation: if not intercepted by nonlipid molecules, a single initiating event can result in sequential oxidation of literally hundreds of molecules in the primary chain and in secondary branching chains, as shown in Figure 12. The net effect is a noticeable increase in measurable oxidation, as seen in the monomolecular rate period (Figure 11).

Oxygen uptake remains slow and LOOH decomposes monomolecularly (Reactions 62–64) during early stages of lipid oxidation as main chains are extending, branches are developing, and hydroperoxide concentrations are low. However, the process becomes more complicated as LOOH accumulates, e.g., in the presence of lipoxygenase or the absence of decomposers at low temperature, in the dark, or with metal chelation. At high [LOOH], i.e., greater than 1% oxidation (114), it has been proposed that LOOH transition dimers form via hydrogen bonding and bimolecular decomposition ensues, leading to greatly accelerated oxidation (277, 351):



Main radical chain



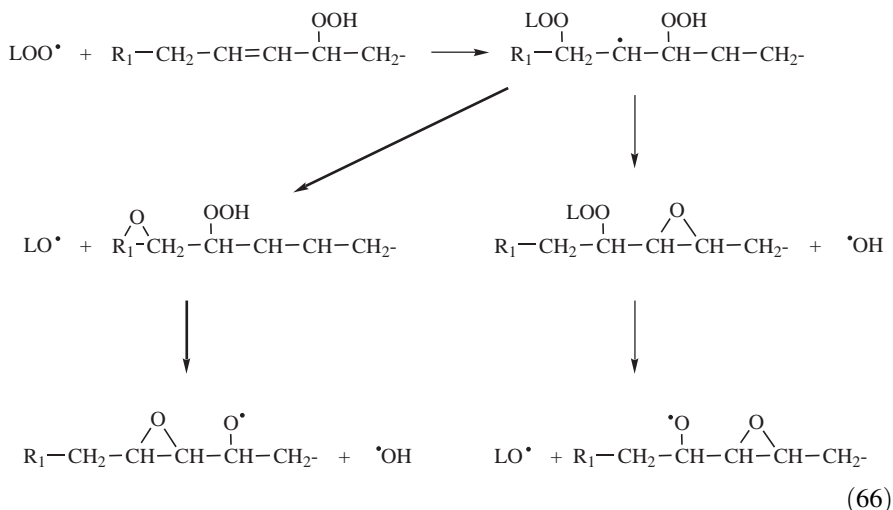
**Figure 12.** Chain propagation and branching in lipid autoxidation. The main chain starts at the *ab initio* radical,  $L_1 \cdot$ , and is driven by cyclical addition of oxygen to form  $LOO \cdot$ , then abstraction of hydrogens to generate new propagating  $L \cdot$  and product  $LOOH$ . Branching reactions are secondary chains originating from radicals produced via a variety of  $LOOH$  decompositions.

Now both alkoxy and peroxy radicals are present equally, and although  $LO \cdot$  dominates kinetically,  $LOO \cdot$  still produces secondary chains. This shift can be seen graphically in the dramatic increase in oxygen consumption rates and production of  $LOOH$  in the bimolecular rate period (B) in Figure 11.

Does bimolecular decomposition actually occur? This mechanism has been widely included in discussions of lipid oxidation, but it is also somewhat controversial. Supporting the theory is the rapid  $O_2$  uptake and shift in product mix (352), as well as the tendency of hydrophilic hydroperoxides to dimerize in nonpolar solutions (353) (e.g., neat lipids) and at high concentrations where bimolecular decomposition is thermodynamically favorable (less endothermic than unimolecular homolysis) (353). Contradicting this are poor fits of oxidation kinetics for some compounds (354). Possible sources of inconsistencies between studies include the measures used to determine kinetics (appearance of product vs. loss of starting material vs. oxygen consumption), specific assumptions made in deriving kinetic equations, and particularly the nature of the oxidizing compound. The rate of reaction increases and the fit of kinetic data improves with a decrease in C—H bond dissociation energies and with increased chain length. For example, methyl oleate, for which the oxidation kinetics are faster, has a better fit with bimolecular breakdown theory than either *n*-decene or ethyl benzene (355).

The latter observations with methyl oleate, together with thermodynamic considerations and EPR evidence for free radical intermediates, suggest an alternative explanation for the dramatic increase in oxidation rates once hydroperoxides accumulate, namely that bimolecular decomposition may be specific to allylic hydroperoxides and proceed via  $LOO \cdot$  radical-induced decomposition rather than by dissociation of hydrogen-bonded dimers (280). Reaction sequence 63 is analogous to Reactions 49 and 50a, where one slowly reacting radical reacts with a

nonpropagating hydroperoxide to generate three very reactive radicals—two LO• and one •OH. The heavy arrows indicate the favored pathway.



Radical-induced decomposition is thermodynamically favorable ( $E_a = 37.5$  kcal), and is also more consistent with the characteristics of bimolecular initiation by hydroperoxides originally proposed by Russell (356), the kinetics measured in lipid oxidation systems, and significant epoxide products reported in many studies. Most importantly, the radical-induced decomposition described in Reaction 63 provides a powerful cascade of reactive radicals to fuel the very rapid increase in oxidation during the bimolecular rate period.

### 3.4. Factors Influencing Propagation Pathways (abstraction vs. scission reactions vs. rearrangement) of LOO• and LO•

The net lipid oxidation observed is a net sum of all the competing reactions occurring in a given system:

LOO• Reactions	LO• Reactions	LOOH Reactions
β-scission of O <sub>2</sub> → isomers	H abstraction	Decomposition →
H abstraction	Addition	chain branching
Cyclization to epioxides	Cyclization to epoxides	
Addition	β-scission → fragment products	
Dismutation		

Hopefully, this chapter has made it clear that there is no fixed sequence of reaction pathways for lipid oxidation. Rather, the pathways most active probably change with reaction system, determined by the type and concentration of lipid,

the solvent, phase distributions of catalysts, surface and interfaces, and numerous other factors. As a consequence, no one standard assay will give a complete or accurate picture of the progress of lipid oxidation. Indeed, one of the difficulties in sorting out controlling factors is that so few lipid oxidation studies have analyzed products quantitatively as well as qualitatively, and even fewer have measured multiple classes of products simultaneously. Several decades of detailed, painstaking product analyses, as discussed above, have now provided a reasonably clear picture of what kinds of compounds are generated during lipid oxidation, but we still need coordinated quantitative analyses of all the classes of products to determine relative contributions of the various pathways under specific reaction conditions. Such information would tremendously improve our ability to tailor oxidation analyses to individual systems as well as to design more effective antioxidant strategies.

Arguments have been presented in the literature that the structure and configuration of the target molecule at the time of radical attack sterically and thermodynamically establish the reaction mechanisms, whereas system conditions, particularly temperature, have relatively little effect. Based on short-term oxidation of simple alkenes, Van Sickle and coworkers (275, 357) proposed that the ratio of H abstraction to addition is determined by the alkene structure and is constant over a very wide temperature range. There is some support for this position in the thermodynamics of H abstraction vs. addition with different double bond structures (Table 13). Clearly, doubly allylic hydrogens are the most susceptible to abstraction, and with this structure, H abstraction has a slight edge over addition most of the time. Allylic hydrogens of isolated double bonds are less susceptible to both H abstraction and addition, reflecting relatively low reactivity (as with oleic acid). In contrast, the conjugated double bond is activated chemically as a result of its extended resonance system: only in this structure is addition competitive with H abstraction, and both reactions are strong. Thus, conditions that favor addition actually develop during lipid oxidation. Although addition is of little importance in early stages, it becomes quite important in secondary stages of oxidation for linoleate and higher PUFAs. However, Van Sickle's theory is not totally applicable to lipids because decades of research has shown quite clearly that system conditions play a major role in determining which propagation mechanisms dominate in lipid oxidation.

Reaction preferences in lipid oxidation have mostly been deduced from product analyses; the few rate constants available for lipid reactions have been determined

**TABLE 13. Ease of Oxy Radical H Abstraction vs. Addition for Different Double Bond Configurations. Data from (279).**

	$\Delta H$ abstraction kcal/mol	$\Delta H$ addition kcal/mol
$RCH_2CH=CHCH_2R'$	-15	-8
$RCH=CH-CH=CHR'$	-19	-20
$RCH=CH-CH_2-CH=CHR'$	-26	-8

in pulse radiolysis studies in the laboratories of Patterson (195–197, 358) and Bors (194, 198, 261, 299, 308). Nevertheless, we can gain some insights from attempts to determine relative contributions of H abstraction, rearrangements, radical additions, and scissions in oxidation of small alkenes that lack the steric complications of fatty acid chain length and polyunsaturation (206, 275, 312, 327, 336, 357). Relevant rate constants are compiled in Table 14. The table includes all fatty acid reaction rates available, and these are supplemented with rates from related compounds, primarily *tert*-butyl and cumyloxy radicals. This approach is justified because model systems have shown that H abstraction rates are determined primarily by the bond strength of the H being abstracted and are relatively independent of the R-group of the abstracting oxy radicals (278). Also, *tert*-butyl peroxy and alkoxy radicals, as well as the corresponding oxy radicals of cumene, have been shown to be reasonable models for unsaturated fatty acids (261, 299, 308, 332, 333). Therefore, consideration of the comparative rate data that has accumulated in defined chemical systems can help elucidate the logic of oxidation processes in lipids. Most critically, it shows how we are usually looking at a totally different process when systems are oxidized under different conditions, and our interpretations of product data and designs of antioxidant strategies must recognize and account for alternative oxidation pathways.

When searching for rate constants to support the product distributions identified under different conditions of lipid oxidation began, numbers were expected that would establish a distinct kinetic hierarchy. Surprisingly, what is most apparent from the rate constants in Table 13 is the lack of clear priority of any of the reactions so that it becomes difficult to establish any “rules” for expected reactivity. Rather, the dominant products in any given reaction must be specifically system dependent. Some of the distinction between reactions may be blurred in ranges of values encompassing multiple sources of oxy radicals that only approximate reactions of lipid radicals, and this argues for more research focused specifically on lipid reactions. Nevertheless, several important patterns do emerge.

1. The literature has long noted that alkoxy radical reactions were faster than peroxy radicals and that reaction rates increased with the solvent polarity. The values in Table 13 reveal the magnitude of those differences—several orders of magnitude in most cases.
2. Both peroxy and alkoxy radicals abstract hydrogens much faster from hydroperoxides than from lipid allylic positions, a fact that has been little appreciated previously and can have great consequences to oxidation kinetics and product distributions.
3. There is a surprising lack of clear preference for one reaction over another, except that H abstraction has a slight priority in general. Thus, most systems should be expected to produce mixtures of products rather than a single class, and only small modifications in reaction conditions (including extent of oxidation) are sufficient to shift the balance between abstraction, cyclization, and scission reactions, altering the product distribution.

**TABLE 14. Rate Constants for Competing Reactions of Lipid or Related Peroxyl and Alkoxy Radicals.<sup>a</sup>**

		ROO•	Reference	LO•	Reference
H abstraction, LH	nonpolar organic	<1–400 M <sup>-1</sup> s <sup>-1</sup>	88, 223, 247, 258, 359	10 <sup>4</sup> –10 <sup>7</sup> M <sup>-1</sup> s <sup>-1</sup>	197, 240, 327, 331
	polar, aqueous			10 <sup>6</sup> –10 <sup>8</sup> L M <sup>-1</sup> s <sup>-1</sup>	198
H abstraction, LOOH	nonpolar organic	600 M <sup>-1</sup> s <sup>-1</sup>	223, 360	2.5 × 10 <sup>8</sup> M <sup>-1</sup> s <sup>-1</sup>	307
	polar, aqueous	NA		NA	
Cyclization	nonpolar organic	10 <sup>1</sup> –10 <sup>3</sup> s <sup>-1</sup>	11, 247	10 <sup>4</sup> –10 <sup>5</sup> s <sup>-1</sup>	307, 361
	polar, aqueous	NA		NA	
Addition	nonpolar organic	NA		10 <sup>4</sup> –10 <sup>8</sup> M <sup>-1</sup> s <sup>-1</sup>	258, 332
	polar, aqueous	NA		NA	
β-scission	oleate	1–8 s <sup>-1</sup>	11, 250, 253	10 <sup>3</sup> –10 <sup>5</sup> s <sup>-1</sup> org	305, 327, 364
	linoleate	27–430 s <sup>-1</sup>	11, 226	10 <sup>4</sup> –10 <sup>5</sup> s <sup>-1</sup> polar org	305, 327
Dismutation	nonpolar organic	10 <sup>6</sup> –10 <sup>9</sup> L M <sup>-1</sup> s <sup>-1</sup>	192, 258, 354, 362, 363	10 <sup>6</sup> –10 <sup>7</sup> s <sup>-1</sup> aq	198, 328, 333
				10 <sup>9</sup> –10 <sup>10</sup> M <sup>-1</sup> s <sup>-1</sup>	361, 362, 364, 365
	polar, aqueous oleate-OO•	10 <sup>7</sup> –10 <sup>8</sup> L M <sup>-1</sup> s <sup>-1</sup> 10 <sup>6</sup> M <sup>-1</sup> s <sup>-1</sup>	261 195, 196, 223	NA	

<sup>a</sup>Data included authentic fatty acids whenever possible, plus primarily cumyl, tetralinyl, and *t*-butyl peroxy and alkoxy radicals.

NA: data not available.

At the risk of being redundant, let me summarize conditions that shift chain propagation mechanisms in lipid oxidation:

- a. Hydrogen abstraction from other fatty acid chains by  $\text{LOO}^\bullet$  and  $\text{LO}^\bullet$  is favored under conditions providing close contact between lipid chains without competition from other H sources—i.e., in aprotic environments such as neat lipids and the lipid interior of membranes, where lipid chains are closely associated. In solvents, H abstraction is favored at moderate lipid concentrations where enough substrate is present to supply hydrogens. However, at low lipid concentrations, cyclization or scission dominate, whereas at high concentrations, radical additions and recombinations become more important (279).
- b. Hydrogen abstraction rates increase with solvent polarity and temperature—but under these conditions, accelerated propagation of lipid oxidation as in (a) must compete with H abstraction from solvent or other nonlipid sources and also with increased rates of scission.
- c. Cyclization is favored when oxygen is limited and abstractable hydrogens are not available, i.e., in neat lipids, aprotic solvents, and low lipid concentrations. Cyclization is facilitated by polyunsaturation, radical formation at internal positions, and iron chlorides. As temperature increases, cyclization diminishes in importance as a propagation mechanism because it is less affected by temperature than other propagation processes and because epidioxide peroxy radicals have an increasing tendency to dimerize rather than abstract hydrogens.
- d. Scission is favored over H abstraction in polar protic solvents that provide the protons necessary to stabilize the scission products, but an excess of water shifts propagation to termination as protons for stabilization of secondary products are drawn from nonlipid sources and increased hydrolysis yields tertiary lipid oxidation products. Scission also increases markedly with temperature as thermal energy facilitates bond rupture.
- e. Propagation by addition is generally a minor reaction whenever hydrogen sources are readily available, but increases when abstractable hydrogens are limited in aprotic solvents, particularly when there is a conjugated double bond. Thus, addition becomes more important once oxidation chains are established. Addition also increases with lipid concentration, but under these conditions it also must compete with increased rates of H abstraction.

All the pathways outlined above eventually lead to H abstraction to form intermediate products that then breakdown to secondary products. Why, then, is the distinction between propagation mechanisms important, other than as an academic exercise? The answer is that shifting among propagation pathways critically affects the kinetics of oxidation, whether determined by oxygen consumption or appearance of specific products, and can induce large differences in the ultimate mix of products, particularly volatiles. This has several important implications and consequences. The first is analytical. If the dominant pathway is not being monitored, an

inaccurate picture of the rate, extent, and character of lipid oxidation is generated and reactivity is misinterpreted. For example, when peroxide values alone are used to follow oxidation under conditions favoring cyclization or scission, much of the lipid change may be missed altogether. Second, changes in the product distributions critically alter flavors and odors from lipid oxidation, and also the potential for secondary effects such as nonenzymatic browning and reactions with proteins. Finally, without information about dominant and active propagation pathways, the most effective strategies for inhibition of the oxidation may not be applied. For example, using only phenolic antioxidants in systems where scission is dominant will probably not be sufficient to stop production of off-flavors and odors. To achieve long-term stability, antioxidant approaches must be tailored specifically to control all active propagation pathways.

## 4. TERMINATION

Termination is one of those nebulous handwaving terms used to imply that a process is coming to a close. In lipid oxidation, “termination” is an even fuzzier concept in that, from a practical standpoint, the lipid oxidation chains probably never fully stop. In addition, a specific radical may be terminated and form some product, but if this occurs by H abstraction or rearrangement, another radical is left behind so the chain reaction continues. Net oxidation slows down when H abstractions or other radical quenching processes exceed the rate of new chain production, but it would be difficult indeed to totally stop the entire radical chain reaction. Thus, in the discussion below, “termination” refers to an individual radical, not the overall reaction.

Free radicals terminate to form nonradical products by four major mechanisms:

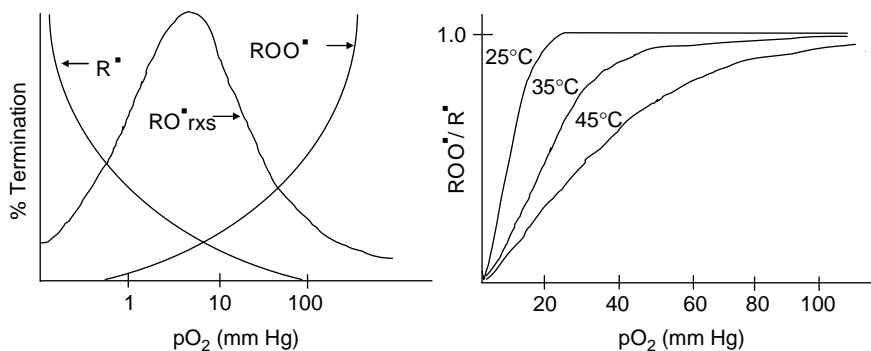
- a. Radical recombinations
- b. A variety of cleavage reactions when proton sources are present to stabilize products
- c. Co-oxidations of other molecules (radical transfer)
- d. Eliminations

LOOH decompositions and rearrangements, sometimes listed as termination reactions, are major sources of propagation  $\text{LOO}^\bullet$ ,  $\text{LO}^\bullet$ , and  $\bullet\text{OH}$  radicals, so were discussed previously in Section 3.3. The mechanisms dominating in a given system are influenced by the nature and concentration of the radicals, the oxygen pressure, and the solvent.

### 4.1. Radical Recombinations

The number of variations possible for radical recombination is nearly limitless, and this accounts, in part, for the broad range of oxidation products detected in lipid





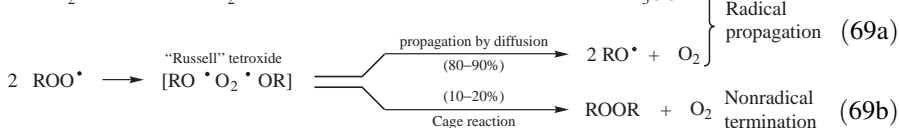
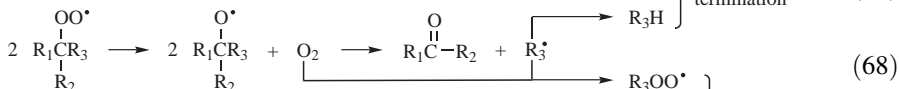
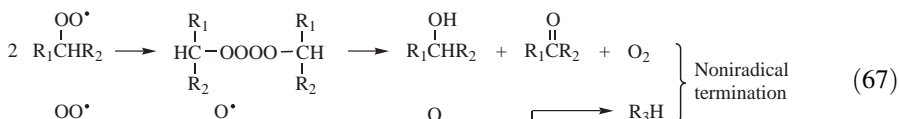
**Figure 13.** Effects of oxygen and temperature on termination processes in lipid oxidation. Adapted (114).

oxidation. Nonetheless, recombinations are not random, and distinct patterns of favored recombinations have been identified.

Temperature and oxygen pressure are key determinants of radical recombination pathways. As shown in the well-known curves of Figure 13 (114, 115),  $L^\bullet$  reactions dominate under low oxygen ( $pO_2 = 1$  to about 80–100 mm Hg) and high temperature (reduced  $O_2$  solubility) conditions, high  $pO_2$  favors  $LOO^\bullet$  reactions (more likely additions than recombinations), and  $LO^\bullet$  contributions to the product mix dominate when  $LOOH$  or  $LOO^\bullet$  decompositions are faster than their formation, i.e., in secondary stages of oxidation and at moderate temperatures and oxygen pressures (15). These oxygen effects on product distributions are indeed striking, but they should not be misconstrued as the only role of oxygen. Oxygen plays critical and complex roles in all three stages of lipid oxidation—initiation, propagation, and termination—although the effects are different in each stage (363), as was implied in discussion of Sections 2 and 3 above.

**4.1.1. Peroxyl Radicals** Secondary peroxyl radicals, as are found in most lipid acyl chains, recombine rapidly ( $2k = 10^8\text{--}10^9 \text{ M}^{-1}\text{s}^{-1}$ ) (192, 362) to form a variety of products, including alcohols and ketones (Reaction 67) (361, 362, 366), ketones and alkanes (Reaction 68) (60, 292), or acyl peroxides and peroxyl radicals (Reaction 69) (264, 367, 369). The alcohols thus produced are indistinguishable from H abstraction products of an original  $LO^\bullet$ , but the ketones and dialkyl peroxides are unique to recombination reactions. As any  $R_3OO^\bullet$  and  $RO^\bullet$  released from Reaction 68 or Reaction 69a react further, peroxyl radical recombinations also have the potential for propagating lipid oxidation (Section 3.1.4).

Concerted addition:



Stepwise addition:



Whether radical or nonradical products dominate depends on the nature of the peroxy radical, the solvent, and the temperature (292). The self-reaction is facilitated in neat oils or aprotic solvents where high  $\text{LOO}^\bullet$  concentrations can accumulate and H abstraction from external molecules is limited; such  $\text{LOO}^\bullet$  recombinations have been extensively cited as the dominant termination product under high  $p\text{O}_2$  conditions. Thus, it is surprising that in reality,  $\text{LOO}^\bullet$  recombination is a major reaction only for oleic acid where the reaction is relatively slow ( $2k = 1\text{--}10 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$ ) (223) even though there are fewer competing reactions (192). Some evidence for Russell mechanism in oxidizing linoleic acid has been presented (367, 368). However, in the higher PUFAs, there is a much stronger tendency toward internal rearrangements to epoxides, etc. (369), as has been discussed above, and the  $\text{LOO}^\bullet$  disappears very rapidly by other reactions [ $2k = 10^7 \text{ L mol}^{-1} \text{ sec}^{-1}$  for L and Ln (196);  $2k = 4.8 \times 10^8 \text{ mol}^{-1} \text{ sec}^{-1}$  for An (192)].  $\text{LOO}^\bullet$  still forms crosslinks, but via addition reactions rather than peroxy recombinations.

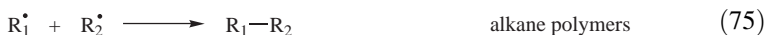
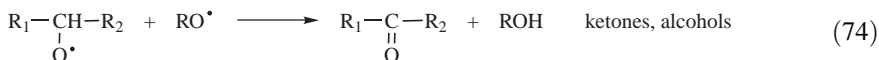
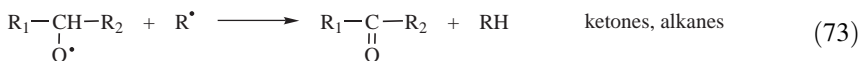
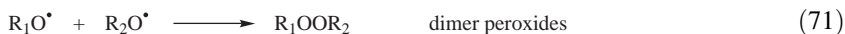
The  $\alpha$ -hydrogen is particularly important for stabilizing products, so secondary  $\text{ROO}^\bullet$  or  $\text{LOO}^\bullet$  (Reaction 67) terminate 100–500 times faster than tertiary  $\text{ROO}^\bullet$  (Reaction 68) (240, 360–362, 364). In oleic acid, most peroxy radicals are *sec*, but *tert* peroxy radicals may derive in secondary oxidations of scission products. This may explain why oleic acid produces Russell products, although in lesser amounts than would be expected (253). Tertiary  $\text{ROO}^\bullet$  produce ketones and release new primary peroxy radicals that can initiate radical chains, rearrange, or be quenched by solvent (294).

There is considerable controversy over whether and how the Russell Mechanism involving tetroxide intermediates (107) actually occurs in lipids, and whether the oxygen is released as  $^1\text{O}_2$ . In early work, Ingold proposed that the Russell mechanism (Reaction 69) was the most important termination process for *sec*

peroxyl radicals (223), and the mechanism has been widely invoked. Nevertheless, although the ketone and alcohol products are found in reactions of small primary peroxyl radicals (366), the prescribed  $O_2$  elimination and alcohol-ketone nonradical products have not always been observed with more complicated lipid peroxyl radicals (367, 370–372). Indeed, Reactions 67 and 68 are probably unimportant at room temperature where 80–90% of the  $ROO^\bullet$  ends up as  $RO^\bullet$  and < 20% of total reaction leads to nonradical products (292). Thus, as long as  $pO_2$  is not limiting,  $LOO^\bullet$  recombination is more active as a propagating reaction than in termination to nonradical species. However, as the temperature increases, this proportion reverses as  $\beta$ -scission of oxygen from  $LOO^\bullet$  predominates and  $LOO^\bullet$  concentrations are decreased below the level required for effective self-reaction (292, 366). Under these conditions, the reaction more likely proceeds via the stepwise radical addition process (Reaction 70) proposed as a general alternative to the Russell mechanism (277).

It should be noted that the tetroxide intermediate proposed as the mechanism for peroxyl radical disproportionation remains somewhat controversial. If it exists, it has been argued that the oxygen should be released as  $^1O_2$  to avoid spin restrictions (291). Some studies claim to have detected  $^1O_2$  from lipid hydroperoxides (366), but the evidence has not been conclusive. One of the difficulties in determining when  $^1O_2$  is produced is that  $O_2^{-\bullet}$  reduces singlet oxygen when water sufficient to provide a hydration shell of five water molecules is present (373).

**4.1.2. Alkoxy and Alkyl Radical Recombinations** A wide variety of alkoxy and alkyl radical recombinations have been proposed to explain lipid oxidation products observed in model reaction systems and in food or biological materials. Many are hypothetical, based on detailed studies with simple compound, but not necessarily verified in lipid oxidation. Nevertheless, the radical recombinations outlined below do provide a pathway to products not generated in the reactions already discussed. Obviously, recombinations lead to polymers. Perhaps just as importantly, however, recombinations of the fragment radicals formed in  $\alpha$  and  $\beta$  scissions of alkoxy radicals generate low levels of volatile compounds and flavor components that augment those produced in scission reactions and provide the undertones and secondary notes that round out flavors (340).



There is little data available to provide a quantitative sense of the contribution of these radical recombinations to the overall mix of lipid oxidation products. The rates of recombinations generally follow the energy of the dimer bond formed (198, 305, 323):

	Bond E	Rate Constant ( $M^{-1} \text{ sec}^{-1}$ )
$R^{\bullet} + R^{\bullet}$	80–90	$10^{10}$ – $10^{11}$
$R^{\bullet} + RO^{\bullet}$	80–90	$10^{10}$ – $10^{11}$
$RO^{\bullet} + RO^{\bullet}$	35–40	$10^7$ – $10^9$

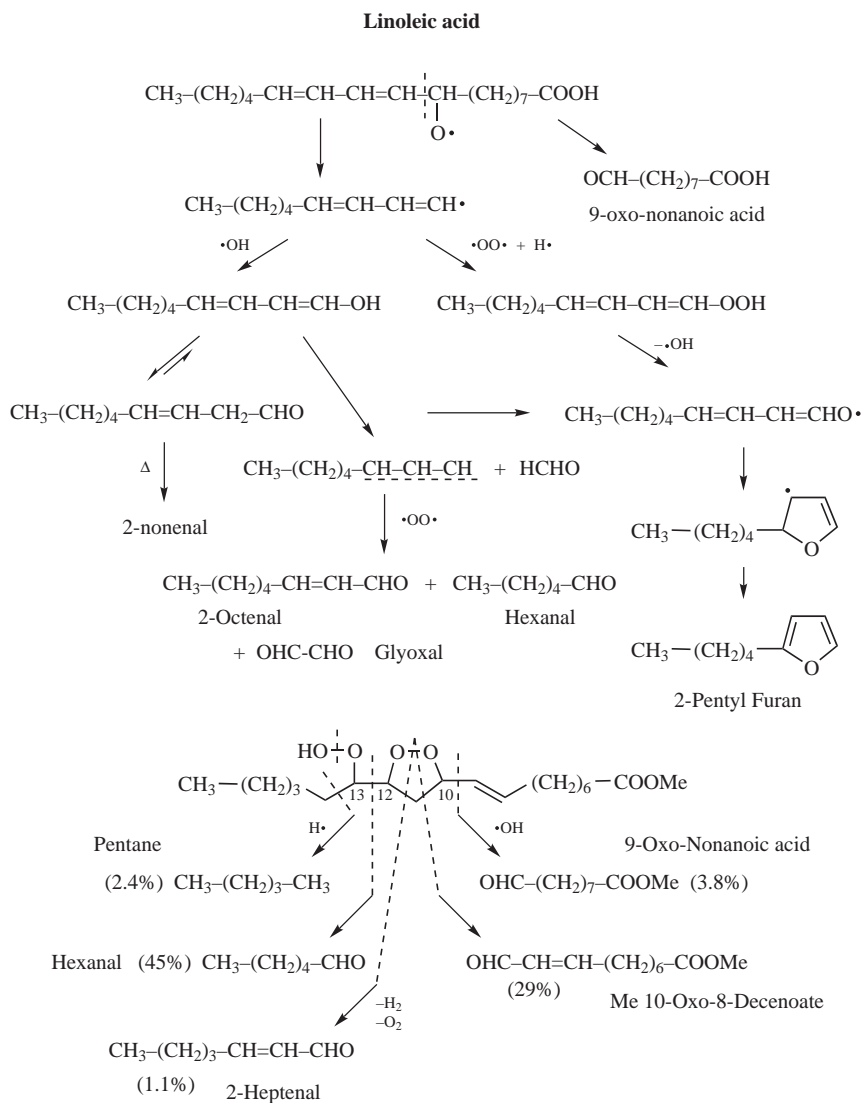
That lipid alkoxy radicals recombine (Reaction 71) at diffusion controlled rates ( $k = 10^9 \text{ M}^{-1}\text{s}^{-1}$ ) (198, 305) probably accounts for the presence of low levels of peroxides even under mild conditions and low levels of oxidation. In one study, oxidation of linoleic acid at 30°C gave  $-C-O-O-C-$  dimers. Reactions 71–73 were found in linolenic acid oxidized under mild conditions to PV 585; this increased to > 50% at PV 4000 and to > 75% after heating to 40°C (276). Alkoxy radicals from hydroperoxyepidioxides heated at 40°C generated > 90% dimers (276).

The reactivity described in the reactions above was determined in neat oils. When oils are in polar solvents or dilute solution in nonpolar solvents,  $\beta$ -scission dominates and radical recombinations are probably unimportant.

## 4.2. Scission Reactions

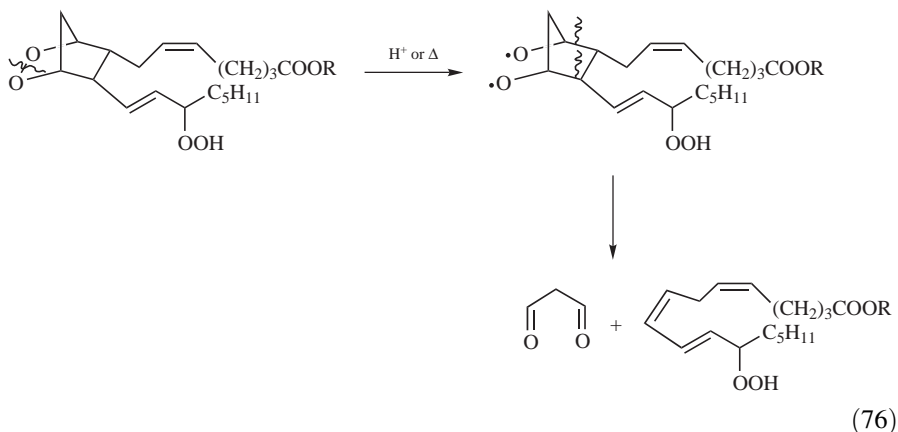
$\beta$ -scissions of alkoxy radicals are the major source of aldehyde products in lipid oxidation. As discussed in Section 3.2.4, a major aldehyde product and a propagating radical are formed via scission of the initial alkoxy radical in a fatty acid. However, products continue to form as unsaturated radical fragments oxidize and undergo secondary scissions to produce carbonyls and alkanes of shorter chain length (224), secondary products that contribute dramatically to the characteristic odors and flavors associated with lipid oxidation. In fact, most evidence suggests that initial oxidation primes fatty acids for additional attack—i.e., oxidation continues on the same molecules rather than randomly attacking new “virgin” acyl chains. The increased susceptibility to oxidation derives both from conjugation and secondary products that oxidize more easily than the parent fatty acids. It is, therefore, not surprising that identifying products of scission reactions of hydroperoxides and various radicals has been the subject of so much research (3, 8, 232, 273, 290, 314, 341, 345, 347–349, 374, 375).

Review of all the scission reactions responsible for the hundreds of volatile products in lipid oxidation is beyond the scope of this chapter. The reader is referred to the available reviews (3, 314, 340, 341, 347) for further details. The scission pattern of hydroperoxide epidioxides from linoleic acid is included here to show how the decompositions can become quite complex (Figure 14), and lists of typical products resulting from scission reactions of oleic, linoleic, and linolenic acids are presented in Table 12.



**Figure 14.** Secondary scissions of intermediate products make important contributions to the total mix of compounds generated during lipid oxidation, shown here for linoleic acid and esters. Top: Oxidation and subsequent scission of radicals released in scissions of initial alkoxy radicals augment some of the original scission aldehydes, although by different routes, and produce some different compounds as well, including the pentyl furan responsible for reversion flavor in oils. Similarly, decomposition of epidioxides formed during photosensitized oxidation of linoleate increase yields of major aldehydes and also produce longer chain aldehydes. Adapted from (273, 314).

This discussion would be incomplete without some mention of the most notorious scission product of lipid oxidation, namely malondialdehyde (MDA). MDA is a downstream scission product from five-membered cyclic hydroperoxides, which can only be formed in linolenic and higher fatty acids (376, 377). Reaction 76 shows only one positional isomer of malonaldehyde, although at least four peroxides give comparable structures (376). Thus, formation of MDA first requires appropriate conditions to generate cyclic peroxide precursors (251), i.e., internal hydroperoxides, aprotic solvents, low lipid concentrations, and limited oxygen pressures. Then conditions for cleavage of the endoperoxide must be supplied, usually mild heat and acid (374). Yields of authentic MDA determined by GC-MS in autoxidized fatty acids are usually less than 0.1% (374, 378), although up to 5% MDA was found in photosensitized fatty acids (374) in which internal hydroperoxides are formed in high concentrations.



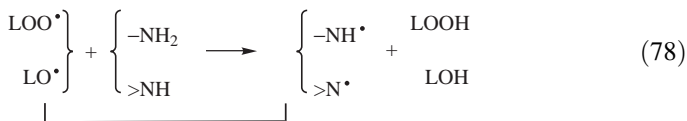
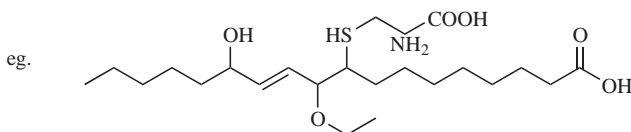
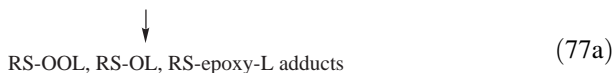
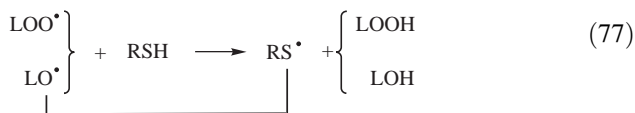
### 4.3. Co-Oxidations with Non-Lipid Molecules

Lipid alkoxy and peroxy radicals abstract H's from any available sources, including nonlipid molecules such as amino acids (346, 379–382), proteins (99, 383, 384), nucleic acids (385, 386), antioxidants (318), carotenoids and other pigments, and even carbohydrates (387). As was noted in the discussion above (Sections 3.1.1 and 3.2.1), this quenches the lipid radical and stops propagation of the immediate radical chain. However, there is increasing evidence that the radicals transferred to proteins and carbohydrates, in particular, may follow processes similar to lipids, i.e., add oxygen to form peroxy radicals that abstract H's and initiate new radical chains. In this way, lipids serve to “broadcast” oxidation damage to other molecules in foods and biological systems (186, 390).

What is important in the context of termination reactions is that radicals formed in nonlipid molecules combine with lipid radicals to generate co-oxidation products (Reactions 77 and 78) that provide footprints of LOOH reactions (389) and should not be ignored in consideration of lipid oxidation kinetics, mechanisms,

and overall effects in foods and biological systems. Co-oxidation products limit extractability of lipids for analysis and, in addition, often remove lipids from product streams normally analyzed. Consequently, these products have probably been severely underestimated in studies of lipid oxidation in complex systems.

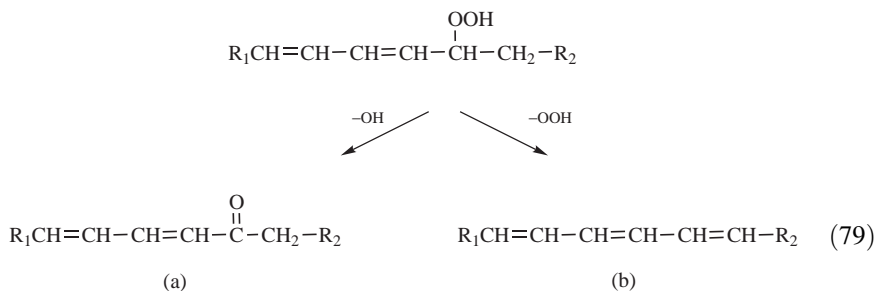
Cysteine (379, 380)



Lipid radical transfer has been demonstrated for trp, arg, his, and lys (99, 383, 384), all of which have reactive N groups on their side chains, and radical decomposition products from these amino acids have been identified (381, 382, 390). Tyrosine and methionine degradation by oxidizing lipids has also been demonstrated (390), but the intermediate radicals in the reaction may be too unstable for detection. Lipid radical adducts to amino acids are important flavor precursors (340) and also may play critical roles in pathological processes in vivo (186, 388).

#### 4.4. Elimination Reactions

$\text{HO}^-$  and  $\text{HOO}^-$  can be eliminated from LOOH, respectively yielding an internal carbonyl (ketone) (Reaction 79a) and a desaturated product with an additional double bond (Reaction 79b) (391, 392). These are not major reactions, but nevertheless account for some of the lipid oxidation products identified under various conditions.



## 5. EXPANDED INTEGRATED REACTION SCHEME

The classic free radical chain reaction mechanism used for more than five decades to understand and track oxidation reactions was developed from product analyses that were somewhat crude compared with the sophisticated chromatography and spectroscopy available today. The reaction scheme is not wrong, but it may be incomplete, at least for complex molecules such as polyunsaturated fatty acids.

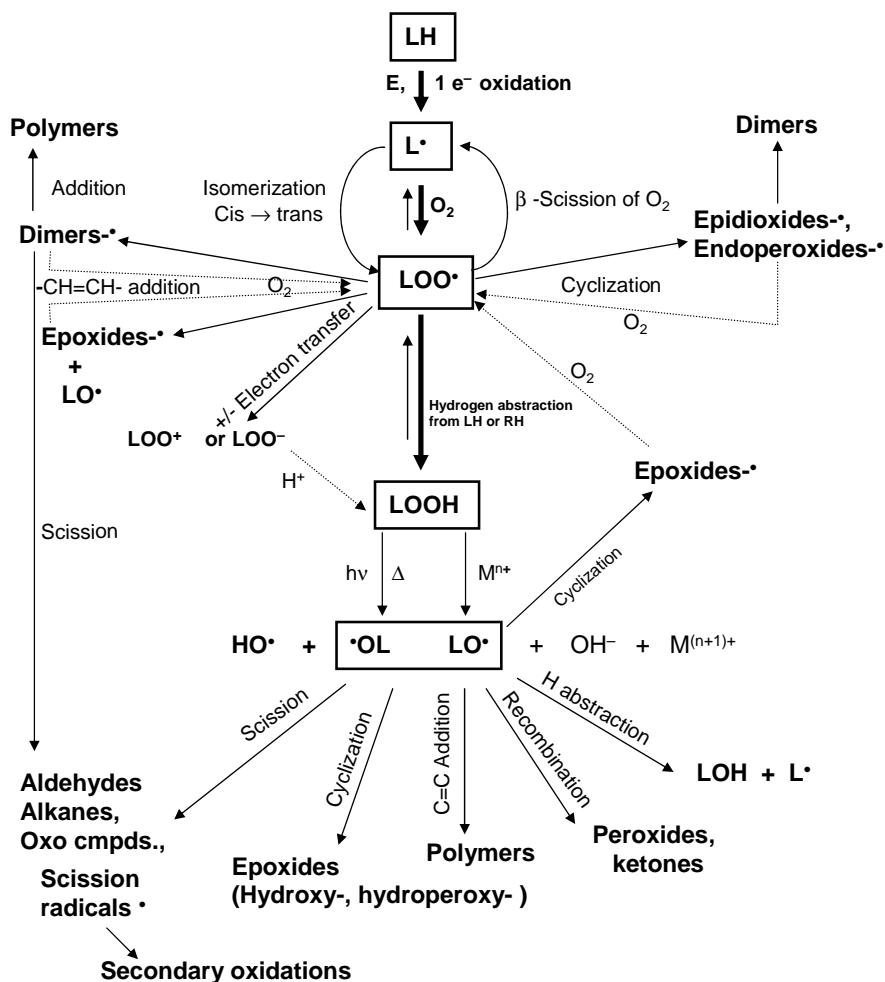
Current information raises questions about the literal application of the classic free radical chain sequence to lipid oxidation. Observed products do not match those predicted: Many studies have now shown that hydroperoxides are not exclusive products in early stages and lipid alcohols are not even major products after hydroperoxide decomposition. Product distributions are consistent with multiple pathways that compete with each other and change dominance with reaction conditions and system composition. Rate constants show no strong preference for H abstraction, cyclization, addition, or scission, which partially explains the mixture of products usually observed with oxidizing lipids. It could be argued that the reactions in Figure 1 accurately describe early processes of lipid oxidation, but  $\text{LOO}^\bullet$  rate constants considerably higher for cyclization than for abstraction contradict this.

The picture emerging from integration of all these observations is that lipid oxidation has multiple pathways available and that the balance of pathways taken in a given system depends on solvent, fatty acid composition and concentration, initiation mechanisms and catalysts present, temperature, oxygen pressure, and especially on availability of abstractable hydrogens from lipids and other sources. These multiple pathways must be considered in determining appropriate analyses for lipid oxidation, designing more effective strategies for stabilization of foods where lipid oxidation is a major mode of deterioration, and understanding how lipid oxidation may mediate pathological processes *in vivo*.

Therefore, a new integrated paradigm for lipid oxidation is proposed in which the major alternative pathways are added to the classic free radical chain (Figure 15). The traditional reaction sequence involving hydrogen abstractions is presented vertically down the center of the scheme because most radicals formed in alternative reactions ultimately abstract hydrogens to propagate the chain. This is the core of the oxidation process. Pathways that compete with H abstraction are



## AN INTEGRATED SCHEME FOR LIPID OXIDATION



**Figure 15.** Integrated scheme for lipid oxidation accounting for multiple reactions pathways competing with the classic hydrogen abstraction. Dotted lines indicate paths for oxygen addition to secondary radicals formed in cyclic and addition products, with formation of new peroxy radicals.

shown for both peroxy and alkoxy radicals, and the H abstractions that are associated with these alternative reactions and propagate the oxidation chain are either designated specifically (dotted lines) or implied in the production of reactive radicals. Cyclization and addition yield intermediate products with radicals at new sites. These radicals can add oxygen and form peroxides that either enter the traditional H abstraction flow, designated by the dotted lines, or undergo further addition

or scission reactions outside the traditional scheme. Some of the products resulting from these alternative pathways are the expected aldehydes, etc., but some are not. Thus, alternative reaction paths increase the complexity of both the kinetics and the product mix of lipid oxidation. In addition, an attempt has been made to distinguish termination of individual radicals from termination of the oxidation chain by including side radicals produced in each reaction. Products are generated by oxidation and have impacts on the system, whether food or biological, but the process nevertheless continues. Any radical deriving during lipid oxidation has the potential to start a separate chain of its own, equivalent to the entire reaction scheme. This approach more accurately portrays the perpetuity of lipid oxidation reactions in the absence of antioxidants or interceptors.

This integrated scheme is a first step to broader recognition of the complexities of lipid oxidation and should be considered a work in progress. The lack of rate constants for lipid reactions, in itself, shows there is still much that we do not know, and factors shifting the balance between pathways are only beginning to be understood. The past twenty years have brought great progress in our understanding of the details of lipid oxidation reactions, and increasing sophistication and sensitivity of analytical techniques promise to advance our knowledge even faster in the next few years. Demands for increased stability in foods and control of lipid oxidation in vivo will force us to look beyond the traditional hydroperoxides and consider the multiple pathways and products that may contribute critically to system deterioration and toxic side reactions.

Hopefully, this chapter will stimulate and encourage broader consideration of the multiple pathways of lipid oxidation, as well as more collaborative research between food chemists, biochemists, and organic chemists to obtain the reaction details that will ultimately be needed to control lipid oxidation in any system.

## REFERENCES

1. M. G. Simic and M. Karel, ed., *Autoxidation in Food and Biological Systems*, Plenum Press, New York, 1980.
2. M. G. Simic, *J. Chem. Ed.*, **58**(2), 125–131 (1981).
3. E. N. Frankel, *Prog. Lipid Res.*, **23**, 197–221 (1985).
4. H. W.-S. Chan, ed., *Autoxidation of Unsaturated Lipids*, Academic Press, London, 1987.
5. N. A. Porter and D. G. Wujek, in A. Quintanilla, ed., *Reactive Oxygen Species in Chemistry, Biology, and Medicine*, Plenum Publishing Corp., New York, 1987, pp. 55–79.
6. H. W. Gardner, *Free Radic. Biol. Med.*, **7**, 65–86 (1989).
7. N. A. Porter, in C. Vigo-Pelfrey, ed., *Membrane Lipid Oxidation, vol. 1*, CRC Press, Boca Raton, Florida, 1990, pp. 33–62.
8. E. N. Frankel, *J. Sci. Food Agric.*, **54**, 495–511 (1991).
9. M. G. Simic, S. V. Jovanovic, and E. Niki, in A. J. St. Angelo, ed., *Lipid Oxidation in Food*, American Chemical Society, Washington, D.C., 1992, pp. 14–32.

10. A. J. St. Angelo ed., *Lipid Oxidation in Food*, American Chemical Society, Washington, D.C., 1992.
11. N. A. Porter, S. E. Caldwell, and K. A. Mills, *Lipids*, **30**(4), 277–290 (1995).
12. N. Pilpel and B. F. J. Hunter, *J. Colloid Interfac. Sci.*, **33**(4), 615–622 (1970).
13. N. M. Emanuel, ed., *The Oxidation Of Hydrocarbons In The Liquid Phase*, Pergamon Press, New York, 1965.
14. E. H. Farmer, *Trans. Faraday Soc.*, **42**, 228–236 (1946).
15. J. L. Bolland, *Quart. Rev.*, **3**, 1–21 (1949).
16. J. L. Bolland, *J. Chem. Soc.*, 445–447 (1945).
17. D. Swern, in W. O. Lundberg, ed., *Autoxidation and Antioxidants*, vol. 1, Interscience, New York, 1961, pp. 1–54.
18. E. H. Farmer, H. P. Koch, and D. A. Sutton, *J. Chem. Soc.*, 541–547 (1943).
19. S. M. Hyde and D. Verdin, *Trans. Faraday Soc.*, **64**(1), 144–154 (1968).
20. J. P. Cosgrove, D. F. Church, and W. A. Pryor, *Lipids*, **22**, 299–304 (1987).
21. J. K. Kochi, in J. K. Kochi, ed., *Free Radicals*, vol. 2, John Wiley, New York, 1973, pp. 665–710.
22. Anonymous. *Chem. & Eng. News*, 18–20 (1981).
23. N. Uri, in W. O. Lundberg, ed., *Autoxidation and Antioxidants*, Interscience, New York, 1961, pp. 55–106.
24. G. Haase, and W. L. Dunkley, *J. Lipid Res.*, **10**, 561–567 (1969).
25. K. M. Schaich, *Lipids*, **23**(6), 570–578 (1988).
26. K. M. Schaich, *Lipids*, **27**(3), 209–218 (1992).
27. F. W. Heaton and N. Uri, *J. Lipid Res.*, **2**(2), 152–160 (1961).
28. W. A. Waters, *Discussions Faraday Soc.*, **46**, 158–163 (1968).
29. P. Smith, and W. A. Waters, *J. Chem. Soc. B., Phys. Org. Chem.*, 462–467 (1969).
30. M. Hirano, K. Nakamura, and T. Morimoto, *J. Chem. Soc., Perkin II*, 817–820 (1981).
31. W. G. Dauben, M. Lober, and D. S. Fullerton, *J. Org. Chem.*, **34**, 3587–3592 (1969).
32. N. Ikeda and K. Fukuzumi, *J. Amer. Oil Chem. Soc.*, **54**(3), 105–109 (1977).
33. A. J. Chalk and J. F. Smith, *Trans. Faraday Soc.*, **53**, 1214–1234 (1957).
34. A. J. Chalk and J. F. Smith, *Trans. Faraday Soc.*, **53**, 1235–1245 (1957).
35. C. E. H. Bawn, *Discuss. Faraday Soc.*, **14**, 181–190 (1953).
36. A. A. Clifford and W. A. Waters, *J. Chem. Soc.*, 2796–2804 (1965).
37. A. A. Clifford and W. A. Waters, *J. Chem. Soc. (B)*, 462–473 (1969).
38. T. A. Cooper and W. A. Waters, *J. Chem. Soc.*, 1538–1543 (1964).
39. C. E. H. Bawn, T. P. Hobin, and L. Raphael, *Proc. Roy. Soc. A*, **237**, 313–324 (1956).
40. W. A. Waters, *J. Amer. Oil Chem. Soc.*, **48**(9), 427–433 (1971).
41. C. Walling, *Acc. Chem. Res.*, **8**, 125–131 (1975).
42. J. F. Black, *J. Amer. Chem. Soc.*, **100**(2), 527–536 (1978).
43. C. E. H. Bawn and J. E. Jolley, *Proc. Royal Soc. (London)*, **A237**, 297–312 (1956).
44. E. J. Y. Scott and A. W. Chester, *J. Phys. Chem.*, **76**(11), 1520–1524 (1972).
45. K. L. Rollick and J. K. Kochi, *J. Amer. Chem. Soc.*, **104**, 1319–1330 (1982).
46. C. Copping and N. Uri, *Discuss. Faraday Soc.*, **46**, 202–212 (1968).

47. T. J. Wallace and R. M. Skomorski, *Chem. Ind.*, 348–349 (1965).
48. K. Fukuzawa, K. Kishakawa, T. Tadokoro, A. Tokumura, H. Tsukatani, and J. M. Gebicki, *Arch. Biochem. Biophys.*, **260**(1), 153–160 (1988).
49. K. M. Schaich and Z. Rajfur, *J. Amer. Oil Chem. Soc.*, in preparation.
50. W. G. Barb, J. H. Baxendale, P. George, and P. R. Hargrave, *Trans. Faraday Soc.*, **47**, 591–616 (1951).
51. W. G. Barb, J. H. Baxendale, P. George, and P. R. Hargrave, *Trans. Faraday Soc.*, **47**, 462–500 (1951).
52. O. K. Borggard, O. Farver, and V. S. Andersen, *Acta Chem. Scand.*, **25**, 3541–3543 (1971).
53. A. Banks, E. Eddie, and J. G. M. Smith, *Nature (London)*, **190**, 908–909 (1961).
54. E. Ochiai, *Tetrahedron*, **20**, 1819–1829 (1964).
55. A. Tkac, K. Vesely, and L. Omelka, *Coll. Czech. Chem. Commun.*, **39**, 3504–3514 (1974).
56. A. Tkac, K. Vesely, L. Omelka, and R. Prikryl, *Coll. Czech. Chem. Commun.*, **40**, 117–128 (1975).
57. R. Prikryl, A. Tkac, L. Omelka, and K. Vesely, *Coll. Czech. Chem. Commun.*, **40**, 104–116 (1975).
58. Y. Kamiya, S. Beaton, A. Lafortune, and K. U. Ingold, *Can. J. Chem.*, **41**, 2020–2032 (1963).
59. J. K. Kochi, *Tetrahedron*, **18**, 483–497 (1962).
60. R. Hiatt, T. Mill, and F. R. Mayo, *J. Org. Chem.*, **33**, 1416–1420 (1968).
61. W. C. Bray and M. H. Gorin, *J. Amer. Chem. Soc.*, **54**(5), 2124–2125 (1932).
62. C. Walling, *Acc. Chem. Res.*, **31**, 155–157 (1998).
63. R. A. Leising, B. A. Brennan, L. Que Jr., B. G. Fox, and E. Münck, *J. Amer. Chem. Soc.*, **113**, 3988–3990 (1991).
64. A. L. Feig and S. L. Lippard, *Chem. Rev.*, **94**, 759–805 (1994).
65. J-U. Rohde, *Science*, **299**, 1037–1039 (2003).
66. B. H. J. Bielski and M. J. Thomas, *J. Amer. Chem. Soc.*, **109**, 7761 (1987).
67. H. Taube, *J. Gen. Physiol.*, **49**(1) (Pt 2), 29–50 (1965).
68. J. D. Rush and B. H. J. Bielski, *J. Amer. Chem. Soc.*, **108**, 523–525 (1986).
69. H. Sugimoto and D. T. Sawyer, *J. Amer. Chem. Soc.*, **106**, 4283–4285 (1984).
70. S. Rahhal and H. W. Richter, *J. Amer. Chem. Soc.*, **110**, 3126–3133 (1988).
71. J. D. Rush and W. H. Koppenol, *J. Amer. Chem. Soc.*, **110**, 4957–4963 (1988).
72. V. K. Sharma and B. H. J. Bielski, *Inorg. Chem.*, **30**, 4306–4310 (1991).
73. H-C. Tung, C. Kang, and D. T. Sawyer, *J. Amer. Chem. Soc.*, **114**, 3445–3455 (1992).
74. B. Ensing and E. J. Baerends, *J. Phys. Chem. A*, **106**, 7902–7910 (2002).
75. B. Ensing, F. Buda, and E. J. Baerends, *J. Phys. Chem.*, **107**, 5722–5731 (2003).
76. F. Buda, B. Ensing, M. C. M. Gribnau, and E. J. Baerends, *Chem. J. Eur.*, **9**, 3436–3444 (2003).
77. J. Kaizer, M. Costas, and L. Que, Jr., *Agnew. Chem. Int. Ed.*, **42**, 3671–3673 (2003).
78. J. Kaizer, E. J. Klinker, N. Y. Oh, J-U. Rohde, W. J. Song, A. Stubna, J. Kim, E. Münck, W. Nam, and L. Que, Jr., *J. Amer. Chem. Soc.*, **126**, 472–473 (2004).

79. S. H. Bossman, E. Oliveros, S. Göb, S. Siegwart, E. P. Dahlen, L. J. Payawan, M. Straub, M. Wörner, and A. M. Braun, *J. Phys. Chem.*, **102**, 5542–5550 (1998).
80. C. M. Jones and M. J. Burkitt, *J. Amer. Chem. Soc.*, **125**, 6946–6954 (2003).
81. A. Bassan, M. R. A. Blomberg, P. E. M. Siegbahn, and L. Que, Jr., *J. Amer. Chem. Soc.*, **124**, 11056–11063 (2002).
82. D. C. Borg, K. M. Schaich, and A. Forman, in W. Bors, M. Saran, and D. Tait, ed., *Oxygen Radicals in Chemistry and Biology*, Walter de Gruyter, Berlin, Germany, 1984, pp. 123–129.
83. A. Wada, S. Ogo, S. Nagatomo, T. Kitagawa, and Y. Watanabe, *Inorg. Chem.*, **41**(4), 616–618 (2002).
84. E. Hecht, *Physics: Calculus*, 2nd ed., Brooks/Cole, Pacific Grove, California, 2000.
85. P. W. Atkins and J. A. Beran, *General Chemistry*, 2nd ed., Scientific American Books (W.H. Freeman & Co.), New York, 1990.
86. L. W. Fine, *Chemistry*, Appleton-Century-Crofts, New York, 1972.
87. R. V. Bensasson, E. J. Land, and T. G. Truscott, *Excited States and Free Radicals in Biology and Medicine*, Oxford Science Publications, Oxford, United Kingdom, 1993.
88. J. A. Howard, in J. K. Kochi, ed., *Free Radicals*, vol. 2, Wiley-Interscience, New York, 1973, pp. 3–62.
89. N. Baker and L. Wilson, *J. Lipid Res.*, **7**, 341–348 (1966).
90. M. Geoffroy, P. Lambelet, and P. Richert, *J. Agric. Food Chem.*, **48**, 974–978 (2000).
91. T. Chiba, K. Fujimoto, and T. Kaneda, *J. Amer. Oil Chem. Soc.*, **58**(5), 587–590 (1981).
92. T. Chiba, K. Fujimoto, and T. Kaneda, *Agric. Biol. Chem.*, **48**(11), 2701–2705 (1984).
93. H. R. Rawls and P. J. Van Santen, *J. Amer. Oil Chem. Soc.*, **47**(4), 121–125 (1970).
94. K. M. Schaich, *CRC Crit. Rev. Food Sci. Nutr.*, **13**, 131–159 (1980).
95. Y. S. R. Sastry and G. Lakshminarayana, *J. Amer. Oil Chem. Soc.*, **48**(9), 452–454 (1971).
96. J. Terao and S. Matsushita, *Agric. Biol. Chem.*, **41**(12), 2467–2468 (1977).
97. C. S. Foote, in W. A. Pryor, ed., *Free Radicals in Biology*, Vol. 2, Academic Press, New York, 1976, pp. 85–134.
98. R. W. Murray, in H. Wasserman and R. W. Murray, eds., *Singlet Oxygen*, Academic, New York, 1979, pp. 59–114.
99. K. M. Schaich, *CRC Crit. Rev. Food Sci. Nutr.*, **13**, 189–244 (1980).
100. T. Schulte-Herbrüggen and E. Cadenas, *Photochem. Photobiol.*, **10**, 35–51 (1985).
101. E. A. Mayeda and A. J. Bard, *J. Amer. Chem. Soc.*, **96**, 4023–4024 (1974).
102. B. A. Svingen, F. O. O’Neal, and S. D. Aust, *Photochem. Photobiol.*, **28**, 803–809 (1978).
103. A. U. Khan, *Science*, **168**, 476–477 (1970).
104. A. U. Khan, *Photochem. Photobiol.*, **28**, 615–627 (1978).
105. A. U. Khan, *J. Photochem.*, **25**, 327–334 (1985).
106. E. W. Kellogg and I. Fridovich, *J. Biol. Chem.*, **250**, 8812–8817 (1975).
107. G. A. Russell, *J. Amer. Chem. Soc.*, **79**, 3871–3877 (1957).
108. B. H. J. Bielski and A. O. Allen, *J. Phys. Chem.*, **81**(11), 1048–1050 (1977).

109. T. Matsuura, N. Yoshimura, A. Hishinaga, and I. Saito, *Tetrahedron*, **28**, 4933–4938 (1972).
110. R. Nilsson and D. R. Kearns, *J. Phys. Chem.*, **78**, 1681–1683 (1974).
111. W. W. Nawar, *J. Agric. Food Chem.*, **17**(1), 18–21 (1969).
112. W. W. Nawar, in O. R. Fennema, ed., *Food Chemistry*, 2nd ed., Academic, New York, 1966, pp. 225–320.
113. R. Marcuse and P-O. Fredriksson, *J. Amer. Oil Chem. Soc.*, **45**(5), 400–407 (1968).
114. T. P. Labuza, *CRC Crit. Rev. Food Sci. Nutr.*, **2**(3), 355–405 (1971).
115. W. O. Lundberg and J. R. Chipault, *J. Amer. Chem. Soc.*, **69**, 833–836 (1947).
116. H. Aoshima, T. Kajiwara, A. Hatanaka, and H. Hatano, *J. Biochem.*, **82**(6), 1559–1565 (1977).
117. M. R. Egmond, J. F. G. Vliegthart, and J. Boldingh, *Biochim. Biophys. Res. Commun.*, **48**(5), 1055–1060 (1972).
118. J. J. M. C. deGroot, G. J. Garssen, J. F. G. Vliegthart, and J. Boldingh, *Biochim. Biophys. Acta*, **377**(1), 71–79 (1975).
119. J. J. M. C. deGroot, G. J. Garssen, J. F. G. Vliegthart, and J. Boldingh, *FEBS Lett.*, **56**(1), 50–54 (1975).
120. H. Aoshima, T. Kajiwara, and A. Hatanaka, *Agric. Biol. Chem.*, **45**(10), 2245–2251 (1981).
121. M. E. Robinson, *Biochem. J.*, **18**, 255–264 (1924).
122. B. M. Watts and I. Chang, *Food Technol.*, **3**, 332–336 (1949).
123. J. Kendrick and B. M. Watts, *Lipids*, **4**, 454–458 (1969).
124. B. M. Watts and B. T. Lehmann, *Food Res.*, **17**, 100–108 (1952).
125. M. T. Younathon and B. M. Watts, *Food Res.*, **24**, 728–734 (1959).
126. A. L. Tappel, *Arch. Biochem. Biophys.*, **44**, 378–394 (1953).
127. A. L. Tappel, *Food Research*, **18**, 560–573 (1953).
128. A. L. Tappel, *J. Biol. Chem.*, **217**, 721–733 (1955).
129. A. L. Tappel, in W. O. Lundberg, ed., *Autoxidation and Antioxidants*, vol. 1, Interscience Publishers, New York, 1961, pp. 326–366.
130. A. L. Tappel, in H. Schultz, E. D. Day, and W. O. Sinnhuber, eds., *Symposium on Foods: Lipids and Their Oxidation*, AVI Publishing, Westport, Connecticut, 1962, pp. 122–138.
131. V. P. Maier and A. L. Tappel, *J. Amer. Oil Chem. Soc.*, **36**, 12–15 (1959).
132. V. P. Maier and A. L. Tappel, *J. Amer. Oil Chem. Soc.*, **36**, 8–12 (1959).
133. J. D. Love and A. M. Pearson, *J. Agric. Food Chem.*, **22**, 1031–1034 (1974).
134. M. M. Verma, V. Paranjape, and D. A. Ledward, *Meat Sci.*, **14**, 91–104 (1985).
135. W. D. Brown, L. S. Harris, and H. S. Olcott, *Arch. Biochem. Biophys.*, **101**(1), 14–20 (1963).
136. C. E. Eriksson, P. A. Olsson, and S. G. Svensson, *J. Amer. Oil Chem. Soc.*, **48**(9), 442–447 (1971).
137. R. M. Kaschnitz and Y. Hatefi, *Arch. Biochem. Biophys.*, **171**, 292–304 (1975).
138. J. D. Love, *Food Technol.*, **37**(7), 117–120 (1983).
139. T. A. Dix, R. Fontana, A. Panthani, and L. J. Marnett, *J. Biol. Chem.*, **260**(9), 5358–5364 (1985).

140. K. S. Rhee, Y. A. Ziprin, and G. Ordonez, *J. Agric. Food Chem.*, **35**, 1013–1017 (1987).
141. R. Labeque and L. J. Marnett, *J. Amer. Chem. Soc.*, **111**, 6621–6627 (1989).
142. P. Gatellier, M. Anton, and M. Renerre, *J. Agric. Food Chem.*, **43**, 651–656 (1995).
143. W. Nam, H. J. Han, S.-Y. Oh, Y. J. Lee, M.-H. Choi, S.-Y. Han, C. Kim, S. K. Woo, and W. Shin, *J. Amer. Chem. Soc.*, **122**(36), 8677–8684 (2000).
144. S. I. Rao, A. Wilks, M. Hamberg, and P. R. Ortiz de Montellano, *J. Biol. Chem.*, **269**, 7210–7216 (1994).
145. M. Hamberg, *Lipids*, **10**(2), 87–92 (1975).
146. E. Ochiai, *J. Inorg. Nucl. Chem.*, **37**, 1503–1509 (1975).
147. T. A. Dix and L. J. Marnett, *J. Amer. Chem. Soc.*, **103**, 6744–6746 (1981).
148. T. A. Dix and L. J. Marnett, *J. Amer. Chem. Soc.*, **105**, 7001–7002 (1983).
149. V. Ullrich, in W. Bors, M. Saran, D. Tait, eds., *Oxygen Radicals in Chemistry and Biology*, Walter de Gruyter, Berlin, 1984, pp. 391–404.
150. T. G. Traylor and F. Xu, *J. Amer. Chem. Soc.*, **109**, 6201–6202 (1987).
151. R. Labeque and L. J. Marnett, *J. Amer. Chem. Soc.*, **109**, 2828–2829 (1987).
152. T. C. Bruice, P. N. Balasubramanian, R. W. Lee, and J. R. Lindsay Smith, *J. Amer. Chem. Soc.*, **110**(23), 7890–7892 (1988).
153. R. Labeque and L. J. Marnett, *Biochemistry*, **27**, 7060–7070 (1988).
154. T. G. Traylor and F. Xu, *J. Amer. Chem. Soc.*, **112**, 178–186 (1990).
155. I. Yamazaki and L. Piette, *J. Amer. Chem. Soc.*, **113**, 7588–7593 (1991).
156. A. L. Wilcox and L. J. Marnett, *Chem. Res. Toxicol.*, **6**, 413–416 (1993).
157. Y. S. Choe, S. I. Rao, and P. R. Ortiz de Montellano, *Arch. Biochem. Biophys.*, **314**(1), 126–131 (1994).
158. A. C. Onuoha, X. Zu, and J. F. Rusling, *J. Amer. Chem. Soc.*, **119**, 3979–3986 (1997).
159. C. P. Baron and H. J. Andersen, *J. Agric. Food Chem.*, **50**, 3887–3897 (2002).
160. W. Nam, M. H. Lim, H. J. Lee, and C. Kim, *J. Amer. Chem. Soc.*, **122**, 6641–6647 (2000).
161. M. J. Davies, *Biochim. Biophys. Acta*, **1077**(1), 86–90 (1991).
162. M. J. Davies, *Free Radical Res. Commun.*, **10**(6), 361–370 (1990).
163. D. Tew and P. R. Ortiz de Montellano, *J. Biol. Chem.*, **263**, 17880–17886 (1988).
164. M. R. Gunther, D. J. Kelman, J. T. Corbett, and R. P. Mason, *J. Biol. Chem.*, **270**(27), 16075–16081 (1995).
165. H. Kühn, R. Götze, T. Schewe, and S. M. Rappaport, *Eur. J. Biochem.*, **120**, 161–168 (1981).
166. D. Galaris, A. Sevanian, E. Cadenas, and R. Hochstein, *Arch. Biochem. Biophys.*, **281**, 163–169 (1990).
167. W. Nam, S.-E. Park, I. K. Lim, M. H. Lim, J. Hong, and J. Kim, *J. Amer. Chem. Soc.*, **125**, 14674–14675 (2003).
168. W. H. Koppenol and J. F. Liebman, *J. Phys. Chem.*, **88**, 99–101 (1984).
169. N. Hogg, C. A. Rice-Evans, U. V. Darley, and M. T. Wilson, *Arch. Biochem. Biophys.*, **314**(1), 39–44 (1994).
170. P. J. O'Brien and C. Little, *Can. J. Biochem.*, **47**, 493–499 (1969).
171. H. B. Dunford, *Free Radic. Biol. Med.*, **3**, 405–421 (1987).
172. M. P. Richards and M. A. Dettmann, *J. Agric. Food Chem.*, **51**, 3886–3891 (2003).

173. S. J. Yang and W. Nam, *Inorg. Chem.*, **37**, 606–607 (1998).
174. W. Nam, H. J. Choi, H. J. Han, S. H. Cho, H. J. Lee, and S-Y. Han, *Chem. Commun.*, 387–388 (1999).
175. S. E. Lewis and E. D. Wills, *Biochim. Biophys. Acta*, **70**, 336–338 (1963).
176. K. Nakamura and T. Nishida, *J. Lipid Res.*, **12**, 149–154 (1971).
177. C. P. Baron, L. H. Skibsted, and H. J. Andersen, *J. Agric. Food Chem.*, **50**, 883–888 (2002).
178. C. E. Catalano, Y. S. Choe, and P. R. Ortiz de Montellano, *J. Biol. Chem.*, **264**(18), 10534–10541 (1989).
179. K. Whang and I. C. Peng, *J. Food Sci.*, **53**(6), 1863–1865 (1988).
180. M. Williams, B. B. H. Krootjes, J. van Steveninck, and J. van der Zee, *Biochim. Biophys. Acta*, **1211**, 310–316 (1994).
181. F. D. Gunstone and F. A. Norris, *Lipids in Foods: Chemistry, Biochemistry, and Technology*, Pergamon Press, Oxford, United Kingdom, 1983.
182. B. D. Goldstein, O. J. Balchum, H. B. Demopolous, and P. S. Duke, *Arch. Environ. Health*, **17**, 46–49 (1968).
183. O. S. Privett and M. L. Blank, *J. Amer. Oil Chem. Soc.*, **39**(11), 465–469 (1962).
184. O. S. Privett and E. C. Nickell, *J. Lipid Res.*, **58**, 208–211 (1963).
185. O. S. Privett and E. C. Nickell, *J. Amer. Oil Chem. Soc.*, **43**(6), 393–400 (1966).
186. W. A. Pryor, *Photochem. Photobiol.*, **28**, 787–801 (1978).
187. O. J. Balchum and J. S. O'Brien, *Arch. Environ. Health*, **22**, 32–34 (1971).
188. E. C. Nickell, M. Albi, and O. S. Privett, *Chem. Phys. Lipids*, **17**, 378–388 (1976).
189. W. A. Pryor, J. P. Stanley, E. Blair, and G. B. Cullen, *Arch. Environ. Health*, **31**(4), 201–210 (1976).
190. M. E. Kurz and W. A. Pryor, *J. Amer. Chem. Soc.*, **100**(25), 7953–7959 (1978).
191. W. A. Pryor, *Ann. Rev. Physiol.*, **48**, 657–667 (1986).
192. P. S. Rao, S. M. Ayres, and H. S. Mueller, *Biochem. Biophys. Res. Commun.*, **104**(4), 1532–1536 (1982).
193. B. H. J. Bielski, D. E. Cabelli, R. L. Arudi, and A. B. Ross, *J. Phys. Chem. Ref. Data*, **14**(4), 1041–1100 (1985).
194. M. Erben-Russ, C. Michael, W. Bors, and M. Saran, *Radiat. Environ. Biophys.*, **26**, 289–294 (1987).
195. L. K. Patterson and K. Hasegawa, *Ber. Bunsenges. Phys. Chem.*, **82**, 951–956 (1978).
196. K. Hasegawa and L. K. Patterson, *Photochem. Photobiol.*, **28**, 817–823 (1978).
197. R. D. Small, Jr., J. C. Scaiano, and L. K. Patterson, *Photochem. Photobiol.*, **29**, 49–51 (1979).
198. M. Erben-Russ, C. Michael, W. Bors, and M. Saran, *J. Phys. Chem.*, **91**, 2362–2365 (1987).
199. M. V. Piretti, C. Cavani, and F. Zeli, *Revue française des Corps Gras*, **25**(2), 73–78 (1978).
200. J. M. Gebicki and B. H. J. Bielski, *J. Amer. Chem. Soc.*, **103**, 7020–7022 (1981).
201. R. J. Hamilton, C. Kalu, E. Prisk, F. B. Padley, and H. Pierce, *Food Chem.*, **60**(2), 193–199 (1997).



202. M. J. Thomas, K. S. Mehl, and W. A. Pryor, *J. Biol. Chem.*, **257**(14), 8343–8347 (1982).
203. D. H. Giamalva, D. F. Church, and W. A. Pryor, *J. Amer. Chem. Soc.*, **108**, 6646–6651 (1986).
204. C. Schöneich and K-D. Asmus, *Radiat. Environ. Biophys.*, **29**, 263–271 (1990).
205. F. H. Doleiden, S. R. Fahrenholtz, A. A. Lamola, and A. M. Trozzolo, *Photochem. Photobiol.*, **20**(6), 519–521 (1974).
206. W. A. Pryor, J. W. Lightsey, and D. F. Church, *J. Amer. Chem. Soc.*, **104**, 6685–6692 (1982).
207. M. G. J. Heijman, H. Nauta, and Y. K. Levine, *Radiat. Phys. Chem.*, **26**(1), 73–82 (1985).
208. M. Al-Sheikhly and M. G. Simic, *J. Phy. Chem.*, **93**, 3103–3106 (1989).
209. Y. S. Ilan, G. Czapski, and D. Meisel, *Biochim. Biophys. Acta*, **430**, 209–224 (1976).
210. J. M. C. Gutteridge, *FEBS Lett.*, **172**(2), 245–249 (1984).
211. B. H. J. Bielski, R. L. Arudi, and M. W. Sutherland, *J. Biol. Chem.*, **258**, 4759–4761 (1983).
212. R. Overend and G. Paraskevopoulos, *J. Phys. Chem.*, **82**(12), 1329–1333 (1978).
213. Y. A. Ilan, J. Rabani, and A. Henglein, *J. Phys. Chem.*, **80**, 1558–1562 (1976).
214. H. W. Gardner, R. Kleiman, and D. Weisleder, *Lipids*, **9**(9), 696–706 (1974).
215. P. Schieberle, Y. Trebert, J. Firl, and W. Grosch, *Chem. Phys. Lipids*, **41**, 101–116 (1986).
216. P. Schieberle, Y. Trebert, J. Firl, and W. Grosch, *Chem. Phys. Lipids*, **48**, 281–288 (1988).
217. Y. Yamamoto, S. Haga, E. Niki, and Y. Kamiya, *Bull. Chem. Soc. Jpn.*, **57**(5), 1260–1265 (1984).
218. K. M. Schaich and D. C. Borg, *Lipids*, **23**(6), 570–579 (1988).
219. K. M. Schaich, Unpublished data.
220. K. M. Schaich and D. C. Borg, in M. G. Simic and M. Karel, eds., *Autoxidation in Food and Biological Systems*, Plenum Publishing Corp, New York, 1980, pp. 45–70.
221. J. A. Kerr, *Chem. Rev.*, **66**, 465–500 (1966).
222. G. Scott, *Atmospheric Oxidation and Antioxidants*, Elsevier, London, 1965.
223. K. U. Ingold, *Acc. Chem. Res.*, **2**, 1–9 (1969).
224. E. Schauenstein, *J. Lipid Res.*, **8**, 417–428 (1967).
225. E. N. Frankel, in H. Schultz, E. D. Day, and W. O. Sinnhuber, eds., *Symposium on Foods: Lipids and Their Oxidation*, AVI Publishing, Westport, Connecticut, 1962, pp. 51–76.
226. K. A. Tallman, D. A. Pratt, and N. A. Porter, *J. Amer. Chem. Soc.*, **123**, 11827–11828 (2001).
227. D. T. Coxon, K. R. Price, and H. W-S. Chan, *Chem. Phys. Lipids*, **28**, 365–378 (1981).
228. W. E. Neff, E. N. Frankel, and D. Weisleder, *Lipids*, **16**, 439–448 (1981).
229. W. A. Pryor and J. P. Stanley, *J. Org. Chem.*, **40**(24), 3615–3617 (1975).
230. N. A. Porter, M. O. Funk, D. Gilmore, R. Isaac, and J. Nixon, *J. Amer. Chem. Soc.*, **98**(19), 6000–6005 (1976).
231. R. F. Garwood, B. P. S. Khambay, B. C. L. Weedon, and E. N. Frankel, *J. Chem. Soc. Chem. Commun.*, 364–365 (1977).
232. E. N. Frankel, W. E. Neff, and T. R. Bessler, *Lipids*, **14**(12), 961–967 (1979).
233. J. Terao and S. Matsushita, *J. Food Process. Preserv.*, **3**, 329–337 (1980).
234. F. Haslbeck and W. Grosch, *Lipids*, **18**(1), 706–713 (1983).

235. H. W-S. Chan and G. Levett, *Lipids*, **12**(10), 837–840 (1977).
236. J. Terao and S. Matsushita, *Agric. Biol. Chem.*, **45**(3), 587–593 (1981).
237. M. VanRollins and R. C. Murphy, *J. Lipid Res.*, **25**, 507–517 (1984).
238. L. J. Parr and P. A. T. Swoboda, *J. Food Technol.*, **11**, 1–12 (1976).
239. S. L. Boyd, R. J. Boyd, Z. Shi, R. C. Barclay, and N. A. Porter, *J. Amer. Chem. Soc.*, **115**, 687–693 (1993).
240. J. A. Howard and K. U. Ingold, *Can J. Chem.*, **46**, 2655–2660 (1968).
241. E. G. Janzen, F. J. Johnston, and C. A. Ayers, *J. Amer. Chem. Soc.*, **89**, 1176–1183 (1967).
242. I. M. Campbell, R. B. Caton, and D. N. Crozier, *Lipids*, **9**(11), 916–920 (1974).
243. H. W-S. Chan, G. Levett, and J. A. Matthew, *Lipids*, **24**, 245–256 (1979).
244. N. A. Porter and J. R. Nixon, *J. Amer. Chem. Soc.*, **100**(22), 7116–7117 (1978).
245. N. A. Porter, D. H. Roberts, and C. B. Ziegler, Jr., *J. Amer. Chem. Soc.*, **102**, 5912–5913 (1980).
246. N. A. Porter, B. A. Weber, H. Weenan, and J. A. Kahn, *J. Amer. Chem. Soc.*, **102**, 5597–5601 (1980).
247. N. A. Porter, L. S. Lehman, B. A. Weber, and K. J. Smith, *J. Amer. Chem. Soc.*, **103**, 6447–6455 (1981).
248. H. Weenan and N. A. Porter, *J. Amer. Chem. Soc.*, **104**, 5216–5221 (1982).
249. A. N. Roe, A. T. McPhail, and N. A. Porter, *J. Amer. Chem. Soc.*, **105**, 1199–1203 (1983).
250. N. A. Porter and D. G. Wujek, *J. Amer. Chem. Soc.*, **106**, 2626–2629 (1984).
251. N. A. Porter, L. S. Lehman, and D. G. Wujek, in W. Bors, M. Saran, and D. Tait, eds., *Oxygen Radicals in Chemistry and Biology*, Walter de Gruyter, Berlin, 1984, pp. 235–237.
252. N. A. Porter, *Acc. Chem. Res.*, **19**, 262–268 (1986).
253. N. A. Porter and J. S. Wujek, *J. Org. Chem.*, **52**, 5085–5089 (1987).
254. K. A. Mills, S. E. Caldwell, G. R. Dubay, and N. A. Porter, *J. Amer. Chem. Soc.*, **114**, 9689–9691 (1992).
255. N. A. Porter, K. A. Mills, S. E. Caldwell, and G. R. Dubay, *J. Amer. Chem. Soc.*, **116**, 6697–6705 (1994).
256. N. A. Porter, K. A. Mills, and R. L. Carter, *J. Amer. Chem. Soc.*, **116**, 6690–6696 (1994).
257. D. A. Pratt, J. H. Mills, and N. A. Porter, *J. Amer. Chem. Soc.*, **125**, 5801–5810 (2003).
258. J. A. Howard and K. U. Ingold, *Can. J. Chem.*, **45**, 793–802 (1967).
259. R. A. Sheldon and J. K. Kochi, *Metal-Catalyzed Oxidations of Organic Compounds*, Academic Press, New York, 1981.
260. M. Simic, in G. E. Adams E. M. Fielden and B. D. Michael, eds., in *Fast Processes in Radiation Chemistry and Biology*, Wiley, New York, 1975, pp. 162–179.
261. W. Bors, M. Erben-Russ, and M. Saran, *J. Electroanal. Chem.*, **232**, 37–49 (1987).
262. T. M. Bockman, S. M. Hubig, and J. K. Kochi, *J. Amer. Chem. Soc.*, **120**, 2826–2830 (1998).
263. C. Walling and A. Padwa, *J. Amer. Chem. Soc.*, **85**, 1593–1597 (1963).
264. A. Factor, C. A. Russell, and T. G. Traylor, *J. Amer. Chem. Soc.*, **87**, 3692–3696 (1965).
265. H. W-S. Chan, J. A. Matthew, and D. T. Coxon, *J. Chem Soc. Chem. Commun.*, 235–236 (1980).
266. R. K. Haynes and S. C. Vonwiller, *J. Chem. Soc. Chem. Commun.*, 1102–1104 (1990).

267. H. W. Gardner, in H. W-S. Chan, ed., *Autoxidation of Unsaturated Lipids*, Academic Press, London, 1987, pp. 51–93.
268. N. A. Porter, P. J. Zuraw, and J. A. Sullivan, *J. Org. Chem.*, **49**(8), 1345–1348 (1984).
269. J. E. Baldwin, *J. Chem. Soc. Chem. Commun.*, 734–736 (1975).
270. M. O. Funk, R. Isaac, and N. A. Porter, *J. Amer. Chem. Soc.*, **97**, 1281–1282 (1975).
271. M. Hamberg and B. Samuelsson, *J. Amer. Chem. Soc.*, **88**, 2349–2350 (1966).
272. N. A. Porter and M. O. Funk, *J. Org. Chem.*, **40**, 3614–3615 (1975).
273. E. N. Frankel, W. E. Neff, E. Selke, and D. Weisleder, *Lipids*, **17**(1), 11–18 (1982).
274. E. D. Mihelich, *J. Amer. Chem. Soc.*, **102**, 7143–7145 (1980).
275. D. E. Van Sickle, F. R. Mayo, E. S. Gould, and R. M. Arluck, *J. Amer. Chem. Soc.*, **89**(4), 977–984 (1967).
276. W. E. Neff, E. N. Frankel, and K. Fujimoto, *J. Amer. Oil Chem. Soc.*, **65**(4), 616–623 (1988).
277. R. Hiatt and T. McCarrick, *J. Amer. Chem. Soc.*, **97**, 5234–5237 (1975).
278. F. R. Mayo, *J. Amer. Chem. Soc.*, **80**, 2500–2507 (1958).
279. F. R. Mayo, *Acc. Chem. Res.*, **1**(7), 193–201 (1968).
280. I. H. Elson, S. W. Mao, and J. K. Kochi, *J. Amer. Chem. Soc.*, **97**(2), 335–341 (1975).
281. S. E. Lewis and F. R. Mayo, *J. Amer. Chem. Soc.*, **70**(4), 1533–1536 (1958).
282. J. K. Kochi, *J. Amer. Chem. Soc.*, **84**, 2785–2793 (1962).
283. D. G. Hendry and D. Schuetzle, *J. Org. Chem.*, **41**, 3179–3182 (1976).
284. I. A. Witting, S. S. Chang, and F. A. Kummerow, *J. Amer. Oil Chem. Soc.*, **34**, 470–473 (1957).
285. R. P. Sims and W. H. Hoffman, in W. O. Lundberg, ed., *Autoxidation and Antioxidants* vol. 2, Interscience, London, 1962, pp. 629–694.
286. O. S. Privett, *J. Amer. Oil Chem. Soc.*, **36**(10), 507–512 (1959).
287. R. Hiatt, C. W. Gould, and F. R. Mayo, *J. Org. Chem.*, **29**, 3461–3466 (1964).
288. K. Miyashita, K. Fujimoto, and T. Kaneda, *Agric. Biol. Chem.*, **46**(3), 751–755 (1982).
289. T. L. Mounts, D. J. McWeeny, C. D. Evans, and H. J. Dutton, *Chem. Phys. Lipids*, **4**, 197–202 (1970).
290. E. N. Frankel, W. E. Neff, E. Selke, and D. D. Brooks, *Lipids*, **23**(4), 295–298 (1988).
291. J. R. Thomas, *J. Amer. Chem. Soc.*, **87**, 3935–3940 (1965).
292. D. A. Lindsay, J. A. Howard, E. C. Horswill, L. Iton, K. U. Ingold, T. Cobbley, and A. Li, *Can. J. Chem.*, **51**(6), 870–880 (1973).
293. K. Adamic, J. A. Howard, and K. U. Ingold, *Can. J. Chem.*, **47**, 3803–3808 (1969).
294. T. G. Traylor and C. A. Russell, *J. Amer. Chem. Soc.*, **87**(16), 3698–3706 (1965).
295. C. Walling, H. P. Waits, J. Milanovic, and C. G. Pappiaonnu, *J. Amer. Chem. Soc.*, **92**(16), 4927–4932 (1970).
296. R. Hiatt and T. G. Traylor, *J. Amer. Chem. Soc.*, **87**(16), 3766–3768 (1965).
297. H. W. S. Chan, F. A. A. Prescott, and P. A. T. Swoboda, *J. Amer. Oil Chem. Soc.*, **53**(9), 572–576 (1976).
298. S. S. Kim, S. Y. Kim, S. S. Ryou, C. S. Lee, and K. H. Yoo, *J. Org. Chem.*, **58**, 192–196 (1993).

299. W. Bors, C. Michael, and M. Saran, *Biochim. Biophys. Acta*, **796**, 312–319 (1984).
300. E. Bascetta, F. D. Gunstone, C. M. Scrimgeour, and J. C. Walton, *J. Chem. Soc. Chem. Commun.*, 110–112 (1982).
301. R. Hiatt and L. Zigmund, *Can. J. Chem.*, **48**, 3967–3969 (1970).
302. R. Hiatt and S. Szilagyi, *Can. J. Chem.*, **48**, 616–627 (1970).
303. B. C. Gilbert, R. G. G. Holmes, and R. O. C. Norman, *J. Chem. Res.*, **7**, 1949–1973 (1977).
304. C. Walling and A. Padwa, *J. Amer. Chem. Soc.*, **1963**, **85**, 1597–1601 (1963).
305. Y. P. Tsentalovich, L. V. Kulik, N. P. Gritsan, and A. V. Yurkovskaya, *J. Phys. Chem.*, **102**, 7975–7980 (1998).
306. D. V. Avila, K. U. Ingold, and J. Kusztyk, *J. Amer. Chem. Soc.*, **117**, 2929–2930 (1995).
307. H. Paul, R. D. Small, Jr., and J. C. Scaiano, *J. Amer. Chem. Soc.*, **100**(14), 4520–4527 (1978).
308. W. Bors, D. Tait, C. Michel, M. Saran, and M. Erben-Russ, *Isr. J. Chem.*, **24**, 17–24 (1984).
309. J. K. Kochi, *J. Amer. Chem. Soc.*, **84**, 1193 (1962).
310. C. Walling and P. J. Wagner, *J. Amer. Chem. Soc.*, **86**, 3368–3375 (1964).
311. W. E. Neff, E. N. Frankel, C. R. Schofield, and D. Weisleder, *Lipids*, **13**(6), 415–421 (1978).
312. E. Selke, E. N. Frankel, and W. E. Neff, *Lipids*, **13**(7), 511–513 (1978).
313. F. R. Mayo, *J. Amer. Chem. Soc.*, **80**, 2497–2500 (1958).
314. E. N. Frankel, *Prog. Lipid Res.*, **22**, 1–33 (1982).
315. G-S. Wu, R. A. Stein, and J. F. Mead, *Lipids*, **12**(11), 971–978 (1977).
316. G-S. Wu, R. A. Stein, and J. F. Mead, *Lipids*, **13**(8), 517–524 (1978).
317. B. Acott and A. L. J. Beckwith, *Austral. J. Chem.*, **17**(12), 1342–1353 (1964).
318. H. W. Gardner, K. Eskins, G. W. Grams, and G. R. Inglett, *Lipids*, **7**(5), 324–34 (1972).
319. H. W. Gardner and R. Kleiman, *Biochim. Biophys. Acta*, **665**, 113–125 (1981).
320. H. W. Gardner and R. Kleiman, *Biochim. Biophys. Acta*, **665**, 126–133 (1981).
321. W. E. Neff, *Lipids*, **15**(8), 587–590 (1980).
322. H. W. Gardner and R. Kleiman, *Lipids*, **14**(10), 848–851 (1979).
323. J. K. Kochi, ed., *Free Radicals*, vol. 1, John Wiley and Sons, New York, 1973.
324. C. Walling and W. Thaler, *J. Amer. Chem. Soc.*, **83**, 3877–3884 (1961).
325. C. A. Russell, *J. Amer. Chem. Soc.*, **21**, 300–302 (1959).
326. C. Walling and P. J. Wagner, *J. Amer. Chem. Soc.*, **85**, 2333–2334 (1963).
327. D. V. Avila, C. E. Brown, K. U. Ingold, and J. Luszyk, *J. Amer. Chem. Soc.*, **115**, 466–470 (1993).
328. B. C. Gilbert, P. D. R. Marshall, R. O. C. Norman, N. Pineda, and P. S. Williams, *J. Chem. Soc.*, 1392–1400 (1981).
329. E. H. Farmer and D. A. Sutton, *J. Chem. Soc.*, 119–122 (1943).
330. P. Neta, M. Dizdaroglu, and M. Simic, Sr. *J. Chem.*, **24**, 25–28.
331. A. Baignee, J. A. Howard, J. C. Scaiano, and L. C. Stewart, *J. Amer. Chem. Soc.*, **105**, 6120–6123 (1983).
332. A. G. Neville, C. E. Brown, D. M. Rayner, J. Luszyk, and K. U. Ingold, *J. Amer. Chem. Soc.*, **111**, 9269–9270 (1989).

333. E. Baciocchi, M. Bietti, M. Salamone, and S. Steenken, *J. Org. Chem.*, **67**, 2266–2270 (2002).
334. A. Loidl-Stahlhofen and G. Spiteller, *Biochim. Biophys. Acta*, **1211**, 156–160 (1994).
335. W. I. Kimoto and A. M. Gaddis, *J. Amer. Oil Chem. Soc.*, **51**(7), 307–311 (1974).
336. A. Boto, D. Hernandez, R. Hernandez, and E. Suarez, *J. Org. Chem.*, **68**, 5310–5319 (2003).
337. R. Ellis, A. M. Gaddis, G. T. Currie, and S. L. Powell, *J. Amer. Oil Chem. Soc.*, **45**(8), 553–559 (1968).
338. J. H. Horner, S.-Y. Choi, and M. Newcomb, *Org. Lett.*, **2**(21), 3369–3372 (2000).
339. W. H. Richardson, M. B. Yelvington, A. H. Andrist, E. W. Ertley, R. S. Smith, and T. D. Johnson, *J. Org. Chem.*, **38**, 4219–4225 (1973).
340. W. Grosch, in H. W-S. Chan, ed., *Autoxidation of Unsaturated Lipids*, Academic Press, London, 1987, pp. 95–139.
341. E. N. Frankel, *Chem. Phys. Lipids*, **44**, 73–85 (1987).
342. S. Fukuzumi and J. K. Kochi, *J. Amer. Chem. Soc.*, **103**, 2783–2791 (1981).
343. S. Wilsey, P. Dowd, and K. N. Houk, *J. Org. Chem.*, **64**, 8801–8811 (1999).
344. G. A. Russell, *J. Amer. Chem. Soc.*, **78**, 1047–1054 (1956).
345. E. N. Frankel, W. E. Neff, and E. Selke, *Lipids*, **19**(10), 790–800 (1984).
346. S. B. El-Magoli, M. Karel, and S. Yong, *J. Food Biochemistry*, **3**, 111–123 (1980).
347. E. N. Frankel, *J. Amer. Oil Chem. Soc.*, **61**(12), 1908–1917 (1984).
348. E. N. Frankel, W. E. Neff, W. K. Rodwedder, B. P. S. Khambay, R. F. Garwood, and B. C. L. Weedon, *Lipids*, **12**, 1055–1061 (1977).
349. E. N. Frankel, W. E. Neff, W. K. Rohwedder, B. P. S. Khambay, R. F. Garwood, and B. C. L. Weedon, *Lipids*, **12**(11), 901–907 (1977).
350. L. Bateman, H. Hughes, and A. L. Morris, *Discuss. Faraday Soc.*, **14**, 190–199 (1953).
351. J. Sliwiok, T. Kowalska, W. Kowalski, and A. Biernat, *Microchem. J.*, **19**(4), 362–372 (1974).
352. M. Hicks and J. M. Gebicki, *Int. J. Radiat. Biol.*, **64**(2), 143–148 (1993).
353. C. Walling and L. Heaton, *J. Amer. Oil Chem. Soc.*, **87**, 48–51 (1965).
354. J. A. Howard and J. E. Bennett, *Can. J. Chem.*, **50**(14), 2374–2377 (1972).
355. M. H. Brodnitz, *Agric. Food Chem.*, **16**(6), 994–999 (1968).
356. G. A. Russell, *J. Chem. Ed.*, **36**, 111–118 (1959).
357. D. E. Van Sickle, F. R. Mayo, R. M. Arluck, and M. A. Syz, *J. Amer. Chem. Soc.*, **89**(4), 967–977 (1967).
358. L. K. Patterson and J. L. Redpath, in K. L. Mittal, ed., *Micellization, Solubilization, and Microemulsions*, vol. 2, Plenum Press, New York, 1977, pp. 589–601.
359. W. Bors, M. Erben-Russ, and M. Saran, *Bioelectrochem. Bioenerg.*, **18**, 37–49 (1987).
360. J. A. Howard, W. J. Schwalm, and K. U. Ingold, *Adv. Chem. Series*, **75**, 6–23 (1968).
361. J. A. Howard and K. U. Ingold, *Can. J. Chem.*, **46**, 2661–2666 (1968).
362. J. A. Howard and K. U. Ingold, *J. Amer. Chem. Soc.*, **90**, 1058–1059 (1968).
363. H. De Groot and T. Noll, *Chem. Phys. Lipids*, **44**, 209–226 (1987).
364. J. A. Howard, in R. F. Gould, ed., *Organic Free Radicals*, American Chemical Society, Washington, D.C., 1978, pp. 413–432.

365. J. A. Howard, K. Adamic, and K. U. Ingold, *Can. J. Chem.*, **47**, 3793–3795 (1969).
366. J. A. Howard and K. U. Ingold, *J. Amer. Chem. Soc.*, **90**, 1056–1058 (1968).
367. P. Scheiberle, B. Tsoukalas, and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **168**, 448–456 (1979).
368. P. Scheiberle, W. Grosch, H. Kexel, and H-L. Schmidt, *Biochim. Biophys. Acta*, **666**, 322–326 (1981).
369. W. Grosch and J. Megele, in W. Bors, M. Saran, and D. Tait, ed., *Oxygen Radicals in Chemistry and Biology*, Walter de Gruyter & Co., Berlin, 1984, pp. 249–256.
370. P. Schieberle and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **173**, 192–198 (1981).
371. P. Schieberle and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **173**, 199–203 (1981).
372. H. W-S. Chan, G. Levett, and J. A. Matthew, *J. Chem. Soc. Chem. Commun.*, 756–758 (1978).
373. A. U. Khan, *J. Phys. Chem.*, **80**(20), 2219–2228 (1976).
374. E. N. Frankel and W. E. Neff, *Biochim. Biophys. Acta*, **754**, 264–270 (1983).
375. W. E. Neff, E. N. Frankel, and D. Weisleder, *Lipids*, **17**(11), 780–790 (1982).
376. W. A. Pryor, J. P. Stanley, and E. Blair, *Lipids*, **11**(5), 370–379 (1976).
377. L. K. Dahle, E. G. Hill, and R. T. Holman, *Arch. Biochem. Biophys.*, **98**(2), 253–261 (1962).
378. R. J. Shamberger, B. A. Shamberger, and C. E. Willis, *J. Nutr.*, **107**, 1404–1409 (1977).
379. H. W. Gardner and D. Weisleder, *Lipids*, **11**(2), 127–34 (1976).
380. H. W. Gardner, R. Kleiman, D. Weisleder, and G. E. Inglett, *Lipids*, **12**(8), 655–660 (1977).
381. S. H. Yong and M. Karel, *J. Amer. Oil Chem. Soc.*, **55**(3), 352–357 (1978).
382. S. H. Yong and M. Karel, *J. Food Sci.*, **22**(2), 568–574 (1979).
383. K. M. Schaich and M. Karel, *J. Food Sci.*, **40**, 456–459 (1975).
384. K. M. Schaich and M. Karel, *Lipids*, **11**, 392–400 (1976).
385. M-H. Yang, and K. M. Schaich, *Free Radic. Biol. Med.*, **20**, 225–236 (1996).
386. K. M. Schaich and D. C. Borg, in W. Bors, M. Saran, and D. Tait, eds., *Oxygen Radicals in Chemistry and Biology*, Walter de Gruyter, Berlin, 1984, pp. 603–606.
387. H. W. Gardner, in J. W. Finley, ed., *Xenobiotics in Foods and Feeds*, American Chemical Society, Washington, D.C., 1983, pp. 63–84.
388. D. C. Borg and K. M. Schaich, *Isr. J. Chem.*, **24**, 38–53 (1984).
389. W. A. Pryor, *Free Radic. Biol. Med.*, **7**, 177–178 (1989).
390. M. Karel, K. M. Schaich, and R. B. Roy, *Agric. Food Chem.*, **23**, 159–164 (1975).
391. J. Terao, T. Ogawa, and S. Matsushita, *Agric. Biol. Chem.*, **39**(2), 397–402 (1975).
392. E. Bothe, M. N. Schuchmann, D. Schulte-Frohlinde, and C. von Sonntag, *Photochem. Photobiol.*, **28**, 639–644 (1978).

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## *Lipid Oxidation: Measurement Methods*

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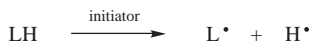
### **1. INTRODUCTION**

Dietary lipids, naturally occurring in raw food materials or added during food processing, play an important role in food nutrition and flavor. Meanwhile, lipid oxidation is a major cause of food quality deterioration, and has been a challenge for manufacturers and food scientists alike. Lipids are susceptible to oxidative processes in the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins, and micro-organisms, giving rise to the development of off-flavors and loss of essential amino acids, fat-soluble vitamins, and other bioactives. Lipids may undergo autoxidation, photo-oxidation, thermal oxidation, and enzymatic oxidation under different conditions, most of which involve some type of free radical or oxygen species (1, 2). Among these, only autoxidation and thermal oxidation are discussed here in detail.

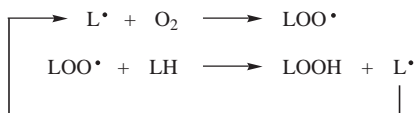
Autoxidation is the most common process leading to oxidative deterioration and is defined as the spontaneous reaction of atmospheric oxygen with lipids (3). The process can be accelerated at higher temperatures, such as those experienced during deep-fat frying, which is called thermal oxidation, with increases in free fatty acid and polar matter contents, foaming, color, and viscosity (4). Unsaturated fatty acids

are generally the reactants affected by such reactions, whether they are present as free fatty acids, triacylglycerols (as well as diacylglycerols or monoacylglycerols), or phospholipids (3). It has been accepted that both autoxidation and thermal oxidation of unsaturated fatty acids occurs via a free radical chain reaction that proceeds through three steps of initiation, propagation, and termination (5). A simplified scheme explaining the mechanism of autoxidation is given below:

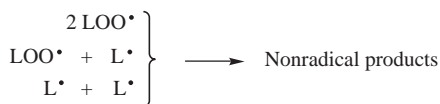
Initiation:



Propagation:



Termination:



As oxidation normally proceeds very slowly at the initial stage, the time to reach a sudden increase in oxidation rate is referred to as the induction period (6). Lipid hydroperoxides have been identified as primary products of autoxidation; decomposition of hydroperoxides yields aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, known as secondary oxidation products. These compounds, together with free radicals, constitute the bases for measurement of oxidative deterioration of food lipids. This chapter aims to explore current methods for measuring lipid oxidation in food lipids.

## 2. METHODS FOR MEASURING LIPID OXIDATION

Numerous analytical methods are routinely used for measuring lipid oxidation in foods. However, there is no uniform and standard method for detecting all oxidative changes in all food systems (7). Therefore, it is necessary to select a proper and adequate method for a particular application. The available methods to monitor lipid oxidation in foods can be classified into five groups based on what they measure: the absorption of oxygen, the loss of initial substrates, the formation of free radicals, and the formation of primary and secondary oxidation products (8). A number of physical and chemical tests, including instrumental analyses, have been employed in laboratories and the industry for measurement of various lipid oxidation parameters. These include the weight-gain and headspace oxygen uptake method for oxygen absorption; chromatographic analysis for changes in reactants;



iodometric titration, ferric ion complexes, and Fourier transform infrared (FTIR) method for peroxide value; spectrometry for conjugated dienes and trienes, 2-thio-barbituric acid (TBA) value, *p*-anisidine value (*p*-AnV), and carbonyl value; Rancimat and Oxidative Stability Instrument (OSI) method for oil stability index; and electron spin resonance (ESR) spectrometric assay for free-radical type and concentration. Other techniques based on different principles, such as differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR), have also been used for measuring lipid oxidation. In addition, sensory tests provide subjective or objective evaluation of oxidative deterioration, depending on certain details.

### 3. MEASUREMENT OF OXYGEN ABSORPTION

#### 3.1. Weight Gain

Consumption of oxygen during the initial stage of autoxidation results in an increase in the weight of fat or oil, which theoretically reflects its oxidation level. Heating an oil and periodically testing for weight gain is one of the oldest methods for evaluating oxidative stability (9). This method requires simple equipment and directly indicates oxygen absorption through mass change. Oil samples are weighed and stored in an oven at a set temperature with no air circulation. To avoid the influence of mass change by volatiles, samples can be preheated in an inert atmosphere. Samples are then taken out of the oven at different time intervals, cooled to ambient temperature, and reweighed; the weight gain is then recorded. The induction period can be obtained by plotting weight gain against storage time. In some cases, the time required to attain a 0.5% weight increase is taken as an index of oil stability (7, 9, 10).

As a physical method for measuring lipid oxidation, the weight-gain method has several drawbacks such as discontinuous heating of the sample, which may give rise to non-reproducible results, and requiring long analysis time and intensive human participation (7). Nevertheless, this method offers advantages such as low instrumentation cost as well as a high capacity and processing speed of samples without limitation (7). Antolovich et al. (9) suggested that this technique may be extended to more sophisticated continuous monitoring of mass and energy changes as in thermogravimetry (TG)/differential scanning calorimetry (DSC). The weight-gain method can also be used for measuring antioxidant activity by comparing the results in the presence and absence of an antioxidant. Nevertheless, this method is useful only when highly unsaturated oils, such as marine oils and vegetable oils containing a high content of polyunsaturated fatty acids, are examined.

#### 3.2. Headspace Oxygen Uptake

In addition to the weight-gain method, oxygen consumption can be measured directly by monitoring the drop of oxygen pressure. Using headspace oxygen method, an oil sample is placed in a closed vessel also containing certain amount of oxygen at elevated temperatures, commonly around 100°C. The pressure reduction in

the vessel, which is due to the oxygen consumption, is monitored continuously and recorded automatically. The induction period as the point of maximum change in rate of oxygen uptake can be calculated (11). A commercial instrument for this method, known as Oxidograph, is available. In the Oxidograph, the pressure change in the reaction vessel is measured electronically by means of pressure transducers (7, 12).

Oxygen consumption can also be measured by electrochemical detection of changes in oxygen concentration. However, the analysis of the graphical data obtained has been the bottleneck for this technique. The use of a semiautomatic polarographic method has been proposed as an improvement for evaluation of lipid oxidation by determination of oxygen consumption (13). As described by Genot et al. (13), this method is based on use of two oxygen meters with microcathode oxygen electrodes, coupled to a computerized data collection and processing unit.

The headspace oxygen method is simple and reproducible and may be the best analytical method to evaluate the oxidative stability of fats and oils (14). Its application in measurement of lipid oxidation in food products other than fats and oils, however, is limited because protein oxidation also absorbs oxygen (15).

#### 4. MEASUREMENT OF REACTANT CHANGE

Lipid oxidation can also be assessed by quantitatively measuring the loss of initial substrates. In foods containing fats or oils, unsaturated fatty acids are the main reactants whose composition changes significantly during oxidation. Changes in fatty acid composition provide an indirect measure of the extent of lipid oxidation (15). In this method, lipids are extracted from food, if necessary, and subsequently converted into derivatives suitable for chromatographic analysis (7). Fatty acid methyl esters (FAME) are the derivatives frequently used for determination of fatty acid composition, usually by gas chromatography (GC) (16). Similarly, iodine value, which reflects the loss of unsaturation, can also be used as an index of lipid oxidation (17).

Measurement of changes in fatty acid composition is useful for identification of lipid class and fatty acids that are involved in oxidation reactions (7). However, because the distribution of unsaturated fatty acids varies in different food systems, for instance, the highly unsaturated fatty acids being located predominantly in phospholipids of muscle foods, separation of lipids into neutral, glycolipid, phospholipid, and other classes may be necessary (7, 15). Moreover, it is an insensitive way of assessing oxidative deterioration. For comparison through calculation, oxidation of 0.4% polyunsaturated fatty acids to monohydroperoxides would represent a change of 16 meq oxygen/kg oil in peroxide value, whereas a change of less than 1.0 meq oxygen/kg oil could readily be detected by measuring peroxide value (12). Additionally, the application of this method is limited because of its inability to serve as an indicator of oxidation of more saturated lipids (7). Nevertheless, its usefulness for measuring oxidation of highly unsaturated oils cannot be underestimated.

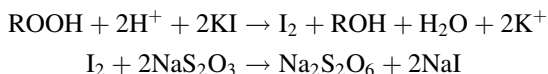
## 5. MEASUREMENT OF PRIMARY PRODUCTS OF OXIDATION

### 5.1. Peroxide Value (PV)

Lipid oxidation involves the continuous formation of hydroperoxides as primary oxidation products that may break down to a variety of nonvolatile and volatile secondary products (8, 15). The formation rate of hydroperoxides outweighs their rate of decomposition during the initial stage of oxidation, and this becomes reversed at later stages. Therefore, the peroxide value (PV) is an indicator of the initial stages of oxidative change (18). However, one can assess whether a lipid is in the growth or decay portion of the hydroperoxide concentration by monitoring the amount of hydroperoxides as a function of time (7).

Analytical methods for measuring hydroperoxides in fats and oils can be classified as those determining the total amount of hydroperoxides and those based on chromatographic techniques giving detailed information on the structure and the amount of specific hydroperoxides present in a certain oil sample (8). The PV represents the total hydroperoxide content and is one of the most common quality indicators of fats and oils during production and storage (9, 18). A number of methods have been developed for determination of PV, among which the iodometric titration, ferric ion complex measurement spectrophotometry, and infrared spectroscopy are most frequently used (19).

**5.1.1. Iodometric Titration Method** Iodometric titration assay, which is based on the oxidation of the iodide ion ( $I^-$ ) by hydroperoxides (ROOH), is the basis of current standard methods for determination of PV (9). In this method, a saturated solution of potassium iodide is added to oil samples to react with hydroperoxides. The liberated iodine ( $I_2$ ) is then titrated with a standardized solution of sodium thiosulfate and starch as an endpoint indicator (7, 9, 20). The PV is obtained by calculation and reported as milliequivalents of oxygen per kilogram of sample (meq/kg). The official determination is described by IUPAC (21). Chemical reactions involved are given below:



Although iodometric titration is the most common method for measurement of PV, it suffers from several disadvantages. The procedure is time-consuming and labor-intensive (18). As described by Ruiz et al. (18), the assay includes six steps: accurate weighing of the sample, dissolution of lipids in chloroform, acidification with acetic acid, addition of potassium iodide, incubation for exactly 5 minutes, and titration with sodium thiosulfate. This technique requires a large amount of sample and generates a significant amount of waste (18, 22, 23). Furthermore, possible absorption of iodine across unsaturated bonds and oxidation of iodide by dissolved oxygen are among potential drawbacks of this method (7, 9). Besides, lack of sensitivity, possible interferences, and difficulties in determining the titration endpoint

are also the main limitations (8, 23). To overcome these drawbacks, novel methods based on the same reaction have been developed, in which some other techniques are adopted as modification of the classical iodometric assay. Techniques such as colorimetric determination at 560 nm (24), potentiometric endpoint determination (25), and spectrophotometric determination of the  $I_3^-$  chromophore at 290 nm or 360 nm (26, 27) have been proposed. In addition, an electrochemical technique has been used as an alternative to the titration step in order to increase the sensitivity for determination of low PV by reduction of the released iodine at a platinum electrode maintained at a constant potential (7).

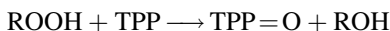
**5.1.2. Ferric Ion Complexes** Other chemical methods based on the oxidation of ferrous ion ( $Fe^{2+}$ ) to ferric ion ( $Fe^{3+}$ ) in an acidic medium and the formation of iron complexes have also been widely accepted. These methods spectrophotometrically measure the ability of lipid hydroperoxides to oxidize ferrous ions to ferric ions, which are complexed by either thiocyanate or xylenol orange (23, 28, 29). Ferric thiocyanate is a red-violet complex that shows strong absorption at 500–510 nm (8). The method of determining PV by colorimetric detection of ferric thiocyanate is simple, reproducible, and more sensitive than the standard iodometric assay, and has been used to measure lipid oxidation in milk products, fats, oils, and liposomes (8, 23).

The ferrous oxidation of xylenol orange (FOX) assay uses dye xylenol orange to form a blue-purple complex with a maximum absorption at 550–600 nm (8). This method is rapid, inexpensive, and not sensitive to ambient oxygen or light (30). It can consistently quantify lower hydroperoxide levels; and good agreement exists between the FOX assay and the iodometric method (30). The FOX method has been successfully adapted to a variety of applications. However, because many factors, such as the amount of sample, solvent used, and source of xylenol orange, may affect the absorption coefficient, knowledge of the nature of hydroperoxides present in the sample, and careful control of the conditions used are required for accurate measurements (8).

**5.1.3. Fourier Transform Infrared Spectroscopy (FTIR)** It has been recognized that hydroperoxides can quantitatively be determined by IR spectroscopy via measurement of their characteristic O-H stretching absorption band (31). An absorption band at about 2.93  $\mu\text{m}$  indicates the generation of hydroperoxides, whereas the replacement of a hydrogen atom on a double bond or polymerization accounts for the disappearance of a band at 3.20  $\mu\text{m}$ . The formation of aldehydes, ketones, or acids gives rise to an extra band at 5.72  $\mu\text{m}$ . Furthermore, *cis*-, *trans*-isomerization and formation of conjugated dienes can be detected through the changes in the absorption band in the range of 10  $\mu\text{m}$  to 11  $\mu\text{m}$  (7).

A rapid Fourier transform infrared spectroscopy (FTIR) method based on the stoichiometric reaction of triphenylphosphine (TPP) with hydroperoxides has been developed and successfully applied to determination of PV of edible oils (32). The hydroperoxides present in oil samples react stoichiometrically with TPP to produce triphenylphosphine oxide (TPPO), which has an intense absorption

band at  $542\text{ cm}^{-1}$  in the mid-IR spectrum (8, 18). The band intensity is measured and converted to peroxide value. The chemical reaction involved is given below:



By using *tert*-butyl hydroperoxide spiked oil standards and evaluation of the band formed at  $542\text{ cm}^{-1}$ , a linear calibration graph covering the range of 1–100 PV was obtained (18). More recently, disposable polymer IR (PIR) cards have been used as sample holders where unsaturated oil samples oxidize at a fairly rapid rate (33). In the FTIR/PIR card method, warm air continuously flows over the sample allowing oxidation to be monitored at moderate temperatures. At periodic intervals, individual cards are removed and the FTIR spectra scanned (33). Another new FTIR approach uses flow injection analysis (FIA), which offers exact and highly reproducible timing of sample manipulation and reaction as well as a closed environment with oxygen and light being easily excluded (18).

The FTIR spectroscopy is a simple, rapid, and highly precise method. It shows excellent correlation with the iodometric method and avoids the solvent and reagent disposal problems associated with the standard wet chemical method (18, 32). The FTIR method provides an automated, efficient and low-cost means of evaluating oxidation in oils undergoing thermal stress and has gained considerable interest for quality control in the industry (8, 20, 34). However, there is a need to characterize the spectral changes, assign wavelengths to more common molecular species produced, and access potential spectral cross interferences (20). Recently, an improved Fourier transform infrared attenuated total reflectance (FTR-ATR) method using the whole FTIR spectral data instead of particular wavenumbers has been proposed (34).

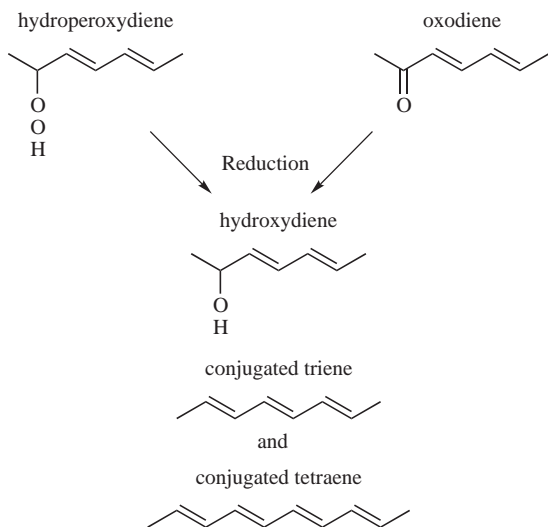
In addition to the three major methods discussed above, other techniques have also been employed in determination of PV, such as chemiluminescence and chromatography. Chemiluminescence method is based on detecting the chemiluminescent products generated during the reaction of hydroperoxides with substances such as luminol and dichlorofluorescein (7, 35). This method was reviewed by Jimenez et al. (36). High correlations have been found between chemiluminescence and other standard methods, indicating that chemiluminescence could serve as an accurate tool for determination of PV (37). However, this method has low sensitivity to *tert*-butyl hydroperoxide, *tert*-butyl perbenzoate, diacyl peroxides, and dialkyl peroxides (35). Chromatographic techniques, mainly gas chromatography (GC) and high-performance liquid chromatography (HPLC), have also been employed for evaluation of lipid oxidation. These methods provide information about specific hydroperoxides, whereas other assays measure their total amount. Chromatographic methods require small amounts of sample, and interference from minor compounds other than hydroperoxides can be easily excluded (8). HPLC shows advantages over GC and has become a popular technique for hydroperoxide analysis. It operates at room temperature, thus decreases the risk of artifact formation, and no prior derivatization is required (8). A wide range of hydroperoxides can be analyzed using either normal or reverse-phase HPLC. Thus, hydroperoxides, the primary products

and intermediates in lipid oxidation reaction, provide an important parameter for evaluation of oxidation level. In addition, the inhibition of formation or action of these unstable species by antioxidants can be used as a means of assessing antioxidant activity (9). Measurement of hydroperoxides is also carried out in accelerated tests to establish the oxidative stability of a given oil. A case in point is the active oxygen method (AOM), in which air is bubbled through fat or oil held at 98–100°C and PV is determined periodically (7, 38). The time required to reach a PV of 100 meq/kg is the AOM stability of the oil sample (7). This method is now considered outdated and is replaced by other standard methods in the industry, although product specifications still routinely give AOM values (38).

## 5.2. Conjugated Dienes and Trienes

It was discovered in 1933 that the formation of conjugated dienes in fats or oils gives rise to an absorption peak at 230–235 nm in the ultraviolet (UV) region. In the 1960s, monitoring diene conjugation emerged as a useful technique for the study of lipid oxidation (9). During the formation of hydroperoxides from unsaturated fatty acids conjugated dienes are typically produced, due to the rearrangement of the double bonds. The resulting conjugated dienes exhibit an intense absorption at 234 nm; similarly conjugated trienes absorb at 268 nm (7). An increase in UV absorption theoretically reflects the formation of primary oxidation products in fats and oils. Good correlations between conjugated dienes and peroxide value have been found (39, 40).

Ultraviolet detection of conjugated dienes is simple, fast, and requires no chemical reagents and only small amounts of samples are needed. However, this



**Figure 1.** Chemical reaction steps in conjugable oxidation products (COP) assay.

**TABLE 1. Summary of Methods for Analysis of Primary Oxidation Products.**

Method	Principle	Measurement	Sensitivity	Applications
Iodometric titration (PV)	Reduction of ROOH with KI and measurement of I <sub>2</sub>	Titration with Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	≈0.5-meq/kg fat	Fats and oils
Ferric ion complexes (PV)	Reduction of ROOH with Fe <sup>2+</sup> and formation of Fe <sup>3+</sup> complexes	Absorption at 500–510 nm of the red complex with SCN <sup>-</sup>	≈0.1-meq/kg fat	Fats, oils and food lipids
		Absorption at 560 nm of the blue-purple complex with xylenol orange	≈0.5-meq/kg sample	All samples
FTIR (PV)	Reduction of ROOH with TPP	Absorption at 542 cm <sup>-1</sup> of TPPO	≈0.2-meq/kg fat	Fats and oils
Chemiluminescence (PV)	Reaction with luminol in the presence of heme catalyst	Chemiluminescence emission of oxidized luminol	≈1 pmol	Fats and oils
GC-MS (PV)	Reduction of ROOH to ROH and quantitation of ROH derivatives	ROH derivatives	From ng to fg depending on technical details, amount of sample and detection system	All samples
UV spectrometry (conjugated dienes and trienes)	Estimation of conjugated dienes and trienes	Absorption at 230–234 nm and 268 nm	≈0.2 meq/kg lipid	All samples

NOTE: The oxygen absorption measurement and loss of double bonds for fatty acid analysis are not considered as primary changes in this table. Adapted from (8).

method has less specificity and sensitivity than PV measurement (9, 12). Furthermore, the result may be affected by the presence of compounds absorbing in the same region, such as carotenoids (7). To avoid these interferences, an alternative spectroscopic method measuring conjugable oxidation products (COPs) has been proposed. In this method, hydroperoxides and some decomposition products are converted to more conjugated chromophores by reduction and subsequent dehydration (Figure 1). The concentrations of the resultant conjugated trienes and tetraenes are determined from their respective absorption at 268 nm and 301 nm and expressed as COP values (7, 12).

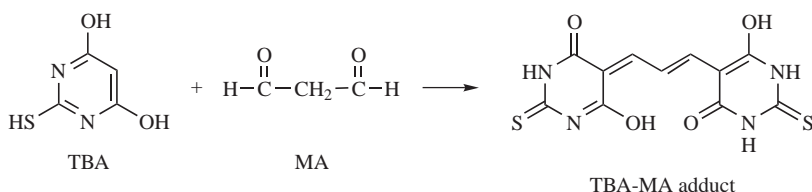
Table 1 summarizes different methods available for analysis of primary oxidation products. Both chemical and instrumental methods are included in this table.

## 6. MEASUREMENT OF SECONDARY PRODUCTS OF OXIDATION

The primary oxidation products (hydroperoxides) are unstable and susceptible to decomposition. A complex mixture of volatile, nonvolatile, and polymeric secondary oxidation products is formed through decomposition reactions, providing various indices of lipid oxidation (5). Secondary oxidation products include aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, among others. Methods for assessing lipid oxidation based on their formation are discussed in this section.

### 6.1. Thiobarbituric Acid (TBA) Test

The thiobarbituric acid (TBA) test was proposed over 40 years ago and is now one of the most extensively used methods to detect oxidative deterioration of fat-containing foods (41). During lipid oxidation, malonaldehyde (MA), a minor component of fatty acids with 3 or more double bonds, is formed as a result of the degradation of polyunsaturated fatty acids. It is usually used as an indicator of the lipid oxidation process, both for the early appearance as oxidation occurs and for the sensitivity of the analytical method (42). In this assay, the MA is reacted with thiobarbituric acid (TBA) to form a pink MA-TBA complex that is measured spectrophotometrically at its absorption maximum at 530–535 nm (Figure 2) (9, 43, 44). The extent of oxidation is reported as the TBA value and is expressed as milligrams



**Figure 2.** Reaction of 2-thiobarbituric acid (TBA) and malonaldehyde (MA).



of MA equivalents per kilogram sample or as micromoles of MA equivalents per gram of sample. It must, however, be noted that alkenals and alkadienals also react with the TBA reagent and produce a pink color. Thus, the term thiobarbituric acid reactive substances (TBARS) is now used instead of MA.

The TBA test can be performed by various procedures, among which four major types have frequently been employed. These include test on the whole sample, test on an aqueous or acid extract of sample, test on a steam distillate, and test on extracted lipid from a sample (45). The test on a steam distillate (distillation method) is the most commonly used method for determining TBA value. Tarladgis et al. (46) found that the distillation of an acidified sample was essential to liberate MA from precursor or bound forms, to produce maximal color development and, especially, to separate TBARS from the food matrix (44). Although the distillation method is the most popular TBA method, it is generally considered less accurate and reproducible than the method using food extracts (15). However, trends obtained in comparative studies always provide useful information that correspond with other measurements. Comparison of different TBA test procedures has been made by Hoyland et al. (46), Shahidi et al. (47), Pikul et al. (48), and Wang et al. (49).

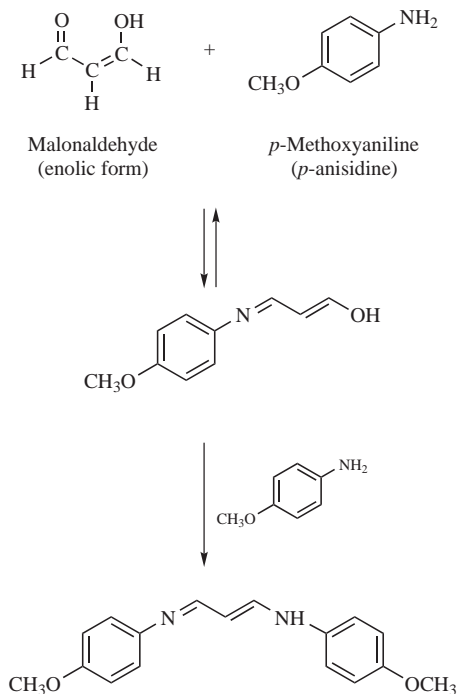
The TBA test is used frequently to assess the oxidative state of a variety of food systems, despite its limitations, such as lack of specificity and sensitivity (44). As already noted, many other substances may react with the TBA reagent and contribute to absorption, causing an overestimation of the intensity of color complex (44). Interferences may come from additional absorption of other alkanals, 2-alkenals, 2,4-alkdienals, ketones, ketosteroids, acids, esters, proteins, sucrose, urea, pyridines, and pyrimidines, also referred to as TBARS (43, 50). For instance, the reaction of TBA with various aldehydes leads to the development of a yellow chromogen (aldehyde-TBA adduct) with an absorption maximum at 450 nm, which overlaps with the pink peak at 532 nm resulting in erroneously high TBA values in certain cases (43, 45, 51). Furthermore, the presence of barbituric acid impurities in the TBA reagent may produce TBA-MA-barbituric acid and MA-barbituric acid adducts that absorb at 513 nm and 490 nm, respectively, indicating that thiobarbituric acid should be purified before use (43). In addition, nitrite can interfere in the TBA test, whereas sulfanilamide could be added to samples to avoid the interference when residual nitrite is present (52). In order to improve the specificity and sensitivity of the TBA test, several modifications to the original TBA procedures have been proposed, including reduction of the heating temperature to stabilize the yellow color aldehyde-TBA complex (53), addition of antioxidants to sample in an attempt to prevent oxidation during the test (54), extraction of the MA prior to the formation of the chromogen (43), direct FTIR analysis of TBARS, and use of HPLC to separate the complex before measurement or to characterize the individual species of TBARS (9, 43).

Despite its limitations, the TBA test provides an excellent means for evaluating lipid oxidation in foods, especially on a comparative basis. However, its use in bulk oils is less common than the so-called *para*-anisidine value (*p*-AnV) detailed below.

## 6.2. *p*-Anisidine Value (*p*-AnV)

The *p*-anisidine value (*p*-AnV) method measures the content of aldehydes (principally 2-alkenals and 2,4-alkadienals) generated during the decomposition of hydroperoxides. It is based on the color reaction of *p*-methoxyaniline (anisidine) and the aldehydic compounds (55). The reaction of *p*-anisidine reagent with aldehydes under acidic conditions affords yellowish products that absorb at 350 nm (Figure 3) (7, 12). The color is quantified and converted to *p*-AnV. The *p*-AnV is defined as the absorbance of a solution resulting from the reaction of 1 g of fat in isoctane solution (100 ml) with *p*-anisidine (0.25% in glacial acetic acid) (12). This test is more sensitive to unsaturated aldehydes than to saturated aldehydes because the colored products from unsaturated aldehydes absorb more strongly at this wavelength (12). However, it correlates well with the amount of total volatile substances (55). The *p*-AnV is a reliable indicator of oxidative rancidity in fats and oils and fatty foods (56). A highly significant correlation between *p*-AnV and flavor scores and PV has been found (57). Nevertheless, some authors have indicated that *p*-AnV is comparable only within the same oil type because initial AnV varies among oil sources (58). For instance, oils with high levels of polyunsaturated fatty acids might have higher AnV even when fresh (59).

This method is used less frequently in North America, but is widely employed in Europe (38), particularly as a part of the Totox number, as explained below. Caution



**Figure 3.** Possible reactions between *p*-anisidine reagent and malonaldehyde.

must be exercised when performing this test because of toxicity of the anisidine reagent (55).

### 6.3. Totox Value

The Totox value is a measure of the total oxidation, including primary and secondary oxidation products. It is a combination of PV and *p*-AnV:

$$\text{Totox value} = 2\text{PV} + p\text{-AnV}$$

During lipid oxidation, it is often observed that PV first rises, then falls as hydroperoxides decompose (38). PV and *p*-AnV reflect the oxidation level at early and later stages of oxidation reaction, respectively. Totox value measures both hydroperoxides and their breakdown products, and provides a better estimation of the progressive oxidative deterioration of fats and oils (38). However, Totox value has no scientific basis because it is a combination of two indicators with different dimensions (7). Recently, Wanasundara and Shahidi used TBA values and defined  $\text{Totox}_{\text{TBA}}$  as  $2\text{PV} + \text{TBA}$  using the TBA test in place of the *p*-AnV assay (60).

### 6.4. Carbonyls

The carbonyl compounds, including aldehydes and ketones, are the secondary oxidation products generated from degradation of hydroperoxides, and are suggested to be the major contributors to off-flavors associated with the rancidity of many food products (9). The analysis of total carbonyl compounds, which is based on the absorbance of the carbonyl derivatives, provides another approach to measure the extent of lipid oxidation in fats and oils. In this method, the total carbonyl content is measured by a colorimetric 2,4-dinitrophenylhydrazone procedure. The carbonyl compounds formed during lipid oxidation are reacted with 2,4-dinitrophenylhydrazine (DNPH) followed by the reaction of the resulting hydrazones with alkali (Figure 4). The final colored products are then analyzed spectrophotometrically

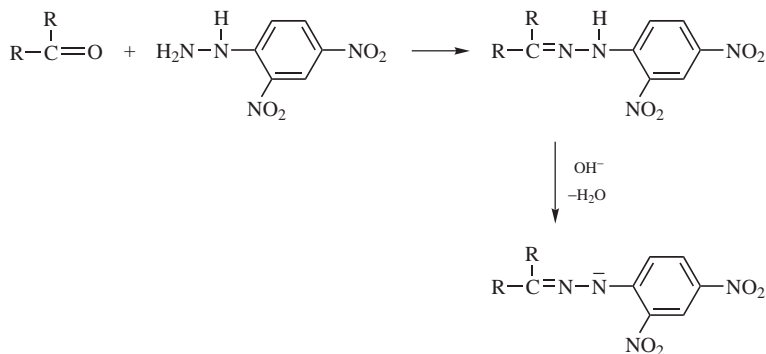


Figure 4. Reactions between carbonyls and 2,4-dinitrophenylhydrazine.

at a given wavelength (7, 15). Many variations of this method using an alternative solvent, reagent, wavelength, or workup have been reported. The determination of total content of carbonyls has been used in different oxidative stability studies. However, it has been criticized because the determination conditions cause degradation of hydroperoxides into carbonyl derivatives, giving erroneous results (58). Carbonyls produced from protein oxidation may also give rise to higher values than those expected from lipid oxidation alone. The addition of triphenylphosphine (TPP) prior to carbonyl determination has been proposed to avoid the interference from hydroperoxides. Hydroperoxides are reduced by TPP, and neither TPP nor TPPO, the oxidation products of TPP, interfere with the measurement of carbonyl content (61). In quality assessment of used frying fats, where short-chain carbonyls are already removed by distillation at the high temperature of the deep-frying, selectivity can be improved by determination of higher carbonyl compounds instead of the total carbonyls. HPLC is used to separate the DNPH derivatives of higher carbonyls from those of short-chain carbonyl compounds (62).

Apart from detection of total carbonyl content, the analysis of individual carbonyl compounds has gained popularity for following lipid oxidation. Hexanal, one of the major secondary products formed during the oxidation of linoleic and other  $\omega 6$  fatty acids, serves as a reliable indicator of lipid oxidation in foods rich in  $\omega 6$  fatty acids (7). A strong linear relationship was reported between hexanal content, sensory scores, and TBA values (63). Moreover, measurement of hexanal offers the advantage of analyzing a single, well-defined end product for antioxidant efficiency studies (9). Hexanal can be quantified by chromatography (64) or as the intensity of the carbonyl band by NIR spectroscopy (65). Nevertheless, these methods may require volatilization of hexanal, whereas hexanal volatilization may be hindered due to covalent or other types of binding between hexanal and proteins in foods and, thus, may affect accurate hexanal quantifications (66). More recently, an indirect enzyme-linked immunosorbent assay (ELISA) has been developed for monitoring lipid oxidation through quantification of hexanal-protein adducts, which are recognized by polyclonal or monoclonal antibodies (66).

Other carbonyl compounds, including propanal, pentanal, decadienal, etc., are also used for evaluating lipid oxidation in foods. For instance, propanal is a recommended indicator for lipid oxidation in foods that are high in  $\omega 3$  fatty acids, such as marine oils (67, 68). In general, it is essential to use appropriate indicators when assessing the oxidative deterioration of different food systems.

## 6.5. Oil Stability Index (OSI)

During lipid oxidation, volatile organic acids, mainly formic acid and acetic acid, are produced as secondary volatile oxidation products at high temperatures, simultaneously with hydroperoxides (20, 69). In addition, other secondary products, including alcohols and carbonyl compounds, can be further oxidized to carboxylic acids (20). The oil stability index (OSI) method measures the formation of volatile acids by monitoring the change in electrical conductivity when effluent from oxidizing oils is passed through water (12). The OSI value is defined as the point of maximal change of the rate of oxidation, attributed to the increase of conductivity

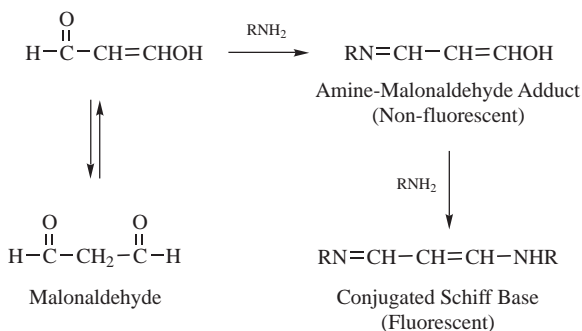
by the formation of volatile organic acids during lipid oxidation (70). However, this method requires a somewhat higher level of oxidation ( $PV > 100$ ) to obtain measurable results than other methods in which hydroperoxides are the most important products formed and detected (71). Therefore, to determine oil stability in the laboratory, especially for some oils that are stable under normal conditions, the oxidation process is accelerated by exposing oil samples to elevated temperatures in the presence of an excess amount of air or oxygen (72, 73). The OSI method differs from ambient storage conditions by using a flow of air and high temperatures to accelerate oxidation (71). The OSI is an automated development of the active-oxygen method (AOM), because both employ the principle of accelerated oxidation. Nevertheless, the OSI test measures the changes in conductivity caused by ionic volatile acids, whereas PV is determined in the AOM (7).

Two pieces of commercially available equipment, the Rancimat (Metrohm Ltd.) and the Oxidative Stability Instrument (Omnion Inc.), are employed for determining the OSI value. Rancimat is a rapid automated method, which agrees well with the AOM (71). In the Rancimat assay, a flow of air is bubbled through a heated oil, usually at  $100^{\circ}\text{C}$  or above. For marine oils, temperatures as low as  $80^{\circ}\text{C}$  are often used. Volatile compounds formed during accelerated oxidation are collected in distilled water, increasing the water conductivity. The change of conductivity is plotted automatically and the induction period of the oil or the time taken to reach a fixed level of conductivity is recorded (20, 74). The Rancimat assay enables continuous monitoring of the oxidation process. As reported by Farooq et al. (75), analysis by the Rancimat method is four to five times more rapid than that by the AOM. Excellent correlation between Rancimat and conjugated dienes has been found (72). However, the main shortcoming of this method is that only eight samples can be included in each batch. Another apparatus, the Oxidative Stability Instrument, operates on the same principle as the Rancimat, and has the capacity of simultaneously analyzing up to 24 samples (20). Various modifications have been proposed for assessing lipid oxidation by the OSI method. These include the use of auxiliary energies, such as microwaves to shorten the analysis time (72) and a combination of the OSI method with chromatography to obtain specific information about volatile products (76). The volatiles trapped during measurement by the Rancimat assay can be analyzed by headspace-GC (HS-GC) with FID and GC-MS for quantification of individual volatiles, thus improving the specificity of the assessment (76).

Although the OSI method is useful for quality control of oils, it is not recommended for measurement of antioxidant activity for certain reasons. The high temperatures used do not allow reliable predictions of antioxidant effectiveness at lower temperatures. Volatile antioxidants may be swept out of the oil by the air flow under test conditions, and also the oils are severely deteriorated when endpoint is reached (12).

## 6.6. Hydrocarbons and Fluorescence Assay

Formation of saturated hydrocarbons, especially short-chain ( $\text{C}_1\text{-C}_5$ ) hydrocarbons such as ethane, propane, and pentane, can be measured for monitoring lipid oxidation when aldehydes are either absent or undetectable (7, 15). Pentane content,



**Figure 5.** Reaction of lipid oxidation products such as malonaldehyde and amines.

determined by GC techniques, has been a useful parameter to assess rancidity of fats and oils as well as freeze-dried muscle foods (7, 15). Significant correlations existed between pentane levels and rancid odor scores (15).

It has been observed that the content of secondary oxidation products, such as malonaldehyde (MA), decreases with increased lipid oxidation, which can be explained by further reaction of MA with proteins. MA reacts with compounds containing primary amino groups (proteins, amino acids, DNA, phospholipids) to form fluorescent products (Figure 5) (37). A fluorescence assay has been successfully used to assess lipid oxidation in muscle foods and biological tissues.

In addition to MA, hydroperoxides and other aldehydes also react with amino compounds generating various fluorescent products with different excitation and emission maxima (37). Significant correlations existed between this method and the TBA value as well as oxygen absorption level, and appears to be a reliable

**TABLE 2. Summary of Methods for Analysis of Secondary Oxidation Products.**

Method	Compounds	Comments	Applications
TBA	TBARS, mainly malonaldehyde	Spectrometry technique It can be carried out on whole sample	All samples, especially fish oils
<i>p</i> -Anisidine	Aldehydes, mainly alkenals	Absorption at 350-nm Standard method	Fats and oils
Carbonyls	Total carbonyls or specific carbonyl compound formed	Spectrometry technique and HPLC for total or specific carbonyl compounds	Fats and oils
OSI method (Rancimat & Oxidative Stability Instrument)	Volatile organic acids	Monitoring changes in conductivity Rapid and automated	Fats and oils
Gas Chromatography	Volatile carbonyls and hydrocarbons	Direct headspace Rapid analysis	All samples

Adapted from (8).

indicator of oxidative deterioration in muscle foods, especially in freeze-dried products (37, 77).

Table 2 exhibits the summary of methods for analysis of secondary oxidation products.

## 7. MEASUREMENT OF FREE RADICALS

The initial steps of lipid oxidation involve chain reactions of free radicals as important short-lived intermediates. Oxidation level of fats and oils can be measured directly by detecting the formation of radicals. Methods based on the detection of radicals or on the tendency for the formation of radicals provide a good indication of initiation of lipid oxidation (78, 79).

Electron spin resonance (ESR), also referred to as electron paramagnetic resonance (EPR) spectroscopy, relies on the paramagnetic properties of the unpaired electrons in radicals and has been developed for assessing the formation of free radicals originating in the early stages of oxidation and the onset of primary oxidation (6, 78). The assay measures the absorption of microwave energy when a sample is placed in a varied magnetic field (7). Quantification of radical concentrations is complicated by comparison with stable paramagnetic compounds, such as transition metals and nitroxyl radicals (78). However, the short lifetimes and low steady-state concentration of the highly reactive lipid-derived radicals make it difficult to detect these radicals at concentrations lower than the minimum detectable concentration of  $10^{-9}$  M (78). To overcome this problem, various approaches have been used, including pulse radiolysis and UV photolysis, continuous flow systems and spin trapping, among which spin trapping has been the most widely employed procedure (9). Spin trapping technique allows the accumulation of detectable concentrations of longer-lived radicals by addition to samples of a spin trapping agent, which reacts with free radicals to form more stable spin adducts, but often at the expense of the ability to identify the original radical (6, 9, 78). Nitroso compounds and nitrones are the most common spin traps, both leading to nitroxyl type spin adducts, such as  $\alpha$ -phenyl-*tert*-butylnitron (PBN) adducts (Figure 6) (78).

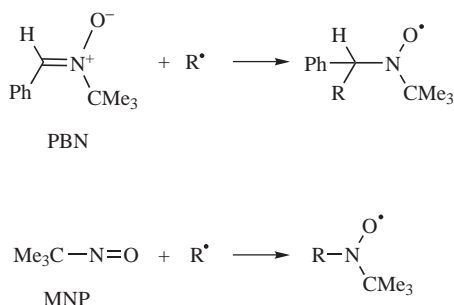


Figure 6. Formation of nitroxyl radical spin adducts.

ESR spectroscopy is of great value for the study of the early stages of lipid oxidation and prediction of oxidative stability of fats and oils. It has high sensitivity and allows mild conditions by applying significantly low temperatures and requires little sample preparation (6, 78, 80). Strong linear correlations were found between ESR and Rancimat and oxygen consumption analyses (6, 79). ESR has also been used for evaluation of antioxidant activity (81). Nevertheless, spin traps used in the ESR assay have been reported to exhibit widely differing trapping efficiencies for different radicals and show both pro-oxidant and antioxidant effects (9, 82, 83). Moreover, spin adducts can act as antioxidants, giving erroneous results of oxidative stability of samples (9). However, even with these limitations, the ESR spectroscopy is a suitable method for measuring lipid oxidation in foods and in biological tissues.

## 8. OTHER METHODS

### 8.1. Differential Scanning Calorimetry (DSC)

During lipid oxidation, fat or oil materials reveal a number of thermally induced transitions, such as the transfer of oxygen molecules to unsaturated fatty acids (exothermic process) (84). Therefore, thermal analysis can be applied in accelerated oil stability tests. The differential scanning calorimetry (DSC) technique, which is based on thermal release of oxidation reactions, has the potential as a nonchemical method for assessing oxidative stability of fats and oils, indicating the onset of advanced oxidation (termination) (6). It provides unique energy profile information, which specifically measures the temperature and heat flows associated with lipid oxidation as a function of time and temperature (85). The method uses isothermal or nonisothermal conditions and a flow of oxygen as purge gas, with a calorimeter measuring the heat flow into (endothermic) or out of (exothermic) an oil sample undergoing oxidation changes (6, 84). The oxidation curves of the sample are obtained with different heating time, and a dramatic increase for the evolved heat can be observed with the appearance of a sharp exothermic curve during initiation of oxidation. The endpoint is taken at the time where a rapid exothermic reaction between oil and oxygen occurs and induction period (IP) determined automatically by intersection of extrapolated baseline and tangent line (leading edge) of the exotherm (Figure 7) (6, 84). The DSC also measures oxidation onset temperature, the temperature at maximum reaction, and the ending temperature (84). The isothermal and nonisothermal DSC show good agreement, suggesting that both isothermal and nonisothermal DSC are suitable for oxidation studies of oils (86). The DSC technique has recently been reviewed by Tan et al. (84, 87).

The DSC is a sensitive, effective, and consistent method for characterization of the quality of oils at different stages of oxidation (20). It is simple and rapid, and it requires no solvent or chemical reagent. As reported by Hassel et al. (89), oils samples, which required 14 days via AOM, could be evaluated in less than 4 hours by DSC. Thus, DSC is a reliable alternative to current methods for monitoring lipid



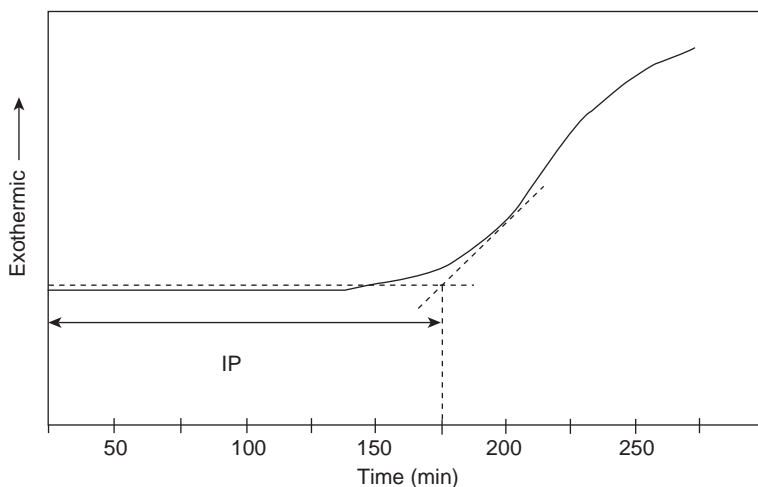


Figure 7. Determination of induction period (IP) by DSC.

oxidation (85). The results from DSC show excellent correlations with other accelerated methods and chemical analyses (6, 73, 85).

## 8.2. Nuclear Magnetic Resonance (NMR) Spectroscopy

High-resolution  $^1\text{H}$  NMR spectroscopy, in which hydrogen atoms (proton,  $^1\text{H}$ ) with various locations in the triacylglycerol (TAG) molecules are determined, has been used to evaluate oxidative deterioration of fats and oils (7). The principle of NMR is that hydrogen atoms in a strong magnetic field absorb energy, in the radiofrequency range, depending on their molecular environment, in which changes occur during the oxidation process (7). These changes may be monitored by NMR spectroscopy as a reflection of oxidation level of food lipids. The oil sample is dissolved in  $\text{CDCl}_3$  to avoid inference from solvent, and its NMR spectrum recorded, with tetramethylsilane (TMS) as an internal standard (7). The spectrum shows several groups of signals, corresponding to the hydrogen atoms in different locations in the TAG molecules (Figure 8). The total number of each of these differently located protons can be calculated, from which ratios of aliphatic to olefinic protons ( $R_{\text{ao}}$ ) and aliphatic to diallylmethylene protons ( $R_{\text{ad}}$ ) may be obtained (7). Both ratios increase steadily during lipid oxidation and may serve as an index of oxidative deterioration of oil samples. This method was reviewed by Guillen et al. (90). NMR spectroscopy has been used by many researchers, and the changes in  $R_{\text{ao}}$  and  $R_{\text{ad}}$  measured by NMR correlated well with Totox values (91, 92), conjugated diene values, and TBA values (93). In addition to  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and  $^{31}\text{P}$  NMR are also powerful tools to predict oxidative stability of oils (94–96).  $^{13}\text{C}$  NMR enables direct observation of carbon atoms. The selectivity and dispersion of  $^{13}\text{C}$  NMR spectra are very high (96).  $^{13}\text{C}$  NMR assesses lipid oxidation by monitoring the changes of carbon chains in TAG molecules, revealing the specific sites that oxidative degradation

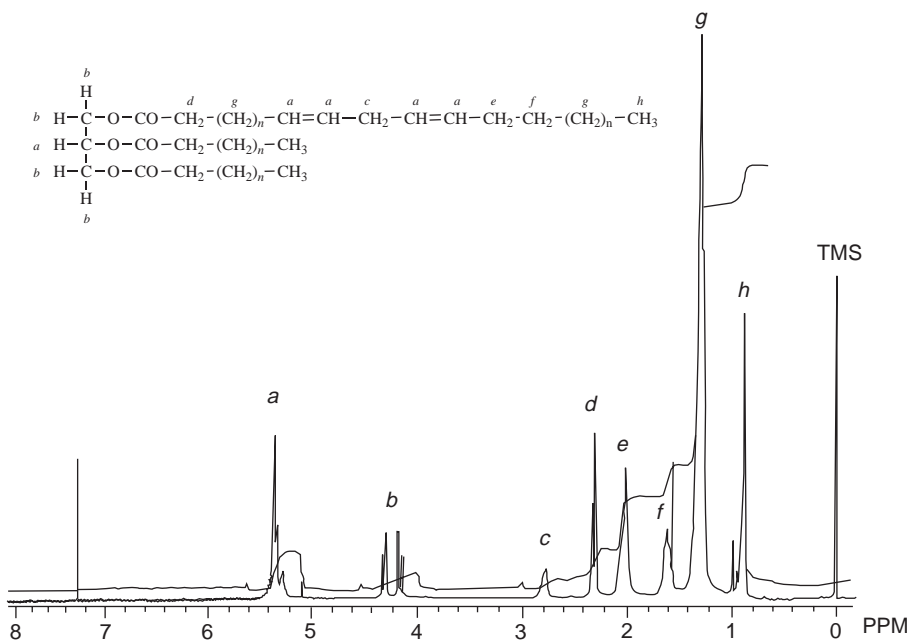


Figure 8.  $^1\text{H}$  NMR spectrum of oxidized canola oil.

occurs (94). However, because the abundance of the NMR active  $^{13}\text{C}$  nucleus isotope is only 1.12% of  $^{12}\text{C}$ , the sensitivity of  $^{13}\text{C}$  NMR is usually much lower than that of  $^1\text{H}$  NMR (96).

NMR spectroscopy is a rapid, nondestructive, and reliable technique for assessing lipid oxidation. It simultaneously measures both the primary and the secondary oxidative changes in oils, and provides specific information on oxidative regions in the TAG molecules. Thus, NMR spectroscopy is considered a more suitable means for estimating lipid oxidation than chemical determinations.

### 8.3. Sensory Evaluation

For the food industry, the detection of oxidative off-flavors by taste or smell is the main method of deciding when a lipid-containing food is no longer fit for consumption (12). Terminologies and methodologies have been developed for sensory evaluation of specific food products such as meats, peanuts, and vegetable oils (97). In the edible oil industry, the AOCS (American Oil Chemists' Society) Flavor Quality Scale (revised) with separate grading and flavor intensity has been employed for describing lipid oxidation (97), as summarized in Table 3. The descriptive analysis, including the detection and the description of both the qualitative and quantitative sensory aspects of a product, is performed by a trained panel, as the sensitivity to the off-flavors varies among different individuals (12, 97). The sensory induction period of the product can be determined.

**TABLE 3. A Partial List of Terms Used to Describe Oxidized Oil.**

Flavor-Related Terms	Process-Oriented Terms
Buttery	Hydrogenated
Nutty	Oxidized
Beany	Reverted
Grassy	Light-struck
Watermelon	Rancid
Painty	
Fishy	

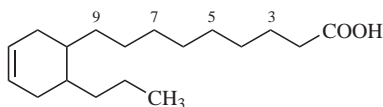
Adapted from (97).

Sensory evaluation of lipid oxidation has been conducted by many researchers (98–100). However, as a subjective method, the reproducibility of sensory analysis is generally considered worse than that of chemical or instrumental methods. More recently, use of an electronic nose to monitor the formation of volatile compounds associated with off-flavors from lipid oxidation has been proposed to supplement information from human sensory panels (101).

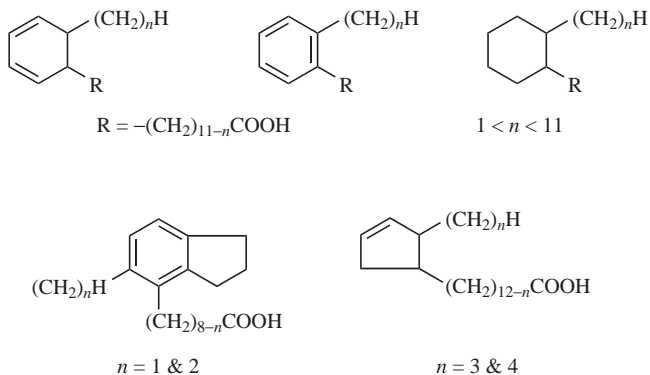
## 9. MEASUREMENT OF FRYING FAT DETERIORATION

Deep-fat frying is a popular method for food preparation, in which vegetable oils not only are used as a heat-exchange medium, but also contribute to the quality of fried products (7). However, lipid oxidation easily occurs at relatively high temperatures, producing a complex series of compounds that exerts undesirable effects on food flavor and quality (4). The measurement of lipid oxidation, therefore, is essential to determine its effect on food and oil quality, as well as the useful life of fats or oils subjected to frying. The oxidative changes in frying fats are characterized by a decrease in the total unsaturation of the fat with increases in the free fatty acid content, foaming, color, and viscosity as well as the content of polar compounds and polymeric material (4). Quality evaluation of frying fats, may be carried out in different ways. Physical methods estimate oxidative degradation by monitoring changes in physical properties of frying fats, such as molecular weight, specific gravity, smoke point, refractive index, chromatic parameter, viscosity, surface tension, and dielectric constant (4). Generally, rejection point of frying fat is established by sensory assessment. Chemical methods include the iodine value, saponification value, free fatty acid content, peroxide value, TBA value, or *p*-anisidine value, among others. PV is less useful because hydroperoxides decompose at about 150°C, and no accumulation of peroxides can be detected.

The extent of oxidation can also be assessed by the analysis of oxidized fatty acids by spectroscopic means such as IR and NMR techniques (102). Moreover, GC-MS for volatile profile analysis (103) and HPLC for determination of DNPH derivatives of nonvolatile higher carbonyl compounds (62) provide qualitative



Double bond may present on the positions of C4, C5, C7, C8, or C9.



**Figure 9.** Chemical structures of cyclic fatty acids formed during deep frying.

and quantitative evaluation of oxidation in frying fats. Cyclic fatty acids (Figure 9), which may contain hydroxy and keto groups, are formed during deep frying and can be measured by chromatography after derivatization (4, 7). Furthermore, determination of polar material in frying fats is a reliable approach for oil quality evaluation and is an official method in Europe. This method involves separation of fat into a polar and nonpolar fraction via silica gel chromatography. Nonpolar fat can be weighed and the total polar material calculated or determined directly by their elution from the silica gel column (4,7).

Routine analysis for frying fat deterioration has been reviewed by Gertz (104). Usually, more than two methods are required when using chemical analysis because no single group of compounds has been identified as a key indicator of oxidative degradation of frying fats.

## 10. METHODS FOR MEASURING ANTIOXIDANT ACTIVITY

A variety of natural and synthetic antioxidants are used in fat-containing foods in order to inhibit lipid oxidation with a wide range of efficiencies, depending on their properties, concentrations, and processing conditions. The need to measure antioxidant activity is well documented. Although numerous methods have been proposed for measurement of antioxidant activity, the essential features of any test are a suitable substrate, an oxidation initiator, and an appropriate measure of endpoint (9). Therefore, certain aspects should be taken into consideration when selecting a test for measuring antioxidant activity. These include the model food system used for

the test, and the means by which oxidation is accelerated and monitored (12). Normally, most assessments of antioxidant activity are performed in oil, or other model systems, giving sensible prediction for the activity in oil or water-in-oil emulsions, whereas the results may be misleading for oil-in-water emulsions (12). Furthermore, stripping of oils may be necessary in such evaluations because the endogenous antioxidants in nonstripped oils are found to enhance the oxidative stability of oils, thus giving rise to erroneous results in the efficiency of antioxidants under investigation (105–107). In addition to oils and fats, lipid substrates used for testing antioxidant activity could be fatty acids, fatty acid ethyl esters or triacylglycerols (9), and  $\beta$ -carotene (108–110). In some cases, such as radical scavenging methods, no substrate is used. Most test procedures involve initiators to accelerate oxidation. The combination of increased temperature and oxygen supply, addition of metal catalysts, and exposure of the reactants to light can reduce the oxidative stability by a large amount (9, 12). Nevertheless, the elevated temperature may bring about changes in the oxidation mechanism, thus causing difficulties in the prediction of

**TABLE 4. Methods of Expressing Results of Antioxidant Activity Tests.**

Method	Dimensions
Induction period	h, d
Time to reach a set level of oxidation (pre-induction period)	h, d
Rate of oxidation (pre-induction period)	$\text{mol kg}^{-1} \text{hr}^{-1}$ , $\text{gL}^{-1} \text{d}^{-1}$
Concentration to produce equivalent effect to reference antioxidant (pre-induction period)	$\text{mol kg}^{-1}$ , $\text{gL}^{-1}$
Concentration of ROOH functional group after set time period	mequiv. $\text{kg}^{-1}$
Concentration of oxidation product after set time period	$\text{mg kg}^{-1}$ (ppm w/w)
Scale reading after set time period	Absorbance, conductivity, etc.
Free stable radical quenching (DPPH)	Percentage inhibition
	$\text{EC}_{50}$ , concentration to decrease concentration of test free radical by 50%
	$\text{T}_{\text{EC}50}$ , time to decrease concentration of test free radical by 50%
Total radical-trapping antioxidant parameter (TRAP)	$\mu\text{mol peroxy radical deactivated L}^{-1}$
ABTS assay, phycoerythrin assay	TEAC (mM Trolox equivalent to 1-mM test substance)
Phycoerythrin assay	ORAC, oxygen radical absorbance capacity; $\mu\text{mol}$ of Trolox equivalents
FRAP assay	Absorbance of $\text{Fe}^{2+}$ complex at 593 nm produced by antioxidant reduction of corresponding tripyridyltriazine $\text{Fe}^{3+}$ complex
Metal chelating assay	Percentage of inhibition of ferrozine- $\text{Fe}^{2+}$ complex formation

NOTE: Also see Tables 1 and 2 for other tests applicable to antioxidant activity determination. Adapted from (9).

antioxidant activity at low temperatures as compared with those at high temperatures (9, 12). After the substrate is oxidized under standard conditions, the oxidation is monitored by chemical, instrumental, or sensory methods. An appropriate measure of endpoint is essential for assessing antioxidant activity. Analytical strategies for endpoint determination include measurement at a fixed time point, measurement of reaction rate, lag phase measurement, and integrated rate measurement (9). The resulting antioxidant activity is expressed using a wide range of parameters (Table 4).

Approaches proposed for testing antioxidant activity include measuring of the current state of oil samples, as discussed above, and radical scavenging assays, which are gaining popularity in the evaluation of antioxidant activity. Radical scavenging methods measure the relative abilities of antioxidants to scavenge synthetic radicals or natural in comparison with the antioxidant potency of a standard antioxidant compound (111). Trolox (6-hydroxy-2,5,7,8-tetramethylchroma-2-carboxylic acid), ascorbic acid, and quercetin are among the standard antioxidants frequently used. The most commonly used synthetic radicals are DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (3-ethylbenzthiazoline-sulfonic acid) radicals. DPPH test (112–116) and ABTS assay (117–122) are simple, rapid, and involve no substrate. However, it has been suggested that these artificial substrate-free methods do not always adequately mimic the processes in food systems, which sometimes makes them less valuable for predicting the effectiveness of the antioxidant in foods (9).

Other measurements of antioxidant activity include FRAP (ferric reducing-antioxidant power) (123–126), TRAP (total radical-trapping antioxidant parameter) (123, 127), phycoerythrin assay (128, 129), and test of metal chelating capacity (130, 131), among others. Reviews on methods for testing antioxidant activity have been published (9, 12).

## 11. CONCLUSIONS AND RECOMMENDATIONS

Lipid oxidation may be assessed in many ways, among which changes in the initial reactants and formation of oxidation products are most commonly assessed. Meanwhile, sensory analysis assesses both the subjective and, in some cases, objective measurements of oxidative changes in foods. Each method shows both advantages and disadvantages, thus it is important to select the most adequate method, depending on the system under investigation and the state of oxidation itself. The use of two or more methods assessing both primary and secondary oxidation products is highly recommended.

## REFERENCES

1. F. Shahidi, *Nahrung*, **44**, 158–163 (2000).
2. J. R. Vercellotti, A. J. St. Angelo, and A. M. Spanier, in A. J. St. Angelo, ed., *Lipid Oxidation in Food*, ACS Symposium Series 500, American Chemical Society, Washington, D.C., 1992, pp. 1–11.

3. M. Gordon, in J. Pokorny, N. Yanishlieva, and M. Gordon, eds., *Antioxidants in Food: Practical Applications*, Woodhead Publishing, Ltd., Cambridge, England, 2001, pp. 7–21.
4. E. G. Perkins, in A. J. St. Angelo, ed., *Lipid Oxidation in Food*, American Chemical Society, Washington, D.C., 1992, pp. 310–321.
5. A. Kamal-Eldin, M. Makinen, and A. M. Lampi, in A. Kamal-Eldin, ed., *Lipid Oxidation Pathways*, AOCS Press, Champaign, Illinois, 2003, pp. 1–36.
6. J. Velasco, M. L. Anderson, and L. H. Skibsted, *Food Chem.*, **85**, 623–632 (2004).
7. F. Shahidi and U. N. Wanasundara, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry, Nutrition and Biotechnology*, Marcel Dekker, Inc., New York, 2002, pp. 465–487.
8. M. C. Dobarganes and J. Velasco, *Eur. J. Lipid Sci. Technol.*, **104**, 420–428 (2002).
9. M. Antolovich, P. D. Prenzler, E. Patsalides, S. McDonald, and K. Robards, *Analyst*, **127**, 183–198 (2002).
10. I. Niklova, S. Schmidt, K. Habalova, and S. Sekretar, *Eur. J. Lipid Sci. Technol.*, **103**, 299–306 (2001).
11. J. Velasco and C. Dobarganes, *Eur. J. Lipid Sci. Technol.*, **104**, 661–676 (2002).
12. M. Gordon, in J. Pokorny, N. Yanishlieva, and M. Gordon, eds., *Antioxidants in Food: Practical Applications*, Woodhead Publishing, Ltd., Cambridge, England, 2001, pp. 71–84.
13. C. Genot, G. Kansci, and M. Laroche, *Sciences des Aliments.*, **14**, 673–682 (1994).
14. H. J. Chung, A. S. Colakoglu, and D. B. Min, *J. Food Sci.*, **69**, 83–88 (2004).
15. S. L. Melton, *Food Technol.*, **37**, 105–111 (1983).
16. G. O. Fruhwirth, T. Wenzl, R. El-Toukhy, F. S. Wagner, and A. Hermetter, *Eur. J. Lipid Sci. Technol.*, **105**, 266–274 (2003).
17. B. J. F. Hudson, in J. C. Allen and J. Hamilton, eds., *Rancidity of Foods*, Applied Science Publishers, London, England, 1983, pp. 47–58.
18. A. Riuz, M. J. Ayora-Canada, and B. Lendl, *Analyst*, **126**, 242–246 (2001).
19. G. Yildiz, R. L. Wehling, and S. L. Cuppett, *J. Amer. Oil Chem. Soc.*, **80**, 103–107 (2003).
20. A. Kiritsakis, A. Kanavouras, and K. Kiritsakis, *Eur. J. Lipid Sci. Technol.*, **104**, 628–638 (2002).
21. Determination of Peroxide Value, in *IUPAC: Standard Methods for the Analysis of Oils, Fats and Derivatives*, *IUPAC Standard Method 2.501*, International Union of Pure and Applied Chemistry, Pergamon, Oxford, England, 1992.
22. D. L. Berner, *INFORM*, **1**, 884–886 (1996).
23. S. Eymard and C. Genot, *Eur. J. Lipid Sci. Technol.*, **105**, 497–501 (2003).
24. T. Asakawa and S. Matsushita, *J. Amer. Oil Chem. Soc.*, **55**, 691–620 (1978).
25. S. Hara and Y. Totani, *J. Amer. Oil Chem. Soc.*, **65**, 1948–1950 (1988).
26. M. Hicks and J. M. Gebicki, *Anal. Biochem.*, **99**, 249–253 (1979).
27. J. M. Gebicki and J. Guille, *Anal. Biochem.*, **176**, 360–364 (1989).
28. Z. Y. Jiang, A. C. S. Woollard, and S. P. Wolff, *Lipids*, **26**, 853–856 (1991).
29. S. P. Wolff, *Methods Enzymol.*, **233**, 182–189 (1994).
30. J. M. DeLong, R. K. Prange, D. M. Hodges, C. F. Forney, M. C. Bishop, and M. Quillian, *J. Agric. Food Chem.*, **50**, 248–254 (2002).

31. J. Sedman, F. R. van de Voort, and A. A. Ismail, in R. E. McDonald and M. M. Mossoba, eds., *New Techniques and Applications in Lipid Analysis*, AOCS Press, Champaign, Illinois, 1997, pp. 283–324.
32. J. Dong, K. Ma, F. R. van de Voort, and A. A. Ismail, *J. AOAC Int.*, **80**, 345–352 (1997).
33. T. A. Russin, F. R. van de Voort, and J. Sedman, *J. Amer. Oil Chem. Soc.*, **80**, 635–641 (2003).
34. B. Innawong, P. Mallikarjunan, J. Irudayaraj, and J. E. Marcy, *Lebensm. Wiss. U. Technol.*, **37**, 23–28 (2004).
35. T. Miyazawa, K. Fujimoto, and T. Kandeda, *Agric. Biol. Chem.*, **51**, 2569–2573 (1987).
36. A. M. Jimenez and M. J. Navas, *Grasas y Aceites*, **53**, 64–75 (2002).
37. J. P. Wold and M. Mielnik, *J. Food Sci.*, **65**, 87–95 (2000).
38. C. Stauffer, in *Fats & Oils*, Eagan Press, St. Paul, Minnesota, 1996, pp. 15–27.
39. F. Shahidi, U. Wanasundara, and N. Brunet, *Food Res. Int.*, **27**, 555–562 (1994).
40. U. N. Wanasundara, F. Shahidi, and C. R. Jablonski, *Food Chem.*, **52**, 249–253 (1995).
41. E. Kishida, S. Tokumaru, Y. Ishitani, M. Yamamoto, M. Oribe, H. Iguchi, and S. Kojo, *J. Agric. Food Chem.*, **41**, 1598–1600 (1993).
42. S. Cesa, *J. Agric. Food Chem.*, **52**, 2119–2122 (2004).
43. D. Jardine, M. Antolovich, P. D. Prenzler, and K. Robards, *J. Agric. Food Chem.*, **50**, 1720–1724 (2002).
44. A. de las Heras, A. Schoch, M. Gibis, and A. Fischer, *Eur. Food Res. Technol.*, **217**, 180–184 (2003).
45. D. V. Hoyland and A. J. Taylor, *Food Chem.*, **40**, 271–291 (1991).
46. B. G. Tarladgis, B. M. Watts, M. T. Younathan, and L. Dugan, *J. Amer. Oil Chem. Soc.*, **37**, 44–48 (1960).
47. F. Shahidi and C. Hong, *J. Food Biochem.*, **15**, 97–105 (1991).
48. J. Pikul, D. E. Leszczynski, and F. A. Kummerow, *J. Agric. Food Chem.*, **37**, 1309–1313 (1989).
49. C. Wang, L. Zhu, and M. S. Brewer, in 1996 IFT annual meeting: Book of Abstracts, pp. 161.
50. R. Guillen-Sans and M. Guzman-Chozas, *Crit. Rev. Food Sci. Nutr.*, **38**, 315–330 (1998).
51. Q. Sun, C. Faustman, A. Senecal, A. L. Wilkinson, and H. Furr, *Meat Sci.*, **57**, 55–60 (2001).
52. F. Shahidi, L. J. Rubin, L. L. Diosady, and D. F. Wood, *J. Food Sci.*, **55**, 274–275 (1985).
53. R. Marcuse and J. Pokorny, *Fett. Wiss. Technol.*, **96**, 185–187 (1994).
54. H. de A. Gomes, E. N. da Silva, M. R. Lopes do Nascimento, and H. T. Fukuma, *Food Chem.*, **80**, 433–437 (2003).
55. F. Doleschall, Z. Kemeny, K. Recseg, and K. Kovari, *Eur. J. Lipid Sci. Technol.*, **104**, 14–18 (2002).
56. G. H. van der Merwe, L. M. du Plessis, and J. R. N. Taylor, *J. Sci. Food Agric.*, **84**, 52–58 (2003).
57. G. R. List, C. D. Evans, W. F. Kwolek, K. Warner, and B. K. Boundy, *J. Amer. Oil Chem. Soc.*, **51**, 17–21 (1974).
58. M. D. Guillen and N. Cabo, *Food Chem.*, **77**, 503–510 (2002).



59. P. J. White, in K. Warner and N. A. M. Eskin, eds., *Methods to Assess Quality and Stability of Oils and Fat-containing Foods*, AOCS Press, Champaign, Illinois, 1995, pp. 159–178.
60. U. N. Wanasundara and F. Shahidi, *J. Food Lipids*, **2**, 73–86 (1995).
61. T. Chiba, M. Takazawa, and K. Fujimoto, *J. Amer. Oil Chem. Soc.*, **66**, 1588–1592 (1989).
62. E. Schulte, *Anal. Bioanal. Chem.*, **372**, 644–648 (2002).
63. F. Shahidi and R. B. Pegg, *J. Food Lipids*, **1**, 177–186 (1994).
64. C. F. Goodridge, R. M. Beaudry, J. J. Pestka, and D. M. Smith, *J. Agric. Food Chem.*, **51**, 4185–4190 (2003).
65. H. Z. Zhang and T. C. Lee, IFT Annual Meeting, 1995, pp. 161.
66. C. F. Goodridge, R. M. Beaudry, J. J. Pestka, and D. M. Smith, *J. Agric. Food Chem.*, **51**, 7533–7539 (2003).
67. U. N. Wanasundara and F. Shahidi, Paper presented at the Canadian Section of American Oil Chemists' Society's Annual Meeting, Guelph, Ontario, Canada, November 15–16, 1995.
68. F. Shahidi and S. A. Spurvey, *J. Food Lipids*, **3**, 13–25 (1996).
69. K. Schwarz, G. Bertelsen, L. R. Nissen, P. T. Gardner, M. I. Heinonen, A. Hopia, T. Huynh-Ba, P. Lambelet, D. McPhail, L. H. Skibsted, and L. Tijburg, *Eur. Food Res. Technol.*, **212**, 319–328 (2001).
70. K. Miura, H. Kikuzaki, and N. Nakatani, *J. Agric. Food Chem.*, **50**, 1845–1851 (2002).
71. S. de la Presa-Owens, M. C. Lopez-Sabater, and M. Rivero-Urgell, *J. Agric. Food Chem.*, **43**, 2879–2882 (1995).
72. M. P. Canizares-Macias, J. A. Garcia-Mesa, and M. D. Luque de Castro, *Anal. Bioanal. Chem.*, **378**, 479–483 (2004).
73. C. P. Tan, Y. B. Che-Man, J. Selamat, and M. S. A. Yusoff, *Food Chem.*, **76**, 385–389 (2002).
74. R. Aparicio, L. Roda, M. A. Albi, and F. Gutierrez, *J. Agric. Food Chem.*, **47**, 4150–4155 (1999).
75. A. Faroop, M. I. Bhangar, and T. G. Kazi, *J. Amer. Oil Chem. Soc.*, **80**, 151–155 (2003).
76. L. C. Boyd, V. C. Nwosu, C. L. Young, and L. MacMillian, *J. Food Lipids*, **5**, 269–282 (1998).
77. A. L. Wilkinson, Q. Sun, A. Senecal, and C. Faustman, *J. Food Sci.*, **66**, 20–24 (2001).
78. M. L. Andersen and L. H. Skibsted, *Eur. J. Lipid Sci. Technol.*, **104**, 65–68 (2002).
79. C. U. Carlsen, M. L. Andersen, and L. H. Skibsted, *Eur. Food Res. Technol.*, **213**, 170–173 (2001).
80. M. K. Thomsen, D. Kristensen, and L. H. Skibsted, *J. Amer. Oil Chem. Soc.*, **77**, 725–730 (2000).
81. L. R. Nissen, Tuong-Huynh-Ba, M. A. Petersen, G. Bertelsen, and L. H. Skibsted, *Food Chem.*, **79**, 387–394 (2002).
82. B. Halliwell and J. M. C. Gutteridge, in *Free Radicals in Biology and Medicine*, Oxford University Press, New York, 1999.
83. D. M. Lee, *Methods Enzymol.*, **234**, 513–523 (1994).
84. C. P. Tan and Y. B. Che Man, *Trends Food Sci. Technol.*, **13**, 312–318 (2002).
85. C. P. Tan and Y. B. Che Man, *Food Chem.*, **67**, 177–184 (1999).

86. G. Litwinienko, A. Daniluk, and T. Kasprzycka-Guttman, *J. Amer. Oil Chem. Soc.*, **76**, 655–657 (1999).
87. C. P. Tan and Y. B. Che Man, *Lipid Technol.*, **14**, 112–115 (2002).
88. B. Kowalski, K. Ratusz, D. Kowalska, and W. Bekas, *Eur. J. Lipid Sci. Technol.*, **106**, 165–169 (2004).
89. R. L. Hassel, *J. Amer. Oil Chem. Soc.*, **53**, 179–181 (1976).
90. M. D. Guillen and A. Ruiz, *Trends Food Sci. Technol.*, **12**, 328–338 (2001).
91. F. Shahidi, U. Wanasundara, and N. Brunet, *Food Res. Int.*, **27**, 555–562 (1994).
92. U. Wanasundara and F. Shahidi, *J. Food Lipids*, **1**, 15–24 (1993).
93. S. P. J. Namal-Senanayake and F. Shahidi, *J. Food Lipids*, **6**, 195–203 (1999).
94. I. Medina, R. Sacchi, I. Giudicianni, and S. Aubourg, *J. Amer. Oil Chem. Soc.*, **75**, 147–154 (1998).
95. F. J. Hidalgo, G. Gomez, J. L. Navarro, and R. Zamora, *J. Agric. Food Chem.*, **50**, 5825–5831 (2002).
96. B. W. L. Diehl, in R. J. Hamilton, ed., *Lipid Analysis in Oils and Fats*, Blackie Academic & Professional, London, 1998, pp. 87–135.
97. G. V. Cinille and C. A. Dus, in A. J. St. Angelo ed., *Lipid Oxidation in Food*, American Chemical Society, Washington, D.C., 1992, pp. 279–289.
98. C. J. Broadbent and O. A. Pike, *J. Amer. Oil Chem. Soc.*, **80**, 59–63 (2003).
99. E. A. Coppin and O. A. Pike, *J. Amer. Oil Chem. Soc.*, **78**, 13–18 (2001).
100. M. Timm-Heinrich, X. Xu, N. S. Nielsen, and C. Jacobsen, *Eur. J. Lipid Sci. Technol.*, **105**, 436–448 (2003).
101. N. Shen, S. Moizuddin, L. Wilson, S. Duvick, P. White, and L. Pollak, *J. Amer. Oil Chem. Soc.*, **78**, 937–940 (2001).
102. S. Khatoon and A. G. G. Krishna, *J. Food Lipids*, **5**, 247–267 (1998).
103. V. Rosales and J. de D, *Dissertation Abstracts International, B.*, **57**, 7290 (1997).
104. C. Gertz, *Lipid Technol.*, **13**, 44–47 (2001).
105. T. Keceli and M. H. Gordon, *J. Sci. Food Agric.*, **81**, 1391–1396 (2001).
106. M. A. Khan and F. Shahidi, *J. Amer. Oil Chem. Soc.*, **77**, 963–968 (2000).
107. V. Povilaityte, M. E. Cuvelier, and C. Berset, *J. Food Lipids*, **8**, 45–64 (2001).
108. F. Shahidi, C. Desilva, and R. Amarowicz, *J. Amer. Oil Chem. Soc.*, **80**, 443–450 (2003).
109. F. Shahidi and R. Amarowicz, *J. Amer. Oil Chem. Soc.*, **73**, 1197–1199 (1996).
110. R. Amarowicz, M. Naczka, and F. Shahidi, *J. Amer. Oil Chem. Soc.*, **77**, 957–961 (2000).
111. O. A. Zaporozhets, O. A. Krushynska, N. A. Lipkovska, and V. N. Barvinchenko, *J. Agric. Food Chem.*, **52**, 21–25 (2004).
112. D. Bandoniene, M. Murkovic, W. Pfannhauser, P. R. Venskutonis, and D. Gruzdiene, *Eur. Food Res. Technol.*, **214**, 143–147 (2002).
113. N. Nenadis and M. Tsimidou, *J. Amer. Oil Chem. Soc.*, **79**, 1191–1195 (2002).
114. H. Matsuura, H. Chiji, C. Asakawa, M. Amano, T. Yoshihara, and J. Mizutani, *Biosci. Biotechnol. Biochem.*, **67**, 2311–2316 (2003).
115. P. J. Park, F. Shahidi, and Y. J. Jeon, *J. Food Lipids*, **11**, 15–28 (2004).
116. R. Amarowicz, B. Raab, and F. Shahidi, *J. Food Lipids*, **10**, 51–62 (2003).

117. A. Podsedek, D. Sosnowska, and B. Anders, *Eur. Food Res. Technol.*, **217**, 296–300 (2003).
118. A. M. Alonso, D. A. Guillen, and C. G. Barroso, *Eur. Food Res. Technol.*, **216**, 445–448 (2003).
119. N. J. Miller and C. Rice-Evan, *Redox Rep.*, **2**, 161–171 (1996).
120. C. E. Rice-Evans and N. J. Miller, *Methods Enzymol.*, **234**, 279 (1994).
121. L. Bramati, F. Aquilano, and P. Pietta, *J. Agric. Food Chem.*, **51**, 7472–7474 (2003).
122. N. J. Miller and C. A. Rice-Evans, *Free Rad. Res.*, **26**, 195–199 (1997).
123. N. Pellegrini, M. Serafini, B. Colobi, D. del Rio, S. Salvatore, M. Bianchi, and F. Brighenti, *J. Nutr.*, **133**, 2812–2819 (2003).
124. I. E. F. Benzie and J. J. Strain, *Anal. Biochem.*, **239**, 70 (1996).
125. I. E. F. Benzie and J. J. Strain, *Methods Enzymol.*, **299**, 15 (1999).
126. J. Chen, H. Lindmark-Mansson, L. Gorton, and B. Akesson, *Int. Dairy J.*, **13**, 927–935 (2003).
127. A. Ghiselli, M. Serafini, G. Maiani, E. Azzini, and A. Fero-Luzzi, *Free Rad. Biol. Med.*, **18**, 29–36 (1995).
128. O. Boxin, M. Hampsch-Woodill, and R. L. Prior, *J. Agric. Food Chem.*, **49**, 4619–4626 (2001).
129. S. McDonald, P. D. Prenzler, M. Antolovich, and K. Robards, *Food Chem.*, **73**, 73–84 (2001).
130. S. N. El and S. Karakaya, *Int. J. Food Sci. Nutr.*, **55**, 67–74 (2004).
131. M. Oktay, I. Gulcin, and O. I. Kufrevioglu, *Lebensm. Wiss. U. Technol.*, **36**, 263–271 (2003).

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## *Flavor Components of Fats and Oils*

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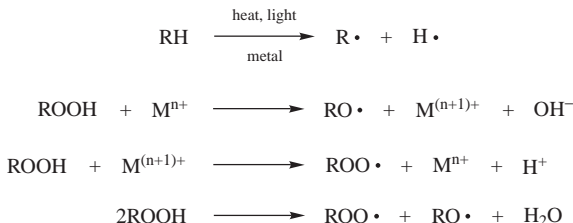
Most of the flavor compounds in fats and oils are produced by the reaction of oxygen with unsaturated fatty acids in triacylglycerols or polar lipids. On the other hand, some flavor compounds such as those present in cocoa butter, roasted sesame oil, or roasted peanut oil are generated by the interaction of reducing sugars with amino compounds during thermal processing.

The development of objectionable flavor compounds by oxidation has significant detrimental effects on consumer acceptability of edible oils. In the last four decades, much progress has been made in the chemistry of volatile products of lipid oxidation, mainly as a result of advances in separation techniques and analytical methodology, particularly gas chromatography-mass spectrometry.

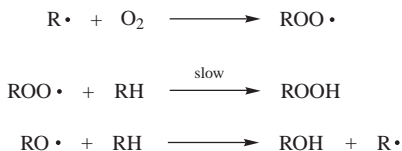
### **1. FREE RADICAL AUTOXIDATION OF LIPIDS**

The reaction of unsaturated lipids with oxygen to form hydroperoxides is generally a free radical process involving three basic steps of initiation, propagation, and termination (1, 2).

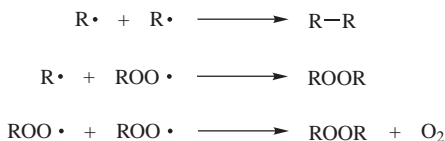
Initiation:



Propagation:



Termination:



RH, R•, RO•, ROO•, ROOH, and M represent an unsaturated fatty acid or ester with H attached to the allylic carbon atom, alkyl radical, alkoxy radical, peroxy radical, hydroperoxide, and transition metal, respectively.

The initiation reaction is the hemolytic abstraction of hydrogen to form a carbon-centered alkyl radical in the presence of an initiator. Under normal oxygen pressure, the alkyl radical reacts rapidly with oxygen to form the peroxy radical, which in turn reacts with more unsaturated lipids to form hydroperoxides. The lipid-free radical thus formed can further react with oxygen to form a peroxy radical. Hence, the autoxidation is a free radical chain reaction. Because the rate of reaction between the alkyl radical and oxygen is fast, most of the free radicals are in the form of the peroxy radical. Consequently, the major termination takes place via the interaction between two peroxy radicals.

The rate of autoxidation increases with the degree of unsaturation. In neat systems without adding initiators, linoleate having two double bonds was 40 times more reactive than oleate, which has only one double bond, linolenate having three double bonds was 2.4 times more reactive than linoleate, and arachidonate having four double bonds was 2 times more reactive than linolenate (2, 3).

## 2. HYDROPEROXIDES OF FATTY ACIDS OR THEIR ESTERS

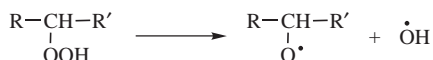
It is well known that the free radical mechanism of hydroperoxide formation involves the abstraction of a hydrogen atom from the  $\alpha$ -methylene group of a lipid

molecule. This result is favored because of the formation of a very stable allyl radical in which the electrons are localized over either three carbon atoms such as in the case of oleate or five carbon atoms such as in the case of linoleate and other polyunsaturated fatty esters. The mechanisms for the formation of isomeric hydroperoxides by autoxidation have been reviewed extensively (4, 5).

For oleate, the hydrogen abstraction on C-8 and C-11 produces two allylic radicals. These intermediates react with oxygen to produce a mixture of 8-, 9-, 10-, and 11-allylic hydroperoxides. Autoxidation of linoleate involves hydrogen abstraction on the doubly reactive allylic C-11, with the formation of a pentadienyl radical. The intermediate radical reacts with oxygen to produce a mixture of conjugated 9- and 13-diene hydroperoxides. In the case of linolenate in which there are two separate 1,4-diene systems, hydrogen abstraction will take place on the two methylene groups, C-11 and C-14. These intermediate free radicals react with oxygen to form conjugated dienes with hydroperoxides on C-9 and C-13, or C-12 and C-16, with the third double bond remaining unaffected.

## 2.1. Decomposition of Hydroperoxides

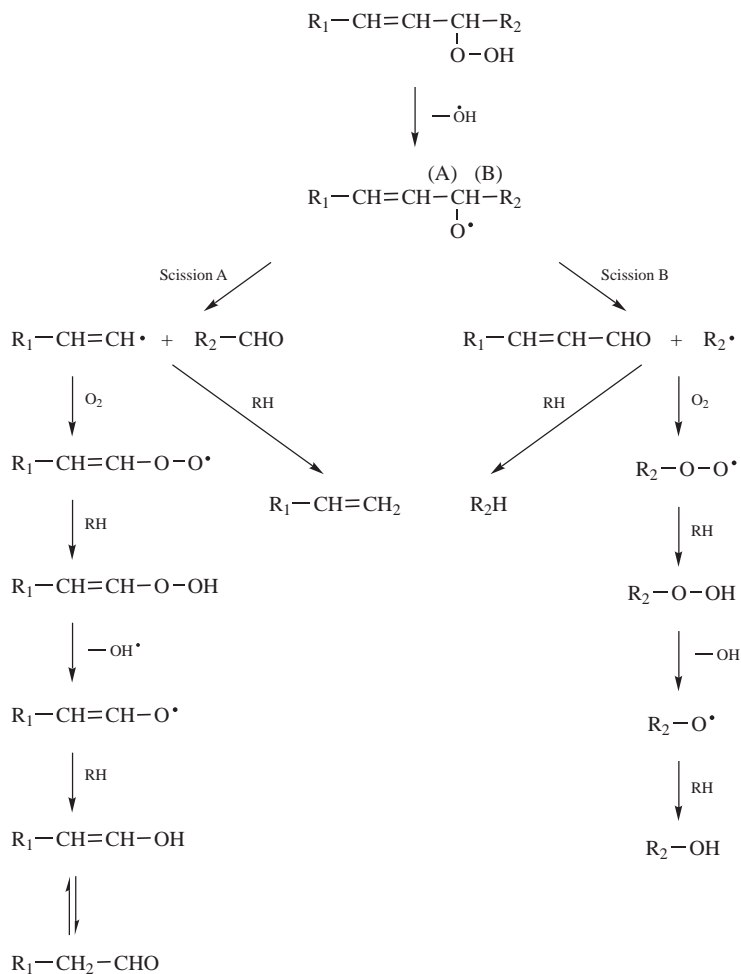
Hydroperoxides of unsaturated fatty acids formed by autoxidation are very unstable and break down into a wide variety of volatile flavor compounds as well as nonvolatile products. It is widely accepted that hydroperoxide decomposition involves homolytic cleavage of the -OOH group, giving rise to an alkoxy radical and a hydroxy radical (5).



The alkoxy radical undergoes  $\beta$ -scission on the C—C bond, with the formation of an aldehyde and alkyl or vinyl radical. A general reaction scheme with the formation of volatile aldehyde, alkene, and alcohol is illustrated in Figure 1 (6).

**2.1.1. Aldehydes** Of the volatiles produced by the breakdown of the alkoxy radicals, aldehydes are the most significant flavor compounds. Aldehydes can be produced by scission of the lipid molecules on either side of the radical. The products formed by these scission reactions depend on the fatty acids present, the hydroperoxide isomers formed, and the stability of the decomposition products. Temperature, time of heating, and degree of autoxidation are variables that affect thermal oxidation (7).

Some volatile aldehydes formed by autoxidation of unsaturated fatty acids are listed in Table 1. The aromas of aldehydes are generally described as green, painty, metallic, beany, and rancid, and they are often responsible for the undesirable flavors in fats and oils. Hexanal has long been used as an index of oxidative deterioration in foods. Some aldehydes, particularly the unsaturated aldehydes, are very potent flavor compounds. Table 2 lists aroma characteristics of some common aldehydes found in fats and oils (8).



**Figure 1.** General reaction pathway for the hemolytic cleavage of hydroperoxides of unsaturated fats (6).

Hexanal and 2,4-decadienal are the primary oxidation products of linoleate. The autoxidation of linoleate generates 9- and 13-hydroperoxides of linoleate. Cleavage of 13-hydroperoxide will lead to hexanal and breakdown of 9-hydroperoxide will lead to 2,4-decadienal (9). Subsequent moisture-mediated retro-aldol reaction of 2,4-decadienal will produce 2-octenal, hexanal, and acetaldehyde (10). 2,4-Decadienal is known to be one of the most important flavor contributors to deep-fat fried foods (11).

2,4-Decadienal can undergo further oxidation to produce *trans*-epoxy-*trans*-decenal. This compound was recently characterized as one of the most potent odorants of soybean oil stored in the dark and has a low odor threshold of approximately 1.5 pg/L (air) (12).

**TABLE 1. Some Volatile Aldehydes Obtained from Autoxidation of Unsaturated Fatty Acids (6).**

Fatty Acid	Monohydroperoxides	Aldehydes Formed	
Oleate	8-OOH	2-Undecenal Decanal	
	9-OOH	2-Decenal Nonanal	
	10-OOH 11-OOH	Nonanal Octanal	
Linoleate	9-OOH	2,4-Decadienal 3-Nonenal	
	13-OOH	Hexanal	
Linolenate	9-OOH	2,4,7-Decatrienal 3,6-Nonadienal	
	12-OOH	2,4-Heptadienal 3-Hexenal	
	13-OOH	3-Hexenal	
	16-OOH	Propanal	
Arachidonate	5-OOH	2,4,7,10-Hexadecatetraenal 3,6,9-Pentadecatrienal	
	8-OOH	2,4,7-Tridecatrienal 3,6-Dodecadienal	
	9-OOH	3,6-Dodecadienal	
	11-OOH	2,4-Decadienal 3-Nonenal	
	12-OOH	3-Nonenal	
	15-OOH	Hexanal	
	Eicosapentaenoate	5-OOH	2,4,7,10,13-Hexadecapentaenal 3,6,9,12-Pentadecatetraenal
		8-OOH	2,4,7,10-Tridecatetraenal 3,6,9-Dodecatrienal
9-OOH		3,6,9-Dodecatrienal	
11-OOH		2,4,7-Decatrienal 3,6-Nonadienal	
12-OOH		3,6-Nonadienal	
14-OOH		2,4-Heptadienal 3-Hexenal	
15-OOH 18-OOH		3-Hexenal Propanal	

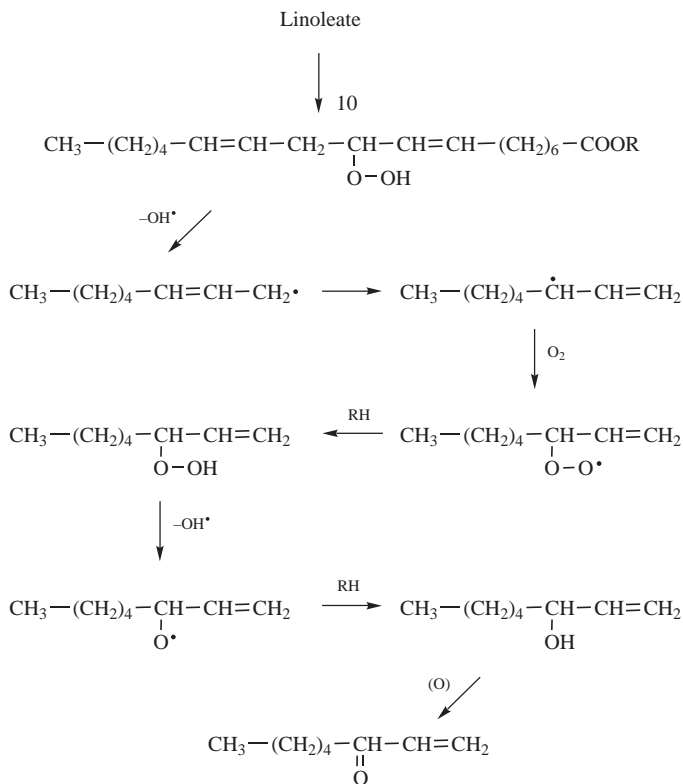
**2.1.2. Ketones** Aliphatic ketones formed by autoxidation of lipids also contribute to the flavor of oils and food products. For example, Guth and Grosch (13) identified 1-octen-3-one as one of the odor-active compounds in reverted soybean oil. This compound was described as metallic and mushroom-like. The reaction pathway for the formation of 1-octen-3-one from the linoleate-10-hydroperoxide via the  $\beta$ -scission route is illustrated in Figure 2. 10-Hydroperoxide of linoleate is not the usual hydroperoxide formed by autoxidation of linoleate; however, it is one of the major hydroperoxides formed by the photosensitized oxidation (singlet oxygen reaction) of linoleate (14).



**TABLE 2. Sensory Characteristics of Selected Aldehydes.**

Volatile Aldehyde	Odor Characteristics
Pentanal	Woody, bitter, oil
Hexanal	Fatty, powerful, oily, grassy
Heptanal	Oily, fatty, heavy, woody, penetrating, nutty
Octanal	Fatty, sharp, citrus
Nonanal	Fatty, waxy, painty, citrus
Decanal	Penetrating, sweet, waxy, painty
Undecanal	Fatty, tallowy
2-Hexenal	Sweet, fragrant, almond. Fruity, green, leafy
2-Heptenal	Oxidized, tallowy, pungent
2-Octenal	Brown beans, herbaceous, spicy
2-Nonenal	Penetrating, Fatty, waxy, nutty, rancid
2-Decenal	Painty, Fishy, fatty
2-Undecenal	Fresh, fruity, orange-peel like
2,4-Heptadienal	Fatty, rancid, hazelnut-like
2,4-Decadienal	Powerful, fatty, citrus

Modified from Morales et al. (8).



**Figure 2.** Mechanism for the formation of 1-octen-3-one from 10-hydroperoxide of linoleate.

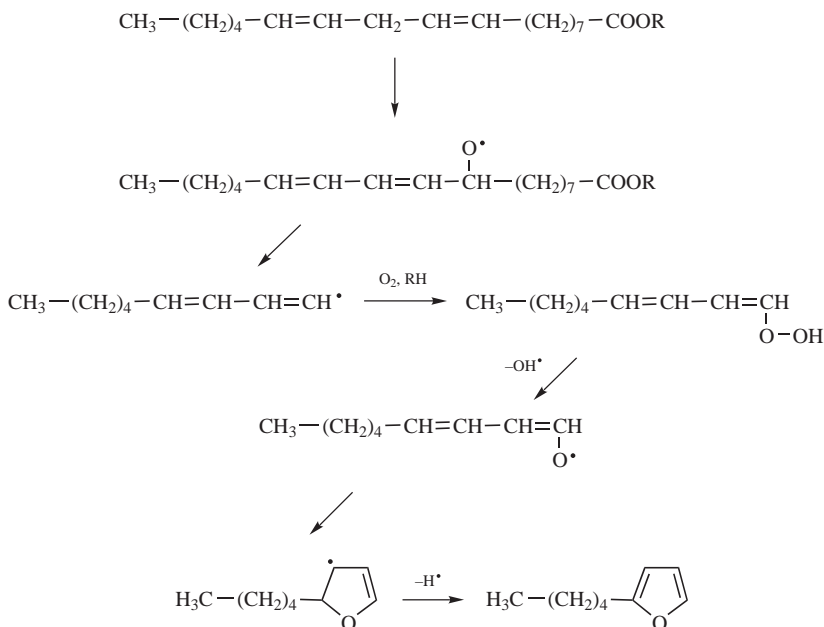


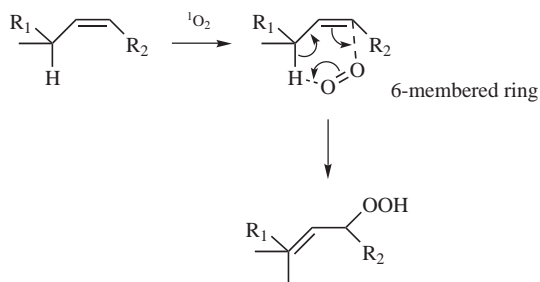
Figure 3. Mechanism for the formation of 2-pentylfuran (6).

**2.1.3. Furan** 2-Pentylfuran has been identified in many fats and oils. It is a well-known autoxidation product of linoleate and has been known as one of the compounds responsible for the reversion of soybean oil (15). Figure 3 shows the probable mechanism for its formation. The conjugated diene radical generated from the cleavage of the 9-hydroxy radical of linoleate may react with oxygen to produce vinyl hydroperoxide. The vinyl hydroperoxide will then undergo cyclization via the alkoxy radical to yield 2-pentylfuran (7).

**2.1.4. Alcohols and Other Compounds** Cleavage of lipid hydroperoxides will also lead to alcohols, alkanes, alkenes, and alkynes. The mechanism for the formation of 1-octen-3-ol, which has a strong mushroom flavor, is also shown in Figure 2. Because of their relative high odor threshold, alcohols and hydrocarbons are generally not considered to be important contributors to the flavors of fats and oils and lipid-containing foods.

## 2.2. Singlet Oxygen Oxidation of Lipids

Oxidation of lipids occurs in the presence of molecular oxygen in both the singlet and triplet states. Atmospheric oxygen that is in the triplet state contains two unpaired electrons, whereas oxygen in the singlet state has no unpaired electrons (16, 17). The electron arrangement of triplet oxygen does not allow for a direct reaction of lipid molecules that exist in the singlet state. Singlet oxygen can be

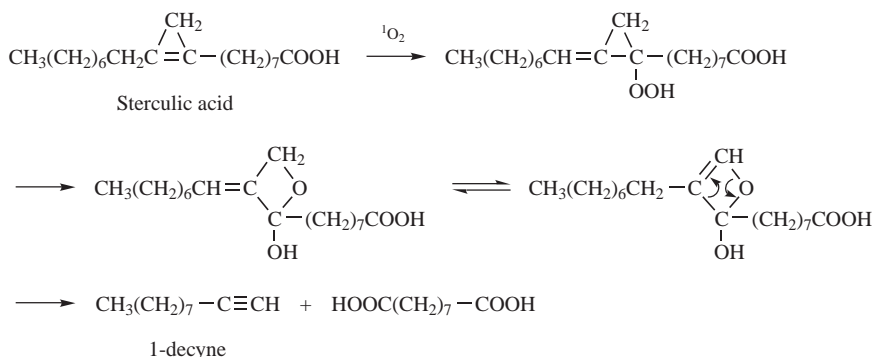


**Figure 4.** The “ene” reaction of singlet oxygen with unsaturated fatty acid.

generated by the interaction of light, photosensitizers, and oxygen. Singlet oxygen has been suggested to be responsible for initiating lipid oxidation of food products because of its ability to directly react with linoleic acid at least 1480 times faster than triplet oxygen (17).

The “ene” reaction of singlet oxygen is important in the photooxidation process in edible oils. In this reaction, singlet oxygen reacts with olefins to form allyl hydroperoxides through a six-membered ring transition state as shown in Figure 4. The lipid hydroperoxides produced from singlet oxygen are different from those generated by autoxidation. In singlet oxygen oxidation, both conjugated and nonconjugated hydroperoxides are formed; in free radical autoxidation, nonconjugated hydroperoxides are not usually formed (17).

One of the major volatile compounds formed from cottonseed oil was 1-decyne, which was reported as a predominant volatile of photooxidized cottonseed oil formed from its precursor, sterculic acid, as shown in Figure 5. The endogenous trace amount of chlorophyll acts as a photosensitizer to produce singlet oxygen. The singlet oxygen then attacks the cyclopropenoid fatty acid, namely, sterculic acid, followed by degradative cleavage and molecular rearrangement to yield 1-decyne (18).



**Figure 5.** Proposed mechanism for the formation of 1-decyne from the photooxidation of sterculic acid (18).

### 3. MAJOR VOLATILE COMPOUNDS OF COMMERCIAL FATS AND OILS

The volatile compounds of eight different vegetable oils, namely, canola, corn, cottonseed, olive, peanut, safflower, soybean, and sunflowerseed oil, have been analyzed and reported by Snyder et al. (19). Table 3 shows the quantitative data of the volatile compounds identified in these vegetable oils after eight days of storage at 60°C.

The volatile compounds in each of the stored vegetable oil samples were related to the main fatty acid components of the oil. Safflower, sunflowerseed, corn, and

**TABLE 3. Volatile Compounds in Vegetable Oils After 8 Days Storage at 60°C (19).**

Volatile Compounds	GC Peak Area							
	Canola	Corn	Cottonseed	Olive	Peanut	Safflower	Soybean	Sunflowerseed
Ethane	1.0	1.0	0.8	0.2	8.0	0.2	0.9	1.0
Propane	0.7	10.6	12.6	0.6	—	10.4	9.1	12.7
Propenal	1.5	2.3	2.1	1.7	2.4	2.3	4.5	2.9
Pentene	0.2	—	—	—	—	—	—	—
Pentane	0.5	24.3	32.7	14.9	19.0	54.1	11.1	41.5
Propanal	1.0	—	—	0.3	0.6	—	0.7	—
Pentene	0.1	—	—	0.1	—	0.1	0.1	—
Hexane	1.1	0.4	0.3	0.3	0.2	0.4	0.3	0.1
2-Butenal	0.5	0.2	0.2	—	0.3	—	0.5	0.1
1-Penten-3-ol	0.4	—	—	—	—	—	0.8	0.1
Pentanal	0.5	1.0	1.3	1.8	2.8	1.9	2.9	2.4
Heptane	0.2	0.2	—	2.2	0.5	0.2	0.1	0.4
Pentenal	0.3	0.1	0.1	—	—	—	0.6	—
Pentanol	0.3	0.5	0.8	0.5	1.4	2.0	1.5	1.3
Octene	—	0.1	0.1	0.2	0.5	0.1	0.2	—
Hexanal	3.1	9.8	10.3	5.8	7.6	11.1	10.7	10.5
Octane	3.7	2.2	3.9	4.1	0.3	0.2	0.3	4.5
Octene	—	0.1	—	0.1	0.2	0.1	0.2	—
<i>t</i> -2-Hexenal	0.2	0.3	0.5	0.2	0.8	0.3	0.5	0.7
Heptanal	0.8	0.6	2.2	1.8	2.0	3.2	2.1	1.8
<i>c</i> -2-Heptenal	0.2	0.2	0.1	0.1	—	0.3	0.2	—
<i>t</i> -2-Heptenal	0.9	1.3	3.6	2.6	1.5	5.6	5.1	3.4
1-Octen-3-ol	0.1	0.3	0.2	0.3	0.3	0.1	0.3	0.3
2-Pentylfuran	0.2	0.5	0.8	0.2	0.7	0.7	1.0	0.6
<i>t,c</i> -2,4-Heptadienal	0.5	—	—	—	—	—	0.6	—
Octanal	0.5	0.3	0.4	1.1	0.6	0.4	0.5	0.3
<i>t,t</i> -2,4-Heptadienal	0.8	—	—	—	—	—	0.8	—
Octenal	0.2	0.5	0.5	0.6	0.7	0.6	0.4	0.5
Nonanal	1.2	0.5	0.6	2.8	0.5	0.4	0.6	0.4
<i>t</i> -2-Decenal	0.2	0.2	0.3	0.7	0.2	0.2	0.3	0.3
Decenol	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1
<i>t,c</i> -2,4-Decadienal	0.3	0.3	0.4	0.1	0.3	0.3	0.6	0.4
<i>t,t</i> -2,4-Decadienal	0.8	1.0	1.2	0.8	0.8	1.5	1.4	1.4
Undecenal	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.2
Total area	25.8	63.1	77.6	49.2	53.6	99.6	40.2	70.4

TABLE 4. Odor Profiles of the Butter Samples (29).

Butter Samples	Odor Quality	Intensity <sup>a</sup>
Irish sour cream (ISC)	Buttery, creamy, sweet	3
Cultured butter (CB)	Typical butter-like, sweet	2–3
Sour cream (SC)	Mild, weakly buttery, sour	1–2
Sweet cream (SwC)	Slightly sour mild	1
Farmer sour cream (FSC)	Rancid, like butanoic acid	3

<sup>a</sup>Intensity: 1, weak; 2, medium; 3, strong.

cottonseed oils, with the highest amount of linoleate, tended to produce the greatest amount of volatiles, especially pentane and hexanal. Canola and soybean oils, which contain linolenate, both formed measurable amounts of 2,4-heptadienal. Olive oil, with the largest quantity of oleate, produced the most octanal and nonanal.

### 3.1. Flavor Compounds of Selected Fats and Oils

**3.1.1. Butter** Because of their commercial significance, the flavor of butter and butter oil has been studied extensively. More than 230 volatile compounds have been identified in different types of butter as well as butter oil (20). The typical flavor of fresh butter is influenced by carbonyl compounds formed by oxidation of unsaturated fatty acids in milk. Critical flavors in butter have recently been reviewed (21, 22).

Using odor activity value (ratio of concentration to odor threshold), Forss et al. (23), Urbach et al. (24), and Stark and co-worker (25–27) reported  $\delta$ -decalactone,  $\delta$ -octalactone, decanoic acid, dodecanoic acid, skatole, and indole as important contributors to the flavor of butter oil. In addition, the data of Siek et al. (28) indicated that in fresh butter, the levels of butanoic acid, caproic acid,  $\delta$ -decalactone were above their taste threshold.

By using aroma extract dilution analysis (AEDA) of the volatile fractions of fresh and stored butter oil, Widder et al. (29) determined diacetyl, butanoic acid,  $\delta$ -octalactone, skatole,  $\delta$ -decalactone, *cis*-6-dodeceno- $\delta$ -decalactone, 1-octen-3-one, and 1-hexen-3-one as potent contributors to the flavor of butter oil. The concentration of 1-octen-3-one, *trans*-2-nonenal, and *cis*-1,5-octadien-3-one increased during the storage of the butter oil at room temperature.

Table 5 shows the sensory evaluation by Schieberle et al. (30) of the different kinds of butter, namely, Irish sour cream (ISC), cultured butter (CB), sour cream (SC), sweet cream (SwC), and farmer sour cream (FSC). It revealed ISC butter and FSC butter with the highest overall odor intensities. Table 5 shows that 19 odor-active compounds were detected by aroma extract dilution analysis (AEDA) in a distillate of the ISC butter. The highest flavor dilution (FD) factors have been found for  $\delta$ -decalactone, skatole, *cis*-6-dodeceno- $\gamma$ -lactone, and diacetyl followed by *trans*-2-nonenal, *cis,cis*-3,6-nonadienal, *cis*-2-nonenal, and 1-octen-3-one.

TABLE 5. Potent Odorants in an Irish Sour Cream Butter (30).

Compound	Flavor Dilution (FD) Factor	Odor Description
Diacetyl	256	Buttery
1-Penten-3-one	32	Vegetable-like
Hexanal	8	Green
1-Octen-3-one	8	Mushroom-like
<i>cis,cis</i> -3,6-Nonadienal	64	Soapy
<i>cis</i> -2-Nonenal	64	Fatty, green
<i>trans</i> -2-Nonenal	128	Green, tallowy
<i>trans,trans</i> -2,4-Nonadienal	8	Fatty, waxy
<i>trans,trans</i> -2,4-Decadienal	32	Fatty, waxy
$\gamma$ -Octalactone	64	Coconut-like
<i>trans</i> -4,5-Epoxy- <i>trans</i> -2-decenal	32	Metallic
Skatole	512	Mothball-like
$\delta$ -Decalactone	4096	Coconut-like
<i>cis</i> -6-Dodeceno- $\gamma$ -lactone	512	Peach-like
Acetic acid	128	Pungent
Butanoic acid	512	Buttery, sweaty
Hexanoic acid	32	Pungent, musty

Aroma extract dilution analysis has also been applied to heated butter (31). Key aroma compounds with highest FD factors have been identified as  $\delta$ -octalactone, skatole, methional,  $\delta$ -decalactone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furanol), and 1-octen-3-one followed by 1-hexen-3-one, *cis*-2-nonenal, *trans*-2-nonenal, *trans,trans*-2,4-decadienal, *trans*-4,5-epoxy-*trans*-2-decenal, and  $\gamma$ -octalactone. Unsaturated triacylglycerols in butterfat are presumed to generate these potent odorants during heating. Additionally, thermal degradation of proteins and Maillard reaction can account for the formation of skatole, methional, and furaneol.

**3.1.2. Cocoa Butter** Cocoa butter is one of the most liked and highly prized food ingredients because of its desirable flavor and unique melting behavior. As early as 1961, van Elzakker and van Zutphen (32) studied and identified 23 volatile compounds in the vacuum steam distillate of cocoa butter. Later, Rizzi (33) identified nine alkylpyrazines including methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-5-methylpyrazine, trimethylpyrazine, 2,5-dimethyl-3-ethylpyrazine, 2,6-dimethyl-3-ethylpyrazine, and tetramethylpyrazine in the basic fraction of a vacuum steam distillate of cocoa butter.

The flavor of cocoa butter depends on the processing conditions to which the cocoa beans are subjected. Cocoa butter obtained from roasted cocoa beans has a strong flavor reminiscent of cocoa. Cocoa butter obtained from unroasted cocoa beans that have been given a steam treatment has a considerable milder, yet distinctive, flavor.

The most comprehensive study on the flavor compounds of cocoa butter was that of Carlin et al. (34–37). They compared the volatile compounds of cocoa butters

**TABLE 6. Some Major Pyrazines Identified in Cocoa Butters from Roasted and Unroasted Cocoa Beans (37).**

Pyrazines	Relative Concentration	
	Roasted	Unroasted
Tetramethylpyrazine	270	3400
Trimethylpyrazine	2620	380
2,5-Dimethyl-3-ethylpyrazine	750	40
2,5-Dimethylpyrazine	710	60
2-Isopropyl-3-methylpyrazine	510	trace
2,6-Dimethylpyrazine	490	—
2-Acetyl-3-methylpyrazine	390	20
2-Ethyl-3,5,6-trimethylpyrazine	310	20
2,6-Diethyl-3-methylpyrazine	10	150
6,7-Dihydro-5H-cyclopentapyrazine	110	—
Methylpyrazine	90	10
2-Acetyl-3-ethylpyrazine	90	10
2,5-Diethyl-3-methylpyrazine	90	—
2-Butyl-3,6-dimethylpyrazine	80	trace
2-Methyl-6,7-Dihydro-5H-cyclopentapyrazine	80	trace
2-Methyl-5-vinylpyrazine	70	—
Isopropenylpyrazine	70	—
2-Methyl-3-pentylpyrazine	50	c
2-Methyl-6-vinylpyrazine	40	30
2,3-Dimethylpyrazine	trace	30
2,3-Dimethyl-5-ethylpyrazine	—	30
2,3-Dimethyl-5-butylpyrazine	30	—
2,5-Dimethyl-5-isobutylpyrazine	30	—
5,6,7,8-Tetrahydroquinoxaline	30	—

from roasted and unroasted cocoa beans. Pyrazines were present in greater numbers and at higher concentrations in the roasted cocoa butter. Of the 62 pyrazines identified, 57 were identified in the roasted cocoa butter and only 27 in the unroasted samples. Table 6 lists the comparison of the major pyrazines identified in cocoa butters from roasted and unroasted cocoa beans.

The most abundant pyrazine identified in cocoa butters was tetramethylpyrazine, which existed at an extremely high concentration in the unroasted cocoa butter but only a moderate level in the roasted cocoa butter. Tetramethylpyrazine accounted for over 90% of the pyrazine content of the unroasted cocoa butter. Besides thermal generation, tetramethylpyrazine could be formed in cocoa beans through biosynthetic reactions. Kosuge and Kamiya (38) identified tetramethylpyrazine as a metabolic product of a strain of *Bacillus subtilis*. Several species of this organism were identified in a fermenting mass of cocoa beans by Ostovar (39).

Table 7 lists the oxazoles and thiazoles identified in the sample of cocoa butter. They were present only in roasted cocoa butter. The sensory characteristics of these compounds shown in Table 7 indicated that oxazoles and thiazoles possessed interesting green, fatty, sweet, and nutty sensory qualities and were high-impact flavor

**TABLE 7. Oxazoles and Thiazoles Identified in Roasted Cocoa Butter (36).**

Compounds	Odor Description
2-Pentylthiazole	Strong, green, fatty, sweet
2-Acetyl-5-methylthiazole	
2-Isopropyl-4,5-dimethylthiazole	
2-Isopropyl-4-methylthiazole	Strong, camphorus
2-Acetyloxazole	
2-Isopropyl-4,5-dimethyloxazole	
2-Isopropyl-4-ethyl-5-methyloxazole	Sweet, fruity
2-Methyl-4,5-dibutyloxazole	
4,5-Dimethyloxazole	
2-Methyl-4-ethyl-5-propyloxazole	Green fatty, vegetable-like
2,5-Dimethyl-4-butyloxazole	Fresh acidic, green, pickle-like
2-Methyl-4-ethyl-5-butyloxazole	Acidic, fatty, sweet, flowery
2-Butyl-4-methyl-5-ethyloxazole	Green, sweet
2-Butyl-4-ethyl-5-methyloxazole	Green, herbal, weak, acidic, slight buttery
4,5-Dibutyloxazole	
2,5-Dibutyl-4-methyloxazole	Sweet, fruity, green

compounds in roasted cocoa butter (36, 37). Of particular interest, 2-pentylthiazole identified had strong fatty, green, and sweet notes and may be an important contributor to cocoa butter flavor. Oxazoles could possibly be formed through the Strecker degradation of aminoketones, which result from the condensation of  $\alpha$ -dicarbonyl compounds with amino acids (40). They might also form through reaction between amino acids (41). Maga (42) has reviewed the occurrence of thiazoles in foods and possible pathways of formation. Thiazoles could possibly form through the interaction of sulfur-containing amino acids and carbonyl-containing compounds.

**3.1.3. Lard** Lard is a traditional edible fat for Chinese people. Lard is generally prepared either by dry-rendering or by wet-rendering. The dry-rendered lard with pork back fat as the raw material usually has better flavor than the wet-rendered lard and is used as cooking fat or shortening. The wet-rendered lard with pork belly fat as the raw material usually has an undesirable flavor and must be refined before further use.

The volatile flavor compounds of lard have been studied by Watanabe and Sato (43–48). They heated the lard at 160–170°C under a stream of air and collected the volatile compounds. They found that 2,4-decadienal and lactones contributed significantly to the flavor of lard. Hwang and Chen (49) compared the volatile flavor compounds generated by heating the crude and refined samples of both dry-rendered and wet-rendered lard at 190°C for 2 hours. Table 8 summarizes the amounts of some flavor-contributing volatiles in different samples of lard. Crude dry-rendered lard showed the highest content of these compounds followed by crude wet-rendered lard, refined dry-rendered lard, and refined wet-rendered lard. Apparently, dry-rendering can yield lard with a stronger flavor.



**TABLE 8. The Amounts of Volatile Flavor Contributing Compounds from Lards of Different Treatments (47).**

Compounds	CDL <sup>a</sup>	RDL	CWL	RWL
	(mg/100-g lard)			
1-Octen-3-ol	2.21	1.83	1.89	1.40
<i>n</i> -Hexanal	10.87	8.13	10.38	7.60
<i>n</i> -Octanal	2.67	2.12	2.07	1.86
<i>trans</i> -2-Heptenal	11.04	8.45	10.44	7.23
<i>n</i> -Nonanal	10.92	8.15	8.70	6.99
<i>trans</i> -2-Octenal	3.24	2.66	2.77	2.40
<i>trans</i> -2-Nonenal	3.14	2.46	2.59	1.89
2,4-Decadienal	17.01	14.34	15.06	12.10
2-Pentylfuran	0.80	0.43	0.43	0.38
$\gamma$ -Octalactone	trace	0.38	trace	trace
Total	61.90	48.95	54.78	41.85

<sup>a</sup>CDL, crude dry-rendered lard; RDL, refined dry-rendered lard; CWL, crude wet-rendered lard; and RWL, refined wet-rendered lard.

**3.1.4. Soybean Oil** Soybean oil is the highest volume vegetable oil produced in the world, as well as in the United States. Because of its commercial importance, the flavor chemistry of soybean oil has been extensively studied and reviewed (50).

The development of a characteristic, objectionable, beany, grassy, and hay-like flavor in soybean oil, commonly known as reversion flavor, is a classic problem of the food industry. Soybean oil tends to develop this objectionable flavor when its peroxide value is still as low as a few meq/kg, whereas other vegetable oils, such as cottonseed, corn, and sunflower, do not (15, 51). Smouse and Chang (52) identified 71 compounds in the volatiles of a typical reverted-but-not-rancid soybean oil. They reported that 2-pentylfuran formed from the autoxidation of linoleic acid, which is the major fatty acid of soybean oil, and contributes significantly to the beany and grassy flavor of soybean oil. Other compounds identified in the reverted soybean oil also have fatty acids as their precursors. For example, the "green bean" flavor is caused by *cis*-3-hexenal, which is formed by the autoxidation of linolenic acid that usually constitutes 2–11% in soybean oil. Linoleic acid oxidized to 1-octen-3-ol, which is characterized by its "mushroom-like" flavor (53).

The most interesting studies on the flavor of soybean oil were those published by Ullrich and Grosch (54) and Guth and Grosch (13, 55). By using aroma extract dilution analysis, they determined some odor compounds that strongly contributed to the off-flavor of soybean oil samples, which were stored at room temperature either in daylight or in the dark. Table 9 lists the FD factor values of various odor compounds in soybean oil samples. 3-Methyl-2,4-nonanedione, *cis*-3-hexenal, *cis*-2-nonenal, *cis*-1,5-octadien-3-one, 1-octen-3-hydroperoxide, 4,5-epoxy-*trans*-2-decenal, 1-octen-3-one, *cis*-1,5-octadien-3-hydroperoxide, and *trans*-2-nonenal were identified as primary odorants of soybean oil, which were exposed to daylight. They also observed that the major differences in the intensity of the reversion odor

TABLE 9. Aroma Extract Dilution Analysis of the Stored Soybean Oils (12).

Compound	Flavor Dilution (FD) Factor	
	SBO (Daylight) <sup>a</sup>	SBO (Dark) <sup>b</sup>
<i>cis</i> -3-Hexenal	2048	8
Hexanal	16	<1
Pentanoic acid	32	<1
<i>trans</i> -2-Heptenal	64	<1
1-Octen-3-one	256	16
<i>cis</i> -1,5-Octadien-3-one	512	<1
2,4-Heptadienal	8	4
Octanal	16	8
<i>cis</i> -2-Octenal	16	<1
<i>trans</i> -2-Octenal	32	8
<i>cis</i> -3-Nonenal	16	8
1-Octen-3-hydroperoxide	512	32
<i>cis</i> -1,5-Octadien-3-hydroperoxide	256	4
<i>cis</i> -2-Nonenal	1024	64
<i>trans,cis</i> -2,4-Nonadienal	16	8
<i>trans</i> -2-Nonenal	256	32
2,4-Nonadienal	32	16
<i>trans,trans</i> -2,4-Nonadienal	16	16
3-Methyl-2,4-nonanedione	16348	16
<i>trans</i> -4,5-Epoxy- <i>trans</i> -2-nonenal	<1	8
<i>trans,trans</i> -2,4-Decadienal	32	16
<i>trans</i> -4,5-Epoxy- <i>trans</i> -2-decenal	512	256

<sup>a</sup>The oil sample was stored for 30 days at room temperature and in daylight.

<sup>b</sup>The oil sample was stored for the sample period in the dark.

of soybean oil samples were mainly caused by an increase in the concentration of 3-methyl-2,4-nonanedione during storage. Guth and Grosch (56) further identified two furanoid fatty acids, namely, 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid and 12,15-epoxy-13,14-dimethyloctadeca-12,14-dienoic acid in soybean oil as precursors for 3-methyl-2,4-nonanedione. The proposed mechanism for the photogeneration of 3-methyl-2,4-nonanedione is shown in Figure 6. However, the work of Kao et al. (57) cannot support the theory that furanoid fatty acids or 3-methyl-2,4-nonanedione contribute strongly to the reversion flavor of soybean

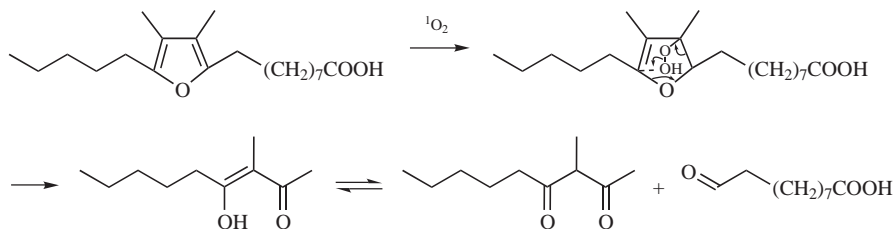


Figure 6. Proposed mechanism for the photogeneration of 3-methyl-2,4-nonanedione (56).

**TABLE 10. Odor Properties of the Hydroperoxides and the Epoxides Identified in Stored Soybean Oils (12).**

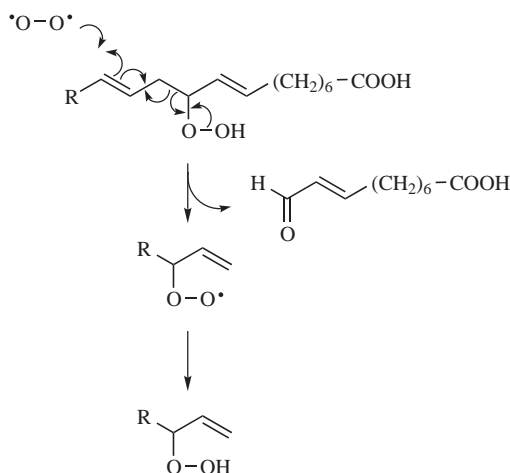
Compound	Odor Description	Odor Threshold
		(ng/L, air)
1-Octen-3-hydroperoxide	Metallic, mushroom-like	0.6–1.2
1-Octen-3-one	Mushroom-like	0.03–0.12
<i>cis</i> -1,5-Octadien-3-hydroperoxide	Geranium-like, metallic	0.03–0.06
<i>cis</i> -1,5-Octadien-3-one	Geranium-like, metallic	0.003–0.006
<i>trans</i> -4,5-epoxy- <i>trans</i> -2-nonenal	Metallic	0.25–1.0
<i>trans</i> -4,5-epoxy- <i>trans</i> -2-decenal	Metallic, green	0.0005–0.005

oil. No significant flavor differences were found when soybean oils with high or low contents of furanoid fatty acids were evaluated during storage for off-flavor intensity of soybean oil (57).

The odor properties of two volatile hydroperoxides, 1-octen-3-hydroperoxide and *cis*-1,5-octadien-3-hydroperoxide, are shown in Table 10. The odor thresholds of these hydroperoxides were 10-fold higher than those of the corresponding ketones. Precursors of both hydroperoxides are presumably the 10-hydroperoxide of linoleic acid and linolenic acid, which are easily formed by photosensitized oxidation of linoleic acid and linolenic acid (48). As shown in Figure 7, a  $\beta$ -scission of the 10-hydroperoxy group, the rearrangement of the double bond and combination of the allylic radical formed with oxygen, followed by abstraction of a hydrogen atom would result in the two allyl hydroperoxides having eight carbon atoms.

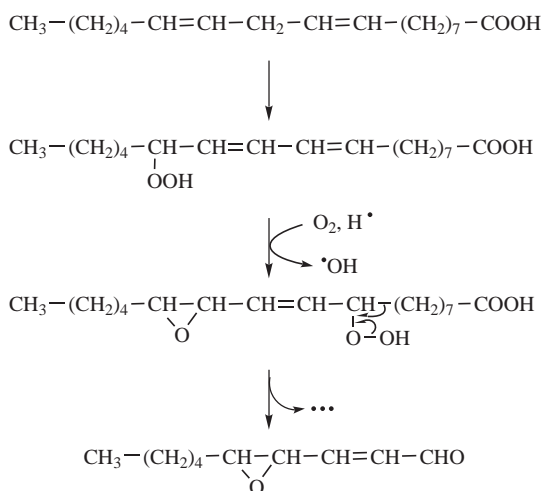
As shown in Tables 9 and 10, on the basis of its high FD factor and its odor properties, the *trans*-4,5-epoxy-*trans*-2-decenal contributed significantly to the green, hay-like overall odor in soybean oil stored in the dark. Guth and Grosch suggested (13) that an epoxyhydroperoxy fatty acid could be the precursor of such epoxy aldehydes. Figure 8 shows the proposed pathway for the formation of *trans*-4,5-epoxy-*trans*-2-decenal from linoleic acid via the *trans*-12,13-epoxy-9-hydroperoxy-*trans*-10-octadecenoic acid intermediate.

**3.1.5. Canola Oil** Canola oil is obtained from low erucic acid, low glucosinolate rapeseed. The unique polyunsaturated fatty acid and low saturated composition of canola oil differentiates it from other oils. It has a higher oleic acid (18:1) content (55%) and lower linoleic acid (18:2) content (26%) than most other vegetable oils, but it contains 8–12% of linolenic acid (18:3) (58). Canola oil is most widely used in Canada and is considered a nutritionally balanced oil because of its favorable ratio of near 2:1 for linoleic to linolenic acid content. Unlike most other edible oils, the major breakdown products of canola oil are the *cis*, *trans*- and *trans*, *trans*-2,4-heptadienals with an odor character generally described as oily, fatty, and putty. Stored canola oil shows a sharp increase in the content of its degradation products, which are well above their odor detection thresholds. The aroma is dominated by *cis*, *trans*-, *trans*, *trans*-2,4-heptadienals, hexanal, nonanal, and the *cis*, *trans*- and



**Figure 7.** Reaction routes proposed for the formation of 1-octen-3-hydroperoxide ( $R = \text{CH}_2-(\text{CH}_2)_4-$ ) and cis-1,5-octadien-3-hydroperoxide ( $\text{CH}_3-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2$ ) (13).

*trans, trans*-decadienals. Octen-3-one with a strong metallic flavor, generally considered an off-flavor in oils, occurs at a concentration of 75 ppb in aged oil, which is approximately 750 times above its odor detection threshold. Despite its relatively low concentration, the metallic note of octen-3-one contributes markedly to the overall flavor character of the aged oil (59).



**Figure 8.** Proposed reaction route to the *trans*-4,5-epoxy-*trans*-2-decenal via the *trans*-12,13-epoxy-9-hydroperoxyoctadec-(*E*)-10-enoic acid (13).

**3.1.6. Olive Oil** Olive oil is commonly used as a table and cooking oil because of its unique flavor and stability. Olive oil comes from the fruits of the olive tree *Olea europaea*, which has been cultivated for many years in the Southern European countries bordering the Mediterranean and in North Africa. Virgin olive oil is extracted under mild conditions and is normally consumed without further treatment, the natural flavor compounds that confer its characteristic aroma are preserved and are uniquely recognized by consumers. In the intact fruit, a high percentage of fatty acids, exists which are mainly bound as triacylglycerols; however, in the oil, there is a resulting high percentage of free fatty acids. Two reasons could account for the high levels of free fatty acid: A high moisture content that is favorable to lipase action, and bruising of the olive fruit during harvest, transportation, and milling of the fruits. The primary free fatty acids are the unsaturated oleic, linoleic, and linolenic acids, with oleic acid constituting 80% of the free fatty acids. These free fatty acids contribute significantly to the taste of the oil and serve as the precursors for the aroma compounds of olive oil.

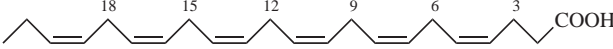
The aroma compounds of olive oil have recently been reviewed by Kiritsakis (60). The most abundant aroma compounds in virgin olive oil are C-6 aliphatic compounds, *trans*-2-hexenal, *trans*-2-hexen-1-ol, hexan-1-ol, *cis*-3-hexen-1-ol, *cis*-2-penten-1-ol, *cis*-3-hexenal, hexyl acetate, and hexanal, accounting for about 80% of total volatile compounds with the prominence of *trans*-2-hexenal (61–64). These C-6 compounds provide the green perception and unique aroma of olive oil. Recently, 2,4-dimethylfuran has been found in olive oil with unpleasant sensory quality (65). As a result, the ratio of *trans*-2-hexenal/2,4-dimethylfuran has been proposed as a quality marker for olive oil and the ratio value of less than 1.5 indicates lower quality olive oil (65).

The stability of olive oil compared with other vegetable oils is attributed to the high-to-low ratio of oleic to linoleic acid, and to the degradation of the chlorophylls to pheophytins (60). In addition, olive oil is also rich in antioxidative phenolic compounds such as hydroxytyrosol (66).

**3.1.7. Marine Oils** Since the use of sardine oil as a food ingredient was discontinued in the 1950s, the Food and Drug Administration (FDA) has determined that fish oils were totally new ingredients for human foods. As a result, 90% of the fish oil produced in the United States was exported to Europe as a food oil and 10% was used domestically in nonfood applications. After lengthy petition, the FDA finally affirmed the GRAS status of partially hydrogenated (PHMO) and hydrogenated menhaden oil (HMO) for direct use as human food ingredients in 1989 (67).

Fish oils are a rich source of  $\omega$ -3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The supplementation of Western diets with fish oils containing EPA and DHA has been recommended (68, 69), and it can be beneficial for ischemic heart disease and thromboembolic events.

In order to explain the reason that marine oils are much more labile than common vegetable oils, basic lipid chemistry, such as oxidation rate, induction period, and oxygen uptake were determined and reported by several researchers (70–72). The relative oxidation rates of fatty esters at 36.5°C were found to be highly



Initial H Abstraction	Hydroperoxide	Aldehydes Formed
C-18	20-OOH	Propanal
	16-OOH	2,4-Heptadienal
C-15	17-OOH	3-Hexenal
	13-OOH	2,4,7-Decatrienal
C-12	14-OOH	3,6-Nonadienal
	10-OOH	2,4,7,10-Tridecatetraenal
C-9	11-OOH	3,6,9-Dodecatrienal
	7-OOH	2,4,7,10,13-Hexadecapentaenal
C-6	8-OOH	3,6,9,12-Pentadecatetraenal
	4-OOH	2,4,7,10,13,16-Nonadecaheptaenal

**Figure 9.** Aldehydes derived from autoxidized decosahexaenoic acid.

correlated to their molecular unsaturation, i.e., oleate (1.0), linoleate (8.0), linolenate (21.7), and EPA + DHA (39.1). Relative oxygen uptake (first two days in air) of oleate, linoleate, linolenate, EPA, and DHA were <1, 1, 99, 743, and 948, respectively. Induction periods (90 lux at 5°C) were also related to the degree of unsaturation of fatty esters. The induction period of oleate was found to be more than 100 days (estimated) and those of linoleate, linolenate, and EPA + DHA, were 50, 20, and 4 days, respectively (68).

The high oxidation rates of EPA and DHA and the instability of their hydroperoxides caused the rapid formation of secondary products such as volatile aldehydes and other compounds, which, in turn, impart flavor reversion in fish oils (56). The hydroperoxides produced from autoxidation of EPA (73) and DHA (74) have been identified but not quantified. They form eight and ten isomers, respectively. Noble and Nawar (75) analyzed the volatile compounds in autoxidized DHA and identified a number of aldehydes. Most of the aldehydes identified could be explained by the  $\beta$ -scission of alkoxy radicals generated by the homolytic cleavage of each isomer of the hydroperoxides as shown in Figure 9.

Meijboom and Stroink (76) found that 2-*trans*, 4-*cis*, 7-*cis*-decatrienal was the compound responsible for the fishy off-flavors occurring in autoxidized oil containing  $\omega$ -3 fatty acids. This trienal was also found in autoxidized methyl DHA by Noble and Nawar (75) and in autoxidized mackerel oil by Ke et al. (77).

The most detailed studies on the flavor of fish oil in recent years were probably those of Hsieh et al. (78, 79), Lin et al. (80), and Lin (81). In their studies, a series of alkanals, alkenals, alkadienals, and alkatrienals were determined by dynamic headspace gas chromatography-mass spectrometry in crude menhaden oils (Table 11). Most of these aldehydes contributed to the characteristic oxidized oily odors, such as green grassy, waxy, and rancid in the crude oils. Alkatrienals, i.e., nonatrienal and decatrienals, were also found at ppb levels in the dynamic headspace of the crude oils. 2-*trans*,4-*trans*,7-*cis*-Decatrienal, 2-*trans*,4-*cis*,7-*cis*-decatrienal,

**TABLE 11. Volatile Aldehydes Identified in the Gulf Menhaden Oil (54).**

Compounds	GC Area (%)
<i>n</i> -Butanal	0.97
<i>n</i> -Pentanal	0.82
<i>cis</i> -2-Butenal	0.55
<i>n</i> -Hexanal	1.56
<i>cis</i> -2-Pentenal	1.48
<i>n</i> -Heptanal	1.42
<i>trans</i> -2-Hexenal	0.97
<i>trans</i> -4-Heptenal	0.24
<i>n</i> -Octanal	0.61
<i>trans</i> -2-Heptenal	0.31
<i>n</i> -Nonanal	0.51
2,4-Hexadienal	trace
<i>cis</i> -2-Octenal	0.13
<i>trans</i> -2-Octenal	0.43
2,4-Heptadienal <sup>a</sup>	0.69
<i>n</i> -Decanal	0.15
2,4-Heptadienal <sup>a</sup>	1.39
Benzaldehyde	0.34
<i>trans</i> -2-Nonenal	0.14
<i>cis</i> -4-Decenal	0.14
2,4-Octadienal <sup>a</sup>	0.11
2,6-Nonadienal <sup>a</sup>	0.06
2,4-Octadienal <sup>a</sup>	0.34
<i>trans</i> -4-Decenal	0.02
2,4-Nonadienal <sup>a</sup>	trace
2,4-Nonadienal <sup>a</sup>	trace
2,4-Decadienal	trace
2,4-Undecadienal <sup>a</sup>	trace
Nonatrienal <sup>a</sup>	trace
Nonatrienal <sup>a</sup>	trace
Decatrienal <sup>a</sup>	trace
Decatrienal <sup>a</sup>	trace

<sup>a</sup>Configuration of geometric isomers were not determined.

and 4-*cis*-heptenal impart a strong fishy odor to oils. Aldehydes have a green or plant-like note; ketones (1-octen-3-ol) have a metallic off-flavor. Besides the aldehydes identified in crude menhaden oil, other compounds identified such as short-chain unsaturated alcohol (1-penten-3-ol), had a medicinal odor, and others had a green unpleasant odor and may also contribute to the flavor of fish oil. Lin et al. (80) also reported that steam-deodorization can effectively remove a total of 99% of most aldehydes in the oils. However, reversion flavor of fish oils during storage can generate pentylfuran and aldehydes that have green and beany odors.

Cadwallader and Shahidi (82) identified the potent odorants of seal blubber oil by direct thermal desorption-gas chromatography-olfactometry (DTD-GCO). In

general, odorants were present at higher odor-potency in the crude oil. Predominant odorants were (Z)-1,5-octadien-3-one (metallic), (E, E, Z)-2,4,7-decatrienal (fatty, fishy), (Z)-3-hexenal (green, cut-leaf), and (E,Z)-2,6-nonadienal, (cucumber). These compounds are among breakdown products of thermally labile hydroperoxides and are responsible for the typical off-flavors encountered in stored marine oils.

Oxidative stability of  $\omega$ -3 fatty acids can be increased using free radical scavengers. TBHQ (*t*-butylhydroquinone) at a concentration of 0.02% has successfully slowed down the oxidation of menhaden oil for up to 40 days, compared with 3 days for the control group (83).  $\alpha$ -Tocopherol and butylated hydroxytoluene (BHT) alone or in combination increased the oxidative stability of EPA and DHA (84). The most notable success in fish oil stabilization has been achieved with ternary antioxidant systems, which contain  $\alpha$ - or  $\gamma$ -tocopherol concentrates, ascorbic acid (or ascorbyl palmitate), and lecithin (85).

**3.1.8. Sesame Oil** Sesame oil has traditionally been used in eastern Asian countries, especially China, Japan, Korea, and Taiwan. It has been prized for its nutritive and health-promoting values. Sesame oil, prepared from roasted sesame seeds, has a distinctive flavor and a long shelflife (86). Several studies have been reported on the flavor components of sesame oil (87–91). The amount of volatile flavor compounds in sesame oil is greatly affected by the roasting process. It has been reported that the ratio of the amount of volatile components in deep-roasted oils was increased by 2–7 times in deep-roasted oil as compared with that of light-roasted oils (90).

Perhaps the most important compounds identified in the roasted sesame oils are 2-furfurylthiol and guaiacol. Using aroma extract dilution analysis method, these two compounds have been characterized by Schieberle (92) to be the most odor-active compounds in roasted sesame seeds. 2-Furfurylthiol, having an intense coffee-like odor, increased from 16 ppb in roasted oil processed at 160°C for 30 min to 158 ppb in the oil processed at 200°C for 30 min (Table 12). Guaiacol has a burnt and smoky odor with an extremely low-odor threshold of 0.02 ppt in

**TABLE 12. Changes in the Content (ppb) of Selected Odor-Active Compounds in Sesame Oils with Sesame Seeds Roasted at 160, 180, 200, and 220°C for 20 min (88, 89).**

Compound	FD Factor in Roasted Sesame Seed	Content (ppb)			
		160°C	180°C	200°C	220°C
2-Furfurylthiol	20480	16	51	105	158
Guaiacol	10240	147	365	537	718
Acetylpyrazine	1280	57	169	201	197
2-Ethyl-3,5-dimethylpyrazine	1280	73	161	276	287
2,3-Diethyl-5-methylpyrazine	640	13	29	57	50
<i>trans,trans</i> -2,4-Decadienal	640	121	169	287	333
2-Ethyl-5-methylpyrazine	320	96	234	301	301



water. The amount of guaiacol increased from 147 ppb in roasted oil processed at 160°C for 30 min to 718 ppb in the oil processed at 200°C for 30 min (Table 12). The extremely high concentration of guaiacol in the high-temperature roasting sample certainly contributes to its smoky and overburnt sensory quality.

## REFERENCES

1. H. W. S. Chan, in H. W. S. Chan, ed., *Autoxidation of Unsaturated Lipids*, Academic Press, London, 1987, pp. 1–16.
2. E. N. Frankel, *Lipid Oxidation*, The Oily Press, Dundee, Scotland, 1998, pp. 13–22.
3. R. T. Holman and O. C. Elmer, *J. Amer. Oil Chem. Soc.*, **24**, 127–129 (1947).
4. E. N. Frankel, *Lipid Oxidation*, The Oily Press, Dundee, Scotland, 1998, pp. 23–41.
5. H. W. S. Chan, in H. W. S. Chan, ed., *Autoxidation of Unsaturated Lipids*, Academic Press, London, 1987, pp. 17–50.
6. C.-T. Ho and Q. Chen, in C.-T. Ho and T. G. Hartman, eds., *Lipids in Food Flavors*, American Chemical Society, Washington, D. C., 1994, pp. 2–14.
7. E. N. Frankel, *Progr. Lipid Res.*, **22**, 1–33 (1982).
8. M. T. Morales, J. J. Rios and R. Aparicio. *J. Agric. Food Chem.*, **45**, 2666–2673 (1997).
9. C.-T. Ho, Y. Zhang, H. Shi, Huang, and J. Tang. *Food Rev. Internat.*, **5**, 253–287 (1989).
10. D. B. Josephson and J. Glinka, in T. H. Parliaent, R. J. McGorrrin and C.-T. Ho, eds., *Thermal Generation of Aromas*, American Chemical Society, Washington, D. C., 1989, pp. 242–.
11. C.-T. Ho, J. T. Carlin, T. C. Huang, I.S. Hwang, and L. B. Hau, in M. Martens, G. A. Dalen, and H. Russwurm, Jr., eds., *Flavour Science and Technology*, Wiley Chichester, England, 1987, pp. 35–42.
12. P. Schieberle and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **192**, 130 (1991).
13. H. Guth and W. Grosch, *Lebensm.-Wiss. U. Technol.*, **23**, 59 (1990).
14. E. N. Frankel, W. E. Neff, and E. Selke, *Lipids* **16**, 279–285 (1981).
15. C.-T. Ho, M. S. Smagula, and S. S. Chang, *J. Amer. Oil Chem. Soc.*, **55**, 233–237 (1978).
16. D. G. Bradley and D. B. Min, *Crit. Rev. Food Sci. Nutr.*, **31**, 211–236 (1992).
17. D. B. Min and H. O. Lee, in R. Teranishi, E. L. Wick, and I. Hornstein, eds., *Flavor Chemistry: Thirty Years of Progress*, Kluwer Academic/Plenum Publishers, New York, 1999, pp. 175–187.
18. L. L. Fan, J. Y. Tang, and A. Wohlman, *J. Amer. Oil Chem. Soc.*, **60**, 1115 (1983).
19. J. M. Snyder, E. N. Frankel, and E. Selke, *J. Amer. Oil Chem. Soc.*, **62**, 1675–1679 (1985).
20. H. Maarse and C. A. Visscher, *Volatile Compounds in Food. Qualitative Data Supplement*, TNO, Zeist, The Netherlands, 1988.
21. T. H. Parliment and R. J. McGorrrin, in S. J. Risch and C.-T. Ho, eds., *Flavor Chemistry: Industrial and Academic Research*, American Chemical Society, Washington, D. C., 2000, pp. 44–71.
22. R. J. McGorrrin, in A. H. Spanier, F. Shahidi, T. H. Parliment, C. Mussinan, C.-T. Ho, and E. T. Contis, eds., *Food Flavors and Chemistry: Advances of The New Millennium*, Royal Society of Chemistry, Cambridge, U.K., pp. 67–84.

23. D. A. Forss, W. Stark, and G. Urbach, *J. Dairy Res.*, **34**, 131– (1967).
24. G. Urbach, W. Stark, and D. A. Forss, *J. Dairy Res.* **39**, 35 (1972).
25. W. Stark, G. Urbach, and J. S. Hamilton, *J. Dairy Res.*, **40**, 39 (1973).
26. W. Stark, G. Urbach, and J. S. Hamilton, *J. Dairy Res.*, **43**, 469 (1976).
27. W. Stark, G. Urbach, and J. S. Hamilton, *J. Dairy Res.*, **43**, 479 (1976).
28. T. J. Siek, J. A. Albin, L. A. Sather, and R. C. Lindsay, *J. Food Sci.*, **34**, 265 (1976).
29. S. Widder, A. Sen, and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **193**, 32 (1991).
30. P. Schieberle, K. Gassenmeier, H. Guth, A. Sen, and W. Grosch, *Lebensm. Wissen. u.-Technol.*, **26**, 347–356 (1993).
31. J. Budin, Ph.D. Thesis, University of Minnesota, Minneapolis, Minnesota, 1998.
32. A. H. M. van Elzakker and H. J. van Zutphen, *Z. Lebensm. Unters. Forsch.*, **115**, 222 (1961).
33. G. P. Rizzi, *J. Agric. Food Chem.*, **15**, 549–551 (1967).
34. J. T. Carlin, M. S. Thesis, Rutgers University, New Brunswick, New Jersey, 1980.
35. J. T. Carlin, K. N. Lee, O. A. L. Hsieh, L. S. Hwang, C.-T. Ho, and S. S. Chang, *Proc. 36th P. M. C. A. Production Conf.*, 1982, pp. 95–104.
36. C.-T. Ho, Q. Z. Jin, K. N. Lee, J. T. Carlin, and S. S. Chang, *J. Food Sci.*, **48**, 1570–1571 (1983).
37. J. T. Carlin, K. N. Lee, O. A. L. Hsieh, L. S. Hwang, C.-T. Ho, and S. S. Chang, *J. Amer. Oil Chem. Soc.*, **63**, 1031–1036 (1986).
38. T. Kosuge and H. Kamiya, *Nature*, **193**, 776 (1962).
39. K. Ostovar, Ph.D. Thesis, The Pennsylvania State University, University Park, PA, 1971.
40. O. G. Vitzum and P. Werkhoff, *Z. Lebensm.-Unters. Forsch.*, **156**, 300–307 (1974).
41. G. Ohloff and I. Flament, *Forsch. Chem. Org. Naturst.*, **36**, 231–283 (1979).
42. J. A. Maga, in T. E. Furia and N. Bellanca, eds., *Fenaroli's Handbook of Flavor Ingredients*, Vol. 1, 2nd ed., CRC Press Inc., Cleveland, Ohio, 1975, pp. 228–238.
43. K. Watanabe and Y. Sato, *Agric. Biol. Chem.*, **33**, 242–249 (1969).
44. K. Watanabe and Y. Sato, *Agric. Biol. Chem.*, **33**, 1411–1418 (1969).
45. K. Watanabe and Y. Sato, *Agric. Biol. Chem.*, **34**, 464–472 (1970).
46. K. Watanabe and Y. Sato, *Agric. Biol. Chem.*, **34**, 1710–1715 (1970).
47. K. Watanabe and Y. Sato, *Agric. Biol. Chem.*, **35**, 278–281 (1970).
48. K. Watanabe and Y. Sato, *J. Zootech. Sci.*, **42**, 393 (1971).
49. L. S. Hwang and C. W. Chen, C.-T. Ho and T. G. Hartman, eds., *Lipids in Food Flavors*, American Chemical Society, Washington, D.C., 1994, pp. 244–255.
50. T. H. Smouse, in D. B. Min and T. H. Smouse, eds., *Flavor Chemistry of Fats and Oils*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 85–116.
51. S. S. Chang, G. H. Shen, J. Tang, Q. Z. Jin, H. Shi, J. T. Carlin, and C.-T. Ho, *J. Amer. Oil Chem. Soc.*, **60**, 553–557 (1983).
52. T. H. Smouse and S. S. Chang, *J. Amer. Oil Chem. Soc.*, **44**, 509–513 (1967).
53. B. S. Bistry and D. B. Min, in G. Charalambous, ed., *Off-flavors in Foods and Beverages*, Elsevier Science Publishers, Amsterdam, The Netherlands, 1992, pp. 171–209.
54. F. Ullrich and W. Grosch, *Fat Sci. Technol.*, **91**, 332 (1988).
55. H. Guth and W. Grosch, *Fat Sci. Technol.*, **91**, 225–230 (1989).

56. H. Guth and W. Grosch, *Fat Sci. Technol.*, **93**, 249 (1991).
57. J. W. Kao, X. Wu, E. G. Hammond, and P. J. White, *J. Amer. Oil Chem. Soc.*, **75**, 831–835 (1998).
58. Z. J. Hawrysh, in F. Shahidi, ed., *Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology*, Van Nostrand Reinhold, New York, 1990, pp. 99–122.
59. S. K. Raghavan, D. R. Connell, and A. Khayat, in C.-T. Ho, and T. G. Hartman, eds., *Lipids in Food Flavors. ACS Symposium Series 558*. American Chemical Society, Washington, D.C., 1994, pp. 292–300.
60. A. K. Kiritsakis, *J. Amer. Oil Chem. Soc.*, **75**, 673–681 (1998).
61. T. M. Morales, V. M. Alonso, J. J. Rios, and R. Aparicio, *J. Agric. Food Chem.*, **43**, 2925–2931 (1995).
62. F. Angerosa, C. Basti, and R. Vito, *J. Agric. Food Chem.*, **46**, 836–839 (1998).
63. T. M. Morales and R. Aparicio, *J. Amer. Oil Chem. Soc.*, **76**, 295–300 (1999).
64. J. Sanchez and J. L. Harwood, *Eur. J. Lipid Sci. Technol.*, **104**, 602–613 (2002).
65. F. Angerosa, *Eur. J. Lipid Sci. Technol.*, **104**, 639–660 (2002).
66. A. Ranalli and G. D. Mattia, *J. Amer. Oil Chem. Soc.*, **74**, 1105–1113 (1997).
67. C. F. Lin, in C.-T. Ho and T. G. Hartman, eds., *Lipids in Food Flavors*, American Chemical Society, Washington, D.C., 1994, pp. 208–232.
68. K. Fujimoto, in D. B. Min and T. H. Smouse, eds., *Flavor Chemistry of Lipid Foods*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 190–195.
69. F. Shahidi and K. R. Cadwallader, in *Flavor and Chemistry of Seafoods*, American Chemical Society, Washington, D.C. 1997, pp. 1–8.
70. N. Ikeda and K. J. Fikuzumi, *Jpn. Oil Chem. Soc.*, **27**, 21–26 (1978).
71. S. Y. Cho, K. J. Miyashita, T. Miyazawa, K. Fujimoto, and T. Kaneda, *J. Amer. Oil Chem. Soc.*, **64**, 876–879 (1987).
72. F. Shahidi, U. N. Wanasundara, Y. He, and V. K. S. Shukla, in C.-T. Ho and T. G. Hartman, eds., *Lipids in Food Flavors*, American Chemical Society, Washington, D.C., 1994, pp. 186–197.
73. R. Yamauchi, T. Yamada, K. Kato, and Y. Uevio, *Agric. Biol. Chem.*, **47**, 2897–2902 (1983).
74. M. Van Rollin and R. C. Murphy, *J. Lipids Res.*, **25**, 507–517 (1984).
75. A. C. Noble and W. W. Nawar, *J. Amer. Oil Chem. Soc.*, **48**, 800 (1971).
76. P. W. Meijboom and J. B. A. Stroink, *J. Amer. Oil Chem. Soc.*, **49**, 555 (1972).
77. P. J. Ke, R. G. Ackman, and B. A. Linke, *J. Amer. Oil Chem. Soc.*, **52**, 349 (1975).
78. T. C. Y. Hsieh, C. F. Lin, J. B. Crowther, and J. P. Bimbo, *Tropical and Subtropical Fisheries Technological Society of the Americas*, 1988, pp. 154–161.
79. T. C. Y. Hsieh, S. S. Williams, W. Vejaphan, and S. P. Meyers, *J. Amer. Oil Chem. Soc.*, **66**, 114–117 (1989).
80. C. F. Lin, T. C. Y. Hsieh, J. B. Crowther, and J. P. Bimbo, *J. Food Sci.*, **55**, 1669–1672 (1990).
81. C. F. Lin, in C.-T. Ho and T. G. Hartman, eds., *Lipids in Food Flavors*, American Chemical Society, Washington, D.C., 1994, pp. 208–232.
82. K. R. Cadwallader and F. Shahidi, in F. Shahidi and J. W. Finley, eds., *Omega-3 Fatty Acids: Chemistry, Nutrition and Health Effects*, ACS Symposium Series 788, American Chemical Society, Washington, D.C., 2001, pp. 221–235.

83. J. K. Farkas, J. D. Floros, D. S. Lineback, and B. A. Watkins, *J. Food Sci.*, **62**, 505–507 (1997).
84. J. Ogata, Y. Hagiwara, H. Hagiwara, and T. Shibamoto, *J. Amer. Oil Chem. Soc.*, **73**, 653–656 (1996).
85. R. J. Hamilton, C. Kalu, G. P. McNeill, F. B. Padley, and J. H. Pierce, *J. Amer. Oil Chem. Soc.*, **75**, 813–822 (1998).
86. K. Kikugawa, M. Arai, and T. Kurechi, *J. Amer. Oil Chem. Soc.*, **60**, 1528–1533 (1983).
87. G. C. Yen, *J. Sci. Food Agric.*, **50**, 563–570 (1967).
88. G. C. Yen, S. L. Shyu, and T. C. Lin, *Food Sci. (ROC)*, **13**, 198–211 (1986).
89. M. Shimoda, H. Shiratsuchi, Y. Nakada, Y. Wu, and Y. Osajima, *J. Agric. Food Chem.*, **44**, 3909–3912 (1996).
90. M. Shimoda, Y. Nakada, M. Nakashima, and Y. Osajima, *J. Agric. Food Chem.*, **45**, 3193–3196 (1997).
91. N. Ryu, S. M. Kim, J. Xi, and C.-T. Ho, in F. Shahidi, and C.-T. Ho, eds., *Flavor Chemistry of Ethnic Foods*, Kluwer Academic/Plenum Publishers, New York, 1999, pp. 229–237.
92. P. Schieberle, in P. Schreier, and P. Winterhalter, eds., *Progress in Flavor Precursor Studies*, Allured Publishing, Carol Stream, Illinois, 1999, pp. 343–360.

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## *Flavor and Sensory Aspects*

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### **1. INTRODUCTION**

Sensory evaluation is a scientific discipline that uses humans to measure the acceptability and sensory properties of food and other materials. Sensory properties important in food products include attributes of appearance, odor, taste, and texture. The use of humans as measuring devices is necessary because only humans can define what is “acceptable,” and in many cases, no instrumental or chemical method can adequately measure or replicate the human response. For this reason, sensory evaluation is a vital component in any quality assessment program. In such programs, sensory evaluation can be used to monitor product quality; determine effects of alternative processing, ingredients, or formulations; evaluate packaging; and determine product shelf life.

### **2. SENSORY METHODS**

Sensory methods are often criticized as being subjective techniques. Part of the problem lies with the failure to acknowledge that two distinct types of sensory tests

exist. Product-orientated tests involve the use of selected and trained panelists under controlled testing conditions to evaluate the attributes of a product. These products are objective because they meet the criteria of objectivity, namely freedom from personal bias and repeatability. Consumer-oriented tests involve the use of consumer panelists to determine degree of liking for a product or product acceptability. These tests, by their very nature, are subjective because it is the subjective information (personal likes and dislikes) that is of interest. Thus, if sensory tests are done under controlled testing conditions, using trained panelists and appropriate sensory methodologies, the procedures are objective.

The sensory method chosen for a test is dependent on the type of information required. The appropriate method can only be selected after the objectives of the test are clearly defined. As illustrated in Table 1, there are a substantial number of test methods available and it is important to be familiar with them in order to apply the correct method to achieve the correct results. Familiarity with the test methods also reduces the likelihood of relying on a single method. Difference and descriptive tests are the main tests used to evaluate fat and oil quality. Both tests require the use of trained panelists, but the level of training is considerably more for descriptive testing. In difference testing, panelists only determine if there is a difference between samples. The degree of difference is not determined. In contrast, descriptive tests allow panelists to rate the intensities of several attributes resulting

**TABLE 1. Summary of Product-Oriented and Consumer-Oriented Sensory Tests.**

Test Type	Test Procedure and Objective	Test Method
Product-oriented	Threshold—measures the level at which a sensory characteristic can be detected	Method of limits Average error Frequency
	Difference—measures difference or similarity between products or attributes of products	Paired comparison Duo-trio Dual-standard Triangle
	Scaling—measures intensity of a product attribute	Ranking Category Magnitude estimation
	Duration—measures the duration for which an attribute is detected	Time-intensity
	Descriptive analysis—measures the intensity of several attributes associated with the product	Flavor profile Texture profile Quantitative descriptive analysis Deviation from reference
	Consumer-oriented	Acceptance—measures acceptance of product
Preference—measures which product is preferred		Paired comparison
Hedonic—measures degree of liking/disliking for a product		Ranking Category

in a full description or profile of the samples. A more thorough discussion of test methodologies is available elsewhere (1, 2).

### **3. FACTORS AFFECTING SENSORY MEASUREMENTS**

Standard practices have been developed for conducting sensory panels in order to minimize psychological errors and physical testing conditions that can influence human judgment. By controlling these factors, reliable sensory results can be achieved. The following sections outline the most common psychological errors and how they can be minimized.

#### **3.1. Expectation Error**

Expectation error occurs when panelists are given information about the samples prior to the test. This information can influence their judgment because panelists usually find what they expect to find. To control for this error, panelists are only given enough information for them to perform the task, samples are coded to conceal identity, the panel is held in a separate room away from the preparation area, and the person conducting the test does not serve as a panelist.

#### **3.2. Suggestion Error**

This error occurs when one panelist's reaction to a sample causes other panel members to change their judgments. To minimize this error, individual booths are used and no discussion is permitted during testing.

#### **3.3. Stimulus Error**

Stimulus error occurs when panelists are influenced by irrelevant sample differences, such as differences in size, volume, and color of the samples. To minimize this error, the samples must be prepared and served under identical conditions. Unwanted color differences should be masked using colored lights.

#### **3.4. Positional Bias or Order Effect**

The order in which samples are evaluated can influence a panelist's judgment. The first sample is often scored higher regardless of the product being tested. To minimize this effect, serve samples to panelists in a randomized order: this way positional bias is balanced for the panel as a whole. A warm-up sample can also be used that calibrates the panel before beginning the test.

#### **3.5. Central Tendency Error**

This error is characterized by panelists avoiding the end points on a scale when rating samples. Use of reference samples and training can minimize this error.

### 3.6. Contrast and Convergence Errors

Contrast error occurs when two samples are very different from each other. Panelists tend to exaggerate the difference in their scores. Convergence error is the opposite effect whereby a sample may mask small differences between two samples causing their scores to converge. To minimize both of these errors, sample and serving orders must be randomized among the panelists.

## 4. EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experimental design that is selected and the statistical analysis that is applied to the data will significantly influence the outcome of a test. In sensory testing, the experimental design dictates the serving order of the samples. Several experimental designs are available to choose from, including completely randomized, balanced complete block, and balanced incomplete block designs. Key elements to good experimental design include randomization, blocking, and replication.

Randomization is done to minimize the effects of uncontrolled sources of variation or error and to eliminate bias. It involves ordering sample treatments in such a way that each treatment has an equal chance of being selected. In sensory testing, the order of sample presentation to each panelist is randomized.

Blocking is used to increase the power of an experiment by removing known sources of variability from the estimate of error. Blocks can include panelists, replications, treatment, or anything else that is a known source of variation. By grouping experimental units into blocks, a more accurate measure of pure or experimental error is obtained.

Replication involves repeating the experiment under identical conditions. It improves the reliability and validity of test results and is necessary to provide an estimate of experimental error. The number of replications completed is determined by time, cost, and sample constraints. However, the more replications completed, the better the estimate of experimental error and the more reliable the results.

Statistics are used in sensory testing to determine whether the responses from panelists are sufficiently similar or represent a random occurrence. Knowing the degree of similarity enables one to draw conclusions about the samples being tested with some measure of confidence in the context of that population of subjects and in the case of consumer-oriented tests to the population in general. Thus, it is important that appropriate statistical methods be applied to sensory data to satisfy the test objectives. For a more thorough discussion of the appropriate statistical methods for each of the various sensory tests, refer to Stone and Sidel (1) and ASTM (2).

## 5. SENSORY TESTING FACILITY

An important requirement of conducting sensory tests is the availability of a facility specifically designed for sensory testing. This is necessary to ensure that tests are



run efficiently and conducted under controlled conditions with minimum distractions. Without proper controls, reliable results may not be achieved. Permanent testing facilities provide the best testing environment. If this is not feasible, it is possible to adapt existing facilities for sensory testing. The types of tests to be conducted, the amount and frequency of testing, and the space and resources available will be deciding factors in the facilities that are developed for sensory testing. In general, space is needed for preparation of samples, testing, and training. A more thorough discussion regarding the design of sensory testing facilities including illustrations of possible layouts can be found elsewhere (1, 3).

The preparation area should provide sufficient counter space for preparation of samples and layout of sample trays. The area should provide refrigerated and frozen storage, electrical ranges and microwave ovens for heating, and sinks and dishwashers for cleaning purposes. Installation of distilled or filtered water should be considered for panelists' rinsing water and preparation of solutions if tap water imparts a flavor and odor. Ventilation hoods with exhaust fans should be installed to remove odors in order to prevent the odors from spreading to the booth area.

The testing facility must provide panelists a setting that minimizes factors that can influence their judgments. For this reason, individual booths are constructed so that a panelist can evaluate the samples without being influenced by other panelists. The booth area should be separate from the preparation area. Ideally, this room should be adjacent to the preparation area to permit samples to be passed through the wall of the preparation area to the panelist. Eight booths should be adequate to run most sensory programs. Each booth should be equipped with a counter, chair, a pass-through opening to the preparation area, individual lighting, and an electrical outlet for warming trays. Individual sinks for expectoration can also be provided but are problematic due to sanitation, noise, and odor problems. The use of covered disposable containers for expectoration is a more acceptable alternative. Some booths are computerized so that panelists utilize a computer screen, keyboard, and mouse rather than a paper ballot to record their judgments.

A booth width of 67–80 cm and a counter depth of 45–60 cm is recommended. Counter height should be the same as the counter height on the other side of the pass-through wall to permit ease of passing trays from one side to the other. Standard counter height is 90 cm. Partitions between booths should be at least 90 cm high and should extend 45 cm beyond the edge of the countertop to provide full privacy for the panelist (3). Walls and booths should be constructed of opaque, nonreflecting material that is neutral in color. Countertops and flooring should be odor-free and easily cleaned. Construction of a soffit above the booth counter is recommended to house lighting fixtures and ventilation ducts (1). Adequate space must be provided so that panelists can enter and exit the room without disturbing others. Pass-throughs from the preparation area should be large enough to accommodate tray passage. The opening can be fitted with a hinged, sliding, flip-up, or bread-box type door. However, more counter space will be required if hinged, flip-up, or bread-box type openings are used.

Each booth should have its own lighting to ensure uniform light distribution. Use of both incandescent and fluorescent lighting offers greater testing flexibility.

Fluorescent lights do not generate heat and are recommended when color evaluation is critical because bulbs can be selected that more closely match natural daylight. In situations when color differences between samples needs to be masked, colored incandescent bulbs or colored filters are used. Red lights are recommended for masking color differences in oil samples.

Room temperature control and adequate ventilation are the most critical factors in designing the panel room. In order to maintain a comfortable temperature in the room, air-conditioning should be installed. A slight positive air pressure should be maintained in the booth area to prevent infiltration of external odors. Individual exhaust ventilation ducts should be located in each booth. Air turnover in the room should occur at least every 30 seconds (1). Recirculated and makeup air should pass through activated carbon filters to remove odors.

If an area specifically designed for sensory testing is not available, a temporary area such as a boardroom can be set up provided that noise, distractions, and odors are minimized. Portable partitions made of heavy cardboard, wood, or metal can be used to provide individual booths for panelists.

The training area should also be adjacent to the preparation area and should allow for group discussion and testing of products. A comfortable, well-lit room with good ventilation and a table for 8–12 panelists is required. A whiteboard or flip chart should be provided to facilitate discussion.

Information pertaining to the design of a facility for evaluating room odor has been published by Mounts and Warner (4). The most important feature of this facility is the air lock room that a panelist must pass through before entering the actual odor room. This helps prevent significant loss of volatile odor components from the test room. Control of the amount of airflow into and out of the rooms is also required.

## 6. SAMPLE PREPARATION AND PRESENTATION

Samples should be prepared and presented to panelists under identical conditions. It is recommended that at least 30 g of solid food or 15 mL of liquid food be served (5). It may be necessary to serve some foods with a carrier or to use dilutions. For example, crackers or white bread may be used as a carrier for the evaluation of margarine. However, it is generally better to avoid the use of a carrier where possible, because they have flavor and textural characteristics of their own that can interfere with the panelists' evaluation of the intended product. Through training, panelists can usually overcome any aversions they might have to sampling a fat-based product on its own. If crude oil is to be evaluated, dilution may be required but the effect of the dilution medium needs to be considered. Warner et al. (6) found that good quality freshly deodorized soybean oil could be used as a diluent at a ratio of 95:5 in the evaluation of crude soybean oils. Stone and Hammond (7) reported on a method for evaluating oils in emulsions stabilized with gum acacia. The method allows for smaller quantities of oil to be evaluated because the total volume is increased through dilution. This can be an issue when evaluating experimental lines in breeding programs. The emulsion also clears the palate sooner and

more thoroughly than a pure oil, thereby reducing sample carryover from one oil sample to the next.

Whenever human subjects are used, every effort must be made to ensure that the panelists are not exposed to any risk associated with the samples. If there is some concern, precautions, such as expectorating the samples or only evaluating odor, should be implemented.

Generally, samples should be served at the temperature at which the food is usually consumed. Soft margarines are served at 4–5°C, whereas hard margarines can be served at 4–5°C or 22–24°C. It is recommended that salad oils be evaluated at a temperature of 50°C (8, 9) because this temperature brings out their characteristic odor and flavor. A circulating waterbath can be used for heating and keeping sample containers warm (9). If a waterbath is not available, an electric warming tray equipped with a pan containing water can be used. Alternatively, AOCS (8) recommends heating aluminum blocks containing the sample containers to the desired temperature.

Glass containers are recommended in the evaluation of salad oils because glass can be cleaned more thoroughly and therefore reused. Small glass beakers or jars work the best, but test-tubes can also be used if necessary. The containers should only be slightly filled with oil and should be capped to permit volatiles to build in the headspace. AOCS (8) recommends using 10 mL of oil in a 50 mL glass beaker covered with a watch glass. Disposable plastic or Styrofoam containers can be used provided the samples are not being heated and they do not impart any flavors or odors of their own. If samples are only being assessed for their odor properties, 10 g of oil can be placed in a 125 mL covered glass jar containing 30 g of glass beads (10). The glass beads are used to enhance the release of odor volatiles when the sample is swirled prior to sniffing. Disposable filter paper sticks used in the perfume industry can also be employed for assessing the odor characteristics of an oil by dipping the stick into the oil and smelling the oil absorbed onto the filter paper.

Margarine can be served by preloading on to plastic serving spoons for flavor evaluations. If margarines are to be evaluated for their spreadability properties, they should be served in a small plastic or paper cup (25–30 mL) with a plastic knife and a slice of white bread. Bland crackers can also be used, although these are less desirable than bread in evaluating spreadability due to their limited size and ease of breaking.

Samples should be coded in such a way that they impart no information to the panelists that might influence their judgment. It is therefore recommended that samples be coded with three digit random numbers. Samples should be served in a random order to each panelist to minimize positional bias or order effect.

The number of samples that can be evaluated in a session is dependent on the amount of training and experience of the panelists and on the nature of the product being evaluated. In the case of fats and oils, sensory fatigue can result from the evaluation of too many samples. This is due, in part, to carryover effects caused by the tendency of fats and oils to coat the mouth, making it difficult to cleanse the palate. Warm water (38–40°C) is necessary to clear the mouth when fats and

oils are being evaluated. Unsalted crackers or white bread can also be used. In some cases, it may be necessary to specify a waiting time between samples. As a result of the nature of fats and oils, most experienced panelists expectorate samples rather than swallow them. If samples are only being evaluated for odor, it is recommended that panelists sniff a sample of water between samples or wait between samples before proceeding to the next sample (20–30 seconds is usually adequate). Panelists need to mutually agree on how the samples should be evaluated and then should consistently use this technique.

## 7. REFERENCE SAMPLES

The use of reference samples or standards is critical in both training and calibration of panelists during testing. Reference samples are used during training to aid panelists in understanding terminology used to describe product quality. They are also used to anchor end-points and mid-points on attribute rating scales. The presence of reference samples during testing permits calibration of panelists before evaluation of the coded samples, thereby ensuring panelists score the coded samples more consistently. This is especially critical when sensory testing takes place over a period of time, such as during storage testing. Reference samples can be either internal or external references or standards. Internal references are selected from the samples that are to be tested. For example, fresh and oxidized oils are used to anchor the bland and strong end-points on an oil intensity scale, respectively. An external reference is a chemical or alternative food product that is selected to represent a specific attribute. An example of an external reference would be the use of a bland oil sample spiked with an artificial butter flavoring to anchor the high-intensity end-point on a buttery intensity scale. Spiking good quality oil with oxidized oil using a series of different ratios can be done to provide a range of oxidized samples that can be used during training. Regardless of the reference used, it is important that the product selected clearly represents the attribute and that it is stable and will not change over the course of the sensory test (11). Terms, definitions, and reference samples suitable for evaluating fats, oils, and fat-containing foods are provided in Table 2.

**TABLE 2. Flavor and Odor Terms, Definitions, and Reference Standards Used in the Evaluation of Fats, Oils, and Oil-Containing Foods.**

Term	Definition	Reference Standard
Acrid	Aromatic characteristic of burnt oil, pungent, sharp.	Canola oil heated to 240°C, acrolein
Beany	Aromatic characteristic of soybeans.	Cooked soybeans, not raw or green
Bland	No aromatics or flavors perceived	
Burnt	Aromatic characteristic of overheating.	Crude corn oil (odor only)
Buttery	Aromatic characteristic of fresh, unsalted butter.	Unsalted fresh butter diluted 1:99 in fresh oil
Cardboard	Aromatic characteristic of slightly oxidized oil or oil containing foods.	Water that has had cardboard or paper soaked in it for 30 min and then filtered to remove paper

TABLE 2. (Continued)

Term	Definition	Reference Standard
Corny	Aromatic characteristic of steeped corn and corn oil.	Crude corn oil diluted 5:95 in good quality deodorized corn oil
Fishy	Aromatic characteristic of oxidized linolenate-containing oils such as linseed, canola, and soybean.	Cod liver oil or canola oil heated to 190°C for 30 min
Fried Food	Aromatic characteristic of food fried in hot oil.	Deep fried potatoes
Fruity	Aromatic characteristic of ripe non-citrus fruit.	Olive oil
Grassy	Aromatic characteristic of freshly mown green grass.	Fresh cut green grass, hexanal
Green	Aromatic characteristic of raw, unripe vegetables.	Raw soybeans
Hay	Aromatic characteristic of dried grass or hay.	Crude soybean oil from heat-processed beans diluted 5:95 in fresh oil
Hull-like	Aromatic characteristic of grain hulls.	Peanut or sunflower hulls
Hydrogenated	Aromatics characteristic of typical hydrogenated oils comprised of attributes of paraffin, fruity, and flowery.	Hydrogenated soybean oil with a iodine value of <100
Melon	Aromatic characteristic of ripe melons.	0.002 ppm <i>cis</i> -6-nonenal in fresh oil
Metallic	Aromatic characteristic of metal coins.	0.01% ferrous sulfate in distilled water
Musty	Aromatic characteristic of damp basement.	Geosmin (odor only)
Nutty	Aromatic characteristic of fresh nuts.	Freshly processed peanut oil
Overheated	Aromatic characteristic of oil overheated during processing	Crude corn oil (odor only)
Oxidized	General term used to describe aromatics characteristic of oxidized oils from slight to intense i.e. from buttery to painty. Not recommended as a descriptor.	
Painty	Aromatic characteristic of oxidized linolenate-containing oils such as linseed, canola, and soybean with a peroxide value >10.	Linseed oil
Pine	Aromatic characteristic of pine needles.	Nondeodorized sunflower oil diluted 5:95 in fresh sunflower oil
Rancid	Aromatic characteristic of oxidized oil.	Safflower or sunflower oil with a peroxide value >5
Raw	Aromatic characteristic of unprocessed grain or vegetables.	Whole wheat flour, raw potato
Reverted	General term used to describe an oxidative process known as reversion. Not recommended as a descriptor.	
Rubbery	Aromatic characteristic of old rubber.	Rubber stoppers used for chemical glassware
Smokey	Aromatic characteristic of overheated oil.	Oil heated to 240°C
Sour	Basic taste characteristic of acids.	0.08% citric acid in water solution
Stale	Aromatics characteristic of old, no longer fresh product with loss of original flavors; usually occurs in initial stages of oxidation.	Potato chips aged 2 weeks at 25°C
Sulfur	Aromatic characteristic of sulfur compounds.	Nondeodorized canola oil diluted 5:95 in fresh oil
Waxy	Aromatic characteristic of melted paraffin.	Paraffin oil
Weedy	Aromatic characteristic of green non-grass weeds.	Fresh cut green weeds
Woody	Aromatic characteristic of dry wood.	Sawdust, wood chips, popsicle sticks, or peanut oil heated to 190°C for 30 min

Adapted from Warner (12).

## 8. SELECTION AND TRAINING OF PANELISTS

Panelists for trained panels can be recruited from the organization where the testing is to be conducted. In many cases, approval to use panelists as human subjects requires prior approval by an internal committee, so the agency can be in compliance with national, regional, or organizational requirements for use of human subjects. Provided there is management support, most employees are willing to participate if they feel their contribution is important. Potential panelists should be asked to complete a simple questionnaire to determine their likes and dislikes of foods, any food restrictions, allergies and medications, and time available for panels. This information is useful in screening individuals in terms of availability and general health. Candidates should be in good health and not prone to frequent colds. Certain medications may alter a panelist's sensitivity to taste and odor compounds. Age, sex, and smoking habits may be other factors to consider in selecting panelists, although they are not critical.

The next step is to screen panelists for their sensory acuity. This is done by having panelists complete tests designed to identify their ability to recognize basic tastes, common odors, and textural characteristics. More information on these tests is provided in guidelines published by ASTM (13).

The final step in the selection of panelists is based on their ability to detect differences in the products that they will be evaluating and to be consistent in their judgments. This is often done using a series of triangle tests. For example, samples of vegetable oils of known differences are presented to potential panelists. Panelists are asked to identify which sample is different to gain a measure of the panelist's ability to discriminate. The test is repeated several times to gain a measure of the panelist's ability to reproduce judgments. A group of 20–25 potential panelists are generally screened in order to select a panel of 8–12 members for training.

The performance of individual panelists and the panel as a whole is improved significantly through training as the goal is to help panelists make valid, reliable, and objective judgments free of personal preferences. Training exercises are designed to teach panelists about the terminology used to describe product quality, the scoring system, and the task they are being asked to perform. They are also exposed to the range of samples they are likely to encounter in the test sessions and to reference samples. Through discussion, panelists learn to develop standardized evaluations such that panelists' responses are consistent and agree with each other. Depending on the needs of the group, training sessions are designed to last 30–60 minutes per day over a period of 1–2 weeks. The exact length of time depends on the nature of the products to be tested, the number of attributes to be measured, and the past experience of the panelists. Training is complete when panelists are in agreement on the attributes to be measured and in the placement of the reference samples on the scale, are comfortable with the procedure, are consistent in their judgments, and are in agreement with each other.

## 9. MONITORING AND MOTIVATION OF PANELISTS

Panelist performance as a group and individually should be monitored throughout the testing period to ensure the panel is performing and does not require recalibration. To determine this, a set of samples of known differences is evaluated by the panel on several occasions.

It is important to maintain the interest and motivation of panelists throughout training and testing because panelists who are highly motivated perform better. Feedback about their performance, especially during training, is critical to keep panelists motivated. Rewards of food or beverage at the conclusion of the session can also be used. At the conclusion of the test, panelists should be provided with information about the test and be assured that their contribution was appreciated. This is important as it will assist in motivating panelists to participate in future panels.

## 10. SENSORY EVALUATION OF OILS

Salad oils are evaluated for their initial odor and flavor and for their stability during storage. During odor evaluations, the sample container should always be covered. Panelists are instructed to gently swirl the container, remove the cover quickly, and take three short sniffs before placing the lid back on the container. Odors disappear from the headspace quickly, so it is important for panelists to make their judgment as rapidly as possible. If retesting of the oil is required, panelists should allow the volatiles to concentrate in the headspace of the covered sample. Generally 3–5 minutes is adequate. The exact waiting time is dependent on the amount of headspace and the aperture of the container. Evaluation of the flavor characteristics of oil requires taking 5–10 mL of oil into the mouth, pulling air through the oil, and exhaling through the nose. This procedure enhances the flow of volatiles to the retronasal area responsible for detection of odors through the oral cavity. Although some researchers suggest that this method is more sensitive than the nasal method of assessing volatiles, by carefully controlling testing conditions, the nasal method can be successfully used to assess oil quality. Indeed, panelists can generally evaluate more samples using this technique because there is no buildup of oil residue as there is when oil is taken into the mouth.

The American Oil Chemists' Society (AOCS) and the American Society for Testing Material (ASTM) have published recommended practices with regard to the serving containers and serving procedures that should be used when assessing oil samples (8, 9). The AOCS practice includes two rating scales based on a 10-point scoring system for measuring the overall quality and intensity of liquid vegetable oils. The intensity scale has a range from 10 = bland to 1 = extremely strong (Figure 1). This scale is recommended for rating oils that have a bland odor and flavor after processing, such as canola, soy, sunflower, safflower, and cottonseed. Oils such as corn, peanut, and olive, which have a naturally distinct

Directions: Take 5-10 mL of warm oil into the mouth; pull air through the oil and exhale through the nose. Rate samples for overall flavor intensity on the 10 point scale; identify flavors and rate as weak (W), moderate (M), or strong (S).

Intensity		Overall Intensity Scores			
		492	716	258	931
10	Bland	___	___	___	___
9	Trace	___	___	___	___
8	Faint	___	___	___	___
7	Slight	___	___	___	___
6	Mild	___	___	___	___
5	Moderate	___	___	___	___
4	Definite	___	___	___	___
3	Strong	___	___	___	___
2	Very Strong	___	___	___	___
1	Extreme	___	___	___	___

Descriptions	Intensity			
Nutty	___	___	___	___
Buttery	___	___	___	___
Corny	___	___	___	___
Beany	___	___	___	___
Hydrogenated	___	___	___	___
Burnt	___	___	___	___
Weedy	___	___	___	___
Grassy	___	___	___	___
Rubbery	___	___	___	___
Melon	___	___	___	___
Rancid	___	___	___	___
Painty	___	___	___	___
Fishy	___	___	___	___
Other _____	___	___	___	___

**Figure 1.** Flavor intensity ballot (8).

yet desirable odor and flavor, should be rated on the quality scale (Figure 2). After rating the overall flavor of the sample, panelists are instructed to rate the oil for individual flavor characteristics on a 3-point scale from weak to strong using a checklist of possible flavor attributes. The practice of having panelists rate the overall intensity or quality, followed by rating individual attributes, suggests that a single measurement is not always adequate to profile an oil. In such instances, it may be more practical and informative to have panelists rate the intensity of specific attributes using scales that are less restrictive than the 3-point scales used in the AOCS method. Malcolmson et al. (14) have successfully used



Directions: Take 5-10 mL of warm oil into the mouth; pull air through the oil and exhale through the nose. Rate samples for overall flavor quality on the 10 point scale; identify flavors and rate as weak (W), moderate (M), or strong (S).

Intensity		Overall Quality Scores			
		492	716	258	931
10	Excellent	_____	_____	_____	_____
9	Good	_____	_____	_____	_____
8		_____	_____	_____	_____
7	Fair	_____	_____	_____	_____
6		_____	_____	_____	_____
5	Poor	_____	_____	_____	_____
4		_____	_____	_____	_____
3	Very Poor	_____	_____	_____	_____
2		_____	_____	_____	_____
1	Bad	_____	_____	_____	_____

Descriptions	Intensity			
Nutty	_____	_____	_____	_____
Buttery	_____	_____	_____	_____
Corny	_____	_____	_____	_____
Beany	_____	_____	_____	_____
Hydrogenated	_____	_____	_____	_____
Burnt	_____	_____	_____	_____
Weedy	_____	_____	_____	_____
Grassy	_____	_____	_____	_____
Rubbery	_____	_____	_____	_____
Melon	_____	_____	_____	_____
Rancid	_____	_____	_____	_____
Painty	_____	_____	_____	_____
Fishy	_____	_____	_____	_____
Other _____	_____	_____	_____	_____

Figure 2. Flavor quality ballot (8).

unstructured line scales to rate the intensity of buttery and painty odors of various canola oils stored under accelerated storage conditions.

Sensory quality of olive oil is currently determined by the European Union regulation (15) or the International Olive Oil Council (IOOC) trade standard (16). Both official methods used trained panelists but differ in the sensory descriptors and the scales employed. The EU standard involves rating the oil for olfactory, gustatory, and tactile attributes on intensity rating scales ranging from 0 (no perception) to 5

(extreme), followed by an overall rating for grade on a 9-point scale from 1 (lowest quality) to 9 (maximum quality). Using these methods, Aparicio and Morales (17) proposed a flavor sensory wheel to show relationships among attributes perceived in olive oils evaluated by Spanish, Italian, Dutch, and British trained panels based on principal component analyses.

Scales reportedly used by the oil industry combine intensity and quality characteristics in one scale. For example, oil with a weak melon flavor is rated as a 5, whereas oil with a weak painty flavor is rated as a 4. These scales are not recommended because information cannot be accurately captured using scales of this nature.

## 11. SENSORY EVALUATION OF OIL-CONTAINING FOODS

Evaluation of oil-containing foods, such as salad dressings, mayonnaise, and margarines, requires highly trained panelists. During training, panelists agree on the attributes important in a good quality product. Attributes of appearance, taste, flavor, and texture need to be considered as well as physical attributes such as pourability and spreadability. Definitions, references, and evaluation procedures must also be established during training. The use of dilutions or carriers may be required. However, trained panelists generally prefer to evaluate these products without the use of carriers or dilutions as they find the task easier without the presence of interfering ingredients. Attributes are rated for their intensities using either category/structured scales or unstructured line scales. Using unstructured line scales, Kok et al. (18) measured the spreadability, graininess, and waxiness of margarines served at room temperature while Rousseau and Marangoni (19) measured cold spreadability, butter flavor, off-flavors, and texture (oily, lingering coating in mouth to clean, rapid melt-in-mouth). Structured scales were used by Jacobsen et al. (20) to evaluate the aroma, flavor, and textural properties of mayonnaise.

## 12. SENSORY EVALUATION OF FRYING OILS/ROOM ODOR

Methods to evaluate the odor characteristics of heated oils were developed by Evans et al. (21), which involved heating the oil to 190°C and having panelists enter the room and rate the odor of the room for overall quality and individual odor characteristics using 0–10 point intensity scales where 2 = weak, 5 = moderate, and 8 = strong. This method was later upgraded by constructing a small room with controlled air flow and temperature (4).

## 13. SENSORY EVALUATION OF FRIED FOODS

The sensory properties of fried foods are evaluated either immediately after frying, e.g., French fries, chicken, fish, or after storage, e.g., potato chips and other snack

foods. Bread cubes have also been used as an alternative by some researchers (22, 23). Similar to the evaluation of oils, fried foods can be evaluated for flavor quality using a scale similar to the quality scale used for oils (Figure 1). However, additional information can be obtained by having panelists rate individual flavor or off-flavor characteristics using less restrictive category scales, i.e., scales greater than 3 points or by using unstructured line scales. Petukhov et al. (24) reported a technique for evaluating the odor properties of stored potato chips that involved presenting panelists with individual bags of chips. The bags were opened by the panelist and immediately rated for painty and stale/musty odor using unstructured line scales. French fries are also evaluated for off-flavors/off-odors as well as textural properties such as greasiness and crispiness (25).

## 14. ELECTRONIC NOSE

Recent advances in the technology of multisensor arrays and neural computing have made the development of the electronic nose of great interest to the food industry for discrimination between odors (26). Provided the instrument has been calibrated properly, the technique is rapid, nondestructive, and objective. Shen et al. (27) found the electronic nose was capable of measuring changes in volatile compounds associated with lipid oxidation in canola, corn, and soybean oils stored under accelerated conditions and Aparicio et al. (28) found the electronic nose could be calibrated to detect rancidity levels in good quality olive oil spiked with rancid olive oil.

## 15. GAS CHROMATOGRAPHY–OLFACTOMETRY

Gas chromatography–olfactometry (GC-O) provides a sensory profile of odor active compounds present in an aroma extract by sniffing the GC effluent. Several techniques have been developed to collect and process GC-O data and to estimate the sensory contribution of individual odor active compounds, including dilution analysis (29, 30), time intensity (31), and detection frequency (32) methods. GC-O has successfully been used to evaluate the odor active compounds of olive oil (33), soybean oil (34), and fish oil enriched mayonnaise (35).

## 16. CONCLUSIONS

Sensory evaluation can play an important role in quality assessment programs. By controlling and using proper testing conditions, and by using trained panelists and appropriate sensory methodologies, sensory evaluation can provide essential information necessary to make an informed decision on quality, and can contribute to a better understanding of product behavior. Sensory evaluation is also necessary in validating instrumental methods such as the electronic nose and for determining key odorants using GC-O techniques.

## REFERENCES

1. H. Stone and J. L. Sidel, *Sensory Evaluation Practices*, Academic Press, San Diego, California, 1993.
2. American Society for Testing and Materials, *MNL 26 Sensory Testing Methods*, 2nd ed., ASTM, Philadelphia, Pennsylvania, 1996.
3. American Society for Testing and Materials, *STP 913 Physical Requirement Guidelines for Sensory Evaluation Laboratories*, ASTM, Philadelphia, Pennsylvania, 1986.
4. T. L. Mounts, and K. Warner, in D. R. Erickson et al., eds., *Handbook of Soy Oil Processing and Utilization*, American Soybean Association and American Oil Chemists' Society, Champaign, Illinois, 1980.
5. American Society for Testing and Materials, *STP 434 Manual on Sensory Testing Methods*, ASTM, Philadelphia, Pennsylvania, 1968.
6. K. Warner, E. N. Frankel, and K. J. Moulton, *J. Amer. Oil Chem. Soc.*, **65**:386 (1988).
7. R. Stone, and E. G. Hammond, *J. Amer. Oil Chem. Soc.*, **60**:1277 (1983).
8. Official Methods and Recommended Practices of the American Oil Chemists' Society, AOCS, Champaign, Illinois, 2002, Method Cg 2-83.
9. American Society for Testing and Materials, *Standard Practice for the Bulk Sampling, Handling, and Preparing of Edible Oils for Sensory Evaluation Laboratories*, ASTM, Philadelphia, Pennsylvania, 2000, Method E1346-90 (2000).
10. R. Przybylski, L. J. Malcolmson, N. A. M. Eskin, S. Durance-Tod, J. Mickle, and R. Carr, *Lebensm. Wiss.u. Technol.*, **26**:205 (1993).
11. B. A. Rainey, *J. Sensory Studies*, **1**:149 (1986).
12. K. Warner, in K. Warner and M. Eskin, eds., *Methods to Assess Quality and Stability of Oils and Fat-Containing Foods*, AOCS Press, Champaign, Illinois, 1995.
13. American Society for Testing and Materials, *STP 758 Guidelines for the Selection and Training of Sensory Panel Members*, ASTM, Philadelphia, Pennsylvania, 1981.
14. L. J. Malcolmson, M. Vaisey-Genser, R. Przybylski, D. Ryland, N. A. M. Eskin, and L. Armstrong, *J. Amer. Oil Chem. Soc.*, **73**:1153 (1996).
15. EC 1991 Commission Regulation EC 2568/91, July 1991, on the Characteristic Methods of Olive Oils and Their Analytical Methods. EC Journal L 8121.10.91 to the Regulation EEC/2568/91.
16. IOOC. International Olive Oil Council. COI/T.2o/Document 15/Rev. 1 (Organoleptic Assessment of Olive Oil. Resolution RES-3/75-IV/96, 20 November), Madrid, Spain, 1996.
17. R. Aparicio and M. T. Morales, *J. Sci. Food Agric.*, **67**:247 (1995).
18. L. L. Kok, W. R. Fehr, E. G. Hammond, and P. J. White, *J. Amer. Oil Chem. Soc.*, **76**:1175 (1999).
19. D. Rousseau and A. G. Marangoni, *Food Research Int.*, **31**:381 (1999).
20. C. Jacobsen, J. Adler-Nissen, and A. S. Meyer, *J. Agric. Food Chem.*, **47**:4917 (1999).
21. C. D. Evans, H. A. Moser, G. R. List, H. J. Dutton, and J. C. Cowan, *J. Amer. Oil Chem. Soc.*, **48**:711 (1971).
22. E. N. Frankel, K. Warner, and K. J. Moulton, *J. Amer. Oil Chem. Soc.*, **62**:1354 (1985).
23. H-R. Liu and P. J. White, *J. Amer. Oil Chem. Soc.*, **69**:533 (1992).

24. I. Petukhov, L. J. Malcolmson, R. Przybylski, and I. Armstrong, *J. Amer. Oil Chem. Soc.*, **76**:889 (1999).
25. X-Q., Xu, V. H. Tran, M. V. Palmer, K. White, and P. Salisbury, *Food Aust.*, **52**:77 (2000).
26. P. N. Barlett, J. M. Elliot, and J. W. Gardner, *Food Technol.*, **51**:44 (2001).
27. N. Shen, S. Moizuddin, L. Wilson, S. Duvick, P. White, and L. Pollak, *J. Amer. Oil Chem. Soc.*, **78**:937 (2001).
28. R. Aparicio, S. M. Rocha, I. Delgadillo, and M. T. Morales, *J. Agric. Food Chem.*, **48**:853 (2000).
29. F. Ullrich, and W. Grosch, *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, **184**:277 (1987).
30. T. E. Acree, J. Barnard, and D. Cunningham, *Food Chem.*, **14**:273 (1984).
31. R. Miranda-Lopez, L. M. Libbey, B. T. Watson, and M. McDaniel, *J. Food Sci.*, **57**:985 (1992).
32. J. P. H. Linssen, J. L. G. M. Janssens, J. P. Roozen, and M. A. Posthumus, *Food Chem.*, **8**:1 (1993).
33. J. Reiners and W. Grosch, *J. Agric. Food Chem.*, **46**:2754 (1998).
34. S. M. van Ruth, E. S. Shaker, and P. A. Morrissey, *Food Chem.*, **75**:177 (2001).
35. K. Hartvigsen, P. Lund, L. Hansen, and G. Holmer, *J. Agric. Food Chem.*, **48**:4858 (2000).

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## *Antioxidants: Science, Technology, and Applications*

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### **1. AN ANTIOXIDANT—DEFINITION**

In a biological system, an antioxidant can be defined as “any substance that when present at low concentrations compared to that of an oxidizable substrate would significantly delay or prevent oxidation of that substrate” (1). The oxidizable substrate may be any molecule that is found in foods or biological materials, including carbohydrates, DNA, lipids, and proteins. Food is a multicomponent system composed of a variety of biomolecules, and therefore, this definition describes well an antioxidant. However, regulatory bodies that overlook the food-supply categorize antioxidants under food additives and define them as “substances used to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation” (Code of

Federal Regulations, Food and Drug Administration). In foods, much of the work on antioxidants has emphasized retardation of lipid oxidation, which eventually triggers and transforms to the oxidation of other macromolecules such as proteins. It is the intention of this chapter to summarize the available information on the chemistry, technology, and regulatory aspects of compounds that can delay oxidation of unsaturated fats and lipids in food.

## 2. HISTORY OF ANTIOXIDANTS AND THEIR USE

Antioxidants may occur as natural constituents of foods, and may intentionally be added to products or formed during processing. Use of substances to enhance quality of food by means of delaying lipid oxidation has been in practice for centuries, although it was not chemically defined or understood. The first recorded scientific observation on oxidation inhibitors came from Berthollet in 1797 (2) and later from Davy (3). Their theory was described as “catalyst poisoning” in oxidative reactors, and this was well before the free radical theory of peroxidation had been proposed. Duclaux (4) first demonstrated participation of atmospheric oxygen in oxidation of free fatty acids. Later, it was found that oxidation of unsaturated acylglycerols can generate rancid odors in fish oils (5).

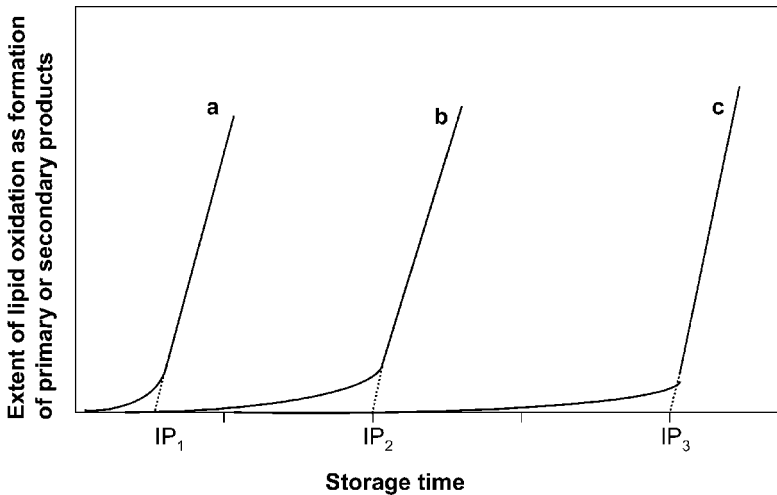
The earliest reported work on the use of antioxidants to retard lipid oxidation appeared in 1843, in which Deschamps showed that an ointment made of fresh lard containing gum benzoin (contains vanillin) or populin (from polar buds, contains saligenin and derivatives) did not become rancid as did the one with pure lard (2). Interestingly, the first reports on antioxidants employed for food lipids were about using natural sources; in 1852, Wright (6) reported that elm bark was effective in preserving butterfat and lard. Chevreul (7) showed that wood of oak, poplar, and pine (in the order of decreasing efficacy) retarded the drying of linseed oil films applied on them, and on all three, it took much longer time to dry than on glass. Moureu and Dufraise (8–11) first reported the possibility of using synthetic chemicals, especially phenolic compounds, to retard oxidative decomposition of food lipids. Their work provided the basic information leading to theories of lipid oxidation and antioxidants, which they referred to as “inverse catalysis.” Systematic investigation of antioxidant activity based on the chemistry of radical chain peroxidation of “model” chemicals was reported by Lowry and his colleagues (12) and Bolland and tenHave (13) of the British Rubber Producers Research Association. Antioxidant synergism in food was first reported by Olcott and Mattill (14), and this was significant in achieving oxidative stability in food by using a combination of antioxidants found in the unsaponifiable fraction of oils. They described the antioxidants as inhibitors and grouped them into acid type, inhibitols, and hydroquinone and phenolics. Bailey (15) and Scott (16) have provided the history and a descriptive analysis of the development of antioxidants in their books, “The Retardation of Chemical Reactions” and “Antioxidants and Autoxidation”, respectively.

Since the early 1960s, the understanding of autoxidation of unsaturated lipids and antioxidative mechanisms have advanced significantly as a result of development of

effective analytical tools. The last two decades have been very important to the antioxidant research. Around the world a revival is seen in studying the natural antioxidants in foods and the potential health benefits of natural antioxidants in relation to prevention and therapy of oxidative stress and related diseases. The emphasis has largely been on their implications on vital biological reactions that have a direct relationship to tissue injury and degenerative diseases. Enough scientific evidences have already been accumulated in relation to these conditions with free radicals and reactive oxygen species. Therefore, not only enhancing the shelf life stability of foods has been examined, but also control of lipid oxidation by suppressing free radical formation in foods to prevent their deleterious health effects has become important. The quest for understanding the oxidation of lipids and its prevention and control has continued since historical times and is still on.

### 3. SCOPE OF USING ANTIOXIDANTS IN FOOD

The function of an antioxidant is to retard the oxidation of an organic substance, thus increasing the useful life or shelf life of that material. In fats and oils, antioxidants delay the onset of oxidation or slow the rate of oxidizing reactions. Oxidation of lipids chemically produces compounds with different odors and taste and continues to affect other molecules in the food. The main purpose of using an antioxidant as a food additive is to maintain the quality of that food and to extend its shelf life rather than improving the quality of the food. Figure 1 illustrates how antioxidants can affect the quality maintenance of food in terms of oxidative rancidity



**Figure 1.** Typical curves for oxidation of lipids (a) No antioxidant added; (b) and (c) represent added or endogenous antioxidants. Antioxidant activity of (c) is higher than (b). IP<sub>1</sub>, IP<sub>2</sub>, and IP<sub>3</sub> are induction period in hours or days.

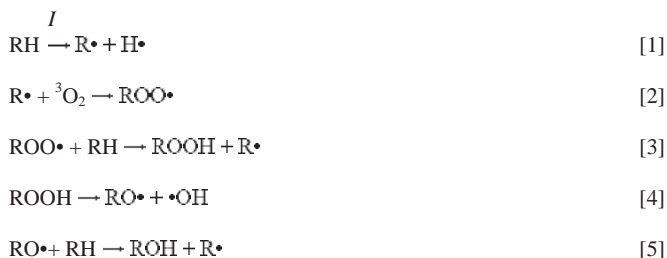


development. Use of antioxidants reduces raw material wastage and nutrition loss and widens the range of fats that can be used in specific products. Thus, antioxidants are useful additives that allow food processors to use fats and oils economically in their product formulation.

#### 4. OXIDATION OF FATS AND OILS AND MECHANISM OF ANTIOXIDANTS

In fats and oils, the process of oxidation is similar to that oxidation of any other unsaturated organic material and requires an initiation process, in order to generate free radicals from the substrate. As antioxidants inhibit oxidation or autoxidation process, the mechanism(s) involved need(s) to be discussed. Figure 1 explains the relationship of antioxidant activity and oxidation of a lipid as examined by a typical evaluation method.

Autoxidation is the oxidative deterioration of unsaturated fatty acids via an autocatalytic process consisting of a free radical chain mechanism. This chain includes initiation, propagation, and termination reactions that could be cyclical once started. The initiation process generates free radicals from the substrate. The  $\alpha$ -methyleneic H atom is abstracted from the unsaturated lipid molecule to form a lipid (alkyl) radical ( $R\cdot$ ) (Scheme 1, Equation [1]). The lipid radical is highly reactive and can react with atmospheric oxygen ( ${}^3O_2$ ), a facile reaction resulting from the diradical nature of the oxygen molecule, and it produces a peroxy radical ( $ROO\cdot$ ) (Scheme 1, Equation [2]). In the propagation reactions, the peroxy radical reacts with another unsaturated lipid molecule to form a hydroperoxide and a new unstable lipid radical (Scheme 1, Equation [3]). As a new free radical is generated at each step, more oxygen is incorporated into the system. The newly propagated lipid radical will then react with oxygen to produce another peroxy radical, resulting in a self-catalyzed, cyclical mechanism (Scheme 1, Equation [4]).



**Scheme 1.** Possible reactions of the autoxidation process. "R" is an alkyl group of an unsaturated lipid molecule. "H" is an  $\alpha$ -methyleneic hydrogen atom easily detachable because of the activating influence of the neighboring double bond or bonds. "RO" is alkoxy radical, "ROO" is peroxy radical, and "I" is an initiator.

Hydroperoxides are unstable and may degrade to radicals that accelerate propagation reactions. These are branching steps of lipid autoxidation process (Scheme 1, Equations [5] and [6]). This chain reaction proceeds, and termination occurs only when two free radicals combine to form a nonradical product. Autoxidation can break down the substrate molecules as well as forming new molecules causing gross changes in the chemical and physical properties of the oxidizing substrate (17–19). Degradation of hydroperoxides may generate new molecules that have undesirable odors and flavors, associated with oxidative rancidity of unsaturated lipids. Such sensory perceivable changes are noted when oxidation of unsaturated lipids has been progressed to advanced stages. This is only a brief description of autoxidation process.

A lipid that contains double bonds undergoes autoxidation induced by various ways. It is now clear that metal-catalyzed decomposition of preformed hydroperoxides is the most likely cause for the initiation process. The direct oxidation of unsaturated lipids by triplet oxygen ( $^3\text{O}_2$ ) is spin forbidden. This is because of the opposite spin direction of ground state lipid of single multiplicity and oxygen of triplet multiplicity, which does not match. When initiators are present, this spin barrier between lipids and oxygen can readily be overcome and produce radicals by different mechanisms. Ground state oxygen may be activated in the presence of metal or metal complexes and can initiate oxidation either by formation of free radicals or singlet oxygen. Exposure of lipids to light, metals, singlet oxygen and sensitizers (chlorophyll, hemoproteins, and riboflavin), or preformed hydroperoxide decomposition products causes generation of primary hydroperoxides. Photosensitized oxidation or lipoxygenase-catalyzed oxidation also produces hydroperoxides.

Thermal oxidation is also autocatalytic and considered as metal-catalyzed because it is very difficult to eliminate trace metals (from fats and oils or food) that act as catalysts and may occur as proposed in Equation 4. Redox metals of variable valency may also catalyze decomposition of hydroperoxides (Scheme 2, Equations [6] and [7]). Direct photooxidation is caused by free radicals produced by ultraviolet radiation that catalyzes the decomposition of hydroperoxides and peroxides. This oxidation proceeds as a free radical chain reaction. Although there should be direct irradiation from ultraviolet light for the lipid substrate, which is usually uncommon under normal practices, the presence of metals and metal complexes of oxygen can become activated and generate free radicals or singlet oxygen.



**Scheme 2.** Possible reactions of generating hydroperoxides ( $\text{M}^{n+}$  is the metal ion with transitional valency).

Photosensitized oxidation is a direct reaction of light-activated, singlet oxygen with unsaturated fatty acids, and subsequently hydroperoxides are formed. Photosensitized

oxidation happens because of the presence of molecules that can absorb visible or near UV light to become electronically excited (sensitizers) (Equation 8). Pigments initiating photosensitized oxidation in foods include chlorophylls, hemoproteins, and riboflavin. The type I sensitizer serves as photochemically activated free radical initiator, and type II sensitizers in the triplet state interact with oxygen by energy transfer to form singlet oxygen ( $^1\text{O}_2$ ) that reacts further with unsaturated lipid (Equation 9). Under photosensitized oxidation conditions, the reaction of unsaturated lipids with singlet oxygen ( $^1\text{O}_2$ ) leads to rapid formation of hydroperoxides (Equations 10) (17, 19, 20).



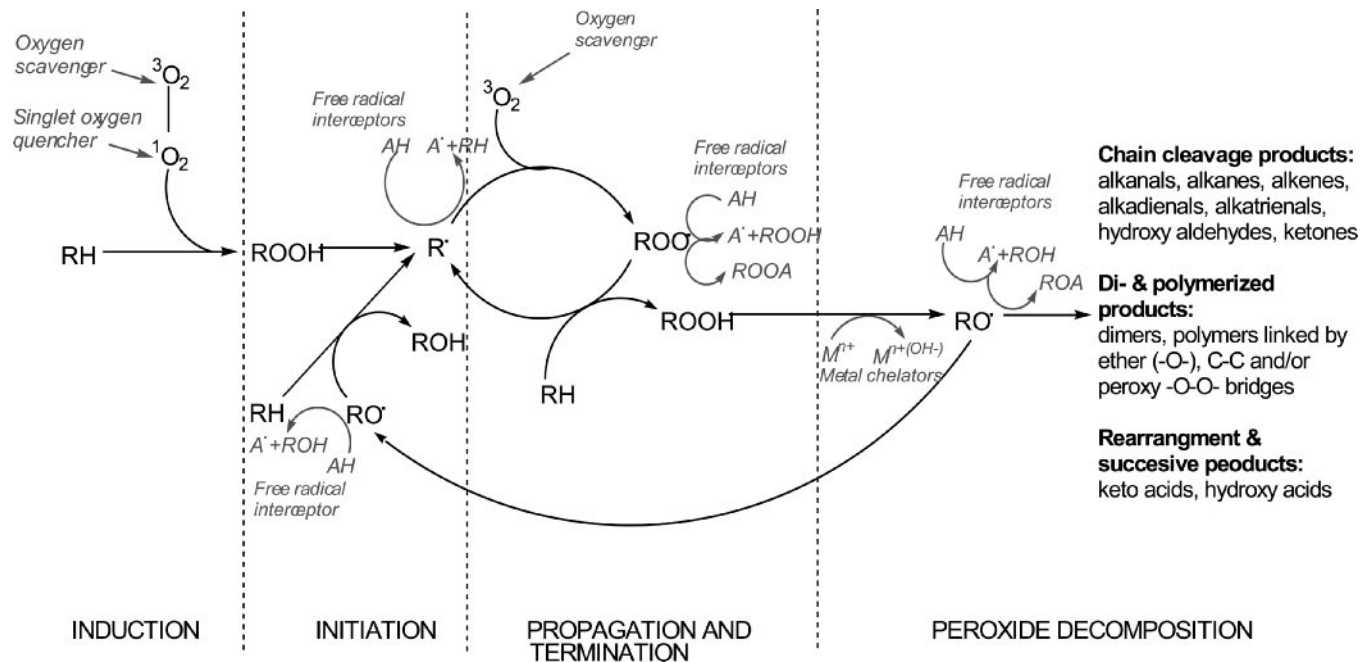
**Scheme 3.** Formation of hydroperoxides by photooxidation of a lipid with a sensitizer ( $h\nu$  is energy in the form of UV light, sensitizers that are naturally present in photosensitive pigments, their degradation products, or polycyclic aromatic hydrocarbons capable of transferring energy from light to chemical molecules).

## 5. CLASSIFICATION OF ANTIOXIDANTS

Antioxidants may be broadly grouped according to their mechanism of action: primary or chain breaking antioxidants and secondary or preventive antioxidants. According to this classification, some antioxidants exhibit more than one mechanism of activity, therefore, referred to as multiple-function antioxidants. Another commonly used classification categorizes antioxidants into primary, oxygen scavenging, and secondary, enzymatic and chelating/sequestering antioxidants. However, synergistic antioxidants are not included in this classification. During the past two decades, several naturally occurring compounds have been added into the list of antioxidants that are effective against oxidation of unsaturated fats and oils and most of them fall into the multifunctional category. Classification of antioxidants according to the mode of activity as primary and secondary is preferred in this discussion.

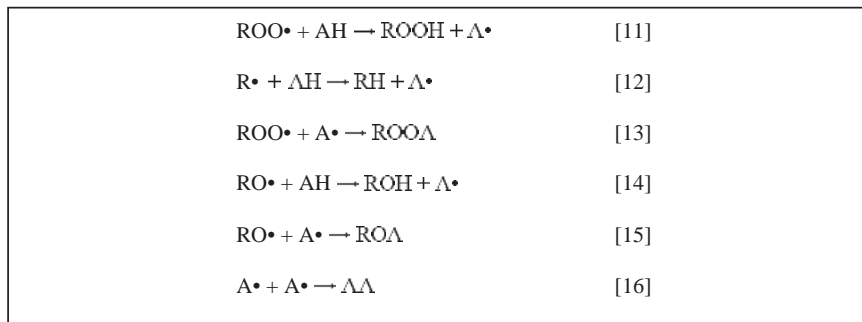
### 5.1. Primary Antioxidants

Primary antioxidants are also referred to as type 1 or chain-breaking antioxidants. Because of the chemical nature of these molecules, they can act as free radical acceptors/scavengers and delay or inhibit the initiation step or interrupt the propagation step of autoxidation. Figure 2 illustrates possible events that primary



**Figure 2.** Possible interactions of primary and secondary antioxidants with lipid oxidation pathway in foods.

antioxidants may interfere along the lipid autoxidation pathway. Primary antioxidants cannot inhibit photosensitized oxidation or scavenge singlet oxygen.



**Scheme 4.** Mechanism of primary antioxidant activity (AH is an antioxidant molecule).

The first kinetic study of antioxidant activity was conducted by Boland and tenHave (13) who postulated the Equations 11 and 12. The primary antioxidants (AH) react with lipid and peroxy radicals ( $ROO\cdot$ ) and convert them to more stable, nonradical products as shown in Scheme 4, Equations 13 and 14. These antioxidants are capable of donating a hydrogen atom to lipid radicals and produce lipid derivatives and antioxidant radicals ( $A\cdot$ ) that are more stable and less readily available to participate in propagation reactions (Equation 12). Primary antioxidants have higher affinities for peroxy radicals than lipids and react predominantly with peroxy radicals. The following reasons have been listed for their high affinity. Propagation is the slow step in lipid oxidation process; thus, peroxy radicals are found in comparatively larger quantities than other radicals. In addition, peroxy radicals have lower energies than alkoxy radicals; therefore, they react more readily with the low-energy hydrogen of primary antioxidants than unsaturated fatty acids. As the free radical scavengers are found in low concentration, they do not compete effectively with initiating radicals (e.g., hydroxyl radicals) (21, 22). Therefore, primary antioxidants inhibit lipid oxidation more effectively by competing with other compounds for peroxy radicals, and they are able to scavenge peroxy- and alkoxy-free radicals formed during propagation (Equation 3) and other reactions (Equations 4 and 5) in autoxidation.

The antioxidant radical produced because of donation of a hydrogen atom has a very low reactivity toward the unsaturated lipids or oxygen; therefore, the rate of propagation is very slow. The antioxidant radicals are relatively stable so that they do not initiate a chain or free radical propagating autoxidation reaction unless present in very large quantities. These free radical interceptors react with peroxy radicals ( $ROO\cdot$ ) to stop chain propagation; thus, they inhibit the formation of peroxides (Equation 13). Also, the reaction with alkoxy radicals ( $RO\cdot$ ) decreases the decomposition of hydroperoxides to harmful degradation products (Equation 14).

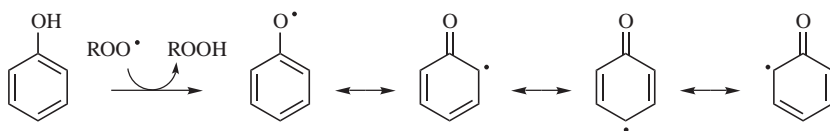
Most of the primary antioxidants that act as chain breakers or free radical interceptors are mono- or polyhydroxy phenols with various ring substitutions.

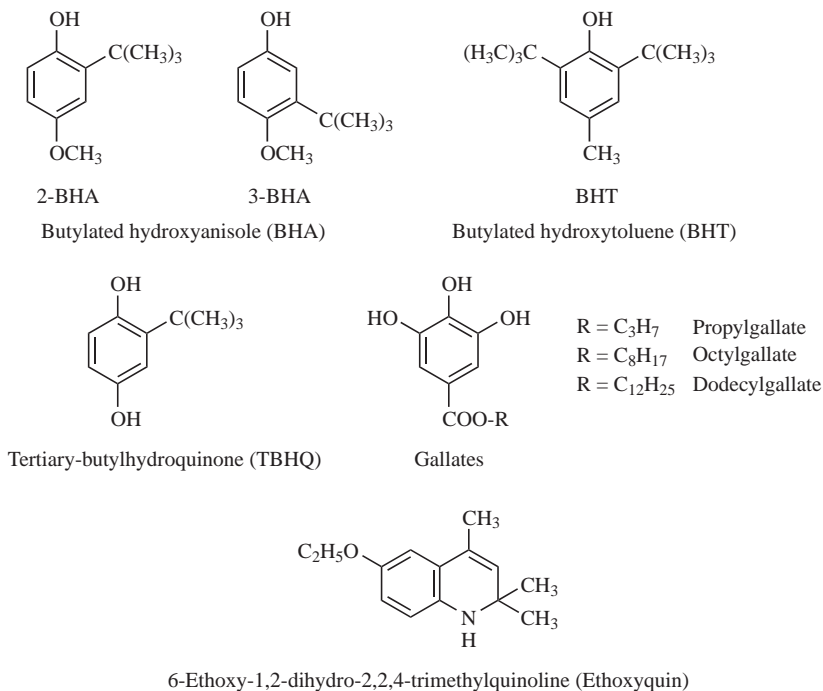
**TABLE 1. Standard One-Electron Reduction Potential ( $E^{\circ}$ ) at pH 7 for Selected Radical Couples (Adapted from Ref. 21).**

Couple	$E^{\circ}$ (mV) at pH 7.0
HO $\cdot$ , H $^+$ /H $_2$ O	2310
RO $\cdot$ , H $^+$ /ROH (alkoxy)	1600
ROO $\cdot$ , H $^+$ /ROOH (peroxy)	1000
PUFA $\cdot$ , H $^+$ /PUFA-H (polyunsaturated fatty acid)	600
$\alpha$ -Tocopheroxyl $\cdot$ , H $^+$ / $\alpha$ -tocopherol	500
Ascorbate $\cdot$ , H $^+$ /ascorbate $^-$	282
Dehydroascorbic/ascorbate $^{2-}$	-174

The antioxidant effectiveness is influenced by the chemical properties of the compound including hydrogen bond energies, resonance delocalization, and susceptibility to autoxidation. The ability of the primary antioxidant molecule to donate a hydrogen atom to the free radical is the initial requirement. The ability of the free radical interceptor (scavenger) to donate a hydrogen atom to a free radical can be predicted from standard one-electron potentials (Table 1). According to Buettner (21), each oxidizing species is capable of stealing an electron (or H atom) from any reduced species listed below it. That means when the standard one-electron reduction potential is concerned, the free radical scavengers that have reduction potential below peroxy radicals are capable of donating an H atom to peroxy radical and form a peroxide. The resulting antioxidant radical should be of low energy, ensuring the lesser possibility of catalyzing the oxidation of other molecules. The formed antioxidant radical is stabilized by delocalization of the unpaired electron around the phenol ring to form a stable resonance hybrid (Figure 3) and as a result attained low-energy levels (18, 22, 23).

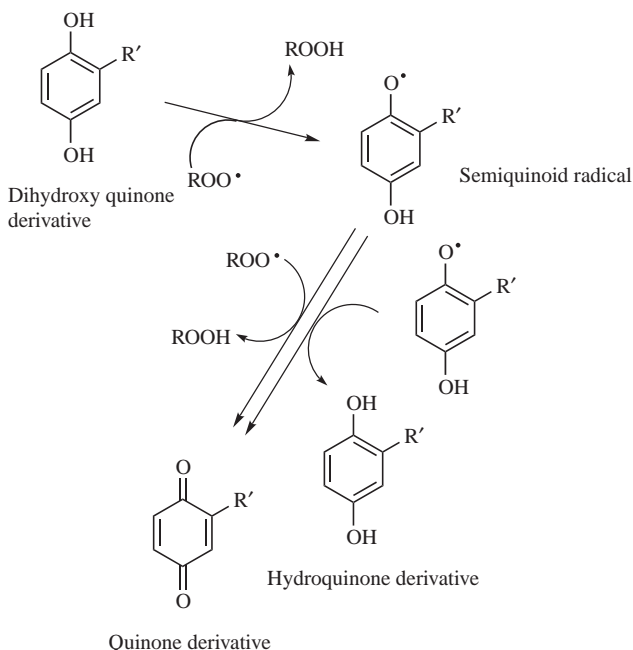
Antioxidant radicals are capable of participating in termination reactions with peroxy (Equation 13), alkoxy (Equations 14 and 15), or antioxidant (Equation 16) radicals removing reactive free radicals from the system. In fats and oils containing phenolic antioxidants, dimers of antioxidant molecules are usually found. This is a good indication that antioxidant radicals readily undergo termination reactions and form dimers as proposed in Equation 13. When considering all of these, the primary antioxidants or free radical scavengers can inactivate at least two free radicals, the first one during the interaction with peroxy radical and the second in the termination reaction with another peroxy radical.

**Figure 3.** Stable resonance hybrids of phenoxyl radical of phenolic antioxidant [adapted from (18)].



**Figure 4.** Chemical structures of synthetic phenolic antioxidants commonly used in fats and oils.

The compounds that exhibit primary antioxidant activity include polyhydroxy phenolics as well as the hindered phenolics. There are several synthetic ring-substituted phenolics as well as naturally occurring phenolic compounds that may perform via the primary antioxidant mechanism, as discussed later in this chapter. The common feature of all of these antioxidants is that they are mono- or polyhydroxy phenols with various ring substitutes (Figure 4). Substitution with an electron-donating group/s *ortho* and/or *para* to the hydroxyl group of phenol increases the antioxidant activity of the compound by an inductive effect (e.g., 2,6-di-tert-butyl-4-methylphenol or BHA). Thus, the presence of a second hydroxyl group in the 2- (*ortho*) or the 4-position (*para*) of a phenol increases the antioxidant activity (e.g., TBHQ). In the dihydroxybenzene derivatives, the semiquinoid radical produced initially can be further oxidized to a quinone by reacting with another lipid radical (Figure 5). This semiquinoid radical may disproportionate into a quinone and a hydroquinone molecule, and the process of this conversion contributes to antioxidant activity as peroxy radical scavenging potential (18, 24). Table 2 summarizes most commonly used primary antioxidants in fats and oils and lipid-containing foods. Substitution with butyl or ethyl group/s *para* to the hydroxy groups also enhances the antioxidant activity. Substitution of branched alkyl groups at *ortho* positions enhance the ability of the molecule to form a stable resonance structure that reduces the antioxidant radical's participation in propagation reactions (18, 23).



**Figure 5.** Possible mechanism of antioxidant activity of dihydroxybenzene derivative.

To be most effective, primary antioxidants should be added during the induction or initiation stage of the autoxidation reaction cascade. Antioxidants can scavenge the formed free radicals, as the cyclical propagation steps have not occurred at this stage. Addition of primary antioxidants to a lipid that already contains substantial amounts of lipid peroxides may result in loss of antioxidant activity (25).

## 5.2. Secondary Antioxidants

Secondary antioxidants are also classified as preventive or class II antioxidants. They offer their antioxidant activity through various mechanisms to slow the rate of oxidation reactions. The main difference with primary antioxidants is that the

**TABLE 2. Primary Antioxidants that are Commonly Used in Foods.**

Natural	Synthetic
Carotenoids	Butylated hydroxyanisole (BHA)
Flavonoids	Butylated hydroxytoluene (BHT)
Phenolic acids	Ethoxyquin
Tocopherols and tocotrienols	Propyl gallate (PG)
	Tertiary-butylhydroquinone (TBHQ)



**TABLE 3. Compounds that Exhibit Secondary Antioxidant Activity.**

Mode of Activity	Compounds in use
Metal chelation	Cirtic, Malic, Succinic and Tartaric acids Ethylenediaminetetraacetic acid, Phosphates
Oxygen scavenging and reducing agents	Ascorbic acid, Ascorbyl palmitate, Erythorbic acid, Sodium erythorbate, Sulfites
Singlet oxygen quenching	Carotenoids ( $\beta$ -Carotene, Lycopene and Lutein)

secondary antioxidants do not convert free radicals into stable molecules. They act as chelators for prooxidant or catalyst metal ions, provide H to primary antioxidants, decompose hydroperoxide to nonradical species, deactivate singlet oxygen, absorb ultraviolet radiation, or act as oxygen scavengers. They often enhance the antioxidant activity of primary antioxidants. Table 3 provides examples of some of these compounds that exhibit secondary antioxidant activity.

**5.2.1. Sequestering/Chelating Agents or Metal Deactivators** Heavy metals with two or more valency states with a suitable oxidation-reduction potential between them (e.g., Co, Cu, Fe, Mn, etc.) shorten the induction period and increase the maximum rate of oxidation of lipids. Trace amounts of these metal ions are present in the lipid-containing foods coming from naturally present compounds or included during processing operations. The effectiveness of copper as a catalyst for hydroperoxide decomposition has been reported (26–28). Transition metals such as iron exhibit low solubility at pH values near neutrality (29). That means in foods, transition metals may exist chelated to other compounds; many compounds form complexes with these metals and change their catalytic activity. Chelation can increase the prooxidant activity of transition metals by making them more nonpolar (increase solubility in lipids; 30), and some can increase oxidative reactions by increasing metal solubility or altering redox potential (31). According to Graf and Eaton (32), chelators may exert antioxidant activity by prevention of metal redox cycling, occupation of all metal coordination sites, and formation of insoluble metal complexes and steric hindrance of interactions between metals and lipids or oxidation intermediates (e.g., peroxides). Chelation of these metal ions or use of metal deactivators reduces the pro-oxidant activity by raising the energy of activation for initiation reactions. The most effective form of chelating agents as secondary antioxidants, which form  $\sigma$ -bonds with metal ions because they reduce the redox potential and stabilize the oxidized form of the metal ion. Chelating agents such as heterocyclic bases that form  $\pi$ -complexes raise the redox potential and may accelerate metal-catalyzed hydroperoxide decomposition (Equations 7 and 8) and act as prooxidants.

Multiple carboxylic acid compounds such as citric acid, ethylenediaminetetraacetic acid (EDTA), and phosphoric acid derivatives (polyphosphates and phytic acid) are commonly used in extending the shelf life of lipid-containing foods because of their metal chelating properties. Typically these chelators are water soluble, but citric acid exhibits solubility in lipids, which allows it to inactivate metals

in the lipid phase (33). Chelator's activity depends on pH and the presence of other chelatable ions (e.g., Ca). Most food grade chelators are unaffected by food-processing operations and storage; however, polyphosphates may decrease their antioxidant activity because of possible hydrolysis by endogeneous phosphatases in foods, especially in raw meat (22).

Several proteins that exist in food (e.g., lactoferrin, ferritin, transferritin, heme protein) possess strong binding sites for iron. Reducing agents (ascorbate, cysteine, superoxide anion) to low pH causes release of iron from proteins and accelerates lipid oxidation (34). Some amino acids and peptides found in muscle foods (e.g., carnosine) are capable of chelating metal ions and inhibit their prooxidant activity (35, 36).

**5.2.2. Oxygen Scavengers and Reducing Agents** As oxygen is essential and is one of the reactants in the autoxidation process, scavenging of oxygen molecular species is one way of providing antioxidant activity. Ascorbic acid acts as a reducing agent and as an oxygen scavenger. The mechanism of antioxidant activity of ascorbic acid is discussed elsewhere in this chapter.

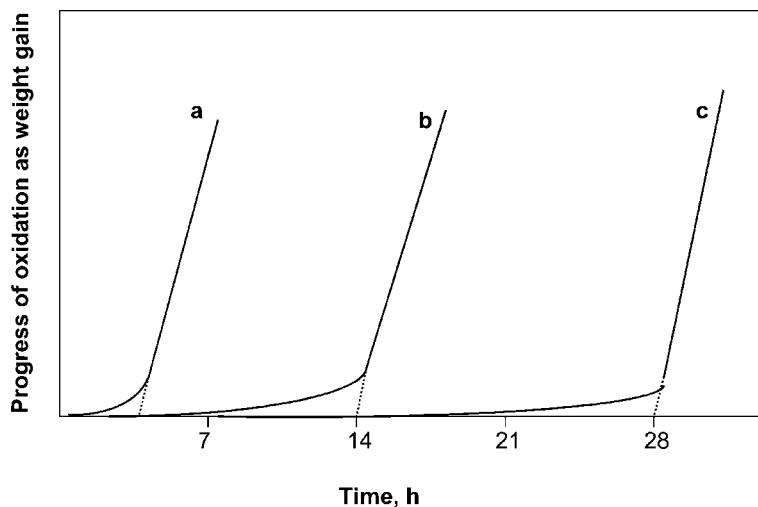
Singlet oxygen is the excited state oxygen, and its inactivation is an effective way of preventing initiation of lipid oxidation. Carotenoids are capable of inactivating photoactivated sensitizers by physically absorbing their energy to form the excited state of the carotenoid. Later, the excited state carotenoid returns to ground state by transferring energy to the surrounding solvent (37, 38). Other compounds found in food, including amino acids, peptides, proteins, phenolics, urates, and ascorbates also can quench singlet oxygen (20).

Compounds such as superoxide anion and peroxides do not directly interact with lipids to initiate oxidation; they interact with metals or oxygen to form reactive species. Superoxide anion is produced by the addition of an electron to the molecular oxygen. It participates in oxidative reactions because it can maintain transition metals in their active reduced state, can promote the release of metals that are bound to proteins, and can form the conjugated acid, perhydroxyl radical depending on pH, which is a catalyst of lipid oxidation (39). The enzyme superoxide dismutase that is found in tissues catalyzes the conversion of superoxide anion to hydrogen peroxide.

Catalase is capable of catalyzing the conversion of hydrogen peroxides to water and oxygen (40). Glucose oxidase coupled with catalase is well used commercially to remove oxygen from foods, especially fruit juices, mayonnaise, and salad dressings (19). Glutathione peroxidase that is found in many biological tissues also helps to control both lipid and hydrogen peroxides (41, 42). These enzymic reactions help to reduce various types of radicals that could be formed in lipid-containing biological systems.

### 5.3. Synergism and Synergists

Synergism is the cooperative effect of antioxidants or an antioxidant with other compounds to produce enhanced activity than the sum of activities of the individual



**Figure 6.** Synergistic effect of antioxidants: (a) 0.32% (w/w) dipalmitoyl phosphatidylethanolamine, (b) 0.02% (w/w) propyl gallate, and (c) is (a)+(b), evaluated in lard at 120°C, and the induction period was used to compare antioxidant activity [modified and redrawn from (44)].

component when used separately (43). Figure 6 illustrates how synergistic effect is expressed as antioxidant activity. Two types of synergism are observed, one involving primary antioxidants only and the other involving a combination of primary antioxidants with metal chelators or peroxy scavengers.

In a combination of two or more free radical scavengers, rapid reaction with free radicals occurs because of the differences in bond dissociation energies or steric hindrance of free radical scavenger/ROO• interactions (23). These differences result in one scavenger being used faster than the other. Also, it is possible to regenerate the primary antioxidant by transferring its radical to another scavenger. Ascorbic acid together with  $\alpha$ -tocopherol also shows a good synergism, which is explained by the regeneration and recycling of the tocopheroxyl radical intermediate to the parent phenol,  $\alpha$ -tocopherol (44, 45).

In the combination(s) of free radical scavenger and metal chelator, the chelator decreases the oxidation rates by inhibiting metal-catalyzed oxidation; thus, fewer free radicals are generated in the system. Inactivation of antioxidants via termination reaction or participation in autoxidation occurs to a lesser extent in such situations. This makes the concentration of antioxidant, which is available to scavenge free radicals, to be always greater at a given time than when no metal chelator is present. Therefore, the combination of chelator and radical scavenger decreases free radical generation and increases radical scavenging potential (22). Strong synergistic activity has been observed in the mixtures of natural tocopherols and citric acid. The synergistic effect of this mixture is caused by the chain-breaking ability of tocopherols and metal chelation of citric acid (19).

In an antioxidant combination that contains compounds exhibiting different mechanisms of action and physical properties, inhibition of oxidation occurs in

many different phases. This suggests that food antioxidants should be carefully selected considering such factors as the type of oxidation catalyst, physical state of lipid (bulk, emulsified), pH, temperature, and the ability to interact with other components in the food.

## 6. EVALUATION OF ANTIOXIDANT ACTIVITY

Antioxidant activity of a given compound is assessed as resistance to oxidation of lipids in the presence of that particular compound. Therefore, most of the methods described and used to assess antioxidant activity follow oxidation and the stages of oxidation of unsaturated lipid substrates. Many techniques have been developed to determine the antioxidant efficacy of the compounds of interest, but all of these have to be employed and interpreted carefully. Frankel (19, 46) has listed following parameters that are fairly important in choosing methods to evaluate antioxidants.

**Substrate:** Should be relevant to foods. Triacylglycerols and phospholipids, in the bulk, emulsion, or liposome form represent the closest model in biological systems including foods.

**Conditions:** Test under various conditions. Under different temperatures, with metal catalysts, with surface exposure, etc., select these conditions to mimic conditions in food.

**Analysis:** Measure relatively low levels of oxidation (below 1%) and include measurement of initial or primary products of lipid oxidation (e.g., hydroperoxides, conjugated dienes) as well as secondary decomposition products of lipid oxidation (e.g., carbonyls, volatiles, dialdehydes).

**Concentrations:** Compare antioxidants at the same mole concentration of active compound, and an appropriate reference compound should also be used, which may be a structurally related reference compound. With crude extracts (e.g., natural antioxidants), compositional data are needed to compare samples.

**Calculations:** Use the induction period, percentage inhibition or rates of hydroperoxide formation or decomposition, or  $IC_{50}$  value (concentration required to achieve 50% inhibition) based quantification (this is discussed further in a later section).

Obviously attention should also be paid to the system under examination. Thus, bulk oil, water-in-oil, or oil-in water emulsions behave differently under similar oxidation conditions.

An updated review by Antolovich et al. (47) discusses the methods of determining antioxidant activity extensively. The methods used in measuring antioxidant activity may be categorized into three groups, which directly or indirectly measure the rate or extent of the following:

1. Decay of substrate, probing compound, or oxygen consumption
2. Formation of oxidation products by the oxidizing substrate
3. Formation or decay of probing free radicals.

Methods that use approaches (1) and (2) measure antioxidant activity as an inhibitory effect exerted by the test compound on the extent or rate of consumption of reactants or the formation of oxidation products. The antioxidant activity (AA) of a compound or a component mixture that is a function of many parameters of the assay method employed may be defined using the following mathematical expressions (47; Schemes 5 and 6):

$$AA = f(\text{time or rate; temperature; substrate; concentration of antioxidant; concentration of other substances}^a; \text{partitioning behavior})$$

<sup>a</sup>e.g., oxygen, peroxides, or other antioxidants or prooxidants

For a fixed set of assay conditions, AA could be defined independent of the test method. Scheme 5 provides equations for a situation that measures time as the independent variable.

$$AA = (t_{AH} - t_{CONTROL}) / ([AH]t_{CONTROL})$$

where

$t_{AH}$  is time taken by the substrate to reach a predetermined level of oxidation based on the test method

$t_{CONTROL}$  is the time for untreated substrate or control to reach the same level of oxidation

[AH] is the concentration of antioxidant in appropriate units

After rearranging,

$$AA = [(t_{AH} / t_{CONTROL}) - 1] / [AH]$$

**Scheme 5.** Proposed expressions to calculate antioxidant activity.

According to the equations in Scheme 5, if:

$t_{CONTROL} = t_{AH}$ , no antioxidant activity is exerted

$t_{CONTROL} < t_{AH}$ , an antioxidant activity is exhibited

$t_{CONTROL} > t_{AH}$ , a prooxidant activity is observed and AA has a negative value

A similar expression can be formulated for rate of oxidation.

Another expression that can be used is relative antioxidant activity (RAA; Scheme 6).

$$RAA_{AH} = AA_{AH}/AA_{REFERENCE}$$

where

$AA_{AH}$  is antioxidant activities of test compound

$AA_{REFERENCE}$  is the antioxidant activity of the reference antioxidant at the same molar concentration

Rearranging gives,

$$AA_{AH} = (RAA_{AH}) AA_{REFERENCE}$$

**Scheme 6.** Proposed expressions to calculate relative antioxidant activity.

RAA represents the activity equivalence of the test compound relative to the reference antioxidant, which is suitable for activity comparison.

Methods of category (3) tract the capacity of the test compound to capture radicals or to inhibit radical formation rather than monitoring the actual oxidation product formation or substrate oxidation. Several new methods are developed based on this concept, and a variety of new parameters for expressing results are used. It is expected that a high correlation exists between these two types of measurements. It should be noted here that there are no standard units for reporting the antioxidant activity because such activity (assay, capacity, efficiency, effectiveness, etc.) is independent of the test procedure. Table 4 summarizes the methods available for measuring antioxidant activity and how the results of such determinations are expressed.

Another way of categorizing the methods of determining antioxidant activity is (1) accelerated stability tests, and (2) free radical-based methods. Most of the studies that are currently used tend to employ accelerated test systems and try to relate them to real food systems.

### 6.1. Methods Based on Lipid/Substrate Oxidation (Stability Tests)

These methods are based on lipid (substrate) oxidation and specific to the analysis of oxidation that occurs in food lipids. The tests employed strongly correlate to the conditions that oils and fats are subjected to during processing, food preparation, and storage. The substrate is a model compound that could be a pure triacylglycerol, fatty acid methyl ester, or an actual edible oil/lipid. Favorable conditions for substrate oxidation (e.g., high temperature) are provided to facilitate increased rate of oxidation reactions in a controlled environment. The end point is determined

**TABLE 4. Methods, Entities Tested, and Units to Express Results in Determining Antioxidant Activity.**

Method/Test	Reference/s	Measurement	Results and Units
<i>Substrate oxidation or oxidation product formation</i>			
Active oxygen method	49, 50	Change of mass, peroxide value or hydroperoxides, conjugated dienes, 2-thiobarbituric acid reactive substances, anisidine value, formation of hexanal, ethane, or pentane	Induction period (h, d) Time to reach a set level of oxidation during preinduction period (h, d) Rate of oxidation during preinduction period ( $\text{mol kg}^{-1}\text{hr}^{-1}$ , $\text{g L}^{-1}\text{d}^{-1}$ ) Concentration required to produce equivalent effect to reference antioxidant during preinduction period ( $\text{mol kg}^{-1}$ , $\text{g L}^{-1}$ ) Concentration of a functional group after a set time period (mequiv. $\text{kg}^{-1}$ ) Concentration of an oxidation product after a set time period ( $\text{mg kg}^{-1}$ , ppm w/w) Scale reading after a set time period (absorbance, conductivity, etc.)
Oven storage test	51, 52		
Shelf storage test	48		
<i>Free radical capturing or suppression of formation</i>			
DPPH quenching assay	53, 54	Ability to quench DPPH radical in solution	Percentage inhibition, $\text{EC}_{50}$ (concentration of test compound required to decrease the concentration of test free radical by 50%), $T_{\text{EC}_{50}}$ (time to decrease concentration of test free radical by 50%).
Hydroxyl radical quenching assay	55	Ability to quench hydroxyl radicals generated in a model system	
Superoxide radical quenching assay	56–58	Ability to quench superoxide radicals generated in a model system	

Electron paramagnetic resonance (EPR) spectrometry/spin trap tests	59–61	Detects free radical involved in autoxidation and related process	Intensity or rate of change in EPR signal
Ferric Reducing Antioxidant Power	62–64	Spectrophotometric measurement of Fe (II) complex formed due to reducing ability of the test compounds	Change of absorbance
Oxygen Radical Absorption Capacity	65–67	Based on Phycoerythrin assay	Fluorencence intensity, $\mu\text{mol}$ of Trolox equivalents
Total Radical-trapping Antioxidant Parameter	68, 69	Measure oxygen consumption during controlled lipid oxidation induced by thermal decomposition of 2,2'Azobis(2-aminopropane) hydrochloride; AAPH	$\mu\text{mol}$ peroxy radical deactivated $\text{L}^{-1}$
Trolox Equivalent Antioxidant Capacity	70, 71	Based on inhibition of production of 2,2'Azinobis(3-ethylbenzthiazoline)-6-sulfonic acid; ABTS radical cation and mM concentration of a Trolox solution having antioxidant capacity equivalent to 1.0-mM solution of test substance	$\text{mM L}^{-1}$ Trolox equivalents

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**TABLE 5. Commonly Used Accelerated Stability Tests for Oils in Evaluating Antioxidants (72).**

Method/Test	Conditions and characteristics
Ambient storage	Atmospheric pressure and room temperature, too slow and time consuming
Active oxygen method (AOM)	Bubbling air in a closed environment, 98°C, do not represent normal storage <i>Rancimat</i> is the automated version, also <i>OSI</i> instrument
Light	Atmospheric pressure and room temperature, rapid screening test, photo-oxidation occurs
Metal catalysts	Atmospheric pressure and room temperature, rapid screening test, more decomposition occurs
Oxygen uptake	Atmospheric pressure, 80–100°C, do not represent normal storage
Oxygen bomb	65–115 psi, O <sub>2</sub> , 99°C, do not represent normal storage
Schaal oven	Atmospheric pressure, 60–70°C, generally correlates well with actual storage
Weight gain	Atmospheric pressure, 30–80°C, not always very sensitive

by measuring chemical (e.g., primary or secondary oxidation products) or physical changes (e.g., change of mass or energy) of the oxidizing substrate. Table 5 provides a summary of methods commonly used for stability testing of edible oils.

**6.1.1. Shelf Storage Test** The test material is stored under similar conditions as in retail and is evaluated for the effectiveness of antioxidants in prolonging the premium quality of the product. Periodic evaluation of the lipid oxidation products (primary or secondary) by chemical tests (e.g., peroxide value, conjugated diene value, 2-thiobarbituric acid reactive substances, hexanal content) or sensory evaluation will be used to find out the onset of oxidation. The main drawback of this kind of evaluation is the time taken; therefore, rapid evaluation or accelerated methods are often preferred (19, 51).

**6.1.2. Active Oxygen Method (AOM)** This is one of the widely used methods for evaluating antioxidant activity. This test involves bubbling air through the heated lipid sample to accelerate its oxidation. Periodic analysis of peroxide value is carried out to determine the time required for the fat to oxidize under the conditions provided by AOM. This method has also been referred to as the Swift stability test. The fully automated version of this method is available as *Rancimat* apparatus (Metrohm Ltd, Herisau, Switzerland) and is accepted as a standard method by ISO (ISO6886) and American Oil Chemists' Society (AOCS Cd 12b-92) (47–49, 73). Similarly, the Oxidative Stability Instrument (OSI, Omnion, Inc., Rockland, MA) uses a similar principle as AOM. This instrument is sensitive to the change of conductivity of water, which receives the air passed through the oxidizing lipid. OSI uses induction period or oxidative stability index as the measure of stability of

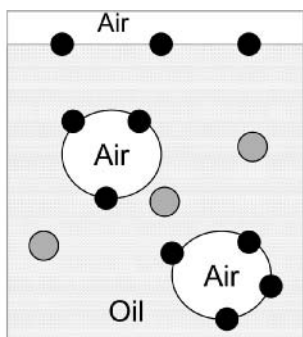
the antioxidant-containing lipid, and it is accepted by the AOCS (73) [AOCS method Cd-126-92 (97)].

**6.1.3. Oven Storage Test** The lipid with or without antioxidants is allowed to oxidize in an electrically heated convection oven (60–70°C). The oil is periodically assessed for change of its mass and its formation of primary oxidation products (hydroperoxides; peroxide value, conjugated dienes; conjugated diene value) or secondary products of oxidation (aldehydes; hexanal, dialdehydes, 2-thiobarbituric acid reactive substances; TBARS) or off-odor formation (50–52). This method is commonly referred to as the *Schaal oven method* and is widely used for bulk oil substrates. Conditions provided in this process are suitable for a low degree of oxidation; thus, the results correlate well with actual shelf stability of the antioxidant-containing lipids.

**6.1.4. Multiphase Systems** Antioxidant activity depends very much on the lipid substrate used for evaluation and the hydrophilic/lipophilic nature of the antioxidative compound. Solubility and partition properties of the compound in the medium affect the activity of antioxidants in the bulk lipid systems. As most foods cannot be related to bulk oil systems (e.g., meat, fish, eggs, mayonnaise, salad dressings, etc.), evaluation of antioxidants in multiphase systems is more relevant to their physical and chemical nature. Because of the very same reasons, several studies have found that compounds exhibiting strong activity against oxidation of lipids in bulk systems are often inefficient in colloidal and emulsion systems.

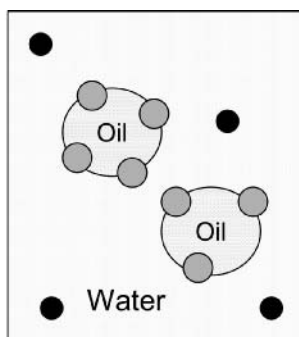
Three systems are generally used for such evaluations: emulsions (water-in-oil, oil-in-water), liposomes (uni- or multilamellar vesicles formed with aqueous phase and phospholipids), and micelles (emulsions formed with free fatty acids and aqueous phase). A lengthy discussion about how to use these multiphase systems in evaluating antioxidant activity is provided by Frankel (19).

Porter (74) has proposed a theory for distinguishing the effectiveness and behavior of antioxidants in bulk oils and emulsions and membranes. The “polar paradoxical behavior” (75, 76) describes the anomalous effect of antioxidants on lipids when they are in different physical systems. Work carried out by Frankel et al. (77) used the interfacial partitioning phenomenon to explain the reciprocal effect of antioxidants in bulk oil versus multiphase/colloidal systems. This phenomenon recognizes the discrete phases of the oxidative stability and antioxidative mechanism; however, exact details are not yet fully understood. The oxidative stability of food lipids varies according to their colloidal location because of the exposure of the lipid to the antioxidant/proxidant is different in such an environment. The partitioning of the antioxidant between the aqueous and nonaqueous phases depends on their solvent properties. Also, the partitioning of antioxidants into the nonaqueous phase can exert an important effect on activity by protecting the lipid oxidizable substrate. Figure 7 explains probable interfacial distribution of antioxidative compounds based on their hydrophobicity and hydrophilicity in monophasic and biphasic systems. Surfactants can improve the solubility of lipophilic antioxidants in the interface and exert a significant effect on activity (77, 78). Thus, it is important

**Bulk oil**

- Hydrophilic components  
Concentrated on oil-air interface  
due to insolubility  
Provide more protection to oil

- Lipophilic components  
Very diluted in oil phase  
Less protection to oil

**Oil-in water emulsion**

- Hydrophilic components  
Very diluted in water phase  
Provide less protection to oil

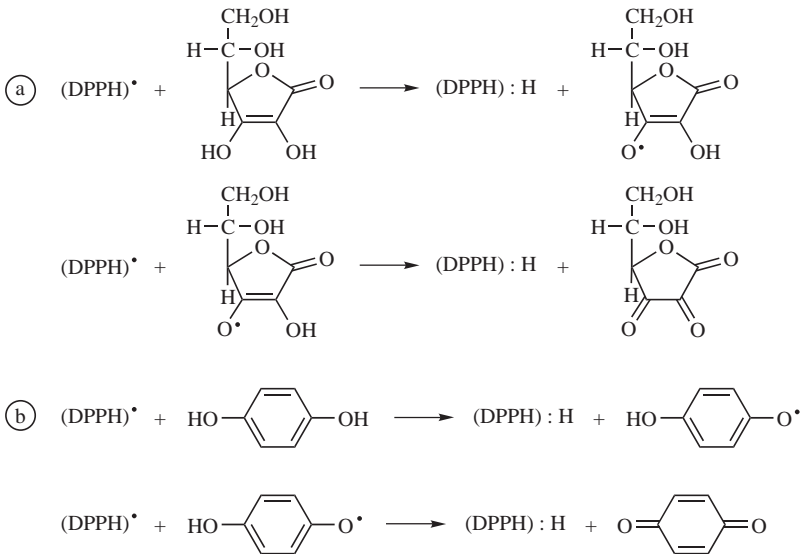
- Lipophilic components  
Concentrated on oil-water  
interface due to surface activity  
Provide more protection to oil

**Figure 7.** A schematic of probable distribution of hydrophilic and hydrophobic antioxidants in bulk oil (oil-air interface) and oil-in-water emulsion interface [adapted from (46)].

to use several methods to measure different products of oxidation under various conditions, including multiphase systems, especially in evaluating natural compounds as antioxidants in foods or biological systems (46).

## 6.2. Methods Based on Radical Scavenging Ability

Several methods have been described and used based on the fact that antioxidants are radical scavengers in aqueous and lipid phases. The radicals employed in these methods do not necessarily originate from lipid oxidation. In general, two approaches are used in these methods. One involves the generation of a free radical species and direct measurement of its inhibition caused by the test compound; such methods do not require an actual substrate to be oxidized. The other approach uses assay systems that involve oxidation of a substrate that is coupled with generated free radicals, which is actually an indirect measurement. The ability of a compound to inactivate radicals that are generated in a model system is extrapolated to its potential as an antioxidant in lipid and lipid-containing foods. These methods are widely and effectively used as screening and comparison tests in search for naturally occurring antioxidants. They are simple and easy to use but should be carefully interpreted. An extensive discussion of the methods of antioxidant activity



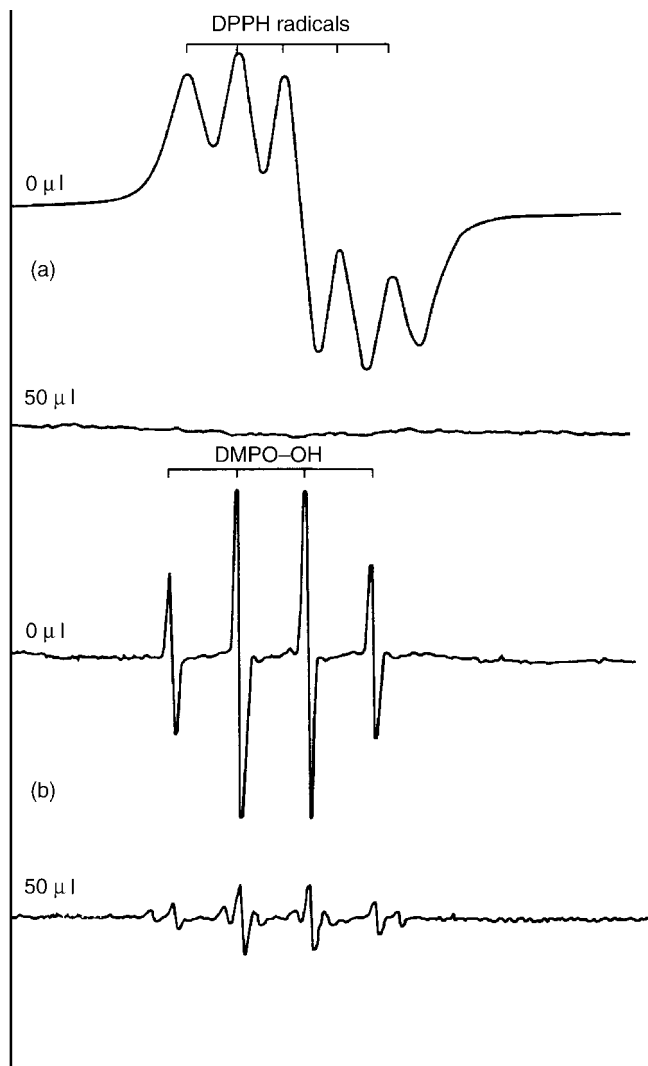
**Figure 8.** Proposed reaction between  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydroxyl (DPPH) radical and Oxidizable groups. (a) oxidation of conjugated group of ascorbic acid to the dehydro form and (b) oxidation of hydroquinone [adapted from (53)].

determination that are based on free radical scavenging is found in the review by Antolovich et al. (47).

**6.2.1. DPPH Free Radical** Blois (53) showed that  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl radical (DPPH $\cdot$ ) can be used for determining antioxidant activity of ascorbic acid, tocopherol, and quinones (Figure 8a and b). DPPH in ethanol shows a strong absorption band at 517 nm (independent of pH from 5.0 to 6.5), and the solution appears to be deep violet in color. As the DPPH radical is scavenged by the donated hydrogen from the antioxidant, the absorbance is diminished according to the stoichiometry.

As DPPH radical is paramagnetic and can become a stable diamagnetic molecule by accepting an electron or hydrogen radical, it can exhibit a change of its spin resonance using electron paramagnetic resonance (EPR). If the compound in question is able to scavenge DPPH radicals, the EPR signal of DPPH is attenuated (Figure 9a), and this can be quantified by integration into an appropriate calculation program. Use of DPPH radical in combination with monitoring EPR signal has been commonly used to assess natural antioxidants (54, 79–81). Although DPPH is a comparatively stable free radical at room temperature, it is not water soluble and the reaction mechanism between the antioxidant and DPPH radical depends on the structural conformation of the antioxidant (82).

**6.2.2. Oxygen Radicals** The oxygen radical absorbing capacity (ORAC) method (66, 67) is developed based on the ability of antioxidant compounds to scavenge



**Figure 9.** Electron paramagnetic resonance of (a)  $\alpha, \alpha$ -diphenyl- $\beta$ -picrylhydroxyl (DPPH) radical and (b) hydroxyl radical as spin adduct of DMPO-OH. (A test solution composed of 50  $\mu$ l of 1:10 (v/v) diluted methanolic extract of *Elusine coracana* was added as a radical quencher, and splitting constant of N and H was 14.791 G.) [From (79), with permission.]

oxygen (e.g., peroxy) radicals. The peroxy free radical generated using 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH; as the generator) in a buffered system is targeted to damage  $\beta$ -phycoerythrin ( $\beta$ -PE, a phycobilliprotein containing a red photoreceptor pigment) molecule. The fluorescent signal of  $\beta$ -PE is recorded and interpreted as ORAC (as micromole Trolox equivalents per weight of material). An automated system of ORAC coupled with chromatographic systems is available for measuring total antioxidant capacity of natural products (67).

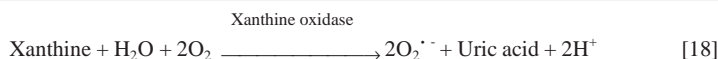
**6.2.3. Hydroxyl Radicals** Hydroxyl radical generated from Fenton reaction (Equation 17) in a buffered system can be used to evaluate hydroxyl radical scavenging ability of an antioxidant. Shi et al. (59) have shown that EPR may be used to assess the hydroxyl radical scavenging ability of compounds when an appropriate spin trapping agent (i.e., 5,5-dimethyl-1-pyrroline-*N*-oxide; DMPO) is used (see Figure 9b).

Halliwell et al. (55) have described a model that uses hydroxyl radicals generated from Fenton reaction to degrade 2-deoxy-D-ribose. The decomposed products of deoxyribose are 2-thiobarbituric acid-reactive substances (TBARS). If the antioxidant present in the system scavenges hydroxyl radicals generated, deoxyribose is protected and the amount of TBARS produced is less.



**Scheme 7.** Hydroxyl radical generation via Fenton reaction.

**6.2.4. Superoxide Radical** The scavenging ability of antioxidants for superoxide radical anions generated in a model reaction is also employed to assess antioxidant activity. Superoxide radical anions can be generated in vitro by enzymatic (Equation 18) or nonenzymatic reactions using a xanthine-xanthine oxidase system, which requires oxygen for the reaction to generate superoxide radicals. Quantification of superoxide radicals can be achieved using chemiluminescence (58) with an appropriate agent (e.g., lucigenin) or by using the oxygen consumption kinetics (83). The nonenzymic system uses reduction of tetrazolium salt induced by superoxide radicals generated in a phenazine methosulfate/NADH mixture (56, 57). Meyer et al. (84) showed that scavenging of superoxide anion radical is not necessarily effective in preventing lipid oxidation by phenolic compounds in natural extracts. Also, no equilibrium can be achieved when superoxide radicals are generated continuously during the test, which is seen as a shortcoming when using  $\text{O}_2^{\cdot-}$ -scavenging ability to assess antioxidant activity.



**Scheme 8.** Generation of superoxide radical by an enzymatic reaction.

## 7. COMMONLY USED ANTIOXIDANTS IN FOODS

This discussion is carried out based on the origin of the antioxidative compound: synthetic (manufactured chemical molecules) and natural (originated from food related material), which is widely used by the food industry. Although compounds

**TABLE 6. Physical Properties of Synthetic Antioxidants Used in Foods (18, 88, 89).**

Property/Characteristic	BHA	BHT	Gallates		TBHQ
			Dodecyl	Propyl	
Appearance	Waxy solid	White crystals	White crystals	White crystals	White-tan crystals
Carry through properties	Very good	Fair–Good	Fair–Good	Poor	Good
Boiling point (°C)	264–270	265	—	Decompose above 148	300
Melting point (°C)	50–52	69–70	146–148	146–148	126–128
Solubility (% w/w) in					
Corn oil	30	40	0	0	5–10
Glycerol	1	0	—	25	<1
Lard	30–40	50	—	1	5–10
Methyl linoleate	very soluble	very soluble	1	1	>10
Propylene glycol	50	0	6.5	4	30
Water	0	0	<1	<1	<1
Synergism	BHT & gallates	BHA	BHA	BHA	—

such as  $\alpha$ -tocopherol and D-ascorbic acid are synthesized, they are considered as naturally existing compounds; thus, they are considered as “natural” and are discussed under natural antioxidants.

### 7.1. Synthetic Antioxidants

Synthetic antioxidants are manmade and are used to stabilize fats, oils, and lipid-containing foods and are mostly phenolic-based. Many compounds are active as antioxidants, but only a few are incorporated into food because of strict safety regulations. These phenolic derivatives usually contain more than one hydroxyl or methoxy group. Ethoxyquin is the only heterocyclic, N-containing compound that is allowed for use in animal feeds.

Synthetic phenolic antioxidants are *p*-substituted, whereas the natural phenolic compounds are mostly *o*-substituted. The *p*-substituted substances are preferred because of their lower toxicity. The *m*-substituted compounds are inactive. Synthetic phenolic antioxidants are always substituted with alkyl groups to improve their solubility in fats and oils and to reduce their toxicity (24, 85, 86). The primary mechanism of activity of these antioxidants is similar to those of primary antioxidants. An antioxidant molecule reacts with a peroxy radical produced by the oxidizing lipid, thus forming a hydroperoxide molecule and an antioxidant free radical. A similar path of reaction may occur with the alkoxy free radicals formed during the decomposition of hydroperoxides. The antioxidant free radical so formed may be deactivated by a lipid peroxy or an alkoxy radical or with another antioxidant radical. Dimers and even trimers of antioxidant molecules are formed because of the reaction of antioxidant radicals, and these may have a modest antioxidant activity of their own. With the help of synergists such as ascorbic acid, some of these original antioxidant molecules may be regenerated. Quinones are formed from phenolic antioxidants by reaction with peroxy radicals. When antioxidants are present in excess, the reaction of antioxidant free radicals with oxygen may become important; even their reaction with polyunsaturated fatty acids has some impact on the course of oxidation. Therefore, at high concentrations, phenolic antioxidants may act as pro-oxidants.

In most countries, use of synthetic antioxidants is regulated and the safety of the compounds involved has been tested based on long-term toxicity studies. The ability of an antioxidant to withstand thermal treatment (e.g., frying or baking) and to retain sufficient stabilizing activity for the food (fried or baked) is termed as “carry through property.” Table 6 provides a summary of physical properties of commonly used synthetic antioxidants. Several researchers have studied the effectiveness of these compounds in suppressing lipid oxidation in fats and oil, and Tables 7 and 8 provide comparative effects of synthetic antioxidants (82).

**7.1.1. Butylated Hydroxyanisole (BHA)** This monophenolic compound exists as a mixture of two isomers (Figure 4), 3-tertiary-butyl-4-hydroxyanisole (90%) and 2-tertiary-butyl-4-hydroxyanisole (10%). The 3-isomer shows a higher antioxidant activity than the 2-isomer. BHA is commercially available as a white, waxy



**TABLE 7. Effect of Different Antioxidants on Oxidation of Soybean Oil [Adapted from (90)].**

Antioxidant (at 0.02% level)	Antioxidant Activity (as time in hours taken to reach peroxide value of 70)	
	45°C <sup>a</sup>	98°C <sup>b</sup>
Control (no added antioxidant)	168	5
Ascorbyl acid	288	43
Ascorbyl palmitate	456	13
BHA	216	9
BHT	240	11
PG	360	14
TBHQ	544	28

<sup>a</sup>Oxidation at 45°C as a thin layer of oil.

<sup>b</sup>Oxidation at 98°C at AOM conditions.

flakes that is lipid soluble. BHA exhibits good antioxidant activity in animal fats as compared to vegetable oils. It has good carry through properties but is volatile at frying temperatures. When BHA is included into packaging materials it easily migrates to the containing food and delays lipid oxidation (18, 74, 91).

**7.1.2. Butylated Hydroxytoluene (BHT)** BHT is also a monohydroxyphenol (Figure 4) and is widely used in foods. This fat-soluble antioxidant is available as a white crystalline compound. BHA is less stable than BHT at high temperatures and has lower carry-through properties. BHA and BHT act synergistically, and several commercial antioxidant formulations contain both of these antioxidants. BHT is effectively used in oxidation retardation of animal fats. It is postulated that BHA

**TABLE 8. Effect of Antioxidants and Metal Inactivators on the Oxidation of Soybean Oil [Adapted from (87)].**

Antioxidant or Combination	Antioxidant Activity (hours based on peroxide value of soy bean oil)
Control <sup>a</sup> (contain 1500 ppm tocopherol)	26
C-treated <sup>b</sup> (contain 45 ppm tocopherol)	17
C-treated + ascorbic acid <sup>c</sup>	43
C-treated + BHA <sup>c</sup>	18
C-treated + BHA + 0.01% citric acid <sup>c</sup>	56
C-treated + BHT <sup>c</sup>	23
C-treated + BHT + 0.01% citric acid <sup>c</sup>	53
C-treated + citric acid <sup>c</sup>	50
C-treated + propyl gallate <sup>c</sup>	26
C-treated + $\alpha$ -tocopherol <sup>c</sup>	21
C-treated + $\alpha$ -tocopherol + 0.01% citric acid <sup>c</sup>	53

<sup>a</sup>Naturally present mixed tocopherol.

<sup>b</sup>Carbon black treated oil to remove natural tocopherol partially.

<sup>c</sup>Antioxidant concentration is 0.57 mmol/kg.

interacts with peroxy radicals to produce a BHA phenoxy radical. This BHA phenoxy radical may abstract a hydrogen atom from the hydroxyl group of BHT. BHA is regenerated by the H radical provided by BHT. The BHT radicals so formed can react with a peroxy radical and act as a chain terminator (92, 53).

**7.1.3. Ethoxyquin** Ethoxyquin, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (Figure 4), is used as an antioxidant in animal feeds primarily to protect carotenoid oxidation. It may also be used in fish products, fish oil, poultry fats, potatoes, apples, and pears during storage and especially to protect pigment oxidation in ground chili and paprika (93). Ethoxyquin may act as a free radical terminator. Dimerization of the radical may inactivate the antioxidant (94).

**7.1.4. Gallates** Esters of gallic acid (Figure 4), namely, n-propyl, n-octyl, and n-dodecyl gallates, are approved antioxidants for food use. Propyl gallate (PG), the most commonly used gallate, is slightly water soluble and is available as a white crystalline powder. PG is not suitable for use in frying oils because it is volatile at the high temperatures of frying (18, 92). Octyl and dodecyl gallates are more lipid soluble and heat stable and have better carry-through properties. Gallates can chelate metal ions effectively, thus retarding lipid oxidation catalyzed by metal ions. However, this may negatively effect the esthetic appeal of the food, because of the dark color of the metal-gallate complexes. Therefore, gallate formulations are always available with a metal chelator such as citric acid to prevent any discoloration in the incorporated food. Gallates show synergistic activity with both primary and some of the secondary antioxidants (94). Propyl gallate works well with BHA and BHT because of a synergistic action, however, its use together with TBHQ is not permitted in the United States (25).

**7.1.5. Tertiary-Butylhydroquinone (TBHQ)** This is a diphenolic antioxidant (Figure 4) and is widely used in a variety of fats and oils. TBHQ has excellent carry-through properties and is a very effective antioxidant for use in frying oils. It is available as a beige color powder that is used alone or in combination with BHA or BHT. TBHQ can be used in a variety of lipid-containing foods and fats and oils. Chelating agents such as monoacylglycerols and citrates enhance the activity of TBHQ, mainly in vegetable oils and shortenings.

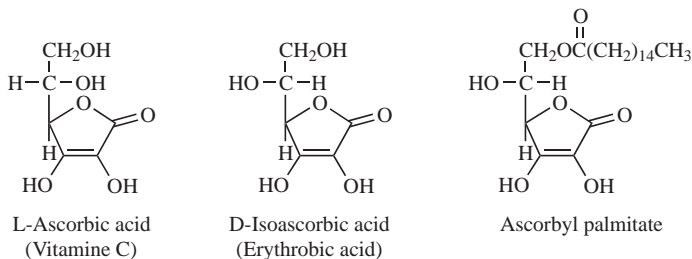
TBHQ reacts with peroxy radicals to form a semiquinone resonance hybrid. The semiquinone radical intermediate may undergo different reactions to form more stable products; they can react with one another to form dimers, dismutate, and regenerate as semiquinones; and they can react with another peroxy radical (Figure 5; 18, 24). A possible mutagenic effect of TBHQ has been the subject of extensive studies, and few countries in the world including Japan and the European Union countries do not yet approve its use in foods. Since 1999, TBHQ is included in the class IV preservative list in Canada, with a maximum usage level of 0.02% (Canada Food and Drug Act).

## 7.2. Natural Antioxidants

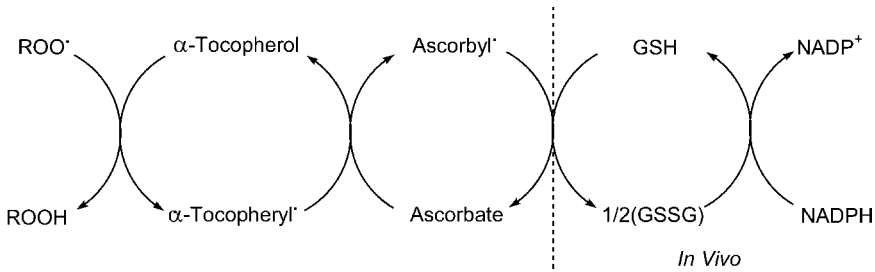
Use of plant parts (bark, leaves, seeds, etc.) and their extracts to preserve food from developing a rancid taste is a practice that has continued since prehistoric time. There is evidence that even for the industrial materials, plant-based components were used as antidrying agents to prevent oxidation and polymerization of polyunsaturated fatty acid-rich plant oils (2, 5, 48). During the past two decades, intensive research has been carried out on naturally occurring antioxidative compounds from different sources. The main drive behind this search was to reduce the use of synthetic compounds as food additives because of their potential negative health effects and as a result of consumer demand.

Plant-based components have increasingly been advocated as “safe and natural” antioxidants considering their existence in regular foods that are consumed. Much of the interest on naturally occurring antioxidants is developed because of the trend to minimize or avoid the use of synthetic food additives. Continuous effort in searching for naturally occurring antioxidative compounds during the past 20 years has helped to develop efficient models for activity screening, structure function relationship assessment, categorizing sources of antioxidant groups, developing methods of isolating purified antioxidative compounds from natural sources, and developing branded foods (e.g., claims for marketing purpose). Again, one has to keep in mind that the “safe it is natural” is based on the fact that these secondary metabolites are present in small concentrations in regular foods. If these are to be added to foods that are largely consumed, they should undergo all safety clearances. There are many naturally occurring compounds that act as antioxidants in fats and lipid-containing foods. Among these, only a few are currently approved and employed in foods. Groups of compounds that are found naturally and exhibit strong antioxidant activity are discussed here.

**7.2.1. Ascorbic Acid and Ascorbic Acid Esters and Salts** Vitamin C or ascorbic acid is widespread in nature but sparingly associated with fats or oils because of its hydrophilic nature (95). Ascorbic acid in the free form, salts of sodium and calcium, and esters of stearic and palmitic are commonly used as antioxidants in foods. Erythorbic acid is the D-isomer of naturally present L-ascorbic acid (Figure 10) and is often used as an antioxidant in dried fruits and a cure



**Figure 10.** Chemical structures of L-ascorbic acid, erythorbic acid, and ascorbyl palmitate.



**Figure 11.** Regeneration path of tocopherol by ascorbic acid and during participation in radical scavenging in biological systems [adapted from (96)].

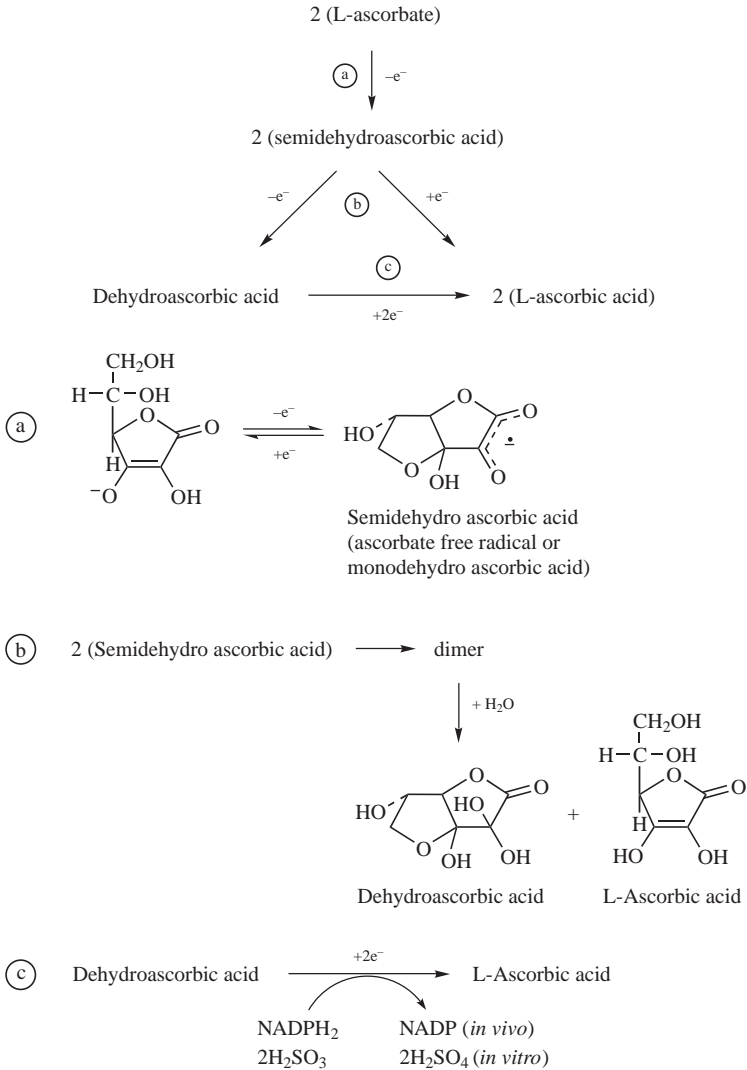
accelerator in cured meat. Unlike ascorbic acid, erythorbic acid is not a natural constituent of foods and has minimal vitamin C activity. Similar to ascorbic acid, erythorbic acid is highly water-soluble but remains insoluble in oils (97).

In foods, water-soluble ascorbic acid acts as a secondary antioxidant and participates in various antioxidative and related functions. Ascorbic acid is capable of quenching various forms of oxygen (singlet oxygen, hydroxyl radicals, and superoxide). When ascorbic acid acts as a hydrogen donor, ascorbyl radical so produced may reduce or terminate radical reactions; hydroperoxides may then be converted into stable products. Ascorbic acid can reduce primary antioxidant radicals and thus act as a synergist. A very good example is donating a hydrogen atom to tocopheryl radical and then regeneration of tocopherol (Figure 11), which is commonly observed in the biological systems. In addition to that, ascorbic acid can shift the redox potential of food systems to the reducing range and can act synergistically with chelators and regenerate primary antioxidants other than tocopherols (98).

*In vivo* ascorbic acid acts as a primary antioxidant and in tissues it is essential for the prevention of oxidative cellular damage by hydrogen peroxide (99). In a solution, ascorbic acid readily oxidizes to dehydroascorbic acid. This formation occurs through one- or two-electron transfer that is due to its enediol structure. It is a reductone and has a very high affinity for oxygen. The 2- and 3-positions of ascorbic acid are unsubstituted. Oxidation happens via the intermediate semidehydroascorbic acid or monodehydroascorbic acid or ascorbate free radical. The semidehydroascorbic acid is either reduced to give ascorbic acid again or oxidized to give dehydroascorbic acid. In nature, these compounds complete a redox system (Figure 12). The redox cycle is completed in living tissues by enzymatic reduction of dehydroascorbic acid to ascorbic acid. Seib (101) has reviewed the oxidation and other reactions of ascorbic acid.

Ascorbyl palmitate and ascorbyl stearate are synthetic derivatives of ascorbic acid. Ascorbic palmitate is soluble in lipid-containing foods because of its relatively good hydrophobicity (88). Ascorbyl palmitate is hydrolyzed by the digestive system to provide nutritionally available ascorbic acid and palmitic acid, but health claims cannot be made for its vitamin C contribution.

As an antioxidant, ascorbic acid is very attractive as it carries GRAS (generally recognized as safe) status with no usage limits; it is a natural or nature-identical



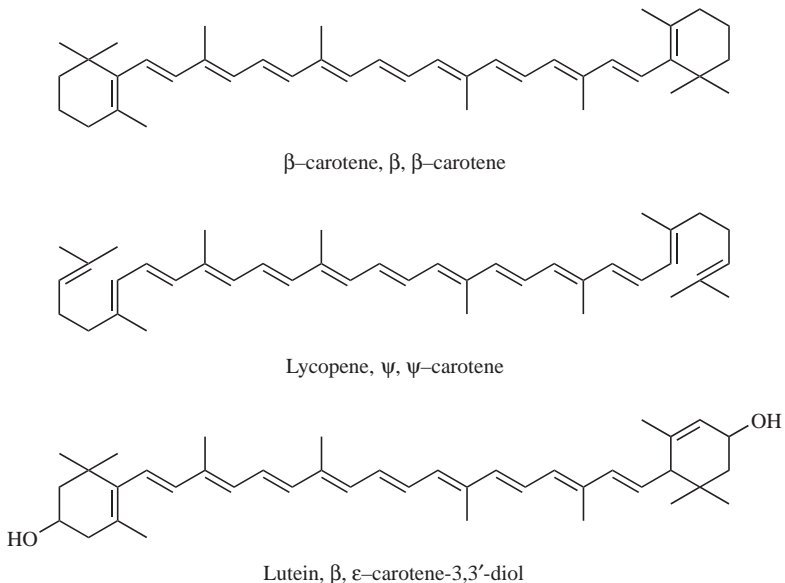
**Figure 12.** Ascorbic acid-dehydroascorbic acid redox system (a) oxidation of ascorbate to semidehydroascorbic acid, (b) disproportionation of semidehydroascorbic acid, and (c) reduction of dehydroascorbic acid [From (100), with permission].

product and is highly recognized as an antioxidant among the nutrient category. Ascorbic acid is also used as an acidulant and flavorant. Heat treatment makes ascorbic acid unstable, and it participates in nonenzymic browning reactions and degrades through reductone formation. Ascorbic acid and its salts (Na- and Ca-ascorbate) are water soluble and are not applicable as antioxidants in oils and fats. These salts are used extensively for stabilizing beverages that contain oxidizable substrates. In fats, ascorbyl palmitate is used mostly because of its superior

solubility. Ascorbyl palmitate also has GRAS status, and there are no restrictions for its usage level.

**7.2.2. Carotenoids** Carotenoids are ubiquitously found lipid-soluble-colored compounds, mainly from green plants, fruits, and vegetables. The two classes of carotenoids, carotenes and xanthophylls, are composed of 40-carbon isoprenoid or tetraterpenes with varying structural characteristics. Carotenes are polyene hydrocarbons and vary in their degree of unsaturation (e.g.,  $\beta$ -carotene, lycopene; Figure 13). Xanthophylls are derived from carotenes by hydroxylation and epoxidation, thus containing oxygen (e.g., astaxanthene, canthaxanthin). Some carotenoids exhibit biological activity of vitamin A and hence are categorized as provitamin A.  $\beta$ -Carotene is the most abundantly found provitamin A. Many fats and oils, especially those from plant sources, contain  $\beta$ -carotene, and it contributes to the deep intense orange red color of many oils (94, 102).

Carotenoids can act as primary antioxidants by trapping free radicals or as secondary antioxidants by quenching singlet oxygen. In foods, carotenoids usually act as a secondary antioxidant; however, at low oxygen partial pressure ( $<150$  mm Hg, in the absence of singlet oxygen), carotenoids may trap free radicals and act as a chain-breaking antioxidant (95, 104). At high oxygen concentrations, the antioxidant activity of  $\beta$ -carotene is diminished. Increased oxygen concentration leads to the formation of carotenoid peroxy radicals because the conjugated double bonds of carotenoid molecules are very susceptible to the attack by peroxide radicals. This reaction favors autoxidation of  $\beta$ -carotene over inactivation of lipid peroxy radicals



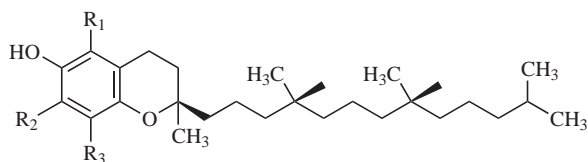
**Figure 13.** Chemical structures of some carotenoids.

(103). At low oxygen concentrations, the lifetime of the carotenoid radical is long enough to permit its reaction with another peroxy radical and to form nonradical species. The unsaturated structure of  $\beta$ -carotene allows the molecule to delocalize electrons in the radical and to produce a resonance-stabilized product with the peroxy radical. This carotene radical participates in termination reactions and converts peroxy radicals to less damaging products. Lieber (104) has provided details of antioxidative reactions of carotenoids. The combination of carotenoids and tocopherols results in synergistic action (105, 106). The stability of carotenoids is affected by oxygen, heat, pH, light, and metals; therefore, care should be taken in their handling as antioxidants.

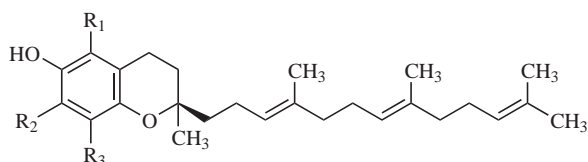
Singlet oxygen, which is unstable, preferentially transfers energy to  $\beta$ -carotene to produce triplet state  $\beta$ -carotene. This occurs through an exchange electron transfer mechanism. Triplet state  $\beta$ -carotene releases energy in the form of heat, and the carotenoid is returned to its normal energy state. This mechanism allows the carotenoid molecule to be an effective quencher of numerous molecules of singlet oxygen (102). The ability of carotenoids to quench singlet oxygen is related to the number of carbon double bonds in their chemical structures. Carotenoids with nine or more conjugated double bonds are very effective antioxidants. Because of the presence of additional functional groups in the hydrocarbon structure, xanthophylls cannot perform as effectively as antioxidants (94).

**7.2.3. Tocopherols and Tocotrienols** Tocopherols and tocotrienols are the natural antioxidative compounds found widely in different tissue, even if it is in trace amounts. Tocopherols are found abundantly in vegetable oil-derived foods. These monophenolic compounds possess varying antioxidant activities. Tocopherols and tocotrienols comprise the group of chromanol homologs that exert vitamin E activity in the diet. These different homologs vary in the extent of methylation of the chromane ring. The  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols contain a saturated phytyl (trimethyltridecyl) side chain (Figure 14). The corresponding tocotrienols have unsaturated phytyl chain at the 3-, 7-, and 11-positions. Only *RRR* isomers are found naturally. Synthetic  $\alpha$ -tocopherol (all *rac*- $\alpha$ -tocopherol) is a combination of eight stereoisomers that are found in equal amounts in the mixture. Biologically, *RRR*- $\alpha$ -tocopherol is the most active vitamin E homolog. The antioxidant activity of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols and tocotrienols decreases in the order of  $\alpha > \beta > \gamma > \delta$  in vivo (107, 108) and  $\alpha > \beta > \gamma > \delta$  in bulk oils and fats (108). Vitamin E activity of tocopherols decreases in the order of  $\alpha > \beta > \gamma > \delta$  (107).

Antioxidant activity of tocopherols is mainly by scavenging peroxy radicals thus interrupting chain propagation (95), which is based on the tocopherol-tocopherylquinone redox system. The active configuration is the phenolic group in the benzene ring, located at the *para* position to the oxygen atom bound next to the dihydropyrone cycle. Alpha-tocopherol donates a hydrogen atom to a peroxy radical resulting in an  $\alpha$ -tocopheryl semiquinone radical (Figure 15). This radical may further donate another hydrogen to produce methyltocopherylquinone or react with another tocopheryl semiquinone radical to produce an  $\alpha$ -tocopherol dimer. The methyltocopherylquinone is unstable and will yield  $\alpha$ -tocopherylquinone (100).



Tocopherol



Tocotrienol

Tocopherol or Tocotrienol	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
α	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>
β	CH <sub>3</sub>	H	CH <sub>3</sub>
γ	H	CH <sub>3</sub>	CH <sub>3</sub>
δ	H	H	CH <sub>3</sub>

**Figure 14.** Chemical structures of tocopherols and tocotrienols.

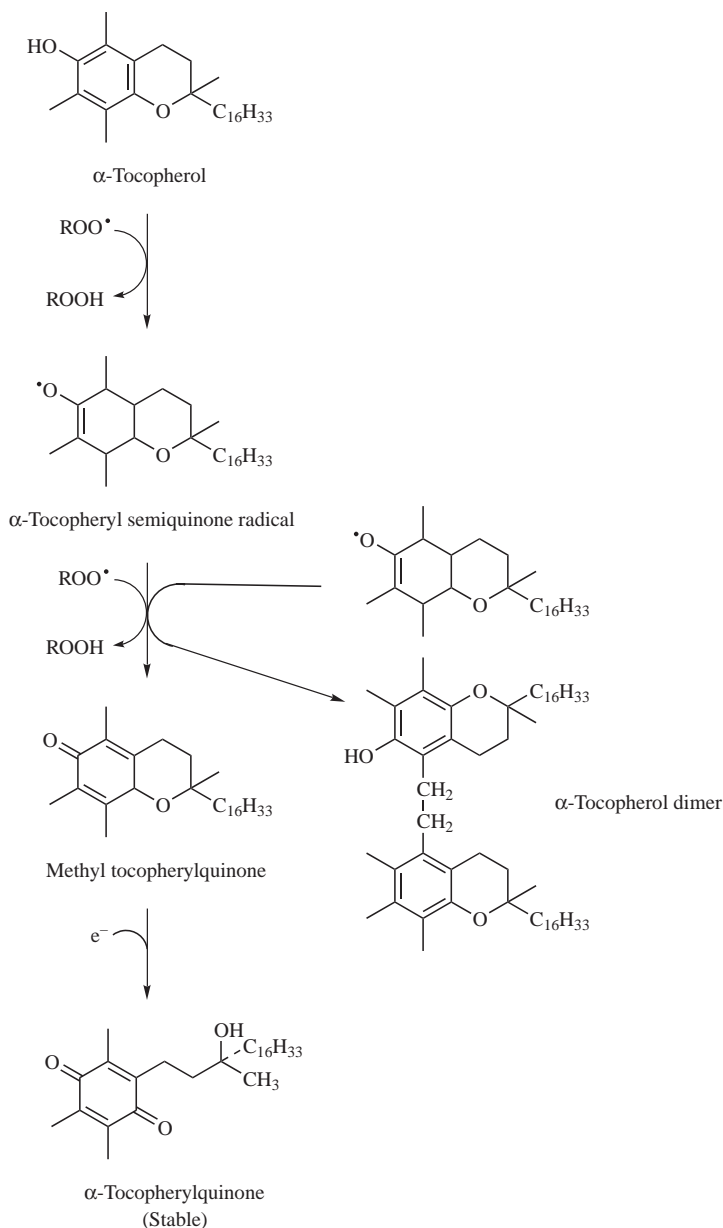
The  $\alpha$ -tocopheryl dimer continues to possess antioxidant activity. Also, two tocopheryl semiquinone radicals can form one tocopheryl quinone molecule and regenerate one tocopherol molecule. The decomposition products of tocopherols (during thermal oxidation) can slowly oxidize and release tocopherol that can act as an antioxidant (100).

Commercially, tocopherol is available as a pure all-rac- $\alpha$ -tocopherol, mixed tocopherols having various contents of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, or  $\delta$ -tocopherols (diluted in vegetable oil) and synergistic mixtures containing tocopherols, ascorbyl palmitate or other antioxidants, and synergists such as lecithin, citric acid, and carriers. Extraction of tocopherols from natural sources and chemical synthesis of tocopherols are well described by Schuler (100).

Tocopherols are considered as natural antioxidants for lipid-containing foods and marketed as “all natural.” They are permitted in food application according to GMP regulations (21CFR 182.3890). Natural tocopherols are limited to 0.03% (300 ppm) in animal fats (9 CFR 318.7). As most vegetable oils naturally contain tocopherols, the addition of this antioxidant may pose prooxidant effects.

**7.2.4. Other Phenolic Antioxidative Compounds from Plants** Higher plants are rich in a myriad of phenolic compounds in their secondary metabolite pool. Among these, phenolic acids and polyphenolic derivatives are found to be the most important series of hydrophilic–hydrophobic antioxidative compounds naturally present. In foods, these polyphenolic compounds act as radical scavengers





**Figure 15.** Possible mechanism of participation of  $\alpha$ -tocopherol in free radical scavenging.

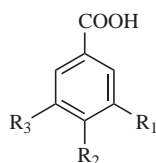
or metal chelators, and some may play a multifunctional role. Numerous plants and their parts have been identified as sources of phenolic acids, flavonoids, and related compounds. Antioxidant activity of phenolic compounds from various plant sources has been reported in several peer-reviewed papers, reviews, and books. Interested

readers are referred to reviews by Shahidi et al. (18, 109–112) about the sources of natural phenolics possessing antioxidative activity that are obtained from plants and have potential applications in oils and fats (as discussed below).

**7.2.4.1. Antioxidants from Cereals, Oilseeds, and Related Sources** Seeds rich in oils are also abundant sources of various types of antioxidative compounds. Among these carotenoids, phenolic acids, and their derivatives, flavonoids, phytic acid, lignans, and tocopherols are predominantly found depending on the plant genera and species. Reviews by Wanasundara et al. (110) and Shukla et al. (111) discuss antioxidants of oilseeds and their products in detail.

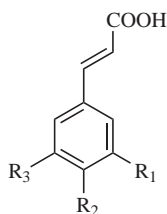
**7.2.4.1.1. PHENOLIC ACIDS AND THEIR DERIVATIVES** Phenolic acids are found in plants and have the basic chemical structure of  $C_6-C_1$  (benzoic acids) and  $C_6-C_3$  (cinnamic acids) (Figure 16). A range of substituted benzoic acid or cinnamic acid derivatives comprise these two major families of phenolic acids that are found in plants. Both of these families occur as free, conjugated, or esterified form, and sometimes as depsides such as chlorogenic acid (3-caffeoyl-quinic acid). Phenolic acids serve as free radical acceptors and chain breakers. The presence of a phenolic ring in the molecular structure and side chains facilitates the radical accepting ability of phenolic acids. According to Chimi et al. (113) and Pokorný (114), monohydroxy phenolic acids are less efficient as antioxidants than polyhydroxy phenolic acids. The presence of the  $CH=CH-COOH$  group in cinnamic acids ensures a greater antioxidative activity than a  $-COOH$  group as in benzoic acids. The participation of the  $C=C$  bond is important in stabilizing the antioxidant radical by resonance.

The antioxidant activity of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule, and this would be strengthened by steric



Benzoic acids

R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	
H	H	H	Benzoic
H	OH	H	<i>p</i> -Hydroxybenzoic
OH	OH	OH	Gallic
OH	OH	OH	Procatechuic
OCH <sub>3</sub>	OH	OCH <sub>3</sub>	Syringic
OCH <sub>3</sub>	OH	H	Vanillic



Cinnamic acids

R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	
OH	OH	H	Caffeic
H	H	H	Cinnamic
H	OH	H	<i>p</i> -Coumaric
OCH <sub>3</sub>	OH	H	Ferulic
OCH <sub>3</sub>	OH	OCH <sub>3</sub>	Sinapic

**Figure 16.** Chemical structures of antioxidative phenolic acids.

hindrance. Hydroxylated cinnamic acids are more effective antioxidants than their benzoic acid counterparts. When the acid group is esterified with a bulky group such as a sugar, the antioxidant potency of the molecule is further enhanced (115). Introduction of a second hydroxyl group in the *ortho* or *para* position increases the antioxidant activity of hydroxylated phenolic acids. Therefore, acids with *ortho* diphenolic groups (caffeic and protocatechuic acids) are more efficient antioxidants than their respective monophenolic acids (*p*-hydrobenzoic and *p*-coumaric acids). Gallic acid that has three hydroxyl groups is more active than protocatechuic acid, but more than three hydroxyl groups in the structure does not appear to improve the antioxidant efficiency in oil systems (114). The aromatic ring substituted with two or three phenolic groups in the *ortho* position are particularly important; some hydroxyl groups may be methoxylated. Substitution of one or two methoxy groups at the *ortho* position relative to the hydroxyl group markedly increases the antioxidant activity of phenolic acids. Therefore, sinapic acid is a more efficient antioxidant than ferulic acid, which is more efficient than *p*-coumaric acid. For the same reason, syringic acid is more active than vanillic acid and *p*-hydroxybenzoic acid (115). *Ortho* substitution of the phenolic acid with electron donor alkyl or methoxy groups increases the stability of aryloxy radical and thus the antioxidant activity. Methoxy substitution strengthens the antioxidant activity than the addition of a hydroxyl group to the molecule (113, 114).

**7.2.4.1.2. LIGNANS** Lignans are compounds with great chemical diversity and found in all parts of the plants. They are dimers of phenyl propanoid ( $C_6-C_3$ ) units linked by the central carbons of their side chains (116). Among these, bisepoxy lignans and cyclo-lignans that occur in oilseeds (sesame and flax) exhibit strong antioxidative activity in aqueous and lipid media. Lignans of sesame (*Sesamum indicum* L.) seed include sesamin, sesamolin, sesaminol, and sesamol, which act as endogenous antioxidants for the oils (117–119). Sesamolin may undergo chemical changes during thermal treatment and under processing conditions (e.g., bleaching) and forms sesaminol and sesamolol (120–122). High oxidative stability of sesame oil obtained from roasted seed may be largely attributed to the presence of lignan compounds (123–125).

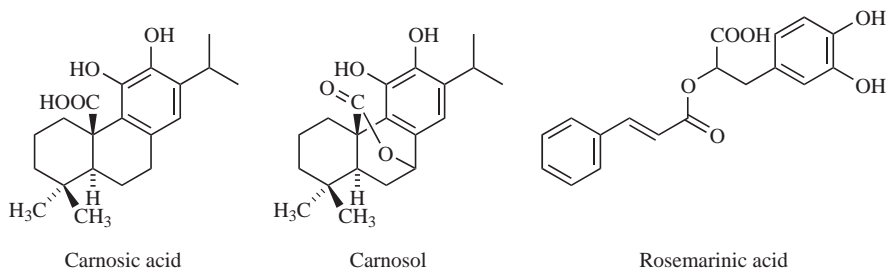
Lignans of flaxseed exist as secoisolariciresinol diglucoside (SDG; 126). SDG is a potent antioxidant in biological systems because of its tendency to associate in the aqueous phase. Much of the work on antioxidant activity of SDG is related to its radical mediated disease prevention (127, 128). Lignans of both sesame (121, 122) and flax (129, 130) have shown hydrogen-donating ability and scavenging activity for various free radicals.

**7.2.4.1.3. STEROLS** Phytosterols are mostly associated with unrefined vegetable oils and exist as derivatives of phenolic acids (e.g., ferulic acid). Several studies are available on antioxidant activity of sterols and their derivatives from sources such as corn fiber, oats, and rice. These compounds can be obtained from the unsaponifiable fraction that is removed during vegetable oil refining. Triterpene alcohols and hydrocarbons (131), or sterols (Figure 20) from oats (132, 133), rice (134, 135),

and corn fiber (135, 136), were able to exert the antioxidative effect on frying oils and as a result displayed antipolymerizing effects. It has been suggested that donation of a hydrogen atom from the allylic methyl group in the side chain of sterols followed by isomerization to a relatively stable tertiary allylic free radical may be the mechanism for sterol antioxidant activity (132). The ethylidene group ( $\text{CH}_3\text{-CH=}$ ) in the side chain of the sterol molecule seems essential for performing the antipolymerizing effect on the frying oils at high temperatures (137, 138). The  $\gamma$ -oryzanol of rice bran performed good antioxidant activity in a linoleic acid model system at  $37^\circ\text{C}$  as opposed to frying temperatures.  $\gamma$ -Oryzanol of rice is composed of at least ten compounds that are mainly ferulic acid derivatives of triterpene alcohols, stigmasterol, campesterol, sitosterol, and cycloartinol (Figure 20; 137). Plant sterol-based compounds are available as physiological antioxidants to prevent certain disease conditions. Commercial preparations of sterol-based natural antioxidants for high-temperature food applications are not abundantly available yet.

**7.2.4.2. Antioxidants from Labiatae Herbs** Among phenolic diterpenes, carnolic acid and carnosine are the very active antioxidants that are commercially available as natural antioxidants for lipid-containing foods. Chemically, carnolic acid has a structure consisting of three six-membered rings, including a dihydric polyphenolic ring and a free carboxylic acid, and carnosol is a derivative of carnolic acid containing a lactone ring (Figure 17). These active antioxidants are found especially in plants of the Labiatae family (oregano, rosemary, sage, and thyme). Commercial extracts of these plants are produced by organic solvent extraction of the plant parts and subsequent deodorization and bleaching. Commercially these antioxidants are available as powder, paste, or liquid and formulations in propylene glycol, medium-chain triacylglycerols, or vegetable oils. Schuler (100) has discussed preparation of rosemary antioxidants and their effectiveness in oils compared with other antioxidants.

Extensive studies (139, 140) on rosemary extracts containing carnosol, carnolic acid, and rosmarinic acid have shown that the activities of these natural antioxidants are system-dependent and that their effectiveness in different food systems is difficult to predict. In bulk vegetable oils (corn, soybean and peanut) and fish oils, carnosol and carnolic acid are effective antioxidants. It has been hypothesized that this



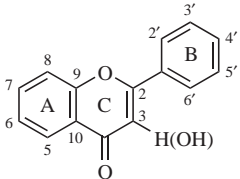
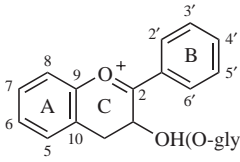
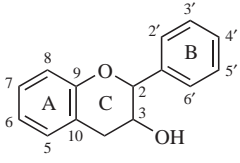
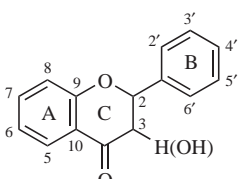
**Figure 17.** Chemical structures of carnosine, carnolic acid, and rosmarinic acid.

behavior of rosemary antioxidants is attributed to the partitioning differences in the biphasic system. These hydrophilic antioxidants are oriented in the oil–air interface and protect the bulk oil phase from oxidation. However, in oil-in-water emulsions, they are less effective in the oxidation of the oil–water interface, where most of the oxidation reactions take place (see illustration in Figure 7). It has been hypothesized that the hydrophilic antioxidants (e.g., rosmarinic acid, gallic acid, catechins, propyl gallate) partitioned more (>90%) into the aqueous phase, thus allowing a considerably low concentration of antioxidants in the oil phase, causing less antioxidative protection (141). Carnosic acid and carnosol are oxidized during oxidation at 60°C and higher temperatures; however, antioxidant activities are maintained; apparently the oxidation products are active antioxidants at high temperatures. These compounds have good carry-through properties and protect frying oils and fried foods.

**7.2.4.3. Flavonoids from Green Tea** Among the natural flavonoids studied as antioxidants for lipid-containing foods, polyphenolic catechins from green tea (*Camellia sinensis* L) have been extensively scrutinized (142, 143). Extracts of immature leaves of the plant (green tea) are rich in flavan-3-ols and their gallic acid derivatives, namely, (+)-catechin, (–)-epicatechin, (+)-gallocatechin, (–)-epicatechin gallate, (–)-epigallocatechin, and epigallocatechin gallate (Figure 18).

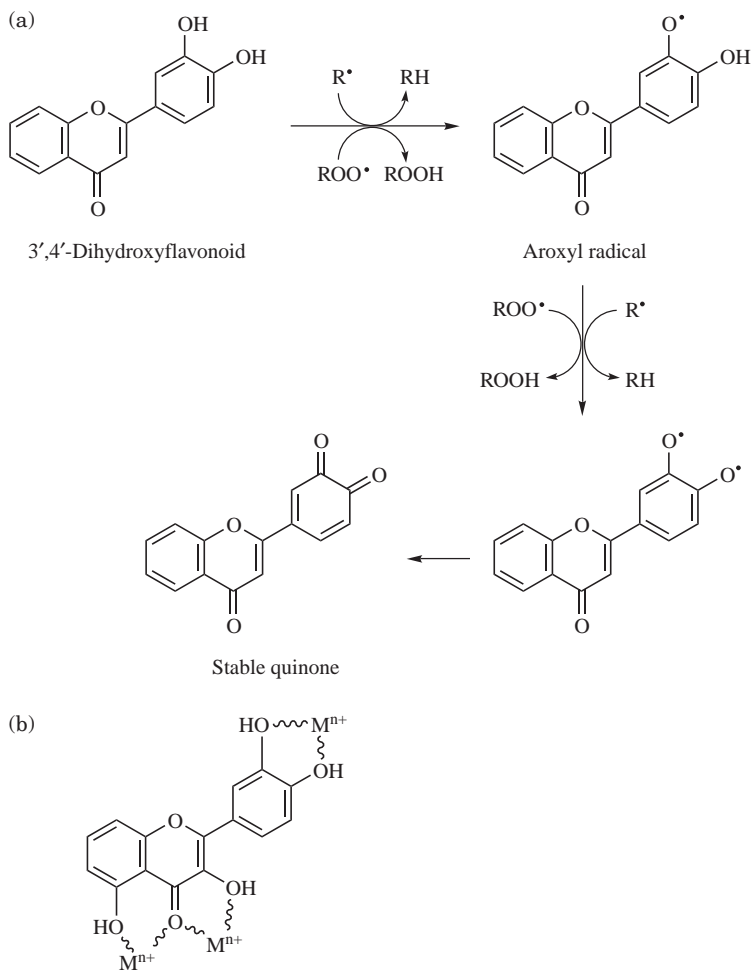
Flavonoids are a heterogeneous group of phenolic compounds having a benzo- $\gamma$ -pyrone structure (Figure 18) in the molecule and occur ubiquitously in plants. Approximately 90% of flavonoids occur in plants in the glycosidic form (143, 144). Antioxidant activity of flavonoids is bimodal, and they are very effective in counteracting lipid oxidation; however, this is very much dependent on the chemical and physical properties of the system. Flavonoids function as primary antioxidants in systems when metal catalyzed oxidation is not present. Because of their lower redox potentials ( $230 < E^0 < 750$  mV), flavonoids (145) are thermodynamically able to reduce highly oxidizing free radicals with redox potential in the range of 2310–1000 mV, such as alkoxy, hydroxyl, peroxy, and superoxide (See Table 1, 21) radicals by hydrogen donation. Flavonoids can form resonance-stabilized radicals while scavenging oxidative free radicals (18). For a molecule that has 3', 4'-dihydroxylation donation of one H atom to a free radical may produce a flavonoid aryloxy radical. This flavonoid aryloxy radical may react with a second radical and acquire a stable quinone structure (Figure 19a; 145, 146). At the same time, flavonoids are good metal chelators that can be used for inhibition of metal-catalyzed oxidation initiation. Metal chelation ability of flavonoids is caused by the ortho-diphenol structure in rings A and B (3-hydroxy-4-keto group or the 5-hydroxy-4-keto) and ketol structure in ring C (Figure 19b). An *ortho* quinol group at the B ring has also demonstrated metal ion chelating activity (18, 147).

The position and the degree of hydroxylation (especially at the A and B rings) are of primary importance in determining the antioxidant activity of flavonoids. Dihydroxylation at *ortho* position of the B ring contributes to antioxidant activity; however, *para* and *meta* hydroxylation of the B ring do not occur naturally. All flavonoids with 3',4'-dihydroxy configuration possess antioxidant activity (18, 147).

		Hydroxylation/substitution							
		3	5	7	3'	4'	5'		
 <p>Flavone (Flavonol)</p>	Flavone	H	OH	OH	H	H	H	Chrysin	
	H	OH	OH	H	OH	H	H	Apigenin	
	H	OH	OH	OH	OH	OH	H	Luteolin	
	Flavonol	OH	OH	OH	OH	OH	H	Quercetin	
	OH	OH	OH	OH	OH	OH	H	Myricetin	
	OH	OH	OH	H	OH	H	H	Kaempferol	
	O-rutinoside	OH	OH	OH	OH	OH	H	Rutin	
	<hr/>								
	 <p>Anthocyanidin (Anthocyanin)</p>		3	5	7	3'	4'	5'	
		OH	OH	OH	H	OH	H	H	Pelargonidin
OH		OH	OH	OH	OH	H	H	Cyanidin	
OH		OH	OH	OCH <sub>3</sub>	OH	H	H	Peonidin	
OH		OH	OH	OH	OH	OH	OH	Delphinidin	
OH		OH	OH	OCH <sub>3</sub>	OH	OH	OH	Petunidin	
OH		OH	OH	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	Malvidin	
<hr/>									
 <p>Flavan-3-ols</p>			3	5	7	3'	4'	5'	Absolute configuration
		OH	OH	OH	OH	OH	H	H	2R:2S
	OH	OH	OH	OH	OH	H	H	2R:3R	(-)-Epicatechin
	OH	OH	OH	OH	OH	OH	OH	2R:3S	(+)-Gallicocatechin
	OH	OH	OH	OH	OH	OH	OH	2R:3R	(-)-Epigallocatechin
	-Gallic acid	OH	OH	OH	OH	H	H	2R:3R	(-)-Epicatechin gallate
	-Gallic acid	OH	OH	OH	OH	OH	OH	2R:3R	(-)-Epigallocatechin gallate
	<hr/>								
 <p>Flavonone (Flavanonol)</p>		3	5	7	3'	4'	5'		
	Flavonone	H	OH	OH	H	OCH <sub>3</sub>	H	H	Hesperetin
	H	OH	OH	H	OH	H	H	H	Naringenin
	Flavanonol	OH	OH	OH	OH	OH	H	H	Taxifolin
	OH	OH	OH	OH	OH	H	H	H	
	OH	OH	OH	OH	OH	H	H	H	

**Figure 18.** Chemical structures of flavonoids according to their hydroxylation.

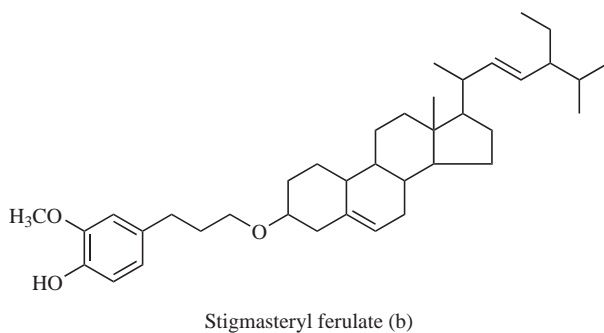
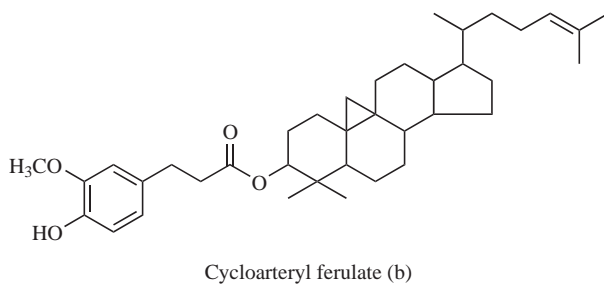
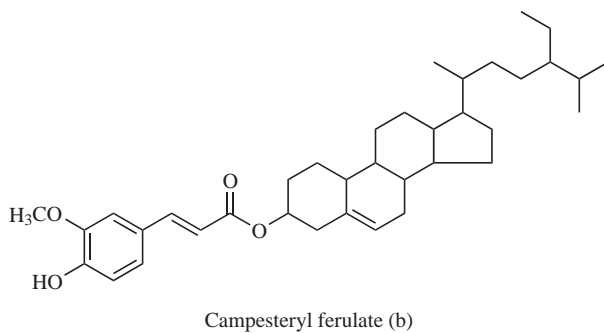
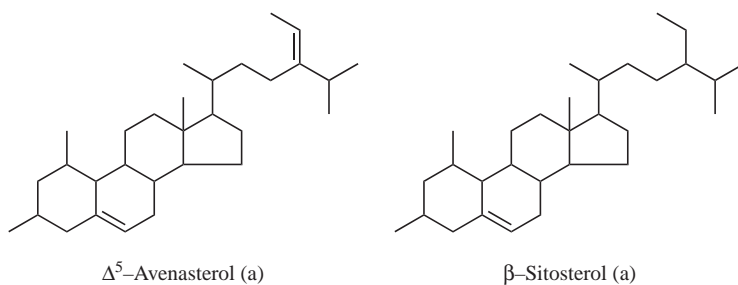
Other important features include carbonyl group at position 4 and a free hydroxyl group at position 3 or 5 (91). Among aglycones, the presence of a free 3-hydroxyl group in the C ring is a requirement for maximal radical scavenging activity of flavonoids (148, 149). When a disaccharide is glycosylated to a flavonoid (e.g., rutin),



**Figure 19.** (a) Free radical scavenging by flavonoids. (b) Binding sites of flavonoids for metal ions.

the substituent at position 3 becomes a poorer leaving group; thus, the molecule becomes less oxidizable and exhibits a lower antioxidant activity in free fatty acid systems than monosaccharide glycosides (e.g., quercetin; 149). Many of the flavonoids and related substances display a significant antioxidant behavior in lipid-aqueous and lipid-lipid food systems (147). Most of these compounds have very low solubility in the lipid phase, and it is a serious disadvantage if the aqueous phase is present to a considerable extent in the food.

The antioxidant activity of these catechins and the derivatives showed a marked difference depending on the substrate used for evaluation. In bulk corn oil that was oxidized at 50°C epigallocatechin, epigallocatechin gallate and epicatechin gallate exhibited better antioxidant activity than epicatechin or catechin. These catechins have been very effective in retarding oxidation of polyunsaturated fatty acids-rich



**Figure 20.** Chemical structure of antioxidative sterols identified from (a) oats, and (b) rice and corn fiber.



vegetable, and marine oils (150–152). In the oil-in-water emulsions, all catechins tested were pro-oxidants; however, in the liposomes comprising lecithin, epigallocatechin gallate was the best antioxidant, followed by epicatechin, epigallocatechin, epicatechin gallate, and catechin (78, 152). When tea catechins were added to noodles and to the frying oils, they were able to improve the oxidative stability of the fried product and the oil used for frying (153). In addition to that, tea polyphenols exhibited protecting ability against  $\beta$ -carotene oxidation; i.e., tea catechins were able to exert an antidiscoloring effect on beverages containing  $\beta$ -carotene that were UV-light irradiated (153).

The antioxidant activity of individual tea polyphenols in different model assays showed a proportional relationship to the number of hydrogen radical donors of catechins. A synergistic effect was observed between tea catechins and caffeine, ascorbic, citric, malic, and tartaric acids and tocopherols (153). Formation of oxidation products of (+)-catechin during the antioxidative process has been observed in oxidation model studies. According to the proposed mechanism, (+)-catechin can scavenge four radicals per molecule (154, 155). Yamamoto et al. (156) have summarized the chemistry and application aspects of green tea, especially in relation to using their catechins.

## 8. ESTIMATION AND ANALYSIS OF ANTIOXIDANTS IN FOODS

Qualitative and quantitative detection of antioxidants and potential antioxidative compounds is of utmost importance to researchers and industry and regulatory agencies. Numerous methods based on colorimetry, spectrometry, fluorometry, voltammetry, polarography, thin-layer chromatography, paper chromatography, gel permeation chromatography, gas chromatography, and high-performance liquid chromatography have been described for both natural and synthetic compounds for their antioxidant activity in foods (157). Almost all of these procedures require considerable sample preparation and estimation of individual antioxidants. A considerable number of procedures have been developed and tested in collaborative studies for determination of commonly used antioxidants, which are strictly regulated for their use. Kochhar and Rossell (157) have provided an elaborative discussion about the methods used to determine BHA, BHT, gallates, tocopherols, and TBHQ. Official methods have been developed to determine phenolic antioxidants under the category of food additives (AOAC method 983.15; 158).

## 9. TECHNOLOGICAL CONSIDERATIONS IN USING ANTIOXIDANTS

The type of food to which antioxidants may be added is variable and ranges from baked goods, biscuits, chewing gum, dry snacks, fruit drinks, mayonnaise, meat products, nuts, and oils and fats, among others. For food applications, the antioxidants must be effective at low concentrations (below 0.02%, w/w) because at high concentrations, they may act as pro-oxidants. The antioxidants should also be nontoxic.

Usually the antioxidant is directly added to the food as a concentrate in lipid/oil, dissolved in a food grade solvent, or in an emulsified form that may be sprayed onto the food product. Antioxidants must be thoroughly blended with the lipid to obtain their maximum potency. To be effective, antioxidant(s) should partition between oil–air interfaces in bulk oil systems or between oil–water interfaces in the emulsion systems. Antioxidants must be added, as soon as possible, to the fresh product as they cannot reverse any oxidation reactions that have already occurred (157). Metal deactivators such as citric acid are added to vegetable oils after heating, during the cooling stage of deodorization step of vegetable oils (19). An effective antioxidant should be stable under processing conditions, especially at high temperatures, and possess a good “carry through property.”

Antioxidants that are added to fats and oils are usually in the form of liquid formulations. The major considerations of devising antioxidant formulations include the following:

1. Antioxidants with different degrees of potency are formulated in an antioxidant combination/mixture.
2. Better control and accuracy in applying of antioxidants should be achieved with the mixture or formulation.
3. Ability to use synergistic effect of antioxidants to enhance their activity.
4. Complete distribution or solvation of antioxidants in fats and oils.
5. Prevent or minimize discoloration associated with specific antioxidants.
6. Ease of handling.

Most synthetic antioxidants are formulated with polypropylene glycol, glyceryl monooleate, mono- and diacylglycerols, or vegetable oils as carriers to enhance their solubility or dispersibility in foods. Several synergistic mixtures are available commercially, especially citric acid with synthetic antioxidants. Commercial preparations of natural antioxidants are predominantly tocopherol- and ascorbic acid ester-based. Few formulations are available with rosemary, sage, and tea chatechin-based antioxidative ingredients. In these formulations, vegetable oils and starches are used as carriers and citric acid is also included. Elliot (159) has extensively discussed the technological considerations when using ascorbic acid,  $\beta$ -carotene, and tocopherols as antioxidants in lipid-containing foods. These antioxidants should be handled differently than the synthetic antioxidants because of their reactive nature.

## **10. REGULATORY STATUS AND SAFETY ISSUES OF SYNTHETIC AND NATURAL ANTIOXIDANTS**

All synthetic antioxidants are generally categorized under direct food additives. They are subjected to careful scrutiny and complex toxicological studies for approval. However, the usage and approval of an antioxidant may differ from

one country to another. Many countries have adopted regulations similar to the United States regarding the usage of these antioxidants; however, significant differences exist among different countries on their type, application, and usage levels. Table 9 provides a summary of regulations governing the use of synthetic antioxidants in Canada and the United.

In Canada, the use of antioxidants is regulated under the Food and Drug Act (Heath Canada), and in United States, it is regulated by the Federal Drug Administration and the U.S. Department of Agriculture. When it comes to the European Economic Community, directives regulate the use of antioxidants; however, individual member countries still have the control of usage levels. In Japan, the Food Sanitation Law specifies the use of antioxidants (48).

Antioxidative compounds naturally present in food are not covered under present regulations; obviously, it is not a controlled substance as it is part of the raw material of food processing. However, if an antioxidative compound isolated from a natural source is to be added to food, the compound should comply with the appropriate regulations and safety clearances.

## 11. SAFETY CONSIDERATIONS OF ANTIOXIDANTS USED IN FOODS

The safety of food additives is always a controversial discussion because of their possible toxic effects during long-term intake. It has become clear that antioxidants may share a number of toxic properties at high doses. However, it is logical to consider using antioxidants at low levels to reduce the deleterious effects of consuming lipid oxidation products that may be produced if no antioxidants are used. The intake amount of each antioxidant is different with the food and the dietary habits. The use of antioxidants in different countries is limited by specific regulations, established on the basis of their safety for use and technological need. Many countries follow the recommendations of the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) on the safe use of food additives. The safety evaluations produced by international bodies such as the Joint FAO/WHO expert committee on food additives (JECFA) and the European Commission (EC) scientific committee for food additive (SCF) are used to establish acceptable daily intake (ADI). ADI is defined as the average amount of the substance that can be consumed daily for a lifetime without health hazards and expressed on the basis of bodyweight. In determining ADI, a range of toxicity tests is carried out. From these tests, the effect that is most sensitive is studied to ascertain the maximum dose at which that effect is no longer observed (no-effect level). A reduction or a safety factor is used to refine the no-effect level, considering the possible difference in sensitivity between species (animals and human) and individuals. To ensure there is an adequate margin of safety for consuming groups, an arbitrary safety factor of 100 is normally used (159). A factor lower than 100 is used if ADI is based on human toxicity study data. For some compounds, ADI is "not specified." According to the Joint FAO/WHO Expert committee recommendations, this is "specified

**TABLE 9. Regulations Governing the Use of Synthetic Antioxidants in Canada and the United States.**

Compound	Canada <sup>a</sup>		United States <sup>b</sup>	
	Item Number and Permitted in or upon	Maximum level of use	Citation and Permitted in	Maximum level of use
Ascorbic acid	Class IV, A.1 In fats and oils, monoglycerides and diglyceride, shortenings, Unstandardized foods	GMP	21 CFR 182.3013	GRAS with GMP
Ascorbyl palmitate	Class IV, A.2 In fats and oils, lard, monoglycerides and diglyceride, Shortenings	GMP	21 CFR 182.3149	GRAS with GMP
	Unstandardized foods except meat and meat byproducts, fish, poultry meat and its byproducts Margarine	GMP  Not to exceed 0.02% of the fat content, alone or in combination with ascorbyl stearate		
Ascorbic stearate	Class IV, A.3 In fats and oils, monoglycerides and diglyceride, Shortenings Margarine	GMP  Not to exceed 0.02% of the fat content, alone or in combination with ascorbyl palmitate		
BHA	Class IV, B.1		21 CFR 172.110	Alone or in combination with BHT
	In fats and oils, shortenings, margarine	Not to exceed 0.02%, alone or in combination with BHT, PG or TBHQ	Dehydrated potato shreds	50 ppm
	Dried breakfast cereals, dehydrated potato products	Not to exceed 0.005%, alone or in combination with BHT or PG	Active dry yeast	1,000 ppm

**TABLE 9.** (Continued)

Chewing gum	Not to exceed 0.02%, alone or in combination with BHT or PG	Beverages and desserts prepared from dry mixes	2 ppm
Essential oils, citrus oil flavors, dry flavours	Not to exceed 0.125%, alone or in combination with BHT or PG	Chewing gum base	1,000 ppm
Citrus oils	Not to exceed 0.5%, alone or in combination with BHT or PG	Dry breakfast cereals	50 ppm
Partially defatted pork or beef fatty tissues	Not to exceed 0.065%, alone or in combination with BHT	Dry diced glazed fruit	32 ppm
Vitamin A liquids for foods	5 mg/1,000,000 international units	Dry mixes for beverages and desserts	90 ppm
Dry beverage mixes, dry dessert and confection mixes	0.009%	Edible fats and oils excluding butterfat and margarine	200 ppm
Active dry yeast	0.1%	Emulsion stabilizers for shortenings	200 ppm
Unstandardized foods except preparations of meat and meat by products, fish, poultry meat and its by products	Not to exceed 0.02% of total fat content of food, alone or in combination with BHT or PG	Essential oils	1,000 ppm
Dry vitamin D preparations for food	10 mg/1,000,000 international units	Margarine	200 ppm
Margarine	Not to exceed 0.01% of the fat content, alone or in combination with BHT or PG or both	Potato flakes	50 ppm
Dried cooked poultry meat	0.015% of the fat content alone or in combination with PG or citric acid or both	Potato granules	10 ppm
	Sweet potato flakes		50 ppm

BHT	Class IV, B.2	21 CFR 172.115	Alone or in combination with BHA
	Fats and oils, lard, shortening	Not to exceed 0.02%, alone or in combination with BHA, PG or TBHQ	Dehydrated potato shreds 50 ppm
	Dried breakfast cereals, dehydrated potato products	Not to exceed 0.005%, alone or in combination with BHA or PG	Dry breakfast cereals 50 ppm
	Chewing gum	Not to exceed 0.02%, alone or in combination with BHA or PG	Chewing gum base 1,000 ppm
	Essential oils, Citrus oil flavors, Dry flavours	Not to exceed 0.125%, alone or in combination with BHA or PG	Edible fats and oils excluding butter fat and margarine 200 ppm
	Citrus oils	Not to exceed 0.5%, alone or in combination with BHA or PG	Emulsion stabilizers for shortenings Essential oils 200 ppm 1,000 ppm
	Partially defatted pork or beef fatty tissues	Not to exceed 0.065%, alone or in combination with BHA	Margarine 200 ppm
	Vitamin A liquids for foods	5 mg/1,000,000 international units	Potato flakes 50 ppm
	Parboiled rice	0.0035%	Potato granules 10 ppm
	Unstandardized foods except preparations of meat and meat byproducts, fish, poultry meat and its byproducts	Not to exceed 0.02% of total fat content of food, alone or in combination with BHA or PG	Sweet potato flakes 50 ppm
	Dry vitamin D preparations for food	10 mg/1,000,000 international units	
	Margarine	Not to exceed 0.01% of the fat content, alone or in combination with BHA or PG or both	

**TABLE 9.** (Continued)

Citric acid	Class IV, C.3			
	In fats and oils, monoglycerides and diglyceride, shortenings,	GMP		
	Unstandardized foods except preparations of meat and meat byproducts, fish, poultry meat and its byproducts	GMP		
	Dried cooked poultry meat		Not to exceed 0.015% of fat content, alone or in combination of BHA or PG or both	
Monoacylglycerol citrate	Class IV, M.1		21 CFR 172.832	Not to exceed 200 ppm of total fat
	In fats and oils, monoglycerides and diglyceride, shortenings	GMP		
	Unstandardized foods except preparations of meat and meat byproducts, fish, poultry meat and its byproducts	GMP		
	Margarine		Not to exceed 0.01% of fat content, alone or in combination of mono isopropyl citrate or stearyl citrate or both	
Mono isopropyl citrate	Class IV, M.2		21 CFR 582.6511	GRAS with GMP
	In fats and oils, monoglycerides and diglyceride, shortenings	GMP		Use as a sequestrant
	Unstandardized foods except preparations of meat and meat byproducts, fish, poultry meat and its byproducts	GMP		

	Margarine	Not to exceed 0.01% of fat content, alone or in combination of mono glyceride citrate or stearyl citrate or both		
Propyl gallate	Class IV, P.1 In fats and oils, lard, shortenings	Not to exceed 0.02%, alone or in combination with BHA, BHT or TBHQ	21 CFR 184.1660	Not to exceed 0.02% of fat content including essential (volatile) oil content
	Dried breakfast cereals, dehydrated potato products	Not to exceed 0.005%, alone or in combination with BHA or BHT	Chewing gum base Edible oils and fats excluding butterfat and margarine	100 ppm 100 ppm
	Chewing gum	Not to exceed 0.02%, alone or in combination with BHA or BHT	Margarine	100 ppm
	Essential oils, dry flavors	Not to exceed 0.125%, alone or in combination with BHA or BHT		
	Citrus oils	Not to exceed 0.5%, alone or in combination with BHA or BHT		
	Unstandardized foods except preparations of meat and meat byproducts, fish, poultry meat and its byproducts	Not to exceed 0.02% of the fat content alone or in combination with BHA or BHT		
	Margarine	Not to exceed 0.01% of the fat content, alone or in combination with BHA or BHT or both		
	Dried cooked poultry meat	0.015% of the fat content alone or in combination with BHA or citric acid or both		



**TABLE 9. (Continued)**

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TBHQ	Class IV, T.1A In fats and oils, lard, shortenings	Not to exceed 0.02% alone or in combination with BHA or BHT or PG	21 CFR 172.185	Not to exceed 0.02% of fat content including essential (volatile) oil, Alone or in combination with BHA and/or BHT GRAS with GMP
Tocopherols ( $\alpha$ -,concentrate or mixed)	Class IV, T.2 In fats and oils, lard, monogly- cerides and diglyceride, shortenings	GMP	21 CFR 182.3890	
	Unstandardized foods except preparations of meat and meat by products, fish, poultry meat and its by products	GMP		

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<sup>a</sup>From Canada Food and Drugs Act.<sup>b</sup>From U.S. Code of Federal Regulations.

**TABLE 10. Acceptable Daily Intake (ADI) Levels of Antioxidants Commonly Used as Food Additives.**

Antioxidant	ADI	Reference
Ascorbic acid and Derivates	No ADI specified for salts of Na, K, Ca	162
	0–1.25 mg/kg bw for ascorbyl palmitate or stearate or sum of both if used together	163
BHA	0–0.5 mg/kg bw (JECFA)	164
	0–0.5 mg/kg bw temporary (SFA)	
BHT	0–0.125 mg/kg bw (JECFA)	161
	0–0.05 mg/kg bw (SFA)	165
TBHQ	0–0.2 mg/kg bw temporary allowed	161
Tocopherols	0.15–2.0 mg/kg bw for dl- $\alpha$ -tocopherol and d- $\alpha$ -tocopherol concentrate	161

on the basis of the available data (biochemical, chemical, toxicological and other), the total daily intake of the substance, from its use at levels necessary to achieve the desired effect and from its acceptable background in food, and does not, in the opinion of the committee, represent a hazard to health. For that reason and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary” (160, 161). For some of the compounds, because of the insufficient information available, an ADI level is not specified or “No ADI allocated.” Table 10 provides information on ADI available for food antioxidants in use. Barlow (160) has provided a very descriptive examination on the toxicological studies on antioxidants used as food additives.

Toxicological risks may develop when the daily doses of a compound rise above a certain threshold limit; therefore, toxicity is a matter of dose as well. Natural antioxidants, especially carotenoids, phenolic acids, flavonoids, and sterols, may also exert in vivo pro-oxidative activity. Rietjens et al. (166) have provided an elaborate discussion on the pro-oxidative chemistry and toxicity of well-known natural antioxidants, including ascorbic acid, tocopherols, carotenoids, and flavonoids.

## REFERENCES

1. B. Halliwell, M. A. Murcia, S. Chirico, and O. I. Aruoma, *Crit. Rev. Food Sci. Nutr.*, **35**, 7 (1995).
2. C. Moureu and C. Dufraisse, *Chem. Rev.*, **3**, 113 (1926).
3. H. Davy, *Ann. Chim. Phys.*, **4**, 347 (1817).
4. M. E. Duclaux, *Compt. rend.*, **102**, 1077 (1886).
5. M. Tsujimoto, *J. Coll. Engl. Imp. Univ. Tokyo*, 4181 (1908).
6. C. W. Wright, *Am. J. Pharm.*, **24**, 180 (1852).
7. E. Chevreau, *Ann. Chim. Phys.*, **47**, 209 (1856).
8. C. Moureu and C. Dufraisse, *Compt. rend.*, **174**, 258 (1922).

9. C. Moureu and C. Dufraisse, *Compt. rend.*, **175**, 127 (1922).
10. C. Moureu and C. Dufraisse, *J. Chem. Soc.*, **127**, 1 (1925).
11. C. Moureu and C. Dufraisse, *Chem. Ind.*, **47**, 819 (1928).
12. C. D. Lowry, G. Eglhoff, J. C. Morrel, and G. C. Dryer, *Ind. Eng. Chem.*, **25**, 804 (1933).
13. J. L. Bolland and P. tenHave, *Discuss. Farad. Soc.*, **2**, 252 (1947).
14. H. S. Olcott and H. A. Mattill, *J. Am. Chem. Soc.*, **58**, 1627 and 2208 (1936).
15. K. C. Bailey, *The Retardation of Chemical Reactions*, Edward Arnold Co., London, 1937, pp. 111, 179.
16. G. Scott, *Atmospheric Oxidation and Antioxidants*, Elsevier, London, 1965, pp. 1 and 15.
17. M. W. Ranney, *Antioxidants Recent Developments*, Noyes Data Corp., Park Ridge, New Jersey, 1979, p. 1.
18. F. Shahidi and P. K. J. P. D. Wanasundara, *Crit. Rev. Food Sci. Nutr.*, **32**, 67 (1992).
19. E. N. Frankel, *Lipid Oxidation*, The Oily Press, West Ferry, Dundee, 1998, pp. 13, 79, 115, 129, and 167.
20. D. G. Bradley and D. B. Min, *Crit. Rev. Food Sci. Nutr.*, **31**, 211 (1992).
21. O. R. Buettner, *Arch. Biochem. Biophys.*, **300**, 535 (1993).
22. E. A. Decker, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry, Nutrition and Biotechnology*, 2nd ed., Marcel Dekker, Inc., New York, 2002, p. 517.
23. W. W. Nawar, in O. Fennema, ed., *Food Chemistry*, 3rd ed., Marcel Dekker, Inc., New York, 1996, p. 225.
24. M. H. Gordon, in B. J. F. Hudson, ed., *Food Antioxidants*, Elsevier Applied Science, New York, 1990, p. 1.
25. D. F. Buck, *Cereal Foods World*, **29**, 301 (1984).
26. N. Uri, in H. M. Sinclair, ed., *Proceedings of International Conference in Biochemistry: Problems of Lipids*, Academic Press, New York, 1958, p. 30.
27. J. K. Kochi, *Free Radicals*, **1**, 591 (1993).
28. J. Pokorný, in H.W.-S. Chan, ed., *Autooxidation of Unsaturated Lipids*, Academic Press, London, 1987, p. 141.
29. H. B. Dunford, *Free Rad. Biol. Med.*, **3**, 405 (1987).
30. C. Ruben and K. Larsson, *J. Dispersion Sci. Technol.*, **6**, 213 (1985).
31. J. R. Mahoney and E. Graf, *J. Food Sci.*, **51**, 1293 (1986).
32. E. Graf and J. W. Eaton, *Free Rad. Biol. Med.*, **8**, 61 (1990).
33. R. S. Lindsay, in O. Fennema, ed., *Food Chemistry*, 3rd ed., Marcel Dekker, Inc., New York, 1996, p. 767.
34. B. Halliwell and J. M. C. Gutteridge, *Arch. Biochem. Biophys.*, **246**, 501 (1986).
35. C. E. Brown, *J. Theor. Biol.*, **88**, 245 (1981).
36. E. A. Decker, V. Ivanov, B. Z. Zhu, and B. Frei, *J. Agric. Food Chem.*, **49**, 511 (2001).
37. P. Palozza and N. I. Krinsky, *Methods Enzymol.*, **213**, 403 (1992).
38. W. Stahl and H. Sies, *Ann. N. Y. Acad. Sci.*, **691**, 10 (1992).
39. J. Kanner, J. B. German, and J. E. Kinsella, *Crit. Rev. Food Sci. Nutr.*, **25**, 317 (1987).
40. A. Claiborne, in C. R. A. Greenwald, ed., *CRC Hand Book of Methods of Oxygen Radical Research*, Vol. I, CRC Press, Boca Raton, Florida, 1985, p. 283.

41. W. A. Günzler and L. Flohé, in R. A. Greenwald, ed., *CRC Hand Book: Methods of Oxygen Radical Research*, Vol 1. CRC Press, Boca Raton, Florida, 1985, p. 285.
42. M. Maioriono, C. Gregolin, and F. Ursini, *Methods Enzymol.*, **186**, 448 (1990).
43. W. W. Nawar, in O. Fennema ed., *Food Chemistry*, 2nd ed., Marcel Dekker, Inc., New York, 1986, p. 139.
44. S. Z. Dziedzic, J. L. Robinson, and B. J. F. Hudson, *J. Agric. Food Chem.*, **34**, 1027 (1986).
45. S. Englard and S. Seifter, *Ann. Rev. Nutr.*, **6**, 365 (1986).
46. E. N. Frankel and A. S. Meyer, *J. Sci. Food Agric.*, **80**, 1925 (2000).
47. M. Antolovich, P. D. Prenzler, E. Patsalides, S. McDonald, and K. Robards, *Analyst*, **127**, 183 (2002).
48. D. E. Pratt, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products, Edible Oil and Fat Products, Products and Application Technology*, 5th ed., Wiley, New York, 1995, p. 523.
49. S. Z. Dziedzic and B. J. F. Hudson, *J. Am. Oil Chem. Soc.*, **61**, 1042 (1984).
50. F. Shahidi and U. N. Wanasundara, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry, Nutrition and Biotechnology*, 2nd ed., Marcel Dekker, Inc., New York, 2002, p. 465.
51. H. S. Olcott and E. Einest, *J. Am. Oil Chem. Soc.*, **35**, 161 (1958).
52. P. Ke and R. G. Ackman, *J. Am. Oil Chem. Soc.*, **53**, 636 (1976).
53. M. S. Blois, *Nature*, **181**, 1199 (1958).
54. T. Yoshida, K. Mori, T. Hatano, T. Okumura, I. Uchura, K. Komagoe, Y. Fujita, and T. Okuda, *Chem. Pharm. Bull.*, **37**, 1919 (1989).
55. B. Halliwell, J. M. C. Gutteridge, and O. I. Aruoma, *Anal. Biol. Chem.*, **63**, 215 (1987).
56. V. Ponti, M. U. Dianzani, K. Cheeseman, and T. F. Slater, *Chem. Biol. Interact.*, **23**, 281 (1978).
57. C. Yuting, Z. Rongling, J. Zongjan, and J. Yong, *Free Rad. Biol. Med.*, **199**, 19 (1990).
58. N. Cotelle, J.-L. Bernier, J.-P. Cateau, J. Pommery, J.-C. Wallet, and E. M. Gaydou, *Free Rad. Biol. Med.*, **20**, 15 (1996).
59. X. Shi, N. S. Dalal, and A. C. Jain, *Food Chem. Toxicol.*, **29**, 1 (1991).
60. A. E. Holley and K. H. Cheeseman, *Brit. Med. Bull.*, **49**, 494 (1993).
61. M. K. Sharma and G. R. Buettner, *Free Rad. Biol. Med.*, **14**, 649 (1993).
62. I. F. F. Benzie and J. J. Strain, *Anal. Biochem.*, **239**, 70 (1996).
63. I. F. F. Benzie and J. J. Strain, *Methods Enzymol.*, **299**, 15 (1999).
64. P. T. Gardner, T. A. C. White, D. B. McPhail, and G. G. Duthie, *Food Chem.*, **68**, 471 (2000).
65. G. Cao, H. M. Alessio, and R. G. Cutler, *Free Rad. Biol. Med.* **14**, 303 (1993).
66. G. Cao, C. P. Verdon, A. H. B. Wu, H. Wang, and R. L. Prior, *Clin. Chem.*, **413**, 1738 (1995).
67. C. Guo, G. Cao, E. Sofic, and R. L. Prior, *J. Agric. Food Chem.*, **45**, 1787 (1997).
68. D. D. M. Wayner, G. W. Burton, K. U. Ingold, and S. Locke, *FEBS Lett.*, **187**, 33 (1985).
69. M. Valkonen and T. Kuusi, *J. Lipid Res.*, **38**, 823 (1997).
70. C. A. Rice-Evans and N. J. Miller, *Methods Enzymol.*, **234**, 279 (1994).

71. C. A. Rice-Evans, N. J. Miller, P. G. Bolwell, P. M. Bramley, and J. B. Pridham, *Free Rad. Res.*, **22**, 375 (1995).
72. E. N. Frankel, *Trends Food Sci. Technol.*, **4**, 220 (1993).
73. *The Official Methods and Recommended Practices of the American Oil Chemists Society*, 5th ed., AOCS Press, Champaign, Illinois, 2000.
74. W. L. Porter, in M. G. Simic and M. Karel, eds., *Autooxidation in Food and Biological Systems*, Plenum Press, New York, 1980, p. 295.
75. W. L. Porter, E. D. Black, and A. M. Drolet, *J. Agric. Food Chem.*, **37**, 615 (1989).
76. W. L. Porter, *Toxicol. Ind. Health*, **9**, 93 (1993).
77. E. N. Frankel, S. W. Huang, J. Kanner, and J. B. German. *J. Agric. Food Chem.*, **42**, 1054 (1994).
78. S. W. Huang and E. N. Frankel, *J. Agric. Food Chem.*, **45**, 3033 (1997).
79. G. Siripriya, K. Chandrasekharan, V. S. Murty, and T. S. Chandra, *Food Chem.*, **57**, 539 (1996).
80. L. A. Santiago, M. Hiramatsu, and A. Mori, *J. Nutr. Sci. Vitaminol.*, **38**, 297 (1992).
81. M. Wettasinghe and F. Shahidi, *Food Chem.*, **70**, 17 (2000).
82. V. Bondet, W. Brand-William, and C. Berset, *Lebensm-wiss.u.-Technol.*, **30**, 609 (1997).
83. N. Darmon, Y. Fernandez, C. Cambon-Gros, and S. Mitiavila, *Food Addit. Contam.*, **7**, 560 (1990).
84. A. S. Meyer, D. S. Rørbæk, and J. Adler-Nissen, *Food Chem.*, **51**, 171 (1994).
85. J. Pokorný, in J. Pospíšil and P. P. Klemchuk, eds., *Oxidation Inhibition of Organic Materials*, Vol. I, CRC Press, Boca Raton, Florida, 1989, p. 349.
86. J. Pokorný, in M. S. Rahman, ed., *Handbook of Food Preservation*, Marcel Dekker, Inc., New York, 1999, p. 309.
87. E. N. Frankel, P. M. Cooney, H. A. Moser, J. C. Cowen, and C. D. Ewan, *Fette Seifen Anstrichm.*, **10**, 1036 (1959).
88. P. P. Coppen, in J. C. Allen and R. J. Hamilton, eds., *Rancidity in Foods*, Applied Science, London, 1983, p. 76.
89. J. D. Dziezak, *Food Technol.*, **9**, 94 (1986).
90. W. M. Cort, *Adv. Chem. Ser.*, **200**, 533 (1983).
91. S. Z. Dziedzic and B. J. F. Hudson, *Food Chem.*, **12**, 205 (1983).
92. H. Y. D. Belitz and W. Grosch, *Food Chemistry*, Springer Verlag, New York, 1987, p. 128.
93. P. Vinas, C. K. Hernandez Cordoba, and C. Sanchez-Pedrenos, *Food Chem.*, **42**, 421 (1991).
94. D. W. Reische, D. A. Lillard, and R. R. Eitenmiller in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry, Nutrition and Biotechnology*, 2nd ed., Marcel Dekker, Inc., New York, 2002, p. 489.
95. E. Niki, *Chem. Phys. Lipids*, **44**, 227 (1987).
96. G. Scott, *Antioxidants in Science, Technology, Medicine and Nutrition*, Albion Publishing, West Sussex, Chichester, England, 1997, p. 202.
97. J. J. Pokorný, *Trends Food Sci. Technol.*, **9**, 223 (1991).
98. D. L. Madhavi, R. S. Singhal, and P. R. Kulkarani, in D. L. Madhavi, S. S. Deshpande, and D. K. Salunkhe, eds., *Food Antioxidants: Technological, Toxicological and Health Perspectives*, Marcel Dekker, New York, 1996, p. 159.

99. C. H. Foyer, in R. G. Alscher and J. L. Hess, eds., *Antioxidants in Higher Plants*, CRC Press, Boca Raton, Florida, 1993, p. 31.
100. P. Schuler, in B. J. F. Hudson, ed., *Food Antioxidants*, Elsevier Applied Science, New York, 1990, p. 99.
101. P. A. Seib, *Int. J. Vit. Nutr. Res. Suppl.*, **27**, 259 (1985).
102. S. S. Desphande, U. S. Desphande, and D. K. Salunkhe, in D. L. Madhavi, S. S. Deshpande, and D. K. Salunkhe, eds., *Food Antioxidants: Technological, Toxicological and Health Perspectives*, Marcel Dekker, New York, 1996, p. 361.
103. G. W. Burton and K. U. Ingold, *Science*, **224**, 569 (1984).
104. D. C. Leiber, *Ann. N. Y. Acad. Sci.*, **691**, 20 (1992).
105. P. Palozza and N. I. Krinsky, *Arch. Biochem. Biophys.*, **297**, 184 (1992).
106. K. M. Haila, S. M. Lievenon, and M. I. Heinonen, *J. Agric. Food Chem.*, **44**, 2096 (1996).
107. C. J. Dillard, V. C. Gavino, and A. L. Tappel, *J. Nutr.*, **113**, 226 (1983).
108. F. V. Timmerman, *Fat Sci. Technol.*, **92**, 201 (1990).
109. F. Shahidi and M. Naczk, *Food Phenolics: Sources, Chemistry, Effects and Applications*, CRC Press, Boca Raton, Florida, 1994, pp. 9, 53, 75, 109.
110. P. K. J. P. D. Wanasundara, F. Shahidi, and V. K. S. Shukla, *Food Rev. Int.*, **13**, 225 (1997).
111. V. K. S. Shukla, P. K. J. P. D. Wanasundara, and F. Shahidi, in F. Shahidi, ed., *Natural Antioxidants: Chemistry, Health Effects and Applications*, AOCS Press, Champaign, Illinois, 1997, p. 97.
112. F. Shahidi and M. Naczk, *Phenolics in Food and Nutraceuticals*, CRC Press, Boca Raton, Florida, 2003.
113. H. Chimi, A. Sadik, B. Le Tutor, and M. Rahmani, *R. Fr. Des Crops Grass.*, **35**, 339 (1988).
114. J. Pokorny, in H. W. S. Chan, ed., *Autoxidation of Unsaturated Lipids*, Academic Press, London, 1988, p. 141.
115. M.-E. Cuvelier, H. Richard, and C. Berset, *BioSci. Biotech. Biochem.*, **56**, 324 (1992).
116. R. D. Howarth, *Anal. Rep. Prog. Chem.*, **33**, 266 (1936).
117. T. Osawa, M. Nagata, M. Namiki, and Y. Fukuda, *Agric. Biol. Chem.*, **49**, 3351 (1985).
118. Y. Fukuda, T. Osawa, M. Namiki, and T. Ozaki, *Agric. Biol. Chem.*, **49**, 301 (1985).
119. H. Katsuzaki, S. Kawakishi, and T. Osawa, *Heterocycles*, **36**, 933 (1993).
120. H. Katsuzaki, T. Osawa, and S. Kawakishi, in *Food Phytochemicals for Cancer Prevention (II)*, American Chemical Society Press, Champaign, Illinois, 1994, p. 275.
121. Y. Fukuda, M. Nagata, T. Osawa, and M. Namiki, *J. Am. Oil Chem. Soc.*, **63**, 1027 (1986).
122. Y. Fukuda, M. Isobe, M. Nagata, T. Osawa, and M. Namiki, *Heterocycles*, **24**, 923 (1986).
123. G. G. Yen and S. L. Shyu, *Food Chem.*, **31**, 215 (1989).
124. H. Yoshida, J. Shigezaki, S. Takagi, and G. Kajimoto, *J. Sci. Food Agric.*, **6**, 407 (1995).
125. F. Shahidi, R. Amarowicz, H. A. Abou-Gharbia, and A. Y. Shehata, *J. Am. Oil Chem. Soc.*, **74**, 147 (1997).
126. J. E. Brekke and H. J. Klosterman, *Proc. North Dakota Agric. Sci.*, **10**, 18 (1956).
127. L. U. Thompson and W. E. Ward, in G. S. Gilvani and J. J. B. Anderson, eds., *Phytoestrogens and Health*, American Oil Chemists' Society Press, Champaign, Illinois, 2002, p. 405.

128. H. Hyvarinen, J.-M. Pihlava, V. Hietanieni, E.-L. Ryhanen, and H. Kohonen, *Innov. Food Technol.*, **15**, 64 and 68 (2002).
129. K. Prasad, *Mol. Cell. Biochem.*, **168**, 117 (1997).
130. D. D. Kitts, Y. V. Yuan, A. N. Wijewickreme, and L. U. Thompson, *Mol. Cell. Biochem.*, **202**, 91 (1999).
131. D. Boskou and H. Katsikas, *Acta Alimentaria*, **8**, 317 (1979).
132. M. H. Gordon and P. Magos, *Food Chem.*, **10**, 141 (1983).
133. P. J. White and L. S. Armstrong, *J. Am. Oil Chem. Soc.*, **63**, 525 (1986).
134. Z. Xu and S. Godber, *J. Am. Oil Chem. Soc.*, **78**, 645 (2001).
135. T. Wang, K. B. Hicks, and R. Moreau, *J. Am. Oil Chem. Soc.*, **79**, 1201 (2002).
136. T. Ohta, S. Yamasaki, Y. Egashira, and H. Sanada, *J. Agric. Food Chem.*, **42**, 653 (1994).
137. Z. Xu and S. Godber, *J. Agric. Food Chem.*, **47**, 2724 (1999).
138. L. L. Tiang and P. J. White, *J. Am. Oil Chem. Soc.*, **7**, 1087 (1994).
139. E. N. Frankel, S.-W. Huang, R. Aeschbach, and E. Prior, *J. Agric. Food Chem.*, **44**, 131 (1996).
140. E. N. Frankel, S.-W. Huang, E. Prior, and R. Aeschbach, *J. Sci. Food Agric.*, **72**, 20 (1996).
141. S.-W. Huang, E. N. Frankel, J. B. German, and R. Aeschbach, *J. Agric. Food Chem.*, **45**, 1991 (1997).
142. D. A. Balentine, S. A. Wiseman, and L. C. Bouwens, *Crit. Rev. Food Sci. Nutr.*, **37**, 693 (1997).
143. S. A. Wiseman, D. A. Balentine, and B. Frei, *Crit. Rev. Food Sci. Nutr.*, **37**, 705 (1997).
144. J. Kühnau, *World Rev. Nutr. Diet.*, **24**, 117 (1976).
145. S. V. Jovanovic, S. Steenken, M. Tosic, B. Marjanovic, and M. G. Simic, *J. Am. Chem. Soc.*, **116**, 4846 (1994).
146. P.-G. Pietta, *J. Nat. Prod.*, **63**, 1035 (2000).
147. D. E. Pratt and B. J. F. Hudson, in B. J. F. Hudson, ed., *Food Antioxidants*, Elsevier Applied Science, New York, 1990, p. 171.
148. W. Bors, W. Hiller, C. Michel, and M. Saran, *Methods Enzymol.*, **186**, 343 (1990).
149. A. Hopia and M. Heinonen, *J. Am. Oil Chem. Soc.*, **76**, 139 (1999).
150. U. N. Wanasundara and F. Shahidi, *Food Chem.*, **50**, 393 (1994).
151. U. N. Wanasundara and F. Shahidi, *J. Am. Oil Chem. Soc.*, **73**, 1183 (1996).
152. U. N. Wanasundara and F. Shahidi, *Food Chem.*, **63**, 335 (1998).
153. E. N. Frankel, S. W. Huang, and R. Aeschbach, *J. Am. Oil Chem. Soc.*, **74**, 1309 (1997).
154. M. Koketsu, in T. Yamamoto, L. R. Juneja, D.-C. Chu, and M. Kim, eds., *Chemistry and Application of Green Tea*, CRC Press, Boca Raton, Florida, 1997, p. 37.
155. Y. Hirose, H. Yamamoto, and M. Nakayama, *Agric. Biol. Chem.*, **54**, 567 (1990).
156. T. Yamamoto, L. R. Juneja, D.-C. Chu, and M. Kim, *Chemistry and Application of Green Tea*, CRC Press, Boca Raton, Florida, 1997.
157. S. P. Kochhar and J. B. Rossell, in B. J. F. Hudson, ed., *Food Antioxidants*, Elsevier Applied Science, New York, 1990, p. 19.
158. W. Horowitz, ed., *AOAC Official Methods, Association of Official Analytical Chemists'* 17th ed., AOAC International, Arlington, VA, 2002, p. 1604.

159. J. G. Elliot, *Food Technol.*, **199**, 46, (1999).
160. S. Barlow, in B. J. F. Hudson, ed., *Food Antioxidants*, Elsevier Applied Science, New York, 1990, p. 253.
161. Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series, World Health Organization, **751**, 18 (1987).
162. Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series, World Health Organization, **669**, 52 (1984).
163. Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series, World Health Organization, **539**, 146 (1974).
164. Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series, World Health Organization, **776**, 14 (1989).
165. R. Haigh, *Food Chem. Toxicol.*, **24**, 1031 (1986).
166. I. M. C. M. Rietjens, M. G. Boersma, L. de Haan, B. Spenkink, H. M. Awad, N. H. P. Cnubben, J. J. van Zanden, H. van der Woude, G. M. Alink, and J. H. Koema, *Environ. Toxicol. Pharmacol.*, **11**, 321 (2002).



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## *Antioxidants: Regulatory Status*

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### **1. INTRODUCTION**

Oxidation of unsaturated lipids is a major cause of food quality deterioration by giving rise to the development of off-flavor compounds and loss of nutritional value of food products (1). Although it has been known for a long time that lipid oxidation can be induced by catalytic systems such as light, temperature, enzymes, metals, and metalloproteins; the mechanism of oxidation reactions remained uncertain until the 1940s when free radicals and reactive oxygen species were found to be involved in oxidation processes by the pioneering work of Farmer et al. (2), Bolland and Gee (3), and Bateman et al. (4–6). Furthermore, antioxidants were found to protect lipids against oxidation either by quenching free radicals or scavenging oxygen, among others (6). Antioxidants are substances that, when present in foods at low concentrations compared with that of an oxidizable substrate, markedly delay or prevent the oxidation of the substrate (7). Antioxidants that fit in this definition include free radical scavengers, inactivators of peroxides, and other reactive oxygen species (ROS), chelators of metals, and quenchers of secondary lipid oxidation products that produce rancid odors (8). Antioxidants have also been

used in the health-related area because of their ability to protect the body against damage caused by ROS as well as reactive nitrogen species (RNS) and those of reactive chlorine species (RCS) (9).

Antioxidants can be broadly classified by their mechanism of action as primary antioxidants, which break the chain reaction of oxidation by hydrogen donation and generation of more stable radicals, and secondary antioxidants, which slow the oxidation rate by several mechanisms, including chelation of metals, regeneration of primary antioxidants, decomposition of hydroperoxides, and scavenging of oxygen, among others. These substances may occur naturally in foods, such as tocopherols and ascorbic acid; however, natural antioxidants are often, at least partially, lost during processing or storage, thus exogenous antioxidants are intentionally added to products or their precursors participate in the formation of antioxidants during processing. Although there are many of compounds that have been proposed to inhibit oxidative deterioration processes, only a few can be used in food products (10). Antioxidants for use in food processing must be inexpensive, nontoxic, effective at low concentrations (0.001–0.02%), capable of surviving processing (carry-through), stable in the finished products, and devoid of undesirable color, flavor, and odor effects. In general, the selection of antioxidants depends on products, compatibility, and regulatory guidelines (11). In this chapter, the properties and applications of antioxidants in foods as well as their regulatory status are discussed.

## 2. SYNTHETIC ANTIOXIDANTS

Although the use of antioxidants dates back to ancient times when herbs and spices were used in food preservation, modern antioxidant technology is only about 60 years old. Since free radicals were found to be responsible for lipid oxidation, hundreds of natural and synthetic compounds have been evaluated for their efficacy as radical scavengers or for their other inhibitory effects. Among them, only four synthetic antioxidants are widely used in foods; namely, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ) (7, 12). Scientists are attempting to develop novel synthetic antioxidants aimed at retarding the effects of free-radical-induced damage in various food products as well as in the human body cells (13). Synthetic antioxidants used in the food industry can be added as direct additives or indirectly through diffusion from packaging material (6).

All antioxidants have points of strengths and weaknesses. Therefore, certain points, such as thermal stability, effective concentration, and synergism, should be taken into consideration when selecting antioxidants for use in particular foods. Regulatory status is another factor that cannot be ignored, especially for some antioxidants that have been reported to show potential adverse health effects. Synthetic antioxidants have been tested for safety and approval for use in food at low concentrations on the basis of complex toxicity studies (10). Allowable limits for use of antioxidants vary greatly from country to country, and depend on the food product under consideration (11).

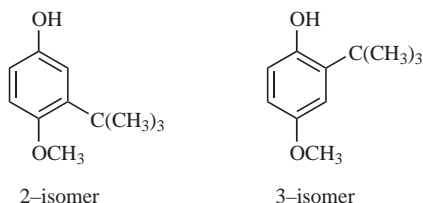


Figure 1. Chemical structures of BHA molecules.

## 2.1. BHA (Butylated Hydroxyanisole) and BHT (Butylated Hydroxytoluene)

Phenolic and polyphenolic compounds are the most active dietary antioxidants (14). The structural variation of phenolic antioxidants directly influences their physical properties, resulting in differences in their antioxidant activity. BHA and BHT are examples of phenols, in which the aromatic ring contains alkyl groups (hindered phenols), which are extremely effective as antioxidants (11).

Chemically, BHA is a mixture of two isomers (2-*tert*-butyl-4-hydroxyanisole and 3-*tert*-butyl-4-hydroxyanisole) (Figure 1). The 3-isomer is generally considered to be a better antioxidant, and accounts for 90% of the commercial BHA (12). BHA is a white, waxy solid that is sold in the form of flake or tablet. It is a highly fat-soluble monophenolic antioxidant that is extensively used in bulk oils as well as oil-in-water emulsions (10–12). It is effective in animal fats and relatively ineffective in vegetable oils. Demonstrating considerable effectiveness in controlling the oxidation of short-chain fatty acids, BHA is frequently used for the preservation of coconut and palm kernel oil in cereal and confectionery products (10). BHA is good in baking because of its stability to heat and its mild alkaline conditions, although its application in frying is limited due to its volatility (11). However, it can be added to packaging materials to provide protection to food products inside the package through volatilization (12). BHA is particularly useful in protecting the odor and flavor of essential oils (10). Furthermore, BHA has been reported to possess antimicrobial activity (15–17) and is known to act synergistically with other antioxidants such as BHT.

BHT (3,5-di-*tert*-butyl-4-hydroxytoluene) (Figure 2) is a white crystalline solid with properties similar to BHA (12). It is appropriate for thermal treatment but not as stable as BHA (11). Being able to regenerate BHA, BHT is commonly used in

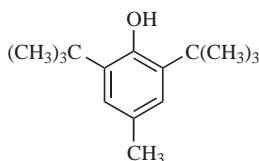


Figure 2. Chemical structure of BHT.

combination with BHA to provide greater antioxidant activity (18). BHT does not have an optimum concentration; usually, BHA/BHT mixtures are added to foods at levels of up to 0.02% (10, 11). Both BHA and BHT have a slight phenolic odor, and may impart undesirable odor in foods when used at high temperature for an extended period of time (10, 11).

Although synthetic antioxidants have widely been used in the food industry, there are some arguments about their safety (19). The use of BHA and BHT in foods has been decreased due to their potential action as promoters of carcinogenesis (20). In addition to the carcinogenicity of BHA in the forestomach of rodents, BHA and BHT have been reported to be cytotoxic (21–23). Furthermore, a suggestion has been made that BHT be withdrawn from use in all foods because of its possible adverse effects on the kidney and liver as well as lung tissues of rat (24, 25). However, some scientists have noted that the metabolism of BHT in rat and man are too widely different to allow a proper hazard assessment of BHT in humans (26). It is generally considered that permitted food antioxidants, such as BHA and BHT, have a considerable safety margin; for instance, the dose for enhancement of carcinogenesis is at least 1500-fold greater than that in human exposure (27, 28). Meanwhile, BHA and BHT have been reported by some researchers to pose no cancer hazard to humans and, on the contrary, have health benefits related to their anticarcinogenic and antimutagenic properties as well as inhibition of cholesterol oxidation (29–32).

Despite positive and negative reports of these synthetic antioxidants on human health, their use is subject to regulation, in the United States, under the Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA); in Canada, the Food and Drug Regulations (National Health and Welfare); in Europe, the European Economic Community (EEC); and in Japan, the Food Sanitation Law. Many other countries have adopted regulations similar to those used in the United States, with significant differences existing both in the antioxidants approved and in their application and level of usage (10, 11). According to the existing food additive regulations published by the FDA, BHA and BHT are lawful for use individually or in combination at a maximum level of 0.02%, or 200 ppm, based on the lipid content of food products, as specified by the Code of Federal Regulations (CFR) (6, 7, 12). Although BHA and BHT are effective at low concentrations, they become prooxidant at high levels in foods (11, 33). As specified in 21CFR, 172.100, and 172.115, limitations for BHA and BHT, alone or in combination for specific products, are as follows: 10 ppm in potato granules; 50 ppm in dehydrated potato shreds, dry breakfast cereals, potato flakes, and sweet potato flakes; and 200 ppm in emulsion stabilizers for shortenings (11). BHA and BHT are not allowed in fish products (5). The summary of regulations, applications, and properties of BHA and BHT are shown in Tables 1 and 2.

The daily dietary intakes of BHA and BHT have been estimated in many countries. The daily intakes of BHA and BHT in Japan in 1998 were 0.119 and 0.109 mg/d/person, which reflect 0.5% and 0.7% of the acceptable daily intake (ADI), respectively (35). The estimates of theoretical maximum daily intake (TMDI) of BHA and BHT in Brazil published in 2001 were in the range of

TABLE 1. Properties, Applications, and Regulations of BHA.

<b>NAME:</b>	<b>Butylated hydroxyanisole (BHA)</b>
<b>CATEGORY:</b>	Antioxidant
<b>FOOD USE:</b>	Bakery products/Meat products/Spices/Cereals/Dehydrated mashed potatoes/Beverage mixes/Dessert mixes/Nuts/Vitamins/Yeast/Vegetable oils/Animal fats/Processed cheeses/Margarine/Essential oils/Chewing gum base
<b>SYNONYMS:</b>	Mixture of two isomers: 3- <i>tertiary</i> butyl-4-hydroxyanisole and 2- <i>tertiary</i> butyl-4-hydroxyanisole/(1,1-dimethylethyl)-4 methoxyphenol/E320/ Antracine 12/Embanox/Nipantiox/Sustane BHA/ Sustane 1-F/Tenox 4B/ Tenox 5B
<b>FORMULA:</b>	$(\text{CH}_3)_3\text{CC}_6\text{H}_3\text{OCH}_3\text{OH}$
<b>MOLECULAR MASS:</b>	180.25
<b>PROPERTIES AND APPEARANCE:</b>	White waxy flakes or tablets
<b>MELTING RANGE IN °C:</b>	48–55
<b>FLASH POINT IN °C:</b>	130
<b>PURITY %:</b>	Not less than 98.5 of 2-isomer and not less than 85 of 3-isomer
<b>SOLUBILITY % AT VARIOUS TEMPERATURE/pH COMBINATIONS:</b>	
in water:	at 20°C Insoluble
in vegetable oil:	at 25°C 30% cottonseed oil 40% coconut, corn, peanut oils 50% soybean oil
in ethanol solution:	100% at 25°C >25%
in propylene glycol:	at 20°C 70%
<b>FUNCTION IN FOODS:</b>	Antioxidant preservative by terminating free radicals formed during autoxidation of unsaturated lipids. It also possesses antimicrobial activity as a phenolic compound.
<b>ALTERNATIVES:</b>	BHT; PG; TBHQ
<b>SYNERGISTS:</b>	BHT; propyl gallate; methionine; lecithin; thiodipropionic acid; citric acid; phosphoric acid
<b>FOOD SAFETY ISSUES:</b>	This antioxidant has not been subjected to great criticism of safety. However, suspected for tumor formation in animals with forestomach.
<b>LEGISLATION:</b>	<b>USA:</b> Maximum usage level approved for general use; FDA 0.02% and USDA 0.01% of weight of fat. Special applications include: Chewing gum base: 0.01% by weight of chewing gum base Active dry yeast or dry material Emulsion stabilizer: 0.02% by weight of emulsion, shortenings, stabilizer Potato flakes, sweet potato flakes: 0.005% by weight, dry breakfast cereal, of food material, packaging material Potato granules: 0.001% by weight of potato granules Dry mixes for beverages: 0.009% of material and desserts Beverages and desserts, prepared from dry mixes: 0.0002% Dry diced glazed fruits: 0.0032% Flavor substances: 0.5% of essential oil content <b>U.K. and EUROPE:</b> approved <b>CANADA:</b> approved <b>AUSTRALIA/PACIFIC RIM and JAPAN:</b> approved

TABLE 2. Properties, Applications, and Regulations of BHT.

<b>NAME:</b>	<b>Butylated hydroxytoluene (BHT)</b>	
<b>CATEGORY:</b>	Antioxidant	
<b>FOOD USE:</b>	Breakfast cereals/Baked goods/Potato chips/Vegetable oils/Snack foods/Butter/Margarine/Frozen seafoods/Chewing gum base	
<b>SYNONYMS:</b>	2,6- <i>bis</i> (1,1-dimethylethyl)-4-methylphenol/2,6-di- <i>tert</i> -butyl- <i>p</i> -cresol/2,6-di- <i>tert</i> -butyl-4-methylphenol/E321/Antracine 8/Ionol CP/Dalpac/Impruvol/Vianol/Tenox BHT/Tenox 8/Sustane BHT	
<b>FORMULA:</b>	[(CH <sub>3</sub> ) <sub>3</sub> C] <sub>2</sub> C <sub>6</sub> H <sub>2</sub> CH <sub>3</sub> OH	
<b>MOLECULAR MASS:</b>	220.34	
<b>PROPERTIES AND APPEARANCE:</b>	White granular crystals with slight odor	
<b>MELTING RANGE IN °C:</b>	69–72	
<b>FLASH POINT IN °C:</b>	118	
<b>PURITY %:</b>	Not less than 99	
<b>SOLUBILITY % AT VARIOUS TEMPERATURE/pH COMBINATIONS:</b>		
<b>in water:</b>	<b>at 20°C</b>	Insoluble
<b>in vegetable oil:</b>	<b>at 25 °C</b>	30% cottonseed, coconut, corn, peanut and soybean oils
<b>in ethanol solution:</b>	<b>100% at 25°C</b>	25%
<b>in propylene glycol:</b>	<b>at 20°C</b>	Insoluble
<b>FUNCTION IN FOODS:</b>	Antioxidant preservative; prevents oxidative rancidity development in oil-containing foods by terminating free radicals formed during autoxidation of unsaturated lipids. It possesses antimicrobial activity as a phenolic compound.	
<b>ALTERNATIVES:</b>	BHA; PG; TBHQ	
<b>SYNERGISTS:</b>	BHA	
<b>FOOD SAFETY ISSUES:</b>	This antioxidant has not been subjected to great criticism over safety.	
<b>LEGISLATION:</b>	<p><b>USA:</b> Maximum usage level approved for general use; FDA 0.02% and USDA 0.01% of weight of fat. Special applications include:</p> <p>Enriched rice: 0.0033%</p> <p>Nonalcoholic beverages, frozen raw breaded shrimp, mixed nuts and margarine: 0.02% based on oil content</p> <p>Dry sausage: 0.003%</p> <p>Fresh pork sausage, brown-and-serve sausage, pre-grilled beef patties, pizza toppings, meatballs, dried meats: 0.01%</p> <p>Rendered animal fat or combination with vegetable fat, poultry fat or various poultry products: 0.01%</p> <p>Dry breakfast cereals: 0.005%</p> <p>Emulsion stabilized for shortening: 0.02%</p> <p>Potato granules: 0.001%</p> <p>Potato flakes, sweet potato flakes, dehydrated potato shreds: 0.005%</p> <p><b>U.K. and EUROPE:</b> approved</p> <p><b>CANADA:</b> approved</p> <p><b>AUSTRALIA/PACIFIC RIM and JAPAN:</b> approved</p>	

0.09–0.15 and 0.05–0.10 mg/kg body weight, respectively, indicating that it is unlikely to exceed the ADI (0.5 and 0.3 mg/kg body weight) (36). In the Netherlands, the mean intake of BHA and BHT in 2000 was 105 and 351  $\mu\text{g}/\text{day}$  (37). In Italy, the likelihood of exceeding the ADI for BHA was very low. However, the TMDI of BHT was above the ADI. The three food categories, “pastry, cake, biscuits,” “chewing gums,” and “vegetable oils and margarine,” were the major sources of BHT and contributed 74% of the TMDI (38).

## 2.2. TBHQ (*tert*-Butylhydroquinone) and Gallates

TBHQ (Figure 3) is a beige powder or is a white-to-tan crystal that is used frequently in frying applications with highly unsaturated vegetable oils. Its solubility in different solvents declines in the order of alcohol > fats > water. As a diphenolic antioxidant, TBHQ is more effective in vegetable oils than BHA and BHT. It is stable to heat and is regarded as the most effective antioxidant in preventing the oxidation of frying oils and an alternative or supplement to oil hydrogenation for increasing oxidative stability (10, 11). TBHQ shows excellent synergism with other antioxidants such as citric acid. A ternary mixture containing TBHQ, monoacylglycerol citrate (MGC), and ascorbyl palmitate (AP) exhibited the highest thermal stability and provides optimum protection for oil during high-temperature processing (39). TBHQ mixed with BHA and BHT can increase the smoke point of fats and oils (40).

Three esters of gallic acid are approved for use in foods, namely propyl gallate (PG), octyl gallate, and dodecyl gallate (Figure 4). PG is a white crystalline powder that is slightly soluble in both water and fat, whereas the higher octyl and dodecyl gallate are practically insoluble in water but dissolve easily in fats and oils (10).

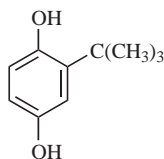
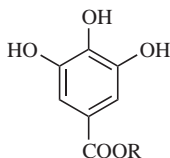


Figure 3. Chemical structure of TBHQ.



- |                                   |                 |
|-----------------------------------|-----------------|
| R = C <sub>3</sub> H <sub>7</sub> | Propyl Gallate  |
| = C <sub>8</sub> H <sub>17</sub>  | Octyl Gallate   |
| = C <sub>12</sub> H <sub>25</sub> | Dodecyl Gallate |

Figure 4. Chemical structures of different alkyl gallates.

Therefore, PG is widely used in foods where lipid-soluble antioxidants such as BHA, BHT, and TBHQ are not suitable. PG is inappropriate for frying due to its poor stability at high temperatures. It decomposes at its melting point of 148°C (11, 12). Gallates can form undesirable, dark-colored complexes with iron and copper; thus, they are sold as a mixture with metal chelators such as EDTA. Gallates also act synergistically with other antioxidants (11, 12).

As synthetic antioxidants, the safety of TBHQ and gallates has been questioned and, similar to BHA and BHT, both drawbacks and benefits of TBHQ and gallates have been reported. According to the literature, TBHQ exhibited a nontypical mode of cell death and proved cytotoxic toward human monocytic leukaemia cells (41). It caused apoptosis and significantly promoted DNA damage (42–44). PG suppressed humoral immunity (45). The coadministration of TBHQ or PG with sodium nitrite promoted forestomach carcinogenesis (46). Meanwhile, TBHQ and PG have been reported to be beneficial to human health. TBHQ was reported to be an effective inhibitor of cholesterol oxidation (32, 47), and PG provided inhibition of foodborne pathogens (48). Anticarcinogenic and antimutagenic activities of TBHQ and PG have also been reported (29, 49).

In accordance with regulations concerning the use of antioxidants in foods, TBHQ is permitted for food use by the FDA and the USDA at less than 0.02% and 0.01%, respectively. At levels higher than 0.02%, TBHQ may exert a pro-oxidant effect. The CFR specifies the maximum addition of TBHQ as 0.02% (200 ppm) (6, 12). However, the combination of TBHQ and PG is illegal (12). In Japan and European countries, addition of TBHQ in foods is not allowed (7, 11), although its use in Canada is quite recent and dates back to 1999. PG is the only gallate permitted in foods in the United States and Canada, but the use of higher alkyl gallates is approved in several European countries (10). Gallates have optimum concentrations for antioxidant activity and may act as pro-oxidants when used at high levels (10). Tables 3 and 4 show detailed information on the regulatory status of TBHQ and PG in various applications, respectively. With respect to daily intake, according to investigations in Brazil and Italy, the estimate of TMDI for TBHQ and gallates was very low and unlikely to exceed the ADI (36, 38).

### 2.3. Erythorbic Acid and Ascorbyl Palmitate

Erythorbic acid (or D-ascorbic acid) (Figure 5) is a white or slightly yellow crystalline powder that is often used in fruits and cured meats to enhance curing action and to stabilize the color of food products (34). Unlike its isomer L-ascorbic acid, erythorbic acid is not a natural constituent of foods and has minimal Vitamin C activity (11).

Ascorbyl palmitate (Figure 6), a synthetic derivative of ascorbic acid, is a white powder with a soapy taste and citrus-like odor (34). It has better lipid-solubility compared with that of ascorbic acid and its salts, and is often used in combination with  $\alpha$ -tocopherol in lipid-containing foods (11). Ascorbyl palmitate prevents oxidative rancidity by quenching singlet oxygen, among other modes of action (34).



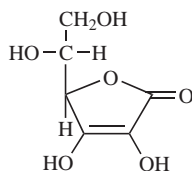
TABLE 3. Properties, Applications, and Regulations of TBHQ.

<b>NAME:</b>	<b><i>Tert</i>-butylhydroquinone (TBHQ)</b>
<b>CATEGORY:</b>	Antioxidant
<b>FOOD USE:</b>	Dry cereals/Edible fats/Margarine/Pizza toppings/Potato chips/Poultry/Dried meats/Sausages/Beef patties/Vegetable oils
<b>SYNONYMS:</b>	2-(1,1-dimethylethyl)-1,4-benzenediol/mono- <i>t</i> -butyl hydroquinone/ Sustane TBHQ/Tenox TBHQ
<b>FORMULA:</b>	$(\text{CH}_3)_3\text{CC}_6\text{H}_3(\text{OH})_2$
<b>MOLECULAR MASS:</b>	295
<b>PROPERTIES AND APPEARANCE:</b>	White to tan color solid crystals, having a characteristic odor
<b>MELTING RANGE IN °C:</b>	126.5–128.5
<b>FLASH POINT IN °C:</b>	171
<b>PURITY %:</b>	99
<b>SOLUBILITY % AT VARIOUS TEMPERATURE/pH COMBINATIONS:</b>	
in water:	at 20°C <1% at 100°C 5%
in vegetable oil:	at 20°C 10% in corn, cottonseed, and soybean oils
in ethanol solution:	100% 25%
in propylene glycol:	at 20°C 30%
<b>FUNCTION IN FOODS:</b>	Prevents oxidative rancidity development in foods by terminating free radicals formation
<b>ALTERNATIVES:</b>	BHA; BHT
<b>SYNERGISTS:</b>	BHA; citric acid
<b>FOOD SAFETY ISSUES:</b>	Has shown mutagenicity <i>in vivo</i> ; therefore, some countries consider that TBHQ does not meet current standards of toxicity testing.
<b>LEGISLATION:</b>	<b>USA:</b> Not allowed to use in combination with PG. For general usage, FDA-0.02%, USDA-0.01%, based on lipid content of food. Special food use: Nonalcoholic beverages Margarine, mixed nuts: 0.02% alone or in combination based on lipid content Dried meats Fresh pork or beef sausages Pre-grilled beef patties Pizza toppings Meatballs: 0.01% based on weight of finished product Rendered animal fats <b>EUROPE, U.K., NORWAY, DENMARK, SWEDEN, SWITZERLAND:</b> not allowed for food use <b>CANADA:</b> allowed for food use <b>AUSTRALIA/PACIFIC RIM:</b> <b>AUSTRALIA, NEW ZEALAND:</b> allowed for food use <b>JAPAN:</b> not allowed for food use

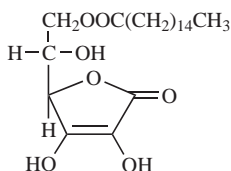
Adapted from (34).

TABLE 4. Properties, Applications, and Regulations of PG.

<b>NAME:</b>	<b>Propyl gallate (PG)</b>
<b>CATEGORY:</b>	Antioxidant
<b>FOOD USE:</b>	Chewing gum base/Nonalcoholic beverages/Margarine/Mixed nuts/Fresh or dry sausages/Pre-grilled beef patties/Rendered animal fat/Pizza toppings and meatball
<b>SYNONYMS:</b>	<i>n</i> -propyl-3,4,5-trihydroxybenzoate/3,4,5-trihydroxybenzoic acid/Gallic acid, propyl ester/E310/Nipa 49/Nipagallin P/Tenox PG/Sustane PG
<b>FORMULA:</b>	$(\text{HO})_3\text{C}_6\text{H}_2\text{COOCH}_2\text{CH}_2\text{CH}_3$
<b>MOLECULAR MASS:</b>	212.20
<b>PROPERTIES AND APPEARANCE:</b>	White crystalline powder with slight odor
<b>MELTING RANGE IN °C:</b>	146–150
<b>FLASH POINT IN °C:</b>	187
<b>PURITY %:</b>	Not less than 98 and not more than 102.5 on the dried basis
<b>SOLUBILITY % AT VARIOUS TEMPERATURE/pH COMBINATIONS:</b>	
in water:	at 20°C <1%
in vegetable oil:	at 20°C 1% in cottonseed oil 2% in soybean oil insoluble in corn oil
in ethanol solution:	100% at 25°C >60%
<b>FUNCTION IN FOODS:</b>	Prevents oxidation rancidity development in lipid-containing foods by terminating free radicals formation during autooxidation of unsaturated lipids. BHA; BHT; TBHQ; octyl gallate; dodecyl gallate BHA; BHT
<b>ALTERNATIVES:</b>	
<b>SYNERGISTS:</b>	
<b>FOOD SAFETY ISSUES:</b>	Not subjected to great criticism over safety.
<b>LEGISLATION:</b>	<b>USA:</b> not allowed to use in combination with TBHQ. For general usage, FDA- 0.02% and USDA – 0.01% alone or in combination with BHT or BHA by weight of lipid proportion of food. Special applications include: Chewing gum base: 0.1% Nonalcoholic beverages: 0.1% Margarine: 0.1% Mixed nuts: 0.02% based on oil content French beef or pork sausages Brown-and-serve sausages Pre-grilled beef patties Pizza toppings and meatballs: for all these 0.01% based on weight of finished product Rendered animal fats or combination of such fats with vegetable fat: 0.01% based on lipid content <b>U.K.:</b> approved <b>EUROPE:</b> listed <b>CANADA:</b> approved <b>AUSTRALIA/PACIFIC RIM and JAPAN:</b> approved



**Figure 5.** Chemical structure of erythorbic acid.



**Figure 6.** Chemical structure of ascorbyl palmitate.

More recently, replacement of the palmitate moiety with oleate has been proposed to increase solubility as well as antioxidant activity in oils (50).

Both erythorbic acid and ascorbyl palmitate have “Generally Recognized as Safe (GRAS)” status with the FDA (11). No restrictions on their usage levels are imposed except for the maximum addition of 0.02% for ascorbyl palmitate in margarine (34).

Table 5 shows the maximum levels permitted by the FDA for the four major synthetic antioxidants (BHA, BHT, PG, and TBHQ) in specific applications (51). The regulatory status for these antioxidants in the USA, Canada, and Europe is given in Table 6; Table 7 summarizes their status in other countries for which a listing could be found. In addition to the major synthetic antioxidants discussed above (BHA, BHT, TBHQ, gallates, erythorbic acid, and ascorbyl palmitate), several other

**TABLE 5. Maximum Levels Permitted for Antioxidants in Specific Applications.**

Food Type	Maximum Permitted Levels (ppm)			
	BHA	BHT	PG	TBHQ
Active dry yeast	1000	—	—	—
Beverages from dry mixes	2	—	—	—
Dehydrated potato shreds	50	50	—	—
Dried meat	100	100	100	100
Dry breakfast cereals	50	50	—	—
Dry diced fruits	32	—	—	—
Dry mixed for beverages and desserts	50	—	—	—
Dry sausage	30	30	30	30
Emulsion stabilizers for shortenings	200	200	—	—
Fresh sausage	100	100	100	100
Potato flakes	50	50	—	—
Poultry products	100	100	100	100

Adapted from (51).

**TABLE 6. Regulatory Responsibility for Major Antioxidants.**

	U.S. FDA	USDA	Canada (NHW)	Europe (EEC)
BHA	21 CFR 182.3169	9 CFR 318.7	Table XI, Part IV, B.1, 320	E320
BHT	21 CFR 182.3173	9 CFR 318.7	Table XI, Part IV, B.2, 321	E321
Gallates	21 CFR 184.1660	9 CFR 381.147	Table XI, Part IV, P.1, 324	E310-312
TBHQ	21 CFR 172.185	9 CFR 361.147	Table XI, Part IV, T.1A, 325	Not approved
Tocopherols	21 CFR 182.3890	9 CFR 318.147	Table XI, Part IV, T.2, 325	E306-309

Adapted from (10).

**TABLE 7. Regulatory Approval Status of Major Antioxidants in Different Countries.**

Country	Antioxidants			
	BHA	BHT	Gallates	TBHQ
Afghanistan	+	+	-	-
Argentina	+	+	-	+
Australia	+	+	+	+
Austria	+	-	-	-
Bahrain	+	+	+	+
Barbados	-	-	-	+
Belgium	+	+	-	-
Brazil	+	+	+	+
Chile	+	+	+	+
China	+	+	+	-
Columbia	+	+	+	+
Cyprus	+	+	-	-
Denmark	+	+	+	-
Ecuador	+	+	-	-
Finland	+	+	+	-
France	+	+	+	-
Germany	+	+	+	-
Gibraltar	+	+	-	-
Greece	-	-	+	-
Hong Kong	+	+	-	-
Hungary	+	+	-	-
Indonesia	+	-	-	-
Iran	+	+	+	+
Ireland	+	+	+	-
Israel	+	+	-	+
Italy	+	+	+	-
Jamaica	-	-	-	-
Japan	+	+	+	-
Kenya	+	-	+	-
Korea, South	+	+	+	+
Luxembourg	+	+	+	-
Malaysia	+	+	+	+
Malta	+	+	-	+
Mauritius	+	+	+	-
Mexico	+	+	+	+
Morocco	-	-	-	+

TABLE 7. (Continued)

Netherlands	+	+	+	-
New Zealand	+	+	+	+
Nigeria	+	+	-	-
Norway	+	+	+	-
Pakistan	+	+	-	-
Panama	+	+	+	+
Papua New Guinea	+	-	-	-
Peru	+	+	-	-
Philippines	+	+	+	+
Portugal	+	+	-	-
Saudi Arabia	+	+	+	+
Singapore	+	+	+	-
South Africa	+	+	+	+
Spain	+	-	-	-
Sweden	+	+	+	-
Switzerland	+	+	+	-
Taiwan	+	+	+	+
Thailand	+	+	+	+
Trinidad/Tobago	+	+	+	-
Turkey	-	-	+	+
United Kingdom	+	+	+	-
Uruguay	+	+	-	-
Venezuela	+	+	+	+
Zimbabwe	+	-	-	-

Adapted from (10).

synthetic antioxidants have been used less frequently in the food and feed industry. These include ethoxyquin, trihydroxybutyrophenone (THBP), and some secondary antioxidants such as thiodipropionic acid and dilauryl thiodipropionate (10, 11). Novel synthetic antioxidants have been created in order to obtain stronger antioxidant activity than that of traditional ones (52). However, the general consumer rejection of synthetic food additives has led to a decrease in their use and an increased interest in their replacement with natural ingredients.

### 3. NATURAL ANTIOXIDANTS

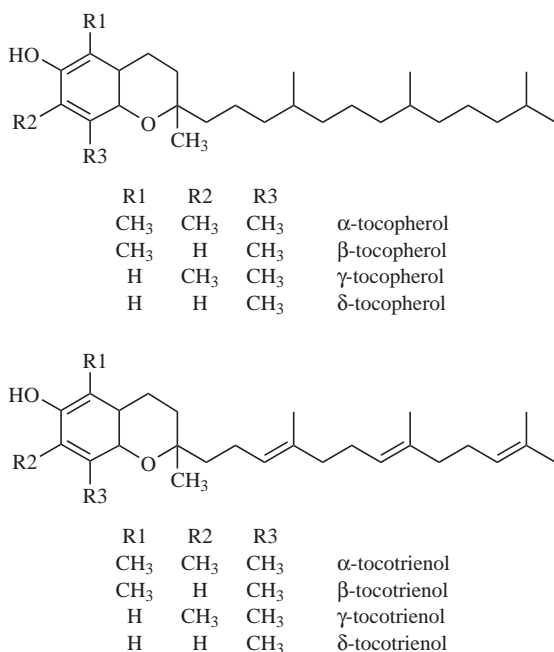
Concerns about the safety of synthetic antioxidants have given rise to a large body of research on natural sources of antioxidants. Natural antioxidants allow food processors to produce stable products with "clean" labels of all-natural ingredients, as described by Reiche (11). They bring less rigorous burden-of-safety proof than that required for synthetic products (11). In addition to their antioxidant activity, some natural antioxidants, such as vitamins, minerals, and enzymes, are also regarded as nutrients due to their bioactivity. However, natural antioxidants may possess several drawbacks, including high usage levels, low antioxidant efficiency, undesirable flavor or odor, and possible loss during processing (11). The safety of natural

antioxidants cannot be taken for granted because of their potential mutagenicity, carcinogenicity, teratogenicity, or other pathogenic activities (10). A case in point is nordihydroguaiaretic acid (NDGA), which was removed from the GRAS list and is no longer of practical use (11, 19). NDGA is a natural constituent of creosote bush.

Ascorbic acid and tocopherols are the most important commercial natural antioxidants. In addition, many naturally occurring phenolic antioxidants have been identified in plant sources and vegetable extracts that may lend themselves for use in a variety of food applications (53). Recent research has focused on isolation and identification of effective antioxidants of natural origin (11).

### 3.1. Tocopherols and Tocotrienols

Tocopherols and tocotrienols, collectively known as tocopherols, are monophenolic and lipophilic compounds that are widely distributed in plant tissues (7). The main commercial source of natural tocopherols is the soybean oil. Tocotrienols, less common than tocopherols, are present in palm oil, rice bran oil, as well as cereals and legumes (11). Tocopherols and tocotrienols are classified into  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -, depending on their chemical structures (Figure 7). In general, tocotrienols have a stronger antioxidant effect on lipid oxidation than tocopherols. The antioxidant activity of tocopherols is dependent on temperature and is in the order of  $\delta$ - >  $\gamma$ - >  $\beta$ - >  $\alpha$ -tocopherol (7). Tocopherols (mixed natural concentrate) are a golden



**Figure 7.** Chemical structure of tocopherols and tocotrienols.

brown colored, slightly viscous liquid with a characteristic odor. However, synthetic tocopherol (mixed  $\alpha$ -,  $\gamma$ -, and  $\delta$ -) is a yellow to brownish viscous oily and odorless liquid (34). Tocols are soluble in vegetable oils but insoluble in water. They function as a free radical terminator in autoxidation reactions, and they are often used in food products deficient in natural antioxidants, such as animal fats, waxes, and butterfat, among others (34, 54). Tocopherols act synergistically with ascorbic acid, citric acid, and phospholipids.

As natural antioxidants, tocopherols have GRAS status, and they are regarded as safe food additives. However, as noted earlier for other antioxidants, excessive addition of tocopherols may lead to pro-oxidant effects (6, 11). Furthermore, the hemorrhagic toxicity of a large dose of  $\alpha$ -tocopherol has been reported (55). Therefore, use of tocopherols as antioxidants in foods is subject to regulations. The FDA (21CFR 182.3890) and the USDA (9CFR 318.147), Canada NHW (Table XI, Part IV, T.2, 325), and the EEC (E306-309) govern the regulations of tocopherols in foods in the United States, Canada, and European countries, respectively (10). In the United States, natural tocopherols are limited to 0.03%, i.e., 300 ppm in animal fats, and 0.02% in combination with BHA, BHT, and PG (9CFR 318.7). In the UK and some other European countries, their maximum addition is not to exceed 500 ppm (10, 34). With respect to ADI of  $\alpha$ -tocopherol, it has been reported that an intake of 1000 mg/day is without risk, and 3200 mg/day is without any consistent risk (56). Actually, in the United States, the majority of men and women fail to meet the current recommendation for Vitamin E intake, according to a recent report on American diets (57).

### 3.2. Ascorbic Acid and Ascorbate Salts

L-ascorbic acid (Vitamin C) and its salts (sodium ascorbate and calcium ascorbate) (Figure 8) are widespread in plant tissues or are produced synthetically in large quantities (11). Ascorbic acid is a white or slightly yellow crystalline powder that is extensively used to stabilize beverages, fruits, and vegetables. Its application in fats and oils, however, is limited because of the insolubility in lipids. It acts as an antioxidant with multiple functions, including quenching various forms of oxygen, reduction of free radicals, and regeneration of primary antioxidants (34). The effect of ascorbic acid on lipid stability in foods is mainly due to synergistic interactions with other antioxidant compounds (58). It shows excellent synergism with  $\alpha$ -tocopherol, citric acid, BHA, BHT, and metal chelators. Ascorbic acid strongly inhibits the depletion of  $\alpha$ -tocopherol by regenerating it (6, 59).

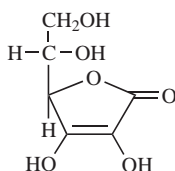


Figure 8. Chemical structure of ascorbic acid.

In addition to antioxidant activity, ascorbic acid also functions as Vitamin C, a flavorant, an acidulant, a color fixing, and a reducing agent in food products (11, 34). Moreover, it can diminish the generation of odor-active compounds in emulsions (60). However, the natural ascorbic acid in foods can be easily destroyed during processing as a result of susceptibility to heat, light, pH, oxygen, acrid smoke, and water activity; thus, it is often added to foods exogenously (61, 62).

Ascorbic acid and ascorbate salts have GRAS status with no usage limits. According to the literature, Vitamin C is safe at supplementation levels of up to 600 mg/day, and higher levels of up to 2000 mg/day are without risk (56). The ascorbic acid and its salts carry GRAS status and with minimal associated organoleptic problems; thus, they are safe, stable, and good antioxidant candidates for use in foods (63). As natural or natural-identical products, they are highly recognized as antioxidant nutrients by consumers (11).

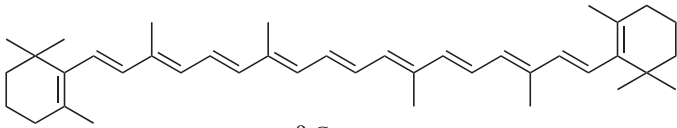
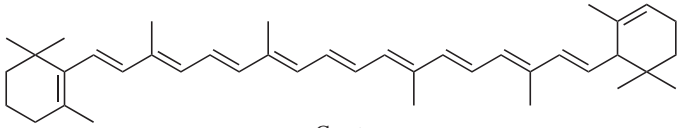
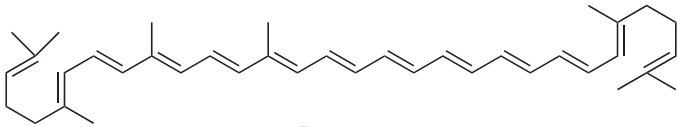
### 3.3. Carotenoids

Carotenoids are yellow, orange, and red lipid-soluble pigments that occur widely in plants, fruits, and vegetables. They are 40-carbon isoprenoids with varying structures (Figure 9), and can be classified as carotenes and xanthophylls (11). Certain carotenoids are also referred to as pro-vitamins such as  $\beta$ -carotene,  $\alpha$ -carotene, and  $\beta$ -cryptoxanthin. Carotenoids are antioxidant nutrients that act mainly as secondary antioxidants in foods by quenching singlet oxygen. They may also prevent oxidation by trapping free radicals in the absence of singlet oxygen (11). Carotenoids are a good synergist with tocopherols.  $\beta$ -Carotene, lutein, lycopene, and isozeaxanthin are typical carotenoids that effectively retard oxidation in foods. Astaxanthin has antioxidant activity that is ten times greater than that of  $\beta$ -caroten, lutein, zeaxanthin, and canthaxanthin, and is often used in fish products (64).

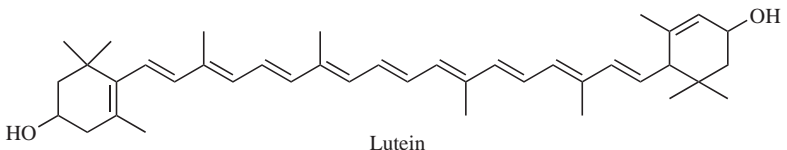
$\beta$ -Carotene is a purple hexagonal prism or a red leaflet that is often used in fruit juices, cheese, dairy products, fats, and oils (34). It has poor solubility in most common solvents, and is highly reactive and unstable to heat, light, pH, oxygen, and the presence of metals, resulting in limited applications as a food antioxidant (11). In a high-oxygen concentration,  $\beta$ -carotene may exhibit a pro-oxidant, rather than an antioxidant effect in food products (61). Carotenoids are natural constituents of foods and have GRAS status. No limitation on their addition level has been stipulated.

In addition to the three major classes of natural antioxidants (tocols, ascorbic acids, and carotenoids), several other natural substances have been identified that show antioxidant activity through different mechanisms; these include phospholipids, flavonoids, protein hydrolyzates, organic acids, sterols, Maillard reaction products, and enzymes. These are naturally occurring constituents of food and act as endogenous antioxidants that help prevent oxidation reactions. They are also regarded as a potential for replacement of synthetic antioxidants. A great deal of research has been conducted on evaluation of their antioxidant activity and methods of extraction. A variety of natural products can serve as sources of natural antioxidants, among which fruits and vegetables, spices and herbs, oilseeds, and animal

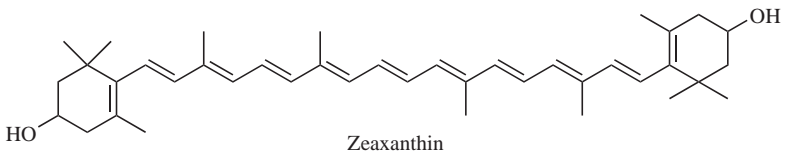


 $\beta$ -Carotene $\alpha$ -Carotene

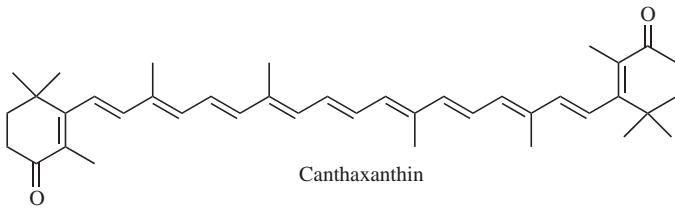
Lycopene



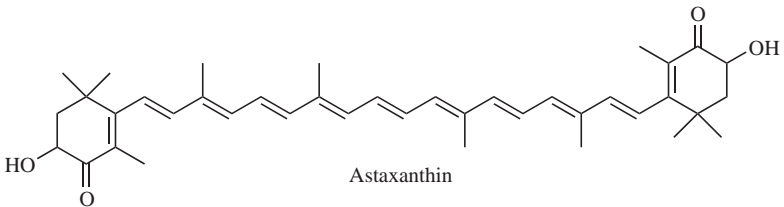
Lutein



Zeaxanthin



Canthaxanthin



Astaxanthin

**Figure 9.** Chemical structures of carotenoids.

and microbial products have been considered (64, 65). Grapes, berry fruits, and citrus are rich sources of antioxidants (65). Among vegetables, garlic, broccoli, mushroom, and pulses have been shown to possess antioxidant effects (65–67); spinach powder has been reported to be capable of improving lipid stability in deep-fat-fried products (68). Aside from fruits and vegetables, several studies have confirmed that many spice and herb extracts show strong antioxidant activity, such as rosemary, sage, oregano, cinnamon, thyme, green tea, and evening primrose extract (65, 69–73). Flaxseed, sunflower, soybean, cottonseed, rapeseed, and sesame seed typify the sources of antioxidants from oilseeds (64, 74, 75). More recently, Shahidi et al. (76) have reported the antioxidant activity of de-fatted Niger seed extract. Animal products can serve as good sources of natural antioxidants, such as protein hydrolyzates (peptides and amino acids), carotenoids, chitosan, and enzymes (64, 77, 78). Furthermore, microbial fermentation is becoming a promising method for producing natural antioxidants (64).

These antioxidants are all-natural ingredients of foods and have GRAS status. However, because of the ability of some natural antioxidants to exhibit pro-oxidant activity, caution should be exercised when adding them to food systems (79). Furthermore, the safety of natural compounds with antioxidant activity should be established.

#### 4. CONCLUSIONS

In foods that may undergo oxidation, antioxidants, endogenous or exogenous, function as an inhibitor to oxidation reactions through various mechanisms. Nevertheless, natural antioxidants are deficient in some foods and can easily deteriorate during processing or in storage, necessitating the use of synthetic antioxidants. However, most synthetic antioxidants are effective at low concentrations, and the addition of higher levels may lead to a pro-oxidant effect. Additionally, large doses of synthetic antioxidants have been reported to impart safety problems. Therefore, caution must be taken when selecting and adding antioxidants in food systems.

**TABLE 8. Antioxidants Conventionally Permitted in Foods.**

ascorbic acid, sodium, calcium salts	glycine
ascorbyl palmitate and stearate	gum guaiac
anoxomer	lecithin
butylated hydroxyanisole (BHA)	ionox-100
butylated hydroxytoluene (BHT)	polyphosphates
<i>tert</i> -butylhydroquinone (TBHQ)	propyl, octyl, and dodecyl gallates
citric acid, stearyl, and isopropyl esters	tartaric acid
erythorbic acid and sodium salt	trihydroxybutyrophenone
ethoxyquin	tocopherols
ethylenediaminetetraacetic acid (EDTA) and calcium disodium salt	thiodipropionic acid, dilauryl and distearyl esters

Adapted from (64).

**TABLE 9. ADI of Some Antioxidants Permitted in Foods.**

Antioxidant	ADI (mg/kg body weight)
propyl gallate	0–2.5
BHA	0–0.5
BHT	0–0.125
TBHQ	0–0.2
tocopherols	0.15–2.0
gum guaiac	0–2.5
ethoxyquin	0–0.06
phosphates	0–70.0
EDTA	2.5
tartaric acid	0–30
citric acid	not limited
lecithin	not limited
ascorbic acid	not limited
sulfites (as sulfur dioxide)	0–0.7
ascorbyl palmitate or ascorbyl stearate (or the sum of both)	0–1.25

Adapted from (64).

Meanwhile, the safety of natural antioxidants should not be taken for granted as antioxidants from natural sources are attracting more and more attention. Adherence to regulatory guidelines remains a necessity.

The most common antioxidants permitted for use in foods in most countries are shown in Table 8. Table 9 presents the ADI of some antioxidants allocated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The food producer has full responsibility for the choice of suitable antioxidants according to the corresponding guidelines governed by regulatory laws of the individual country or the international bodies that declare their safety (64).

## REFERENCES

1. K. R. Cadwallader and C. L. Howard, in C. Mussinan and M. Morello, eds., *Flavor Analysis: Developments in Isolation and Characterization*, ACS Symposium Series 705, American Chemical Society, Washington D.C., 1998, pp. 343–358.
2. E. H. Farmer, G. F. Bloomfield, A. Sundralingam, and D. A. Sutton, *Trans. Faraday Soc.*, **38**, 348–356 (1942).
3. J. L. Bolland and G. Gee, *Trans. Faraday Soc.*, **42**, 236–252 (1946).
4. L. Bateman, H. Hughes, and A. L. Morris, *Disc. Faraday Soc.*, **14**, 190–199 (1952).
5. W. W. Nawar, in O. R. Fennema, ed., *Food Chemistry*, Marcel Dekker, Inc., New York, 1996, p. 255–320.
6. M. van Aardt, S. E. Duncan, T. E. Long, S. F. O’Keefe, J. E. Marcy, and S. R. Sims, *J. Agric. Food Chem.*, **52**, 587–591 (2004).

7. F. Shahidi, *Nahrung.*, **44**, 158–163 (2000).
8. E. A. Decker, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry, Nutrition and Biotechnology*, Marcel Dekker, Inc., New York, 1998, p. 397.
9. F. Shahidi, in F. Shahidi, ed., *Natural Antioxidants: Chemistry, Health Effects and Applications*, AOCS Press, Champaign, Illinois, 1997, pp. 1–11.
10. D. E. Pratt, in Y. H. Hui, ed., *Bailey's Industrial Oil & Fat Products*, vol. 3, John Wiley & Sons, Inc., New York, 1996, pp. 524–545.
11. D. W. Reische, D. A. Lillard, and R. R. Eitenmiller, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry, Nutrition and Biotechnology*, Marcel Dekker, Inc., New York, 1998, pp. 423–448.
12. D. F. Buck and M. K. Edwards, *Food Tech. Int.*, 29–33 (1997).
13. H. H. Hussain, G. Babic, T. Durst, J. S. Wright, M. Flueraru, A. Chichirau, and L. L. Chepelev, *J. Org. Chem.*, **68**, 7023–7032 (2003).
14. F. Shahidi and M. Naczk, in *Food Phenolics: Sources, Chemistry Effects and Applications*, Technomic Publishing Co., Lancaster, Pennsylvania, 1995.
15. S. M. Razavi-Rohani and M. W. Griffiths, *J. Food Safety*, **16**, 59–74 (1996).
16. P. Giridhar and S. M. Reddy, *J. Food Sci. Tech. India*, **38**, 397–399 (2001).
17. B. J. O. Efiuvwevwere and E. Efi, *J. Sci. Food Agric.*, **79**, 1783–1787 (1999).
18. K. Omura, *J. Amer. Oil Chem. Soc.*, **72**, 1565–1570 (1995).
19. F. Shahidi and P. K. Wanasundara, *Crit. Rev. Food Sci. Nutr.*, **32**, 67–103 (1992).
20. R. S. Farag, G. S. El-Baroty, and A. Basuny, *Int. J. Food Sci. Nutr.*, **54**, 159–174 (2003).
21. H. Verhagen, P. A. E. L. Schilderman, and J. C. S. Kleinjans, *Chemico-Biological Interactions*, **80**, 109–134 (1991).
22. T. A. Sarafian, S. Kouyoumjian, D. Tashkin, and M. D. Roth, *Toxicol. Lett.*, **133**, 171–179 (2002).
23. M. Saito, H. Sakagami, and S. Fujisawa, *Anticancer Res.*, **23**, 4693–4701 (2003).
24. C. M. Siman and U. J. Eriksson, *Toxicol. Lett.*, **87**, 103–108 (1996).
25. R. S. Lanigan and T. A. Yamarik, *Int. J. Toxicol.*, **21**, 19–94 (2002).
26. H. Verhagen, H. H. G. Beckers, P. A. W. V. Comuth, L. M. Maas, F. Hoor, P. T. Henderson, and J. C. S. Kleinjans, *Food Chem. Toxicol.*, **27**, 765–772 (1989).
27. M. H. Lessof, *J. R. Soc. Med.*, **85**, 513–515 (1992).
28. J. Whysner, C. X. Wang, E. Zang, M. J. Iatropoulos, and G. M. Williams, *Food Chem. Toxicol.*, **32**, 215–222 (1994).
29. M. Hirose, S. Takhashi, K. Ogawa, M. Futakuchi, T. Shirai, M. Shibutani, C. Uneyama, K. Toyoda, and H. Iwata, *Cancer Lett.*, **143**, 173–178 (1999).
30. D. Slamenova, E. Horvathova, S. Robichova, L. Hrusovska, A. Gabelova, K. Keibl, J. Jakubikova, and J. Sedlak, *Environ. Mol. Mutagen.*, **41**, 28–36 (2003).
31. G. M. Williams, M. J. Iatropoulos, and J. Whysner, *Food Chem. Toxicol.*, **37**, 1027–1038 (1999).
32. A. Valenzuela, J. Sanhueza, and S. Nieto, *Biol. Res.*, **36**, 291–302 (2003).
33. B. Halliwell, M. A. Murcia, S. Chirico, and O. I. Aruoma, *Crit. Rev. Food Sci. Nutr.*, **35**, 7–20 (1995).
34. F. Shahidi, in J. Smish and L. Hong-Shum, eds., *Food Additive Databook*, Blackwell Science, Ltd., Oxford, U.K., 2003, p. 75.

35. H. Ishiwata, M. Nishijima, and Y. Fukasawa, *J. Food Hygienic Soc. Japan*, **44**, 132–143 (2003).
36. G. C. Maziero-C-Baunwart and M. C. F. Toledo, *Food Addit. Contam.*, **18**, 363–373 (2001).
37. A. A. Botterweck, H. Verhagen, R. A. Coldhohm, J. Kleijans, and P. A. Van-den-Brandt, *Food Chem. Toxicol.*, **38**, 599–605 (2000).
38. C. Leclercq, D. Arcella, and A. Turrini, *Food Chem. Toxicol.*, **38**, 1075–1084 (2000).
39. S. S. M. Allan and H. M. A. Mohamed, *J. Food Lipids*, **9**, 277–293 (2002).
40. G. C. Yen, C. H. Shao, C. J. Chen, and P. D. Duh, *Lebensmittel. Wissenschaft. und Technologie*, **30**, 648–652 (1997).
41. T. Okubo, Y. Yokoyama, K. Kano, and I. Kano, *Food Chem. Toxicol.*, **41**, 679–688 (2003).
42. A. N. Kong, P. Yu, W. Lei, S. Mandlekar, T. H. Tan, and D. S. Ucker, *Restor. Neurol. Neurosci.*, **12**, 63–70 (1998).
43. O. A. Badary, R. A. Taha, A. M. Gamal-el-Din, and M. H. Abdel-Wahab, *Drug Chem. Toxicol.*, **26**, 87–98 (2003).
44. Y. Li, Aseacat, P. Kuppusamy, J. L. Zweier, J. D. Yager, and M. A. Trush, *Mutat. Res.*, **518**, 123–133 (2002).
45. H. M. Kim, S. B. Han, W. I. Chang, B. H. Hyun, G. T. Oh, C. J. Ahn, and Y. N. Cha, *J. Toxicol. Sci.*, **21**, 41–45 (1996).
46. M. Miyauchi, H. Nakamura, F. Furukawa, H. Y. Son, A. Nishikawa, and M. Hirose, *Cancer Lett.*, **178**, 19–24 (2002).
47. A. Valenzuela, J. Sanhueza, and S. Nieto, *J. Amer. Oil Chem. Soc.*, **79**, 325–328 (2002).
48. T. C. King, I. W. Cheng, and M. G. Johnson, *Trends Food Sci. Technol.*, **9**, 168–175 (1998).
49. M. Hirose, T. Ito, S. Takahashi, M. Ozaki, T. Ogiso, Y. Nihro, T. Miki, and T. Shitai, *Eur. J. Cancer Prev.*, **7**, 233–241 (1998).
50. F. Viklund, J. Alander, and K. Hulk, *J. Amer. Oil Chem. Soc.*, **80**, 795–799 (2003).
51. N. S. Hettiarachchy and U. Kalapathy, in G. L. Christen and J. S. Smith, eds., *Food Chemistry: Principles and Applications*, Science Technology System, 2000, p. 266.
52. C. X. Zhang, H. Wu, and X. C. Weng, *Food Chem.*, **84**, 219–222 (2004).
53. F. Shahidi, *Lipid Tech.*, **12**, 80–84 (2000).
54. S. J. Byrd, *Cereal Foods World*, **46**, 49–53 (2001).
55. O. Takahashi, *Food Chem. Toxicol.*, **33**, 121–128 (1995).
56. A. T. Diplock, J. L. Charleus, G. Crozier-Willi, F. J. Kok, C. Rice-Evans, M. Roberfroid, W. Stahl, and J. Vina-Ribes, *Br. J. Nutr.*, **80**, 77–112 (1998).
57. J. E. Maras, O. I. Bermudez, N. Qiao, D. J. Bakun, E. L. Boody-Alter, and K. Tucker, *J. Am. Diet. Assoc.*, **104**, 567–575 (2004).
58. M. Makinen, M. Kahkonen, and A. Hopia, *Eur. J. Lipid Sci. Tech.*, **103**, 683–687 (2001).
59. K. Hiramoto, Y. Miura, G. Ohnuki, T. Kato, and K. Kikugawa, *J. Oleo Sci.*, **51**, 569–576 (2002).
60. S. M. van-Ruth, J. P. Roozen, M. A. Posthumus, and F. J. H. M. Jansen, *J. Amer. Oil Chem. Soc.*, **76**, 1375–1381 (1999).
61. J. Lee, N. Koo, and D. B. Min, *Comprehensive Rev. Food Sci. Food Safety*, **3**, 21–33 (2004).
62. S. T. Mayne, *J. Nutr.*, **133**, 933–940 (2003).

63. J. G. Elliott, *Food Technol.*, **53**(2), 46–48 (1999).
64. K. Mikova, in J. Pokorny, N. Yanishlieva, and M. Gordon, eds., *Antioxidants in Food: Practical Applications*, Woodhead Publishing, Ltd., Cambridge, England, 2001, pp. 267–283.
65. C. Kaur and H. C. Kapoor, *Int. J. Food Sci. Technol.*, **36**, 703–725 (2001).
66. F. Shahidi, U. D. Chavan, M. Naczki, and R. Amarowicz, *J. Agric. Food Chem.*, **49**, 926–933 (2001).
67. R. Amarowicz, M. Naczki, R. Zadernowski, and F. Shahidi, *J. Food Lipids*, **7**, 195–205 (2000).
68. J. Lee, S. Lee, H. Lee, K. Park, and E. Choe, *J. Agric. Food Chem.*, **50**, 5664–5669 (2002).
69. J. Mancini-Filho, A. Van-Koijj, D. A. Mancini, F. F. Cozzolino, and R. P. Torres, *Boll. Chim. Farm.*, **137**, 443–447 (1998).
70. F. Shahidi, *Lipid Tech.*, **12**, 80–84 (2000).
71. F. Shahidi, R. Amarowicz, Yuehua-He, and M. Wettasinghe, *J. Food Lipids*, **4**, 75–86 (1997).
72. M. Wettasinghe and F. Shahidi, *J. Agric. Food Chem.*, **47**, 1801–1812 (1999).
73. F. Shahidi and D. M. Alexander, *J. Food Lipids*, **5**, 125–133 (1998).
74. R. Amarowicz, B. Raab, and F. Shahidi, *J. Food Lipids*, **10**, 51–62 (2003).
75. F. Shahidi, R. Amarowicz, H. A. Abou-Gharbia, and A. A. Y. Shehata, *J. Amer. Oil Chem. Soc.*, **74**, 143–148 (1997).
76. F. Shahidi, C. Desilva, and R. Amarowicz, *J. Amer. Oil. Chem. Soc.*, **80**, 443–450 (2003).
77. F. Shahidi and R. Amorowicz, *J. Amer. Oil. Chem. Soc.*, **73**, 1197–1199 (1996).
78. F. Shahidi, J. Kamil, You-Jin-Jeon, and Se-Kwon-Kim, *J. Food Lipids*, **9**, 57–64 (2002).
79. H. T. Osborn and C. C. Akoh, *J. Amer. Oil Chem. Soc.*, **80**, 847–852 (2003).

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## *Toxicity and Safety of Fats and Oils*

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### **1. INTRODUCTION**

For the past half century, healthy eating concepts have evolved around avoiding fat. This is demonstrated in part from the USDA's dietary guideline handbook, which recommends that fats and oils be consumed "sparingly". At present, fat accounts for approximately 35% of the calories in a standard North American diet. With many campaigns directed at low-fat diets to protect consumer health and heart, a current situation exists where efforts to reduce total fat intake have resulted in a shift away from fat foods to high carbohydrate diets that, in turn, contain similar potential health-related concerns. For example, an increased awareness to the hazards of possible elevations in triacylglycerols attributed to a high carbohydrate intake has surfaced with syndrome- *X* profiles and increased risk to heart disease. It is vitally important to acknowledge the importance of fat in the diet as a principal source of energy, essential fatty acids, and fat-soluble vitamins, in addition to expressing

a concern about potential adverse reactions attributed to specific components in fats and oils, that may initiate or promote disease. Moreover, dietary fats provide important organoleptic qualities and a strong satiety signal. Fat is a primary component of cell membranes; for example, the brain alone represents 70% fat.

Safety of fats and oils has mostly centered on the very visible components of the total crude lipid fraction of foodstuffs, such as saturated fat and cholesterol and products of lipid hydrogenation and oxidation. Since the increased awareness of health risks attributed to dietary fats that started in the 1950's by Ancel Keys from University of Minnesota, who initially described a link between dietary saturated fatty acid and cholesterol-induced increase in blood cholesterol, much interest has been given to the source of dietary fat. Saturated and *trans*-fatty acids and cholesterol accelerate atherogenesis, whereas monounsaturated and polyunsaturated fatty acids (PUFA) are associated with reduced incidence of coronary heart disease. With the greater awareness that consumption of n-3 PUFA exert a protective potential against heart disease by lowering concentrations of very low-density lipoprotein, triacylglycerides and reduced blood pressure, platelet aggregation, and thrombosis (1), we are now appreciating that not all fats are equal in health implications. Moreover, relatively less visible components of dietary fat intake may have an even greater role in preventing, or alternatively initiating and propagating, chronic diseases. For example, the introduction of plant sterol and stanol esters into the diet has been shown to effectively lower serum cholesterol and low-density lipoproteins (2); reliable biomarkers for coronary heart disease. Vitamin E is another example of an important lipid soluble nutrient that has antioxidant activity and may, in concert with Vitamin C, protect against LDL oxidation, vascular endothelial dysfunction, and atherosclerosis (3). Alternatively, fat-soluble hydrocarbons with toxic potential must also be realized. Examples of these include the heterocyclic amines derived in cooked foods or the naturally present mycotoxins (e.g., aflatoxins) that represent important determinants of genotoxicity in humans (4) and manmade organic pollutants (e.g., polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OPs) that infiltrate the food chain and bioaccumulate to notable levels in foods consumed by man (e.g., fish and seafoods (5)).

The use of chemical aids and technologies to stabilize lipids also represents a need to evaluate the balance between positive attributes that may reduce the risk of exposure to dietary oxidized lipids, or alternatively, negative consequences, such as generation of *trans*-fatty acids derived from selective hydrogenation of vegetable oils. This chapter is intended to update the information on topics of toxicity and safety of fats and oils described earlier (6), as they relate to: (1) natural constituents of fats and oils; (2) derived products of oxidation and hydrogenation; (3) occurrence of natural and pollutant contaminants; and (4) additives used to preserve the stability, functionality, and nutritional quality of many constituents present in fats and oils.



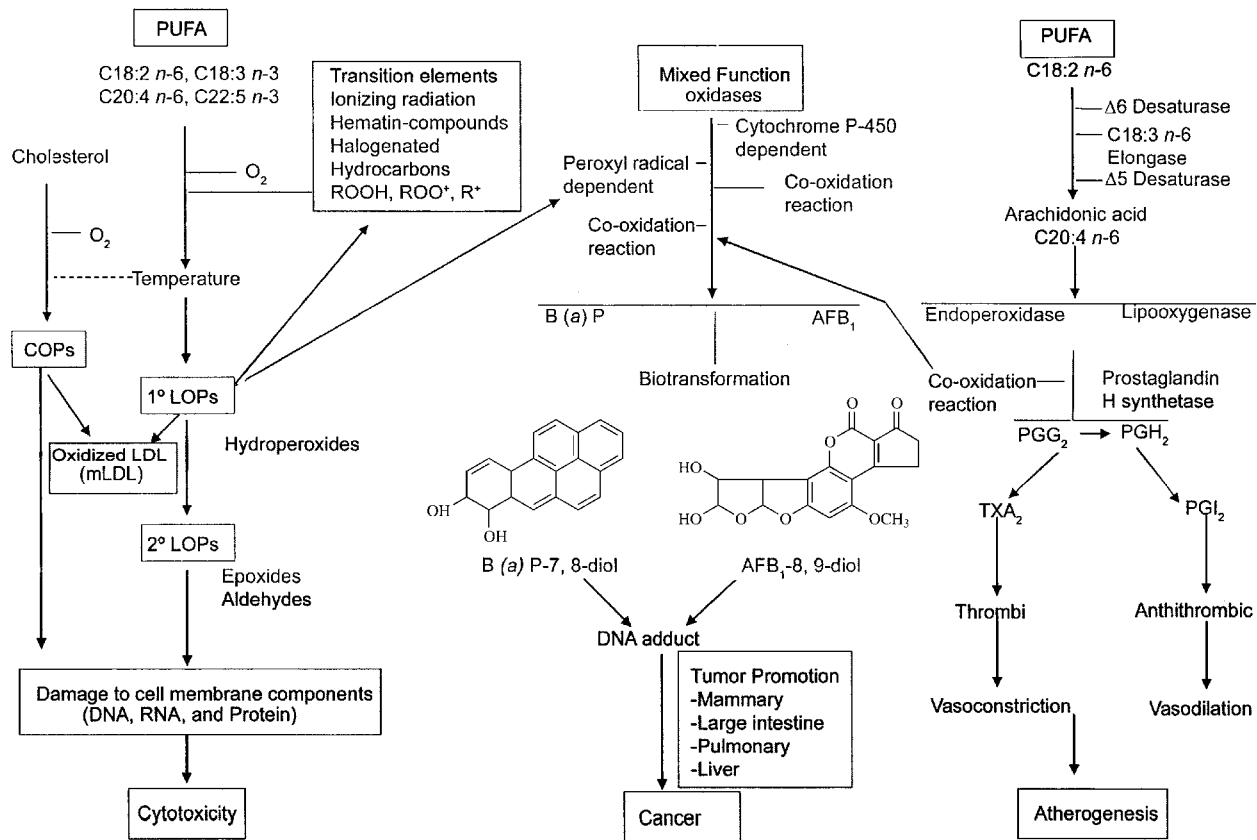
## 2. ADVERSE EFFECTS OF FATS AND ASSOCIATED CONSTITUENTS

### 2.1. Total Fat Intake

The level of dietary fat intake can represent both an initiator and a promoter of many adverse conditions that lead to a health risk (Figure 1). For example, a relationship between dietary fat intake and oxidative status will influence gene expression for drug-metabolizing enzymes, such as phase I and phase II enzymes, as well as glucose-6-phosphate dehydrogenase (G6PDH). Feeding diets containing 20% soybean oil to rats produces greater reductions in G6PDH and glutathione peroxidase activities compared with counterparts receiving 5% soybean oil (7, 8). The subsequent availability of reducing equivalents and glutathione for antioxidant enzyme activity are compromised by the higher fat intake. A completely separate example is seen with exposure to polychlorinated biphenyls (PCB)/dioxins, where the most important route for human exposure is food consumption (e.g., >90% of total exposure). The concentration of these particular contaminants in herbage consumed by cows is controlled by atmospheric deposition, and cows that consume large quantities of herbage can produce milkfat that contains PCB and dioxin contaminants. Therefore, exposure to these specific lipid-soluble xenobiotics in milkfat sources from certain geographic regions will be related to the level of intake of this particular fat source. Total intake of dioxin from dairy fat-containing products is 30% for adults and 50% for children. Background levels of PCBs are actually estimated from typical dietary intakes of food stuffs that include milk, eggs, meats, and fish-lipid matrices that contain these environmental contaminants (9). Using this trend of thought, high intakes of dietary lipids that contain lipid oxidation products in various quantities influence oxidative status, and, therefore, high intakes of total fat could increase the probability of exposure to exogenous- and endogenous-derived lipid oxidation products. Lipid oxidation yields a complex mixture of byproducts that include hydroxyl and dihydroxy fatty acids, hydroperoxides, volatile aldehydes, and alkyl and olefinic radicals (10), which are absorbable through the digestive tract (11) and incorporated into membrane phospholipids whereby they alter membrane fluidity (12, 13). The effect of feeding high-fat diets containing thermally oxidized lipids can lead to peroxidized tissue proteins (14), an up-regulation of peroxisomal-proliferator-activating receptor alpha (15) and increased number of intestinal aberrant crypts that are indicative of a precancerous cellular event (16). Thus, in these examples, both the quantity and the quality of the dietary lipid present a potential health hazard for the consumer.

### 2.2. Saturated Fatty Acids

Medium-chain fatty acids are saturated fatty acids because of the relatively shorter hydrocarbon chain, which does not facilitate unsaturation. The safety of medium-chain triacylglycerol (MCTs) in dietary oil has been debated, and associated effects on cholesterol metabolism remain unclear. Although some studies have shown that



**Figure 1.** Schematic diagram shows the interaction between lipid constituents, products of lipid oxidation, and xenobiotics, which can initiate or promote chronic disease states (6).

MCTs are essentially nontoxic, noncarcinogenic, and nonmutagenic for human consumption with a safety level up to 1 g/kg (17), other studies have indicated that MCT oil-containing diets can increase blood cholesterol levels (18). MCTs, on a percent energy basis, have half the potency of palmitic acid (C16:0) in raising plasma cholesterol (18). Palmitic acid (C16:0) can lead to increases in blood cholesterol levels; however, when ingested in a diet that contains a recommended intake of C18:2,n-6, the effect on both total and LDL cholesterol levels are minimized (19). This has been shown with fat blends, such that hypercholesterolemia was not observed in animals fed either butter or tallow fat sources that were blended with soybean oil in a low-cholesterol-containing diet (20, 21). In gerbils and monkeys, the relative ratio between C14:0 to C18:2 n-6 fatty acids as well as dietary cholesterol are important factors in modulating increases in serum cholesterol levels (22, 23).

Intake of saturated fat sources has also been associated with insulin resistance, leading to altered glucose metabolism, type II diabetes, and impaired glucose tolerance (24). Comparatively, saturated fat has a more deleterious effect on fat-induced insulin sensitivity than both mono- and polyunsaturated fat sources (24). Higher intakes of saturated fat and *trans*-fat adversely affect glucose metabolism and insulin resistance, whereas higher intakes of polyunsaturated fat and possibly long-chain n-3 fatty acids are beneficial (25). Within the category of saturated fats, dietary saturated, short-chain, and  $\omega 6$  fatty acids have been found to have the most deleterious effects on insulin action associated with insulin sensitivity, as opposed to medium- and long-chain fatty acids and  $\omega 3$  fatty acids (26). Intramuscular triacylglycerol (MTG) and elevated plasma free fatty acid (FFA) levels also have roles in insulin-mediated glucose uptake, reflecting a pivotal role of the high saturated fatty acid content in the MTG (27). Changing dietary fat quality by substituting saturated for monounsaturated fat can impair insulin sensitivity, as saturated fat has a greater deleterious impact on insulin sensitivity (28). For example, substituting a monounsaturated fatty acid diet (MUFA diet) for a saturated fatty acid diet (SAFA diet) has been shown to be favorable for only those subjects that had a lower-than-average total fat intake. This intervention improved insulin sensitivity, but had no effect on insulin secretion. Notably, the addition of n-3 fatty acids to MUFA and SAFA diets affected neither insulin secretion nor insulin sensitivity (28).

### 2.3. Monounsaturated Fatty Acids (MUFA)

Dietary MUFA have been found to have several positive effects that include, in addition to lowering human LDL-cholesterol plasma levels, positive effects on lipoprotein oxidation, coagulation, and fibrinolysis (29). Low-fat, monounsaturate-rich diets reduce the susceptibility of low-density lipoproteins to peroxidation *ex vivo* (30). In hypercholesterolemic subjects, diets containing MUFA resulted in favorable alterations in the fatty acid composition and oxidative profile of LDL in hypercholesterolemic subjects that were characterized as an increase in lipid peroxide lag time and a decrease in lipid peroxide formation (30). In animal studies,

rats that were fed long-chain MUFA diets showed only a small, significant increase in peroxisomal  $\beta$ -oxidation, and a slight decrease in mitochondrial oxidation (31). Feeding low-fat, monounsaturated-rich diets that contain high oleic peanuts has been shown to improve human serum lipoprotein profiles (32) and human serum lipid profiles (33). In free-living subjects with impaired glucose tolerance, MUFA diets also seemed to improve glucose metabolism (33).

Several studies have examined the effect of MUFA-containing diets on risk for cardiovascular disease (34). MUFA-containing diets lower both plasma cholesterol and triacylglycerol concentrations, which has favorable effects on the cardiovascular disease risk profile (35). MUFAs can also modify the lipoprotein profile and the mechanism by which fatty acids affect the immune response, which in turn will alter the development of the atherosclerotic lesion by limiting arterial thrombus formation (36). The effects of MUFA on human immune system responses have also been considered. Animals fed MUFA-rich diets, such as those containing olive oil, exhibit a suppressed *in vivo* immune response; the reactions in humans subjects are far more subtle than those reported in animal studies, however (37). The level of MUFA contained in animal feeding studies is much higher than that achievable in human studies, so MUFA still has a negligible effect on the modulation of immune function in humans (37).

## 2.4. Polyunsaturated Fatty Acids (PUFA)

In a comparison of rats fed diets containing either safflower oil or fish oil, it was shown that neither fish oil nor safflower oil intake resulted in an induced increase in phospholipid hydroperoxides and TBARS in rat organs. Thus, the supplementation of a rich source of n-3 PUFA, such as fish oil, compared with an n-6 PUFA containing safflower oil, did not significantly alter lipid peroxidation in rat organs (38). Other studies have investigated the comparative hypocholesterolemic effects of fish oil and soybean or safflower oil, among others, such as evening primrose oil (EPO) and *oenothera biennis* linn oil (OBLO), in cholesterol-fed rats after long-term feeding (39). Both normotensive and hypertensive rats fed menhaden oil-containing diets exhibited lower plasma cholesterol and triacylglycerol concentrations than counterparts fed either saturated (e.g., butter) or n-6 (e.g., soybean oil) diets (20, 21). In other studies where both EPO and OBLO contain n-6 PUFA, gamma-linolenic acid (GLA), it was found that OBLO (linoleic + GLA) and EPO (linoleic + GLA) caused the lowest serum total cholesterol, VLDL-c + IDL-c + LDL-c concentrations (39). EPO has also been found to have both antisecretory and antiulcerogenic effects in rats by inhibiting gastric mucosal damage induced by pylorus ligation, NSAIDs or hypothermic restraint ulcers (40). Crude extracts of meals from evening primrose sources have been compared with crude extracts of meals obtained from borage, for effect of concentration-dependent scavenging of reactive oxygen species and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (41). Along with EPO, other GLA oils, such as black current, borage, and fungal oils, have been noted for their blood pressure lowering effect in spontaneously hypertensive rats. This is likely caused by the alterations in fatty acid profiles of

hepatic and vascular tissue induced by GLA oils (42). A similar result was not observed in spontaneously hypertensive rats fed menhaden oil (21). However, interesting comparative effects of feeding rapeseed (canola) oil and soybean oil on blood pressure of rats showed that systolic blood pressure of rats fed canola oil diets was higher than that of rats fed soybean oil diets. One explanation for this finding was that the intake of canola oil increased plasma sodium and lipid levels and decreased potassium levels compared with soybean oil intake (43). Although the beneficial effects of GLA have been investigated in depth, it is unclear which oils are the best sources of GLA. For example, rats fed equal amounts of GLA, obtained either from transgenic canola plant or from borage plant, reacted similarly in growth and hepatic metabolism of n-6 fatty acids (44).

In other animal studies, where GLA was compared with alpha-linolenic acid (ALA), ALA gave similar effects as GLA in increasing fatty acid oxidation activity in rat livers. GLA and ALA differ, however, in the mechanism of action, as evidenced by the different affect on individual fatty acid oxidation enzymes involved in fatty acid oxidation (45). These results were confirmed by studies that found that, in opposition to dietary saturated fatty acids, both n-6 and n-3 fatty acids, such as GLA and ALA, inhibit the increase of serum total cholesterol and VLDL + IDL + LDL-c concentrations (46). The n-3 long chains PUFAs, EPA, and DHA have also been found to affect lipid peroxidation. The *ex vivo* intake of these highly purified n-3 fatty acids caused an immediate increase in chylomicron peroxidation in plasma (47). Moreover, feeding n-3 PUFA-rich fish oil in the form of menhaden oil reduced both RBC and heart GSH-Px activities (20). The same n-3 PUFA source, when fed at twice the energy equivalent, also can reduce RBC glutathione content and result in enhanced lipid oxidation in RBC, heart, and liver tissues (21). More specifically, n-3 PUFAs EPA and DHA have triacylglycerol-lowering effects (48). EPA and DHA have also been shown to reduce the incidence of mammary tumors that developed in rats. In this case, DHA was found to be slightly more effective than EPA (49).

The relationship between n-3 PUFA and protection against coronary heart disease is closely related to the hypocholesterolemic and hypertriacylglyceridemic responses to consuming this PUFA source (50). Intake of n-6 and n-3 PUFA has been shown to reduce hepatic mRNA and protein levels and the synthesis and activity of G6PHD. The result (51, 52) is reduced hepatic lipogenesis and plasma triacylglycerol content. In addition, n-3 PUFA reduces platelet aggregation and exhibits antithrombotic and fibrinolytic activities, among other functions (53). Several other studies have also been performed to investigate the antiatherogenic potential of GLA (54). GLA has been noted for its positive effects in the modification of atherosclerotic lesions in apolipoprotein E knockout mice. The reduced atherosclerotic lesion size from feeding GLA or n-3 was related to a suppressed smooth muscle cell proliferation *in vivo* and retarded development of diet-induced atherosclerosis (55). In humans, GLA supplementation may also affect cancer cell proliferation via the modification of fatty acid composition. A relationship appears to exist between GLA-induced tumor cell death and the distribution of fatty acids in tumor cells (56). GLA also has anti-inflammatory properties

in humans. The addition of GLA in vitro suppressed IL-1 beta release from human monocytes stimulated with LPS. GLA simultaneously reduced the amplification process of IL-1 beta and left the initial IL-1 beta response to LPS intact (57). The anti-inflammatory and immune parameter effects of GLA have also been reported in rats where GLA induced improvement of inflammatory disorders through the regulation of eicosanoid production. High doses of GLA may exert an anti-inflammatory affect by suppressing leukotriene B4 release and by strengthening the gut immune system, therefore improving responses to allergic reactions (58). As GLA is also known to have anticancer properties, studies have been performed on the effect of GLA on the expression of maspin and the motility of cancer cells. Maspin is a tumor suppressor and GLA has been found to upregulate the expression of maspin, thereby reducing the motility of cancer cells (59). Serum levels of phospholipids dihomo-gamma-linolenic acid were also found to be inversely associated with the risk of death caused by lung cancer (60). In diabetic rats, GLA was found to have beneficial, restorative effects on nerve conduction velocity, Na<sup>+</sup>, K<sup>+</sup> ATPase activity, and membrane fatty acid composition (61). Moreover, bleomycin-induced lung fibrosis in hamsters was altered as a result of elevations in tissue PGE1 and 15-HETrE, both of which have anti-inflammatory properties (62).

In other studies, very long-chain n-3 PUFA, such as those found in fish oil, were suspected of having a hepatotoxic potential in rabbits, which, in turn, was associated with atherosclerosis (63). The feeding of fish oil to rabbits produced an n-3 PUFA concentration dependent increase in aortic plaque surface area, indicating a potential positive relationship between severity of liver pathology and aortic plaque surface area. When fish oil and corn oil were compared for relative effects on chemical-induced hepatic enzyme-altered foci in rats, it was found that dietary fish oil inhibited hepatic enzyme-altered foci formation compared with corn oil. This is important because of possible stimulation of the hepatic detoxification system and enhancement of lipid peroxidation caused by excessive intake of fish oil (64). Conversely, marine oil containing n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) will induce hepatic peroxisomal  $\beta$ -oxidation and upregulate hepatic antioxidant enzymes (e.g., catalase and glutathione peroxidase and reductase activities) and GSH concentration (65).

Different doses of arachidonic oil (C20: 4 (n-6)) have recently been compared with high doses of C20: 4 (n-6) + fish oil with DHA for in utero exposure effects in rats. Higher dosages of C20: 4 (n-6) and C20: 4 (n-6) + DHA decreased alkaline phosphatase activity, lowered serum cholesterol, triacylglycerol, and phospholipids concentrations, while increasing creatinine and urea concentrations and also adrenal, spleen, and liver weights of pups (66). ARASCO, a microfungus source of triglyceride oil enriched in C20: 4 (n-6) was evaluated for its safety in rats. Results showed that high doses of ARASCO increased C20: 4 (n-6) levels in the brain, heart, and liver, thus being readily incorporated into tissue lipids, but without developmental, histopathological, or neuropathological consequences (67). Consistent with these findings regarding the nontoxicity of ARASCO, no genotoxic effects were observed (68).

## 2.5. Sterols

**2.5.1. Cholesterol** Experiments from laboratory animal trials (21, 69, 70) have supported epidemiological studies (71) that link hypercholesterolemia and hyperlipoproteinemia, two risk factors for CVD, with dietary cholesterol intake or atherogenic fatty acid ratios. Common to many of these studies are the findings that consumption of diets rich in cholesterol or saturated fat will result in a reduction of LDL receptors and elevation of LDL cholesterol and total cholesterol.

Studies in nonhuman primates have indicated that the cellular events and tissue morphological changes that occur during the progression of atherosclerosis are similar, whether animals are fed diets inducing modest levels of hypercholesterolemia or inducing extremely high levels of hypercholesterolemia (72). Studies to characterize events involved in the initiation of atherogenesis have suggested the possible involvement of cytotoxic cholesterol oxides in this disease process (73). Moreover, cholesterol and its oxide derivatives have been identified as a significant component of arterial plaque composition in humans (74) and in various animal species (75). There are relatively few studies that have examined the significance of dietary cholesterol intake on aortic plaque composition in atherosclerosis susceptible animals. The characteristic changes in plasma lipids and aortic plaque composition in the atherosclerosis susceptible Japanese quail model has enabled the effects of cholesterol feeding to be evaluated in regard to susceptibility to atherosclerosis (69). Herein, the Wistar rat, which is a noted animal model resistant to atherosclerosis, will be used for comparison with the atherosclerosis susceptible quail in many aspects of cholesterol metabolism and development of atherosclerosis.

Japanese quail exhibit greater plasma total cholesterol concentrations than rat counterparts when fed a similar, basal low-cholesterol diet (Table 1). Species differences in plasma lipids are more pronounced when fed an atherogenic diet (e.g., cholesterol/cholic acid supplemented and saturated fat-containing diet), as evidenced by the marked elevations observed in quail plasma total cholesterol concentrations

**TABLE 1. Plasma Lipids and Aortic Plaque Score and Area Covered in Wistar Rats and Atherosclerosis Susceptible Japanese Quail Fed Low and High Cholesterol Diets.**

Cholesterol Level (% by weight):	Total Cholesterol		Triacylglycerides <sup>2</sup>		Plaque Score		Area Covered (%) <sup>3</sup>	
	0.05	0.5	0.05	0.5	0.05	0.5	0.05	0.5
Animal species:								
Quail	230.4 ± 9 <sup>†</sup>	2049 ± 95 <sup>††</sup>	128 ± 10	359 ± 31	N.D.	3.7 ± 0.2 <sup>†</sup>	N.D.	61 ± 10 <sup>†</sup>
Rat	118.2 ± 5	240 ± 5 <sup>*</sup>	122 ± 18	87 ± 6	N.D.	N.D.	N.D.	N.D.

<sup>1</sup>Values represent mean ± SEM, n = 8. Animals fed tallow fat, semisynthetic diets with low (0.05%) and high (0.5%) cholesterol added.

<sup>2</sup>=mg/dL.

<sup>3</sup>Plaque score based on scale of 0 (N.D.) = clean surface; 1 = ≤5 plaques; 2 = 6–20 plaques; 3 = >20 plaques; 4 = massive atheromas observed. Values represent two judges evaluating in a blinded protocol.

<sup>4</sup>Area covered (%) = percent of aortic epithelium covered by plaque, range 0 (N.D.)–100% (69).

<sup>\*</sup>A significant ( $p \leq 0.05$ ) difference between cholesterol levels.

<sup>†</sup>Significant difference between specie.

compared with the rat (Table 1). Dramatic elevations observed in plasma cholesterol of quail fed cholesterol/cholic acid supplemented diets are directly associated with the level of dietary cholesterol fed to birds and severity of atherosclerosis lesions (Table 1). Although the rat also exhibited increased plasma cholesterol levels when fed the atherogenic diet, absolute cholesterol plasma levels are much lower than the quail, thus possibly reflecting the resistance of this species to the induction of hypercholesterolemia from cholesterol feeding. The concomitant hypertriacylglyceridemia observed in quail fed the atherogenic diet, did not occur in the rat fed a similar atherogenic diet and contributes further to explaining the role of dyslipidemia to the susceptibility of quail to atherosclerosis.

As a result of hypercholesterolemia, an intracellular accumulation of cholesteryl esters occurs, which is associated with the initiating events of atherogenesis, in particular, foam cell generation, which involves the intracellular accumulation of large amounts of cholesterol within macrophages and smooth muscle cells of aorta (76).

Modified (oxidized) lipid species have been identified in the plasma lipoproteins and aortic plaque of atherosclerotic humans (74, 77) and animal models (78, 79). Furthermore, the presence of COPs in circulating lipoprotein has been demonstrated in healthy humans (80) and monkeys. (73) Oxidized LDL has been proposed to have a role in foam cell formation (81) as well as having various proatherogenic properties, such as cytotoxicity and chemotactic activity (73, 82).

**2.5.2. Bile Salts** Bile salts have been linked to colorectal carcinogenesis by a variety of mechanisms (83). On the other hand, bile salt-dependent lipase (BSDL), a digestive enzyme secreted by the pancreas, which hydrolyzes dietary lipid esters, can have a positive effect against atherosclerosis (84). This is because BSDL activity increases with the level of LDL-c and is also positively linked to serum concentration of ApoB100 and ApoA-I. BSDL is associated with LDL in part by a specific interaction with ApoB100, although there appears to be no interaction with ApoA-I. As the increase in LDL-c is a risk factor for atheroma, the associated increase in BSDL, which can metabolize atherogenic LDL, shows that BSDL can have the positive effect against atherosclerosis (84). In another study, in which the cytotoxicity of bile salts against biliary epithelium (BDE) in isolated perfused rat liver was evaluated, it was found that in vitro BDE cells are not damaged by taurine-conjugated bile salts or glycine-conjugated bile salts, but they are very sensitive to the cytotoxicity of hydrophobic unconjugated bile salts (85).

**2.5.3. Phytosterols** The relationship between total dietary phytosterol content and the fatty acid composition of the diet decreases with increasing saturated fatty acids, whereas the total dietary phytosterol content increases with increasing PUFA (86). Phytosterols consist of a mixture of cell membrane constituents that include free sterols, esterified sterols (e.g., esterified to phenolics), steryl glycosides, and acylated steryl glycosides (Table 2).

They are found in varying concentrations in a number of vegetable oils, cereal-based products, and nuts where they function to regulate membrane fluidity



**TABLE 2. Principle Sources of Plant Sterols.<sup>1</sup>**

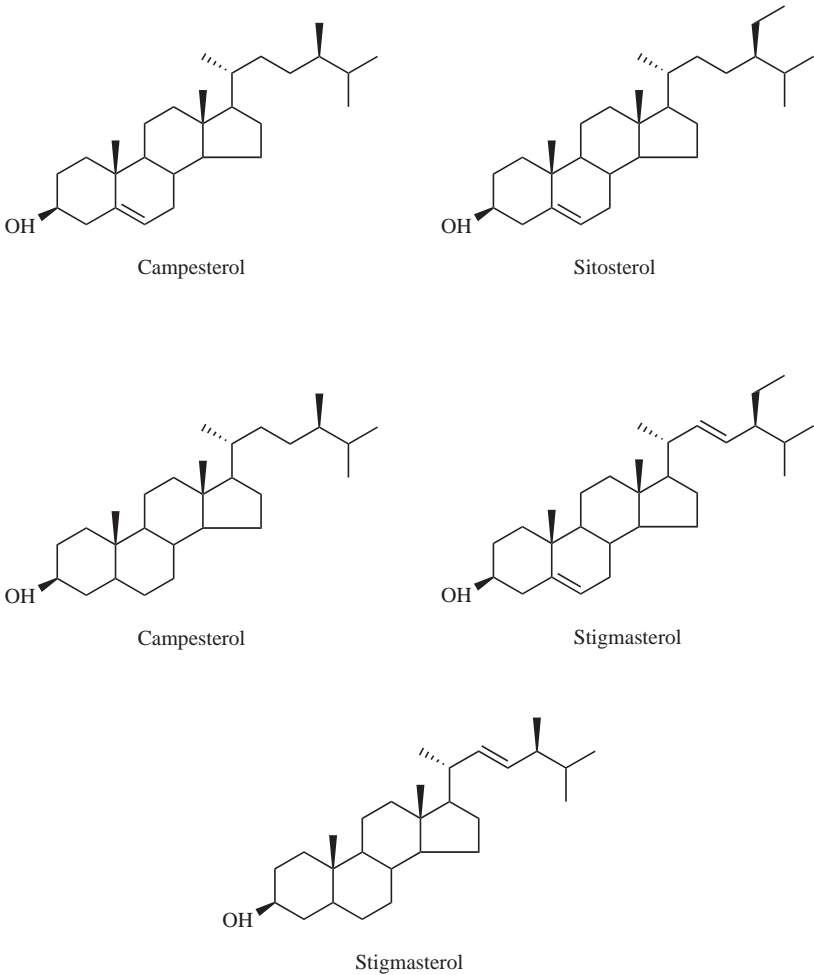
Sterol Source	Concentration (g/kg)
Oil	
Rapeseed	5.13–9.79
Corn	8.09–15.57
Wheat germ	19.70
Rice bran	32.35
Cereals	
Rye	1100
Barley	830
Wheat	760
Oats	520

<sup>1</sup>Ref. (87).

and permeability. Plant sterols (e.g.,  $\beta$ -sitosterol, stigmasterol, campesterol,  $\Delta$ -7 stigmasterol, and  $\Delta$ -5-avenasterol) are present in plant unsaponifiable matter (Figure 2). The main sterol in vegetable oils is 4-desmethylsterol, where sitosterol is the principle sterol and camperstserol, stigmasterol, brassica-, and  $\Delta$ -venasterol are also present in lower concentrations. Total sterol content of soybean oil and safflower oil is 0.29% and 0.39%, respectively, and varies with the source materials (Table 3). This compares with the plant sterol content of 4.1% in rice bran and 10% in rubber seed oils.

A typical Western diet contains approximately 100–300 mg and 20–50 mg of plant sterol and plant stanol, respectively. The relationship between total dietary phytosterol content and the fatty acid composition of the diet decreases with increasing saturated fatty acids, whereas the total dietary phytosterol content increases with increasing PUFA (89). Fortification of lipid foods, such as margarine, with plant sterols will dramatically increase the daily intake of phytosterols and significantly lower serum cholesterol (90). The dietary consumption of large amounts of plant sterols will interfere with cholesterol absorption, thereby leading to an increased daily neutral steroid excretion.

Fat-soluble plant stanols have been approved for fortification into margarine. The sterol solubility is obtained by *trans*-esterification with rapeseed oil fatty acids in the margarine (91). Plant stanol esters lower serum cholesterol more effectively than insoluble free stanol counterparts, which are either in a crystalline, microcrystalline, powdered, or homogenized form (92). The *trans*-esterification process also ensures palatably, with the incorporation of stanols into nutritional fats that are common to a Western diet, such as margarine and mayonnaise (91). Two FDA-approved margarines, Take Control (Unilever) and Benecol (Johnson & Johnson) reached the marketplace in May of 1999. The claim on the Finnish margarine, Benecol, states that 2–3 servings per day of the product, containing 1.5 g plant stanol esters per serving, will lower cholesterol in 2 weeks. In animal studies,  $\beta$ -sitosterol feeding interferes with exogenous and endogenous cholesterol absorption, resulting in the interruption of enterolymphatic circulation of cholesterol, an important regulator of hepatic cholesterol synthesis. Less than 1 g/d of sitostanol esters



**Figure 2.** Plant sterols found in oil seed, vegetable, and wood sources. Of the more than 40 plant sterols identified,  $\beta$ -sitosterol, stigmasterol, and campesterol are the most abundant.

incorporated into mayonnaise significantly lowered cholesterol absorption (92), and a median intake of 2–3 g/d of plant stanol esters have been recommended to lower serum LDL cholesterol by 10–15% (93). The mechanism of action of phytosterol-induced reduction of serum cholesterol involves interference of the relatively insoluble sterol with micellar solubility of cholesterol in the intestinal lumen. Plant stanol esters also mix easily with the oil phase of intestinal content, which, in turn, disrupts cholesterol absorption (93). Plant stanols can also prevent the absorption of other plant sterols (91).

A compensatory increase in cholesterol synthesis attributed to the phytosterol-induced lowering of serum cholesterol occurs, as indicated by a rise in serum

**TABLE 3. GC Quantitation of Cholesterol and Cholesterol Oxidation Products in Aortic Tissue from Wistar Rats and Atherosclerosis Susceptible Japanese Quail Fed Low and High Cholesterol Diets.<sup>1</sup>**

Sterol <sup>2</sup> : (mg/g) Cholesterol level (% by weight):	Cholesterol		7 $\beta$ -hydroxycholesterol		7-ketocholesterol	
	0.05	0.5	0.05	0.5	0.05	0.5
Animal species:						
Quail	3.19 $\pm$ 0.91	11.48 $\pm$ 2.13 <sup>*</sup>	N.D.	0.24 $\pm$ 0.03	N.D.	0.27 $\pm$ 0.04
Rat	0.93 $\pm$ 0.04	0.91 $\pm$ 0.49	N.D.	N.D.	N.D.	N.D.

<sup>1</sup>Values represent mean  $\pm$  SEM, n = 8. Animals fed tallow fat, semisynthetic diets with low (0.05%) and high (0.5%) cholesterol added.

<sup>2</sup>Values expressed on basis of tissue wet weight. See (69) for methodology.

N.D. = none detected.

<sup>\*</sup>A significant ( $p \leq 0.05$ ) difference between cholesterol levels across a row.

cholesterol precursors, lathosterol and desmosterol. This compensatory rise in serum cholesterol caused by absorption inhibition is not necessarily detrimental because the net effect is still a lowering of serum cholesterol (93). The methyl or ethyl side chain on the cholesterol molecule greatly reduces the intestinal absorptibility of plant sterols. The efficiency of sitostanol absorption is only 0–3%, with serum levels being almost undetectable. Campestanol, the other major plant stanol, also has a very low absorption efficiency compared with the unsaturated version, vampestanol (93).

Recently, a seven-part study was conducted that served as a safety evaluation of phytosterol esters. It was shown that phytosterols do not bind to the estrogen receptor (ER) and do not stimulate transcriptional activity of the human ER in a recombinant yeast strain. There was also no indication of estrogenicity from an uterotrophic assay conducted in immature female rats (94). In a rat study, the no-observed-adverse-effect level (NOAEL) of phytosterol esters was 8.1% following a daily oral administration for 90 days, equivalent to a dose of 6.6 g/kg/day. No treatment-related changes that were of toxicological significance occurred in this subchronic study (95). In the ensuing two-generation reproduction study in rats with phytosterol esters that followed, it was found that no effect on the reproduction of parental generation rats, or on the development of second-generation pups and their sexual maturation of the pups, occurred at 8.1% concentration of phytosterol esters (96). The fecal concentrations of bile acids and neutral sterols from healthy normolipidaemic volunteers consuming a controlled diet, either with or without a phytosterol ester-enriched margarine, indicated that a high intake of phytosterol esters increased the amount of neutral sterols in the feces, but did not result in increased formation of bile acids or sterol metabolites (97). Concomitantly, the effect of feeding a controlled diet, either with or without the phytosterol ester-enriched margarine, on fecal short-chain fatty acid and microflora content, fecal bacterial enzyme activity, and serum female sex hormones, indicated that the daily consumption of 8.6 g phytosterol did not affect bacterial profile or metabolic activities of the gut microflora. Moreover, there was no biologically

relevant effect on serum female sex hormones (98). Examination of potential mutagenic activity of phytosterols (99) and the comparative absorption and tissue distribution of phytosterols in the rat (100) disclosed that the most highly absorbed sterol was cholesterol, followed by campesterol,  $\beta$ -sitosterol, and stigmasterol, followed by  $\beta$ -sitostanol and campestanol. The absorption of phytosterols was slightly greater in females than in males (100). None of the phytosterols and phytosterol esters tested showed any evidence of mutagenic activity in bacterial mutation assays, in vitro chromosome aberration assays, or in vitro mammalian cell gene mutation assays (99).

A positive effect of phytosterols from tall oil phytosterols showed a dramatic protection against atherosclerosis in mice (101). Mice fed a cholesterol-enriched diet containing a phytosterol mixture exhibited reduced plasma cholesterol levels and decreased formation of atherosclerotic lesions. In humans, phytosterol treatment resulted in an average of 10% reduction of total cholesterol and a 13% reduction in LDL-c (102).

The safety and tolerability of etherified phytosterols, evaluated with the administration to healthy adult men and women in a reduced-fat spread and salad dressing, indicated that subjects that consumed reduced-fat spread and salad dressing, providing 0 g/day, 3 g/day, 6 g/day, or 9 g/day of phytosterol esters for 8 weeks, displayed normal blood concentrations of all fat-soluble vitamins and no differences in serum vitamin responses among the four groups. Also, total cholesterol, LDL-c, and HDL-c responses did not significantly differ among the groups, although the total cholesterol: HDL-c response in the 9 g/day group was significantly different from control group response. In short, phytosterol esters are well tolerated in humans and show no sign of adverse effects at daily intake up to 9 g/day for 8 weeks (103). Diets enriched with different plant sterol mixtures derived from wood source (e.g., Reducol from Forbes Medi-Tech, Inc. Vancouver, British Columbia) have also been used to lower serum cholesterol. Reducol<sup>TM</sup> is a proprietary mixture of phytosterols composed of  $\beta$ -sitosterol, sitostanol, campesterol, and campestanol derived from tall oil pitch and processed into a crystalline powder. Feeding Reducol at a level of 1.8 g per day (e.g., 6% sterols in margarine) lowered LDL cholesterol by approximately 15% compared with placebo (104). The consumption of phytosterol mixtures increases cholesterol synthesis and lowers cholesterol absorption.

The effects of consuming sitostanol ester margarine in subjects with moderate hypercholesterolemia revealed that subjects showed unaffected Vitamin D and retinol concentrations and  $\alpha$ -tocopherol/cholesterol proportion, but lower serum beta-carotene levels. Alpha-tocopherol and carotenes were related to serum cholesterol and cholesterol absorption (105, 106). Moreover, the frequency of consumption of plant stanol esters in margarines and shortenings does not significantly affect the LDL-c lowering efficacy of the plant stanol esters (107). After standardization for LDL-c, consistent with other findings, it was also found that the sum of the most lipophilic hydrocarbon carotenoids, such as  $\alpha$ -carotene,  $\beta$ -carotene, and lycopene, were only slightly, though not significantly, lowered by the consumption of the plant stanol esters in margarine and shortening.

The ensuing reduction of the most lipophilic antioxidants attributed to phytosterol intake has also been related to a decrease in LDL (108). The increase in dietary carotenoids, when humans consume plant sterols or stanols, is effective in maintaining carotenoid concentrations (109). The addition of more daily servings of high carotenoid-containing fruits or vegetables when consuming sterol or stanol ester-containing spreads maintains plasma carotenoid concentrations while lowering LDL-c (109).

Efforts have attempted to pinpoint the relationship between plant sterol incorporation in human keratinocyte plasma membrane and modulation of membrane fluidity (110). Data from *in vitro* trials on uptake of plant sterols and membrane lipid fluidity in human keratinocytes suggest that, in the presence of sitosterol, the mean fluidity of the membrane is regulated, whereas stigmasterol triggers a looseness of molecular packing of phospholipids acyl chains. Phytosterols also have anticancer dietary components (111); for example, it was found that phytosterols indirectly (*in vivo* as a dietary supplement) and directly (*in tissue culture media*) inhibited the growth and metastasis of human prostate cancer PC-3 cells in mice (112). These findings are not limited to prostate cancer, as it was found that phytosterols might also function in possible protective mechanisms in breast and colon cancer. Phytosterols appear to have an effect on membrane structure and on the function of tumor and host tissue, on the signal transduction pathways that regulate tumor growth and apoptosis, on the immune function of the host, and on the cholesterol metabolism of the host (111).

## 2.6. Fat-Soluble Vitamins (A, E, D, and K)

**2.6.1. Vitamin A** Several studies and reviews have examined the effects of Vitamin A deficiency and toxicity (113). Endogenous retinoid toxicity has a possible role in the pathophysiology of primary biliary cirrhosis (PBC), which is a chronic, cholestatic disease, characterized by progressive destruction of the small intrahepatic bile ducts and portal inflammation, leading to cirrhosis and fibrosis. The major signs and symptoms of PBC resemble the manifestations of hypervitaminosis A. Thus, the hypothesis has been made that exposure to excess endogenous retinoids contributes to the pathogenesis of PBC and may be the cause of some of the symptoms of PBC (114). Retinol has an effect on hepatic and renal drug-metabolizing enzymes (115). Retinol pretreatment potentiates paracetamol-induced hepatotoxicity in BALB/c mice, an organ-specific response. And the potentiation of paracetamol-induced hepatotoxicity is independent of CYP450 and glutathione (115).

Moderate to high doses of Vitamin A given to pregnant mice can result in significant craniofacial, cardiac outflow, and thymic abnormalities (116). These results signal the potential for the induction of birth defects in offspring of women ingesting even moderate to low amounts of supplemental Vitamin A during an early gestational period. Animal studies have been used in order to assess safe dose levels of Vitamin A during human pregnancy (117). In one study, the safe level of Vitamin A during human pregnancy was set at a range of 25,000–37,000 IU/day

(118). In nonpregnant humans, the safe, nonteratogenic dose level of Vitamin A has been determined to be 30,000 IU/day (119). Weaker teratogens than retinol are retinoids, 14-hydroxy-4, 14-retroretinol (14-HRR), and anhydroretinol (AR) (120). The low teratogenicity may be caused by facts that 14-HRR and AR do not contain the terminal carboxylic group involved in binding and activation of the retinoic acid nuclear receptors, and they are not metabolized to acidic retinoids.

Vitamin A, retinol, and derivative retinal, also causes oxidative DNA damage via superoxide generation (121). Beta-carotene, for example, is related to a higher incidence of lung cancer. Retinol and retinal can cause cellular DNA cleavage. This is probably caused by the dismutation of superoxide to  $H_2O_2$ , generated by the auto-oxidation of retinoids in the presence of endogenous metals. Thus, retinol and retinal have capacities to exhibit pro-oxidant activity, which may lead to carcinogenesis of beta-carotene supplements (121). Retinol supplementation also induces DNA damage and modulates iron turnover (122). In animal studies, retinol caused cellular DNA damage involving cellular iron accumulation. These characteristics could be responsible for the increased incidence of lung cancer associated with retinoid supplementation (117, 122). Lycopene, lutein, and zeaxanthin are considered potential antioxidants for the oxidation of carotenoids by free radicals (123). Recently, concerns have been raised as to the retinol equivalents calculated by pro-vitamin A carotenoid conversion factors (124). It has been suggested that these values should be treated with caution until more data on absorption of carotenoids from foods is known.

**2.6.2. Vitamin E (Tocopherols)** In an oral toxicity study using a tocotrienal preparation in rats, the NOAEL for tocotrienal was 0.19% (125). In the presence of copper (II) ions,  $\alpha$ -tocopherol induces oxidative damage to DNA as copper-dependent reactive oxygen species formation occurs from molecular oxygen, thus resulting in DNA base oxidation and backbone cleavage (126). This pro-oxidant activity will induce tumor formation and act as a complete tumor promoter in animals. However, a product of  $\alpha$ -tocopherol oxidation/reduction,  $\alpha$ -tocopherolhydroquinone, (TQH<sub>2</sub>) has antioxidant properties, as it may scavenge peroxy radicals primarily by electron transfer to form TQ and secondarily by addition-elimination to form the epoxyquinones (127).

Vitamin E has been found to be useful in the storage of irradiated fresh meat. Feeding calves a diet rich in PUFA and supplemented with Vitamin E helped to control lipid peroxidation and oxymyoglobin oxidation of ground beef cold pasteurized with electron-beam irradiation during storage (128). Thus, color retention and lipid peroxidation are controlled by the presence of a critically high concentration of  $\alpha$ -tocopherol (TOH) (129). As tocopherol has a peroxy-radical scavenging function (130), the concentration of TOH decreases and the concentrations of alpha-tocopherolquinone and 2,3-epoxy-alpha-tocopherolquinone increase when TOH oxidation occurs in meat, such as in beef and bovine muscle microsomes.

Vitamin E has also been noted for the function of tocopherylquinones as highly cytotoxic agents. Tocopherylquinones escape multidrug resistance in acute lymphoblastic leukemia cell lines (131). Ascorbate and  $\alpha$ -tocopherol also have several poten-

tial antiatherogenic mechanisms, such as the inhibition of LDL oxidation, the inhibition of leukocyte adhesion to the endothelium, and the inhibition of vascular endothelial dysfunction (132). Alpha-tocopherol acts as either antioxidant or pro-oxidant of lipid peroxidation in LDL, but may only protect against atherosclerosis in combination with Vitamin C. Tocopherols and tocotrienols also have antiproliferative and apoptotic effects on normal mouse mammary epithelial cells (133), as mammary epithelial cells uptake of tocotrienols is greater than tocopherols. This suggests that tocotrienols have greater biopotency than tocopherols partly because of greater cellular accumulation. In all,  $\gamma$ - and  $\delta$ -tocotrienols may have important roles in modulating normal mammary gland growth, function, and remodeling. The mechanism underlying the antiatherogenic properties of Vitamin E is its function to decrease the uptake of modified LDL and suppresses acyl-CoA: cholesterol acyltransferase (ACAT) activity, resulting in less cholesterol esterification in macrophages (134).

**2.6.3. Vitamin D** Vitamin D has potential roles in the prevention of some cancers, osteoarthritis progression, multiple sclerosis, and hypertension (135). Feeding low levels of 1,25-hydroxy-vitamin D3 ( $1,25(\text{OH})_2\text{D}_3$ ) supplementation produces no toxicity in laying hens; however, feeding very high levels produces clear toxic symptoms (136). The feeding of excessive amounts of vitamin D3 to rats will lead to bone breakdown and increased levels of zinc in the blood (137). In pot-bellied pigs, Vitamin D toxicity was expressed as anorexia, weight loss, lethargy, polyuria, polydipsia, vomiting, tenesmus, and tremors (138). Similar effects have been observed in weanling pigs as Vitamin D toxicity caused serum calcium and blood urea nitrogen concentrations to increase and a decrease in serum phosphorus (139). Excess Vitamin D3 is toxic, particularly to vascular tissues; a notable pathological feature being arterial calcification (140). Excess Vitamin D is arteriotoxic and it can induce arterial calcification through upregulation of  $1,25(\text{OH})_2\text{D}_3$  receptors and increased calcium uptake in arterial smooth muscle cells (141). Vitamin D toxicity may also be expressed with the wasting and calcification of soft tissues in cattle after ingestion of a plant, *Solanum glaucophyllum* (Sg), which contains high levels of 1,25-dihydroxy-vitamin D3. The Sg-intoxicated cattle showed atrophy of the epidermis, severe involution of hair follicles, sebaceous and sweat glands, and reduced cellular proliferation. In humans, outbreaks of hypervitaminosis D have been linked to the overfortification of milk from home-delivery dairies (142).

Vitamin D has protective effects on diet-induced epithelial cell hyperproliferation (143). Increasing dietary Vitamin D, along with calcium, will prevent hyperproliferation. Amino bisphosphonate ibandronate has been found to prevent Vitamin D toxicity (144) by inhibiting Vitamin D-induced calcification of arteries, cartilage, lungs, and kidney in rats. Thus, in the future, ibandronate may be used to treat patients exposed to toxic levels of Vitamin D. Another protective effect of Vitamin D is the function of 1-alpha-dihydroxy-vitamin D3 ( $1,25(\text{OH})_2\text{D}_3$ ), in particular, in the reduction of the proliferation of human cancer cells (145). It has been shown to increase differentiation in human colon cancer cells, preventing colonic hyperproliferation and oxidative stress.  $1,25\text{-D}_3$  also has other immunosuppressive effects. As a therapy, it prolongs the survival of renal allografts and

preserves graft function in rats (146). These effects are even more apparent when the Vitamin D therapy is combined with cyclosporine A. Vitamin D has several possible noncalcaemic roles, including its role in the immune system and, in particular, on T cell-mediated immunity (142). The role of Vitamin D compounds as selective immunosuppressants is illustrated by an affinity to either prevent or suppress autoimmune disease.

**2.6.4. Vitamin K** Recently, dihydro-vitamin K1 has been identified as a dietary form of Vitamin K produced during the hydrogenation of Vitamin K1-rich vegetable oils (147). Children have the highest intake of dihydro-vitamin K1 (30% of total Vitamin K intake), followed by a progressive decrease in percentage contribution with age. The hydrogenation of vegetable oils decreases the absorption and biological activity of Vitamin K bone (148). The hydrogenated form of Vitamin K, dihydrophyloquinone, is less bioavailable than phyloquinone and has no measurable biological effect on measures of bone formation and resorption. A high-performance liquid chromatographic method for the determination of phyloquinone and menaquinones in foods of animal origin has recently been developed (149). Dietary Vitamin K intakes are associated with hip fractures, but not with bone mineral density in elderly men and women (150). Vitamin K may also play a role in the etiology of colon cancer (151). It has been proposed that bile acids (e.g., deoxycholic acid), K vitamins, iron (II) complexes, and oxygen interact to induce an oncogenic effect in the colon by the generation of free radicals. This may be caused by the function of the reduced K vitamins in initiating generation of superoxide radical ( $O_2^{\bullet-}$ ), leading to an Fe(II)-mediated Fenton reaction in colon stem cells.

### 3. ADVERSE EFFECTS OF SOME NATURAL CONSTITUENTS IN FATS AND OILS

#### 3.1. Erucic Acid

The chain elongation of 22:1n-9 (erucic acid) causes an increase in 24:1n-9 platelet sphingomyelin content (153). This effect of erucic acid has been proven in the transient decrease in platelet counts and increase in platelet size in newborn piglets fed canola oil. Similar findings regarding the effect of erucic acid on platelet counts has been reported in human studies on patients with adrenoleukodystrophy (154). Erucic acid treatment causes decreased platelet counts and morphologic and platelet sizing measurements, suggesting that erucic acid also affects the physical properties of platelets. Speculation has also been prevalent that the erucic acid content in oils, like canola oil, may cause the accumulation of triglyceride in the heart when fed to infants. Recent animal studies have shown, however, that the modest accumulation of erucic acid associated with feeding canola oil is not associated with biochemical evidence of heart triglyceride accumulation (155). Other animal studies have reported that an erucic acid ethyl ester diet induces a



marked increase in free fatty acids and in triglyceride content, as well as marked differences in the fatty acid pattern in triglycerides, free fatty acids, and diglycerides, but only marginal differences in phospholipids (156). The question still remains as to whether dietary erucic acid can be hepatotoxic in pregnancy. Although the erucic acid content in rapeseed oil, for example, is associated with more weight gain and higher proportions of erucic acid in the heart when compared with corn oil, rapeseed oil dietary content also causes lower bile flow in pregnant hamsters (157).

### 3.2. Unconventional Oils

**3.2.1. Rice Bran Oil** Rice bran oil (RBO) and its main components have affinity toward improving the plasma lipid pattern of rodents, rabbits, nonhuman primates, and humans by decreasing total plasma cholesterol and TG levels and increasing HDL-c levels. Other potential properties of rice bran oil and gamma-oryzanol include modulation of pituitary secretion, inhibition of gastric acid secretion, antioxidant action, and inhibition of platelet aggregation (158). Oryzanol is contained in the nonsaponifiable lipid fraction of rice bran oil. Three major components of gamma-oryzanol are cycloartenyl ferulate, 24-methylene-cycloartanyl, and campesteryl ferulate (159). In an aqueous model system, the nonsaponifiable fraction in rice bran has been shown to inhibit cholesterol autoxidation (160). In hamsters, oryzanol decreases cholesterol absorption and aortic fatty streaks (161). Oryzanol treatment results in significant decreases in plasma total cholesterol and the sum of IDL-c, LDL-c, and VLDL-c. Thus, oryzanol is at least partly responsible for the cholesterol-lowering action of RBO. The blending of RBO with other oils, such as safflower oil or sunflower oil, improves lipid profiles by reducing TC, TG, and LDL-c and increasing HDL-c (162). However, where the blending of RBO with safflower oil may magnify a hypocholesterolemic activity (163), the blending of RBO with sunflower oil does not have the same effect (164). This difference is likely caused by the different triacylglycerol structures of safflower and sunflower oil.

**3.2.2. Rubber Seed Oil** Rubber seed oil (RSO), which has a high C18:3, n-3 content (6), has a lower alcoholysis rate than linseed oil, but a higher alcoholysis rate than soybean oil and melon seed oil (165). Studies on the epoxidation of RSO by peroxyacetic acid generated in situ have shown that increase in the process temperature increases the rate of epoxide formation (166). The optimum alcoholysis temperature for RSO is  $245 \pm 2^\circ\text{C}$ .

**3.2.3. Ricinoleic Acid** Ricinoleic acid (RA) can increase mucosal permeability and cause cytotoxicity. It is also associated with the release of eicosanoids, a platelet-activating factor, and nitric oxide (NO). RA disrupts normal intestinal motility and a combination of these effects accounts for the laxative action of RA (167). Consistent with these findings, RA will increase nitric oxide synthetase activity in the rat ileum and colon (168), which likely accounts for the involvement

of NO in the laxative action of RA. Although RA possesses capsaicin-like dual pro-inflammatory and anti-inflammatory properties, unlike capsaicin, RA does not induce an inward current in dorsal root ganglia neurons and it does not have algescic properties in vivo (169). Thus, RA may be viewed as a new capsaicin-like, nonpungent, anti-inflammatory agent suitable for peripheral application.

**3.2.4. Cyclopropenoid Fatty Acids (CPFA)** Animal studies have shown that higher percentages of CPFA in oils are related to retarded growth (170). For example, heating baobab oil, thereby reducing its CPFA content, will caused the oil to increase the cytosolic glutathione transferase activity in rats fed this oil source. These mechanisms of CPFA action might be related to alterations of membrane lipid composition or microsomal proteins. CPFA are classified as toxic nonoils that are found in cottonseed oil (171); albeit cottonseed oil has been found to be an ingredient that is used safely in cosmetic formulations if established limits on gossypol, heavy metals, and pesticide concentrations are not exceeded.

**3.2.5. Structured Triacylglycerols** Exposure to dietary structured triacylglycerols containing docosahexaenoic acid from birth will positively affect the visual and auditory performance and tissue fatty acid profiles of rats (172). Although the feeding of this specifically structured oil will change the fatty acid profiles of rats and cause a higher level of 22:6n-3 in rat brain tissue, these changes will not result in differences in learning ability. However, positive changes in visual function and in auditory brainstem response have been observed. In the brain, 22:6n-3 levels increased in brain phosphatidylcholines and phosphatidylserines (173); 22:6n-3 also increases in the adipose tissue, suggesting that the surplus of dietary 22:6n-3 is stored. Structured triacylglycerols differ from conventional triacylglycerols, because the medium-chain fatty acids esterified to the glycerol skeleton are absorbed in the intestines via portal blood as free fatty acids (174); absorption pathways are also conventional with long-chain triacylglycerols.

Recent animal studies that looked at the effects of the structured lipid, SALATRIM, in enriched biscuits on serum and liver lipid concentration in rats, reported that SALATRIM could be used as a fat substitute in biscuits without influencing lipid metabolism (175). Studies have also shown that SALATRIM can help prevent accumulation of TG and TC in the liver and in white adipose tissue that is induced by a high lard diet fed to rats (176). Synthesis of structured triacylglycerols containing caproic acid by lipase-catalyzed acidolysis have been performed in a batch reactor, with a solvent-free system of structured triacylglycerols containing short-chain fatty acids by Lipozyme RM IM-catalyzed acidolysis between rapeseed oil and caproic acid and optimized using response surface methodology (177).

The effects of Caprenin, another structured lipid, on chylomicron fatty acid composition and postprandial serum lipid concentrations have also been studied (178). It was found that there is a very low uptake of C8:0, C10:0, and C22:0 into chylomicrons. Moreover, a postprandial lipemia after caprenin is comparable with that produced by other dietary fats as opposed to a fat-free meal. There is considerable

contribution of endogenously derived fatty acid to chylomicron lipids, and there are equal effects of saturated fatty acids on pre- and postprandial concentrations of plasma cholesterol.

Olestra is a different example, which is comprised of a fat-derived product made from sucrose that is esterified with fatty acids to generate a virtually unavailable form of calories caused by its very poor digestibility in the gastrointestinal tract. Nevertheless, the similar physical properties of regular oils and fats enabled it to be used as a low-calorie fat source that can be used in high-temperature cooking. A regular intake of Olestra will reduce the uptake of carotenoids (e.g.,  $\beta$ -carotene) and potentially other fat-soluble vitamins. Radio-labelled Olestra experiments have confirmed the fact that Olestra is essentially not absorbed and heating the sucrose polyester will not improve bioavailability (179). Other acute and subacute toxicity studies have indicated that no related effects attributed to Olestra intake in regard to common toxicological endpoints, such as time-to-tumor or tumor incidence, clinical chemistry, haematology, weight changes in specific organs, and tissue morphology, occur when fed up to 10% of the diet (180). Although Olestra has been shown to have no effect on water-soluble vitamins, formulations required the addition of Vitamins A, D, E, and K to avoid depletion in individuals that consume foods containing Olestra.

## 4. BIOACTIVE LIPID-SOLUBLE CONSTITUENTS

### 4.1. Phytoestrogens

The Committee on Toxicity of Chemicals in Foods, Consumer Products and the Environment recommended in 2002 more scientific studies in understanding the relative risk:benefit of phytoestrogens to human health. Studies have reported that isoflavone-containing diets, when fed to C57BL/6 mice, resulted in reduced cholesterol levels, but had no effect on cholesterol levels or on the susceptibility of LDL to oxidative modification in LDLr-null mice (LDL receptor-deficient) (181). Plant estrogen isoflavones also have potentially antiatherogenic effects, in addition to antioxidative and antiproliferative properties (182). Both soy-derived isoflavones and esterified isoflavones reduce *in vitro* oxidation susceptibility of LDL. The lipophilic phytoestrogen derivatives can be incorporated into LDL, thereby increasing the oxidation resistance and antiproliferative efficacy *ex vivo*. It is unclear, however, whether the cholesterol-lowering effect of a soy-rich diet may be associated with the presence of isoflavones (183).

Coumestrol and genistein are principle phytoestrogens, which induce micronuclei containing acentric fragments and DNA strand breaks. Coumestrol also induces hypoxanthine guanine phosphoribosyltransferase mutations in cells where genistein is marginally active at this endpoint (184). The phytoestrogen, daidzein, has no effect, nor does it cause significant toxicity on the reproductive tract of animals or provide a protective effect against chemically induced mammary cancer (185). Antioxidant activity has been shown to include the interference of advanced

glycation end-products-mediated oxidative DNA damage of vascular smooth muscle cells (186). They are also potentially useful against vascular diseases, where reactive oxygen species are involved in hypertension.

Coumesterol exhibits both mutagenic and clastogenic properties in AHH-1 TK<sup>-/-</sup> human lymphoblastoid cells (187). Earlier studies found genestein to have both anticarcinogenic and antiproliferative activities; however, other studies indicate that this phytoestrogen may actually enhance the development of colon cancer (188) and is carcinogenic in neonatal mice (189). Thus, the biological effects of genestein may be organ specific. In the male mouse reproductive tract, genestein exerts estrogen-like effects, comparable with those present in soy-based diets. In neonatal animals, however, higher doses are needed to show estrogen-like effects (190). Neonatal exposure to genestein in mice during critical periods of differentiation may actually increase the incidence of uterine adenocarcinoma and mammary tumorigenesis (189, 191).

## 4.2. Monoterpenes

The cumint herb has been investigated as a new source of essential oil. It contains considerable amounts of oxygenated monoterpenes and small amounts of monoterpene and sesquiterpene hydrocarbons (192). In terms of the antioxidant activity of monoterpenes, one recent study tested 100 pure components of essential oils for antioxidant effectiveness and found that phenol constituents possessed the highest antioxidant activity (193). Additionally, the monoterpene hydrocarbons, terpinolene and  $\alpha$ - and  $\gamma$ -terpinene, showed significant protective action. A number of dietary monoterpenes have chemopreventive activity against rat mammary cancer and the monoterpenes act through multiple mechanisms in chemoprevention of mammary and other cancers (194). The diterpenes found in rosemary leaves have also been found to have antioxidant properties (195). Carnosic acid is a major phenolic diterpene present in rosemary leaves, with lesser amounts of 12-methoxycarnosic acid and carnosol. The antioxidant potency of carnosic acid was more than twice that of any other compound tested. A water-soluble extract of rosemary and its purified component, rosmarinic acid, had an effect on the xenobiotic-metabolizing enzymes in rat liver (196). The induction of xenobiotic-metabolizing enzymes by the water-soluble extract could be attributed to flavones, monoterpenes, or an additive effect of all components, as evidenced by the fact that a water-soluble rosemary extract selectively induced cytochrome P450 and enhanced detoxification enzymes (197). Examinations of the monoterpene d-limonene have shown that d-limonene produces tumors only in kidneys of male rats in association with hyaline-droplet nephropathy, which is because of the accumulation of the rat-specific, low-molecular-weight protein  $\alpha$ 2u-globulin in P2 segment cells of renal proximal tubules (198). There is, however, no risk of cancer for humans from d-limonene, because the binding of d-limonene to  $\alpha$ 2u-globulin would not occur in human cells.

The reproductive toxicity of  $\alpha$ -terpinene has also been reported (199). In rats, embryo and fetal toxicity occurs with doses greater than 30 mg/kg/d. Maternal

toxicity occurs at 125 mg/kg/d.  $\beta$ -Myrcene also has an effect on rat fertility and general reproductive performance (200). In male and female rats, exposure to it will increase liver and kidney weights. In postnatal rats, exposure causes days of appearance of primary coat, incisor eruption, and eye opening to be slightly delayed. The NOAEL for  $\beta$ -Myrcene has been set at 300 mg/kg body weight. The monoterpene, limonene, also causes additive toxicity in human lung cells. The detoxification of limonene in human lung cells occurs mainly by mechanisms not involving the glutathione system because 1,2-epoxide is not the active compound in limonene toxicity (201).

## 5. CHEMICAL REACTIONS IN FATS

### 5.1. Hydrogenation and Isomerization Reactions in Fats and Oils

Moderate amounts of *trans*-fatty acids from partially hydrogenated soybean oil (PHSBO) have an inhibitory effect on PUFA formation (202). In rodents, tissue contents of MUFA increase and PUFA decreases after consuming hydrogenated dietary fat (203). In concordance, total cholesterol (TC) and LDL-c increased after partially hydrogenated fat feedings. Partially hydrogenated fish oil (PHFO) contains a high amount of *trans*-fatty acids. Totally hydrogenated fish oil (THFO) contains minimal *trans*-fatty acids, but a high content of very long-chain saturated fatty acids (VLCSFA). When the absorption and metabolism of VLCSFA from THFO was studied in rats, a low absorption of VLCSFA was observed (204). The PHFO and THFO groups had reduced serum TC, and the PHFO group had increased serum TAG.

Hydrogenated fat consumption affects cholesterol synthesis in moderately hypercholesterolemic women (205). Plasma TC and LDL-c levels increase with increasing degrees of hydrogenation or saturated fat intake. The mechanism by which hydrogenated fat influences plasma lipid levels involves impairment of the catabolic pathway of cholesterol. Hydrogenated fat consumption also affects acylation-stimulating protein levels and cholesterol esterification rates in moderately hypercholesterolemic women (206). The alterations in the circulating lipid levels observed with the consumption of hydrogenated fat-rich diets can be explained partly by changes in acylation-stimulating protein activity and newly synthesized cholesterol. The degree of hydrogenation of dietary fish oil has been shown to have an effect on the *trans*-fatty acid content and enzymatic activity of rat hepatic microsomes (207). Partially hydrogenated vegetable oils have an early mortality effect in stroke-prone spontaneously hypertensive rats (SHRSP) (208). Incorporation and metabolism of *trans*-20:5 in endothelial cells leads to an effect on prostacyclin synthesis (209). In these cases, the survival time-shortening activity of partially hydrogenated soybean oil is exerted by the *cis*- and *trans*-isomers of oleic acid, or by an unidentified factor generated during partial hydrogenation.

**5.1.1. Conjugated Linoleic Acid (CLA)** CLA, present naturally in ruminant lipid sources or derived from thermal processing of oils, may have the capability to prevent cancer and heart disease, improve immune function, and alter body composition for treating obesity or building lean body mass (210). The biologically active isomers of CLA have antiproliferative effects on human colorectal and prostatic cancer cells, with the *trans*-10, *cis*-12-CLA isomer exhibiting the greatest potency against colorectal cancer proliferation. The *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers were moderately effective against prostate cancer (211). An oxidative mechanism appears to be involved in the growth-suppressive effects of *cis*-9, *trans*-11 CLA (212). The mechanism of CLA in decreasing colon tumor incidence in rats may possibly involve increased apoptosis (213). Aside from its anticarcinogenic properties, CLA has also been found to have cytotoxic effects on the antioxidant enzyme defense system in rat hepatocytes (214). The cytotoxic effects of CLA, as described by lactate dehydrogenase leakage and decreased gluconeogenesis, were not mediated by a pro-oxidant action in the hepatocyte, and CLA affected membrane integrity, metabolic function, cellular lipid composition, lipid peroxidation, and activity of antioxidant enzymes. Although CLA has been reported as peroxisome proliferators in mice, it has not been found to act as peroxisome proliferators in rats (215). In general, CLA has been found to have a lack of toxicity in rats, thus supporting the potential determination for the GRAS status of CLA (216). In humans, dietary exposure to CLA may reduce the proportion of body fat and affect fatty acid metabolism (217). The short-term consumption of CLA does not exhibit antithrombic properties in humans (218), nor does it offer health benefits regarding the prevention of atherosclerosis (219), altering blood cholesterol, nor lipoprotein levels. Although CLA does not appear to act as an antioxidant, its affinity to decrease polyenoic fatty acid concentrations could decrease the formation of highly cytotoxic lipid oxidation products such as MDA (220).

**5.1.2. Trans-Isomers and Cancer** A study conducted in postmenopausal women suggested an association between risk of breast cancer and the level of hydrogenated oil derived mono-*trans*-fatty acids was stored in the adipose tissue (221). It was also found that *trans*-fatty acid might cause colorectal neoplasia by interfering with the cell membrane function or eicosanoid metabolism (222). Increased adenoma prevalence was associated with the consumption of sweetened baked goods, oils, and condiments.

**5.1.3. Trans-Isomers and Coronary Heart Disease** An increased risk of developing heart disease has been linked to an intake of *trans*-fatty acids (223). The replacement of dietary saturated fatty acids by *trans*-fatty acids, for example, lowers serum HDL cholesterol and impairs endothelial function in healthy men and women (224). It also impairs flow-mediated vasodilation and decreases the activity of serum paraoxonase, which is an HDL-bound esterase that may protect against atherosclerosis (225).

**5.1.4. Effects of *Trans*-Isomers on Neonatal Growth** Plasma *trans*-fatty acids have been inversely related to birth weight and head circumference, but these results are unclear (226). What is clearer is the hypothesis that dietary *trans*-fatty acids inhibit the biosynthesis of long-chain PUFA with 20 and 22 carbon atoms, and thus affect infant development. These findings are supported by studies showing an inverse correlation of plasma *trans*-fatty acids with n-3 and n-6 long-chain PUFA in infants. *Trans*-fatty acids may inhibit the desaturation of linoleic acid to arachidonic acid and of alpha-linolenic acid to DHA (227). Heightened concern exists over *trans*-fatty acids and infant development, as a result of research showing the importance of n-3 and n-6 fatty acids (228). These studies have shown that *trans*-fatty acids inhibit the delta-6 desaturation of linoleic acid. In fetal, infant, and maternal tissues, there exists an inverse relationship between *trans*-fatty acids and measures of growth and development. Animal studies on the effect of *trans*-fatty acids and behavioral development of pre- and postnatal mice have reported that, although reversal learning in a T-water maze was slower in *trans*-fatty acid-supplemented groups, the long-term effects of *trans*-fatty acids on behavioral development and neural function needs more investigation (229).

## 5.2. Heterocyclic Amines (HAs)

Problems associated with the determination of HAs in cooked foods and with human exposure have been reported. The estimated daily intake of HAs, in different studies, ranges from 0–15 µg/person/day (230). In a study that looked at the heterologous expression of human N-acetyltransferases 1 and 2 and sulfotransferase 1A1 in *Salmonella typhimurium* for the mutagenicity testing of heterocyclic amines, it was found that both O-acetylation and O-sulfonation were important determinants for HA genotoxicity in humans (231).

The heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), is a mutagenic and carcinogenic heterocyclic amine (232). PhIP is metabolically activated by cytochrome P<sub>450</sub>-mediated N-hydroxylation, followed by phase II esterification. The mutagenic metabolite reacts with DNA-forming adducts, proteins, and other cellular constituents, resulting in unstable products that are degraded to 2-amino-1-methyl-6-(5-hydroxy) phenylimidazo[4,5-b]pyridine (5-OH-PhIP). Urinary 5-OH-PhIP can, therefore, be used as a biomarker for the genotoxic dose of PhIP. Studies show that 2-amino-3,8,-dimethylimidazo[4,5-f]quinoxaline (MeIQ<sub>x</sub>) is readily available to tissues for humans and rodents, and adduct levels are linear with an administered dose, except at high chronic doses where adduct levels plateau slightly (233).

HAs content in preprocessed meat cuts produced in Canada were present in 16 different types of processed meat cuts (234). The highest mutagenic activity was found in a smoked turkey breast sample; four samples had low mutagenic activity, and 11 samples were not mutagenic. The only HA found in the samples with mutagenic activity was MeIQ<sub>x</sub>. Conclusions have been made that the consumption of

meat cuts does not present a serious health risk from HAs contaminants. In the American diet, the estimated daily total HAs intakes for children is 11 ng/kg/d and for adults it is 7 ng/kg/d, with PhIP estimated to account for 65% of each intake (235). Pan-fried meats are the largest source of HAs in the average American diet, with chicken being the largest source of HAs among different meat types. In pork, the HAs type and level will vary with the pork product, cooking method, and doneness level (236), but the main HAs found in pork are IQ, MeIQ, MeIQx, DiMeIQx, and PhIP. In chicken, HAs content increases with increasing cooking temperature (237). PhIP formation starts accelerating at temperatures  $>200^{\circ}\text{C}$ . Recent studies have not found any correlation between color developments and HAs content in chicken (237). In pan-fried meat patties, HAs have been shown to induce bacterial mutagenicity and animal carcinogenicity and may be a risk factor for human cancer (238). Fast-food meat products appear to contribute to only a small percent of the estimated daily dietary intake of HAs (239).

The food-derived mutagen 2-amino-9 H-pyrido-[2,3-b]indole (A $\alpha$ C) has been reported to exhibit weak mammary gland carcinogenicity in mice (240). This observation may partly be associated with A $\alpha$ C-DNA adduct formation in the mammary gland epithelium. However, although the acute feeding of MeIQx in mice has produced hepatic tumors, chronic feeding did not cause outward signs of toxicity, although there is a slight increase in sister chromatid exchanges at 400-ppm MeIQx (241).

The carcinogenic effects of HAs can be extended to investigating various cancers in humans. When the duration of meat cooking is taken to be a marker of HAs content, the significantly raised and exposure-related increase in stomach cancer risk occurring in humans was associated with preferences of well-cooked meat as opposed to rare meat. This conclusion was based on the fact that HA content is greater in well-cooked meat (242). For example, the type of meat, method, and extent of cooking has been attributed to a two-fold increase in risk of colon or rectum tumors among those believed to have highest intake of HAs (243). Notwithstanding this, the differences in human cancer risk for HA ranges more than a thousand-fold between individuals based on exposure and genetic susceptibility (244).

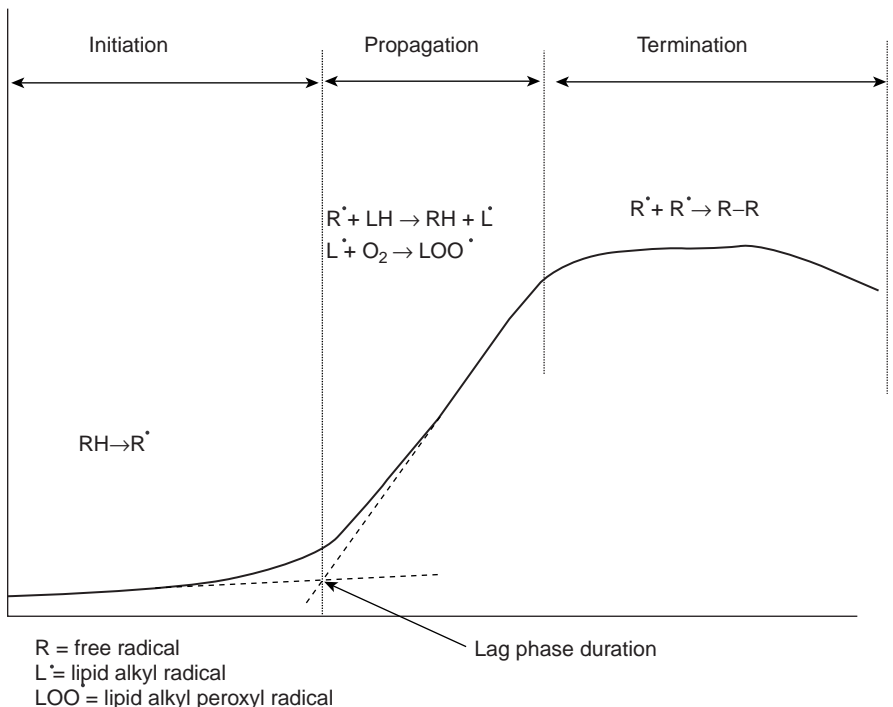
One animal study reported that either the tumor-promoting effects of MeIQx or PhIP were weak, or else the dose of BaP (initiation treatment) in the study was too high and masked the effects of MeIQx or PhIP (245). Several studies have confirmed that PhIP does affect mammary carcinogenesis in various strains of mice and rats (246). Upon the investigation of the formation of mutagenic/carcinogenic heterocyclic amines in dry-heated model systems, meats, and meat drippings, there were nine HAs found at concentrations greater than 0.1 ng/g in model systems, meat, or pan residues (247). Finally, various additives have been shown to have effects on the formation of heterocyclic amines in fried fish fiber (248). For example, HAs formation is retarded by the addition of a high level of sugar, and it is increased with increasing levels of MSG. Antioxidants do not show any consistent effect on HAs formation. Coconut oil, lard, and soybean oil all contribute to high levels of HAs. During heating, HA loss increases with both increasing temperature and heating time (249).



### 5.3. Lipid Oxidation Products (LOPs)

Lipid oxidation in both food systems and biological tissues exhibit the same temporal three-stage pattern of initiation, propagation, and termination (Figure 3).

In Vitamin E-controlled autoxidation of methyl linoleate, the 11-hydroperoxy derivative (11-hydroperoxylinoleate) was identified as the next most prominent primary initial peroxidation product after the 9- and 13-hydroperoxides (250). Feeding a high fish oil diet and associated amounts of n-3 polyunsaturated fatty acid does not propagate increased levels of phospholipid hydroperoxides or TBARS in rat organs (251). The formation of 8-oxo-2'-deoxyguanosine in DNA of intact human diploid fibroblast cells by lipid hydroperoxides are likely due to the generation of reactive species other than superoxide radicals and hydrogen peroxide (252). Feeding oxidized oil to rats causes several changes in lipid and fatty acid metabolism, including: a reduced rate of desaturation of linoleic acid and alpha-linolenic acid by microsomal delta4-, delta5-, and delta6-desaturase; a reduced ratio MUFA/SFA in the liver, suggesting reduced delta9-desaturation; small increases in, liver



**Figure 3.** A schematic diagram of temporal pattern of lipid oxidation in food and biological systems.

weight; and reduced tocopherol concentrations in both liver and plasma. In addition, reduced lipid concentrations in plasma and an increased ratio between phospholipids and cholesterol are biomarkers for the oxidative stress that occurs in the liver (254).

The interaction of lipid peroxides with cellular proteins may contribute to cellular aging. Tubulin, the building block of microtubules, is a potential target for cellular aging, and very low concentrations of phosphatidylcholine hydroperoxides are sufficient to interfere with tubulin and microtubule function (255). When rats are fed a thermally oxidized corn oil diet, higher concentrations of lipid peroxides appear in the liver and kidney (256), which corresponds to an increased liver weight, but decreased body weight gain. The iron redox cycle also catalyzes membrane lipid peroxidation and oxy-myoglobin oxidation, as evidenced by the oxidation of ferrous ions and ascorbic acid with auto-oxidation of myoglobin, and the generation of lipid peroxides by lipid radicals, which, in turn, contributes to the loss of oxy-myoglobin stability (257). Aldehyde lipid oxidation products (LOPs) also have an effect on myoglobin (258). Aldehyde LOPs alter myoglobin stability by increasing oxy-myoglobin oxidation, decreasing the ability of metmyoglobin to be enzymatically reduced, and enhancing the pro-oxidant activity of metmyoglobin. The acceleration of oxy-myoglobin oxidation is triggered by alpha,beta-unsaturated aldehydes via covalent attachment (259). Polyamines and spermidine, along with sulfhydryl-containing compounds glutathione and thiocetic acid, decrease headspace hexanal, a saturated aldehydic lipid oxidation product (260). The generation of lipid peroxy radicals from edible oils and associated biological activities results in a need to quench free radical components. For example, LOOH generated alkylperoxy radical ( $\text{LOO}^*$ ), following reactions with various heme compounds, such as myoglobin, cytochrome c, or hemin, exhibits cytotoxicity and causes DNA damage (261). Lipid hydroperoxides also modify proteins during myocardial ischaemia (262) and can contribute to the pathophysiology of ischaemic injury. Oxidation reactions, however, may not only represent toxicologic events, but rather modulate cell activity and function (263). For example, 4-hydroxynonenal (a LOP present in ox-LDLs) has a role in cell signaling by upregulating AP-1 transcription factors with an induction of a series of genes. Protein modifications are also initiators of oxidant-induced signal transduction pathways. Moreover, evidence exists that implicates a dietary source of plasma lipid peroxides, which becomes elevated in the postprandial state (264); a potential contributing factor for the correlation between postprandial hyperlipidemia and increased risk of CVD. There is also evidence in animal studies of proatherogenic properties of oxLDL and presence in atherosclerotic lesions (265). Reactive aldehydes generated from lipid peroxidation are involved in CVD (266). Another example lies with the role of oxidative stress in the pathophysiology of asthma (267). Lipid peroxidation, as determined by plasma isoprostanes, is related to disease severity in mild asthma. Tumor cell lines are sensitive to PUFA and to associated oxidation products (268). This sensitivity depends on the antioxidant defense mechanism, as well as on culture conditions. Hydroperoxy docosahexaenoic acid is a major metabolite, responsible for the cytotoxicity of DHA.

Uncovering the molecular structures of LOPs using high-resolution, two-dimensional  $^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance techniques have further uncovered the molecular structures of LOPs (269). Advances in chromatography using a combination of normal-phase high-performance chromatography with mass spectrometric detection of nonvolatile LOPs has been used to separate TG oxidation products (270, 271).

#### 5.4. Cholesterol Oxidation Products (COPs)

COPS have been identified and quantitated in a number of food sources that include egg, beef, pork, and butter (6). COPs exhibit cytotoxicity to a wide variety of cells leading to angiotoxic and atherogenic effects (272). They alter vascular permeability to albumin, modify prostaglandin synthesis, and stimulate platelet aggregation. COPs also alter the functionality of LDL receptors, modify cholesterol ester accumulation in various cells, and enrich the LDL particle in cholesterol esters. Oxysterols are also mutagenic and carcinogenic. When the cytotoxicities of cholesterol and a mixture of  $\beta$ -sitosterol/campesterol and related oxides were compared in the C57BL/6 macrophage cell line,  $5\alpha,6\alpha$ -epoxide or cholesterol oxides caused the greatest cell damage, followed by  $\beta$ -sitosterol/campesterol oxides, cholesterol, and  $\beta$ -sitosterol (273). When dietary COPs are absorbed and incorporated into rat lymph chylomicrons, postprandial lipoprotein particle size and composition are influenced (274). These changes may affect the clearance of chylomicrons from plasma, the arterial delivery of COPs, and the possible deposition in arterial lesions.

The oxysterol 7-ketocholesterol is an important COP involved in atherosclerotic lesions and macrophage foam cells (275). There is no direct evidence in humans that COPs contribute to atherogenesis, but it has been found that COP levels are elevated in LDL subfractions that are considered potentially atherogenic (276). In addition, raised levels of  $7\beta$ -hydroxycholesterol may be associated with an increased risk of atherosclerosis. Arterial injury by COPs causes endothelial dysfunction and arterial wall cholesterol accumulation (277). Even under normocholesterolemic conditions, COPs can cause endothelial dysfunction, increased macromolecular permeability, and increased cholesterol accumulation. These are all factors believed to be involved in the development of atherosclerotic lesions. The atherogenic potential of COPs has been demonstrated by *in vitro* cell culture (73, 278), as well as in animal feeding studies (279). Japanese quail fed either purified cholesterol or oxidized cholesterol exhibited greater plasma and liver cholesterol concentrations in association with increased severity of atherosclerotic lesions when fed the oxidized cholesterol (279).

Several COPs have been identified as having cytotoxic, angiotoxic, carcinogenic, and mutagenic bioactivities (280, 281), all or some of which may play a role in the initiation or proliferation of atherosclerotic plaque. Specifically, 25-hydroxycholesterol and cholestane- $3\beta,5\alpha,6\beta$ -triol are particularly toxic to cultured rabbit aortic smooth muscle cells (278). The 25-hydroxycholesterol

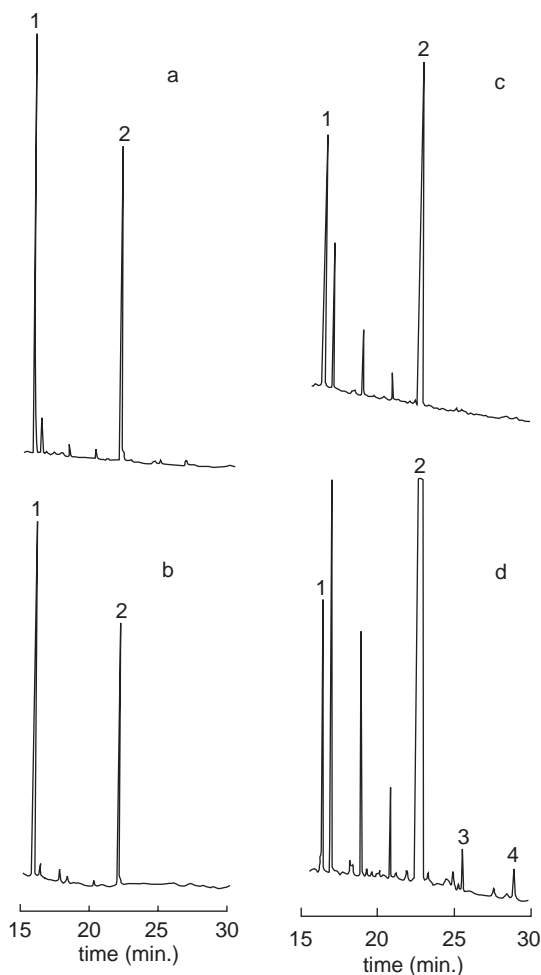
has been found to be preferentially transported in VLDL (34.7%) and LDL (55.1%), with HDL (10.2%) containing a much smaller proportion of the oral dose administered to squirrel monkeys (281). Thus, potentially angiotoxic COPs may be present in the lower density lipoprotein fractions of animals that develop atherosclerotic lesions. In human studies, detectable amounts of COPs, namely the cholesterol-5,6 $\alpha$ - and  $\beta$ -epoxides, the 7-hydroxycholesterol isomers, and 7-ketocholesterol, were reported in aortic tissue (75, 80). These same COPs have also been recovered in the LDL extracted from human atherosclerotic plaques (74). The presence of 7 $\beta$ -hydroxycholesterol in tissues may be caused by the auto-oxidation of cholesterol, whereas the 7 $\alpha$ -hydroxycholesterol isomer is known to be a product of *in vivo* oxidation in bile acid synthesis (280). Other workers have been able to confirm the identity of several COPs (cholest-3,5-diene-7-one, cholestanetriol, 7-hydroxycholesterols, 7-ketocholesterol, 24-hydroxy-, 25-hydroxy-, and 26-hydroxycholesterol) in human aortic specimens (75). In the Japanese quail and rat species comparison study shown herein, the pattern of COPs present in aorta of atherosclerosis susceptible (e.g., quail) was related to the hypercholesterolemia and arterial plaque score, not seen in the resistant (rat) species (Table 4). Using GC-MS, 7 $\beta$ -hydroxycholesterol and 7-ketocholesterol were identified and quantitated in quail aortic tissue exhibiting plaque formation (Figure 4). These COPs are identical to those reported from aortic plaques from both humans as well as other animal models, thereby validating the use of the atherosclerosis susceptible Japanese quail for research in the area of experimental atherogenesis.

The cytotoxicity and apoptosis-inducing potential of commonly occurring oxysterols might not be dependent on the sole generation of an oxidative stress (282). The potential genotoxicity of cholesterol oxidation products in two mammalian fibroblast cell lines showed that none of the COPs detected affected baseline levels of DNA strand breaks or sister chromatid exchanges (283). Thus, in this case, COPs were not genotoxic. Rather, a Ca<sup>2+</sup> influx through plasma membrane channels could be an important signal in the mechanism of COP-induced apoptosis (284). Thus, inflammatory cytokines may increase the cytotoxicity of LOPs.

**TABLE 4. Classification of Plant Sterols.<sup>1</sup>**

Primary	Series	Examples
Cholesterol	4-desmethyl sterols	Campesterol, stigmasterol, $\beta$ -sitosterol, $\Delta^5$ -avenasterol, $\Delta^7$ -stigmasterol, $\Delta^7$ -avenasterol,
4-methyl-cholestane	4-monomeethyl sterols	Atrastandienoligramisterol
Lanostane	4,4'-dimethylsterol	Cycloarterol,

<sup>1</sup>Ref. (90).



**Figure 4.** Gas chromatograph-FID chromatogram of Wistar rat (A&B) and atherosclerosis-susceptible Japanese quail (C&D) aorta derivatized nonsaponifiables. (A) 0.05% low cholesterol, rat; (B) 0.5% high cholesterol, rat; (C) 0.05% low cholesterol, quail; (D) 0.5% high cholesterol quail aorta. 1 = internal standard, 5 $\alpha$ -cholestane; 2 = cholesterol; 3 = 7 $\beta$ -hydroxycholesterol; 4 = 7-ketocholesterol. Samples were analyzed according to Yuan et al. (69).

## 6. ADVERSE PRODUCTS FROM OVERHEATED FATS AND OILS

Some investigators have found gender and organ-specific toxicity in normal and malnourished rats fed thermoxidized palm oil (TPO) (285). The hearts of the first offspring of male and female rats were enlarged, whereas the lung, liver, and kidneys of first filial female offspring were reduced in size. This observation suggests the toxicities of TPO could be cumulative for female offspring. TPO also

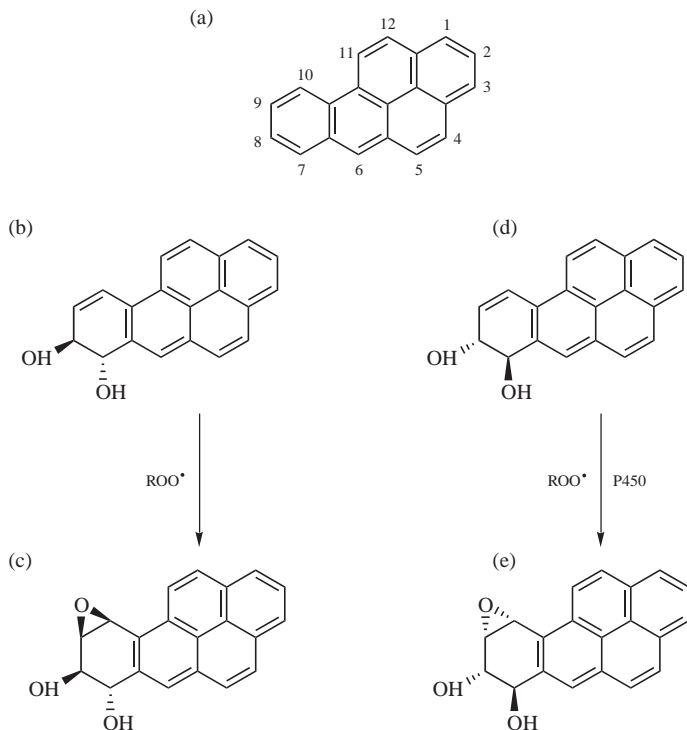
induces reproductive toxicity in healthy and malnourished rats (286). Fetotoxicity was observed in TPO-fed rats, where the neonatal birth weights and litter size were decreased; pregnancy rates in TPO-fed rats were also decreased (55%). The dangers of thermally oxidized dietary fat for colon carcinogenesis in rodents are another relevant effect of heating fats and oils (287). In such animal studies, extensively oxidized beef tallow increased the number of animals with aberrant crypt focus and average ACF per colon. It has also been shown that certain fractions of heated fats ("total polar materials") cause growth retardation, as well as increasing liver and kidney weights, and can cause disorders of the enzyme system if fed in high doses (288). An effect of oxidized dietary oil on plasma cholesterol and thyroid hormone concentrations has been reported in miniature pigs fed on a hyperlipidaemic diet (289). Consistent with rat studies, feeding oxidized oil reduced concentrations of cholesterol in plasma and in LDL and HDL fractions. It also reduced the concentration of  $\alpha$ -tocopherol in the plasma, reduced the concentration of tri-iodothyronine in the plasma, and elevated the ratio between thyroxine and tri-iodothyronine. The only time that long-term feeding effects of heated and fried oils on lipids and lipoproteins in rats does not show any deleterious effect on growth, plasma, and tissue lipid profile of rats is when the conditions of the heating or frying are not too drastic and the oils are not heat-abused (290). In humans, studies have shown impaired endothelial function following a meal rich in used cooking fat (291). For example, the ingestion of a meal rich in fat previously used for deep-frying in commercial fast food restaurant resulted in impaired arterial endothelial function. For red palm oil, the effects of chronic consumption of fresh and heated red palm oil on lipid profile and lipid peroxidation are comparable on serum cholesterol as well as on lipid peroxidation, but prolonged heating of oil increases LDL-c levels, which may make it more atherogenic (292).

The feeding of fried fish that had been fried in the same batch of oil over several days to weanling rats confirmed several adverse effects of reheating dietary oil (293). The weanling rats eventually exhibited a decreased feed consumption and weight gain, a decrease in total lipid and cholesterol content of liver, but an increase in total lipid and cholesterol in heart and serum cholesterol levels. These biochemical changes corresponded to initial stages of cell damage in liver and kidney tissues. More specifically, cyclic fatty acid monomers from heated oil modify the activities of lipid synthesizing and oxidizing enzymes in rat livers (294). The feeding of these cyclic fatty acid monomers has shown evidence of a peroxisome proliferator-like effects. They also induced a coordinated regulation between activities of the lipogenic enzymes studied ( $\Delta^9$ -desaturase, phosphatidate phosphohydrolase) and peroxisomal oxidation. A dose-dependent decrease of  $\Delta^9$ -desaturase with cyclic fatty acid intake accompanied by a similar decrease of MUFA level in the liver and the increase in  $\gamma$ -linolenic acid level suggests an increase in  $\Delta^6$ -desaturase with cyclic fatty acid intake. In short, cyclic fatty acid monomers affect different aspects of lipid metabolism, including a phenotypic peroxisome proliferator response. The frying process has formed cyclic fatty acids (295), and structures formed from oleic, linoleic, and linolenic acids in heated frying oils have been determined.

Aromatic amines from cooking oil fumes are known to be carcinogenic for bladder cancer. Fume samples from three commercial cooking oils commonly used in Taiwan were mutagenic in the presence of an S-9 mix (296). Exposure to cooking oil fumes, such as those from safflower oil, vegetable oil, and corn oil, may also increase exposure to PAHs, which, in turn, has been linked to an increased risk of lung cancer (297). Frying sunflower oil can lead to a potentially toxic product, causing decreased food efficiency ratio, growth retardation, and changes in liver fatty acid composition if fed (298). The use and abuse of frying oil on the quality of frying oil discarded by 16 catering establishments revealed that many discarded oils contained free fatty acids that were higher in concentration than the recommended safe level (299). A recent quality and sensory evaluation of used frying oil from restaurants observed that sensory parameters are reliable indicators of the quality of used frying oil (300). There appears to be a good correlation between sensory evaluation and the actual analysis of total polar components and oxidized acid levels. The frequent addition of fresh oil throughout the frying process of frozen foods, such as prefried potatoes that undergo deep-fat frying in sunflower oil, minimizes fatty acid changes (301).

## **7. TOXIC SUBSTANCES PRODUCED DURING SMOKING, CHARBROILING, AND BARBECUING OF FOODS**

The analysis of 200 food items for benzo[a]pyrene (B(a)P) content indicated that the highest levels of B(a)P were found in grilled/barbecued, very well-done steaks and hamburgers and in grilled/barbecued, well-done chicken with skin (302). The formation of polycyclic aromatic hydrocarbons in the smoke from heated model lipids and food lipids reflects model lipids as being more susceptible to smoke formation than food lipids during heating (303). Methyl linolenate lipid produced the highest amount of PAHs, followed by methyl oleate and methyl stearate. Soybean oil generated larger amounts of PAHs than canola or sunflower oils, and benzene-like compounds were found to be possible precursors for PAHs formation. Numerous studies have been conducted to monitor the formation of PAHs in different smoked meat products and smoke flavor additives (304–306). Smoke flavorings obtained from different types of wood render different concentrations of PAHs (307). Flavoring from poplar wood appears to give the highest number and concentrations of both total and carcinogenic PAHs, but the storage of smoke flavorings in polyethylene flasks reduces the concentration of some PAHs. The removal of PAHs from water by migration into polyethylene is a process that has also been investigated (308). PAHs are primarily absorbed on the polyethylene surface with subsequent migration into the bulk polymer. This transportation of PAHs through the bulk can be described by Fickian laws of diffusion and is consistent with the theory of depth absorption of PAHs in polyethylene. Changes in total fat content, fatty acid composition, tocopherol, ascorbic acid, pH, and oxidation often occur in Atlantic salmon in response to either cold smoking (20°C or 30°C) or electrostatic smoking (309). The leaner the fish, the higher percentile loss in fillet fat with these



**Figure 5.** Reactive oxygen species derived from co-oxidation catalyst of benzy(a)pyrene (B(a)P) diol oxidation. (a) B(a)P; (b) (+)B(a)P diol; (c) (-)anti-diolepoxide B(a)P enantiomer; (d) (-)B(a)P diol; (e) (+)anti-diolepoxide enantiomer.  $ROO^*$  = peroxy radical; P-450 monooxygenase. The (-)B(a)P-7,8 diol enantiomer is generated from B(a)P in vivo from both  $ROO^*$  and cytochrome P-450 (6).

smoking processes. Regardless of smoking temperature, ascorbic acid decreased about 80% from the fresh value in cold-smoked fish, whereas dry-salted and electrostatically smoked fish lost only about 10% of the fresh ascorbic acid content. Electrostatically smoked fish had a smaller drop in fillet pH than cold-smoked fillets.

Investigations that examined the direct mutagenicity of the polycyclic aromatic hydrocarbon-containing fractions of smoked and charcoal-broiled foods treated with nitrite in acid solution indicated that nitrite could convert most samples to direct-acting mutagens toward both strains (310). The treatment of PAHs with nitrite in acid solution produced some non-N-nitroso direct-acting mutagens, which may belong to nitro-PAHs. Thus, consumption of charcoal-broiled and smoked foods, along with nitrite, is generally not recommended. Animal studies have shown that B(a)P treatments will induce squamous cell carcinoma, papiloma, and hyperplasia in the forestomach at incidences of 18% and 91%, respectively (311). B(a)P also produced malignant lymphoma with an incidence of 18%, an increased



leukocyte count and decreased erythrocyte count, and a decreased body weight. In addition, bronchiolar-alveolar hyperplasia in the lung at incidences of 18% and 9%, respectively, were induced. Further risks of frying foods have been identified, such as those caused by the airborne particulates generated during frying of beef, fish, and pork, which can induce carcinogen-metabolizing cytochromes P450 1A1 and 1B1 in human lung-derived cell line CL5 (312). The mutagenic risk posed by simple, well-characterized mixtures of priority PAHs are estimated to be the sum of the risks posed by the mixture components (313). Peroxyl- radical and cytochrome P450 dependent oxidation reactions contribute to co-oxidation processes and increases both the number and the reactive toxicity of products derived from auto-oxidation of xenobiotic agents, such as benzo(a)pyrene (Figure 5).

## 8. POTENTIAL HAZARDS FROM GOVERNMENT APPROVED ANTIOXIDANTS

A review of butylated hydroxyanisole (BHA) and butylated hydroxy toluene (BHT) experimental studies on potential genotoxicity and carcinogenicity concluded that BHA and BHT pose no cancer hazard and may be anticarcinogenic at the current levels of food additive use (314). However, both carcinogenic and anticarcinogenic properties have been reported for BHA and BHT. The association between the dietary intake of BHA and BHT and stomach cancer risk was studied in a Netherlands cohort study that started in 1986 (315). There was no significant association found between usual intakes of BHA and BHT and stomach cancer risk. In a rat study, the forestomach carcinogenicity of BHA was compared in males of the F344, SHR, Lewis, and Sprague-Dawley rat strains (316). Forestomach squamous cell papillomas and hyperplasias were developed in all rats given BHA, but the incidence of squamous cell carcinomas (SCCs) was different, with the highest in the SHR strain. Cytotoxicity was also the most severe in the SHR strain. Thus, rat strain differences in BHA forestomach carcinogenesis can exist and sensitivity to cytotoxicity may be an important factor. The growth modulation of enzyme-altered preneoplastic liver foci (EAF), by BHT feeding to rats bearing enzyme-altering hepatic preneoplastic lesions, has shown that the induction of EAF and BHT treatment resulted in a reduction in the natural killer cell activity of splenocytes (317). This response could be a contributing factor in the enhancement of rodent liver neoplasia by BHT (318). When BHT was fed to rats, an increased liver weight corresponding to a gradual vacolization of liver cells, cytoplasmic disintegration, and hepatocellular necrosis, were detected without any sign of tumorigenicity. In mice, BHT causes lung injury and promotes tumor formation. Thus, a recent study looked at the hydroxylation of a tert-butyl group on BHT, which yields 6-tert-butyl-2-[2'-(2'-hydroxymethyl)-propyl]-4-methylphenol (BHTOH) (319). BHTOH is more potent than BHT. BHT, BHTOH, and other BHT metabolites were compared in respective affinities to kill nontumorigenic and tumorigenic mouse and human lung cell lines. BHTOH was the most toxic and induced apoptosis the greatest. In other animal studies, feeding broiler chickens BHT for

six weeks resulted in hyperaemia, sinusoidal distension, focal necrosis, and mononuclear cell infiltration, along with fatty changes in liver, hyperaemia, tubular degeneration, fibrosis, and cloudy swelling in the kidneys (320). Thus, BHT is hepatotoxic and nephrotoxic in broilers. The effects of BHA and BHT on DNA adduct formation and arylamines in N-acetyltransferase activity in PC-3 cells (human prostate tumor) in vitro showed that the higher the concentration of BHA or BHT, the higher the inhibition of N-acetyltransferase (NAT) activity (321). Exposure to BHA or BHT also decreased DNA adduct formation in PC-3 cells. A Brazilian study that attempted to estimate yields of the theoretical maximum daily intake of phenolic antioxidants BHA, BHT, and TBHQ, found that the current acceptable daily intakes of BHA, BHT, and TBHQ were unlikely to be exceeded by the average Brazilian consumer (322). Oxidative DNA damage and apoptosis can be induced by metabolites of BHT such as BHT-quinone, BHT-OOH, and BHT-CHO (323). It is noteworthy that where BHT-OOH participates in oxidative DNA damage directly, BHT-quinone causes DNA damage through H<sub>2</sub>O<sub>2</sub> generation, which leads to internucleosomal DNA fragmentation.

### 8.1. Allylbenzenes

Allylbenzene analogs, including safrole, eugenol, and estragol, are flavor compounds derived from essential oils and metabolized by biotransformation in the liver to form potentially reactive electrophilic intermediates. The major route of bioactivation is via the hydroxylation of the 1' carbon atom on the allylic side chain. The 2',3'-allylic epoxide derivatives of allylbenzene, estragole, eugenol, and safrole have been used to determine the genotoxicity of epoxidation at the allylic double bond for allylbenzene and its analogs (324). The epoxide formation at the allylic double bond is a potentially genotoxic bioactivation pathway for allylbenzene analogs. Although the epoxidation pathway of allylbenzene poses a potential genotoxic threat to humans, no actual genotoxicity occurs as a result of its further metabolism.

The essential oils of dill (*Anethum graveolens* L.), peppermint (*Mentha piperita* L.), and pine (*Pinus sylvestris* L.) have been found to be cytotoxic for human lymphocytes (325). These oils have different levels of activity for chromosome aberration and sister chromatid exchange in human lymphocytes, with dill seeds usually being the most active. In contrast to this activity, a dose-dependent increase in mutation frequency has been found in pine and dill herb oils, where dill seed oil has been found to be almost inactive. Peppermint oil has been found to have a dose-independent effect on mutations. In terms of the genotoxicity of alkenylbenzenes in rodents, it has been accepted that  $\alpha$ - and  $\beta$ -asarone are hepatocarcinogenic, whereas two other alkenylbenzenes, myristicin and elimicin, are not. Elimicin and  $\alpha$ - and  $\beta$ -asarone, but not myristicin, are genotoxic in an unscheduled DNA synthesis assay using rat hepatocytes (326). Simple allylbenzenes, such as safrole and estragole, are activated by 1-hydroxylation and sulfation, and this is the likely mechanism of the genotoxicity of elimicin. Moreover, the propenyl analogues isosafrole, anethole, and methylisoeugenol, which cannot undergo 1-hydroxylation, are not genotoxic. Another comparative induction of unscheduled DNA

synthesis in cultured rat hepatocytes by allylbenzenes and 1'-hydroxy metabolites 1'-hydroxyestragole, -methyleugenol, and -safrole indicated greater genotoxicity than parent compounds, the allylbenzenes (327).

Pretreatment of mice with *trans*-anethole and eugenol produced antigenotoxic effects against cyclophosphamide (CPH), procarbazine (PCB), N-methyl-N-nitro-N-nitrosoguanidine (MNNG), and urethane (URE). *Trans*-anethole also inhibits the genotoxicity of ethyl methane sulfonate (EMS) (328). Both *trans*-anethole and eugenol gave a dose-related antigenotoxic effect against PCB and URE. Under *in vitro* conditions, *trans*-anethole does not increase the mutant frequency in the Salmonella/microsome test, but a dose-related response occurs in the L5178Y mouse lymphoma TK+/- assay with metabolic activation (329). *Trans*-anethole also induces chromosome aberrations *in vitro* in Chinese hamster ovary cells, but does not induce chromosome aberrations. A continuous intake of high doses of *trans*-anethole in rats will induce a continuum of cytotoxicity, cell necrosis, and cell proliferation (330). Regardless, *trans*-anethole has been reaffirmed as GRAS (generally recognized as safe) because of a functional metabolic detoxication pathway in humans, and its lack of mutagenic or genotoxic potential.

Safrole has been found to be a weak hepatocarcinogen, which is linked to the formation of safrole-DNA adducts. Safrole treatment will induce oxidative damage in rat hepatic tissue (331) and is a genotoxic carcinogen in rat liver *in vivo* (332). These cytogenetic effects may result from covalent DNA modification in the rat liver. The threshold cytotoxic concentration found for safrole is 0.10 µg/ml safrole (333).

## 8.2. Migration of Packaging Materials

The effects of different plastic films (PET, PVC, PP, and PS) on the stability of olive, sunflower, and palm oils at 24°C and 37°C during 60 days storage, indicated that changes in peroxide and TBA values were higher in plastic bottles than in glass (334). Plastic permeability has a major effect on oil stability, with both BHA and BHT capable of leaching out from plastic films into oils. PVC has the highest stability of oil samples, followed by PET, PP, and PS. Increasing storage temperature accelerates the oxidation and limited stability of vegetable oils.

The exposure of pregnant female rats to different concentrations of bis (2-Ethylhexyl) phthalate (DEHP) in drinking water produced a decrease in the kidney and testes weights, and increase in liver weight (335). Signs of histological damage in kidneys, liver, and testes were observed, and pups exposed to the highest DEHP concentration had an increase in time required to perform a learned avoidance test. The NOAEL for DEHP and DNOP, respectively, are estimated to be 50 ppm or 3.7 mg/kg/day for DEHP and 500 ppm or 36.8 mg/kg/day for DNOP (336). Unfortunately, it has been reported that retail packaging of small portions of cheese, even in a "low-migration" PVC cling film, may lead to consumer intakes of DEHA close to or above the tolerable daily intake of 0.3 mg/kg body weight (337). Investigators have evaluated the significance of the potential contamination of polyethylene terephthalate bottles when reused as food packaging material (338). Changes

in product flavor, caused by aroma sorption and the transfer of undesirable flavors from packaging to foods, are important mechanisms of deterioration when foods are packaged in polymer-based materials (339). Product considerations include sensitivity to flavor and related deteriorations, color changes, vitamin loss, microbial activity, and amount of flavor available. Storage considerations include temperature, time, and processing method.

The rate of migration of styrene from general-purpose polystyrene indicates a relatively weak dependence of the diffusion coefficient on the residual styrene content and a strong dependence on temperature. Monitoring the level of styrene migration from PS cups in different foods indicates that the styrene migration is strongly dependent on fat content and storage temperature (340). Styrene monomers, styrene dimers, and styrene trimers that migrated from polystyrene containers into instant food exhibit no endocrine-disrupting effects that include apparent estrogenic, androgenic, anti-androgenic, and thyroid activity (341). Blends of nylon 6 and ethylene-co-vinyl alcohol have been used as innovative food-packaging materials (342).

## **9. MANUFACTURING HAZARDS IN PROCESSING CRUDE OILS AND FATS**

### **9.1. Pesticide Residue, PAH Contamination, and Polychlorinated Biphenyls**

Samples of crude fish oil refined and at each step of the refining process have been analyzed for organochlorine pesticides and PCB (343). The deodorization step causes a decrease in the amount of contaminants, especially the most volatile compounds. Also, the concentrations of less volatile organochlorine pesticides and PCB are reduced to about half the concentration in the crude oil. Cooked lake trout has significantly less PCB and PAH residue than raw (344). Interestingly, smoking results in a greater losses of pesticides and PCBs than other cooking methods, but PAH showed greater formation during smoking. Consumption of large quantities of contaminated fish oils by children may lead to behavioral and neurological effects (345). In food samples obtained from Catalonia, a toxic potency of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) was reported (346). TEQ (PCB) contribution varied from 27% in olive oil to 81% in mussel samples. Thus, regulation of TEQ content in foods should include not only the TEQ(PCDD/F), but also TEQ(PCB). In January 1999, in Belgium, 500 tonne of feed contaminated with PCB and dioxins were distributed to animal farms, resulting in contamination of the food chain (347). The contamination was caused by transformer oil, rather than other environmental sources, and the mean intake/kg body weight for Belgians was estimated to be maximally 25,000 ng PCB and 500 pg international TEQ dioxins. An estimated total number of cancers resulting from this incident ranged between 40 and 8000. Neurotoxic and behavioral effects in neonates were expected, but not quantified,

therefore leading to a reassessment of PCDD/PCDFs and Co-PCBs toxicity in contaminated rice bran oil responsible for the “Yu-Cheng” disease (348). There is evidence for PCBs as neurodevelopmental toxicants in humans from previous studies, such as two U.S. prospective longitudinal studies that analyzed cord, maternal serum, and maternal milk samples, and a Taiwanese study on infants exposed to PCB and polychlorinated dibenzofurans because of maternal ingestion of highly contaminated rice oil (349). The response to shellfish contamination following an oil spill on the Oregon coast and some of the complication factors affecting evaluation of potential health risks from the consumption of oil-contaminated shellfish has been reported (350).

## 9.2. Toxic Oil Syndrome

Oil refined by ITH and distributed by RAELCA was the principle, and probably only, oil responsible for the TOS epidemic that occurred in Spain in 1981 (351). Regarding this incident, the oil contaminants have been detected and a possible chemical link between oil contaminants and those detected in L-tryptophan, implicated in the eosinophilia-myalgia syndrome (EMS), has been found (352). TOS is strongly associated with the consumption of oils containing fatty acid esters of 3-(N-phenylamino)-1,2-propanediol (PAP), but it is unknown whether PAP esters are simply markers of toxicity of oils or have the capability to induce the disease (353, 354). One study assessed the effect of several refining process variables on the formation of PAP esters (355). These variables include storage time prior to refining as well as elevated refining temperatures. Among the survivors of TOS, the prevalence of some chronic conditions (e.g., sclerodermia, neurologic changes) is high twenty years after its onset. 3-(N-phenylamino)-1,2-propanediol PAP esters are absorbed in the gi tract and are distributed and stored in different organs, particularly in the liver and brown adipose tissue (356). PAP in these organs showed different patterns of fatty acids, indicating the ability of the gi tract to modify the fatty acid composition of the parent PAP. Also, some PAP esters, when a long acyl chain was present in the *sn*-1 position of the molecule, showed an inhibitory effect on the PAF synthesis. This is an important observation in line with the systemic nature of TOS. A recent study of a cohort of 758 TOS patients found an increased prevalence of cardiovascular risk factors, such as arterial hypertension (34%), dyslipemias (44%), overweight (40%), obesity (27%), carbohydrate intolerance (9%), and diabetes mellitus (9.4%) (357). The most common reported symptoms were cramps (78%), arthralgias (78%), and paresthesias (70%). Only 2.8% of patients reported to be asymptomatic. The analytical results most common were changes in lipidic and carbohydrate metabolism, overt or subclinical hypothyroidism (6.6%) and respiratory changes in patients with no previous pulmonary disease, changes in spirometry (6%), diffusion test (8%), and hypoxemia (18%).

When guinea pigs were fed diets supplemented with oil related to TOS, the TOS-related oil produced a decrease in lipid peroxidation products with minimal alterations in phospholipids fatty acid composition of liver microsomes (358). TOS is an exogenously induced autoimmune disease in humans, believed to be a

result of the ingestion of oleic acid anilides. When the in vitro effects of anilides on splenocytes and T cells in A/J and B10.S mice were compared, it was shown that anilides are able to affect the immune system in a strain-dependent way and may therefore take part in inducing TOS in humans and mice (359).

## 10. CONCLUSIONS

The combined increased awareness by the consumer concerning the health and safety of fat consumption and the technological advances made by the food industry to provide a safe and nutritious product with sensory appeal for human consumption has resulted in a relatively safe and highly scrutinized food matrix. There are, however, situations of untold consumer exposure to invisible lipid-soluble xenobiotic agents that enter this particular food matrix from both intentional addition, or as a consequence of environmental contamination or reactions of labile constituents. In these cases, risk assessment strategies are required to predict potential harm to consumers exposed to tolerable intakes of these materials. On the other hand, fats and oils contain many important nutrients and “extranutrients” that have important roles in maintaining healthy lifestyles. In particular, with the advent of functional foods and nutraceuticals, many specific food products that contain lipid nutrients or lipid-soluble bioactive constituents will reach the consumer with the purpose of enhancing health and wellness.

## REFERENCES

1. J. E. Kinsella, B. Lokesh, and R. A. Stone, *Amer. J. Clin. Nut.*, **52**, 1 (1990).
2. M. B. Katan, S. M. Grundy, P. Jones, M. Law, T. Miettinen, and R. Paoletti, *Mayo Clin. Proc.*, **78**, 965 (2003).
3. A. C. Carr, B. Z. Zhu, and B. Frei, *Circ. Res.*, **87**, 349 (2000).
4. H. Frandsen, H. Frederiksen, and J. Alexander, *Food Chem. Toxicol.*, **40**, 1125 (2002).
5. M. D. Easton, D. Lusznik, and E. Von der Geest, *Chemosphere*, **46**, 1053 (2002).
6. D. D. Kitts, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, 5th ed., John Wiley & Sons, Inc., New York, 1996, pp. 215–280.
7. L. Y. Sheen, H. W. Chen, Y. L. Kung, C. T. Liu, and C. K. Lii, *Nutr. Cancer.*, **35**, 160 (1999).
8. J. E. Thmlinson, R. Nakayama, and D. Holten, *J. Nutr.*, **118**, 408 (1988).
9. J. F. Focant, C. Pirard, and E. De Pauw, *Chemosphere*, **54**, 137 (2004).
10. E. N. Frankel, *Chem. Phys. Lipids*, **44**, 73 (1987).
11. I. Staprans, D. A. Hardman, X. M. Pan, and K. R. Feingold, *Diabetes Care.*, **22**, 300 (1999).
12. I. Hayam, U. Cogan, and S. Mokady, *J. Nutr. Biochem.*, **4**, 563 (1994).
13. R. Wilson, K. Lyall, L. Smyth, C. E. Fernie, and R. A. Riemersma, *Free Rad. Biol. Med.*, **32**, 162 (2002).

14. I. Hayam, U. Cogan, and S. Mokady, *Biosci. Biotech. Biochem.*, **61**, 1011 (1997).
15. P. M. Chao, C. Y. Chao, and C. Huang, *J. Nutr.*, **131**, 3166 (2001).
16. C. M. Yang, C. W. Kendall, D. Stamp, A. Medline, M. C. Archer, and W. R. Bruce, *Nutr. Cancer*, **30**, 69 (1998).
17. K. Traul, A. Driedger, D. Ingle, and D. Nakhasi, *Food Chem. Toxicol.*, **38**, 29 (2000).
18. N. B. Cater, *Food Chem. Toxicol.*, **35**, 428 (1997).
19. Y. Tsai, S. Park, J. Kovacic, and J. Snook, *Lipids*, **34**, 895 (1999).
20. A. Lichtenstein, and U. Schwab, *Atherosclerosis*, **150**, 227 (2000).
21. Y. V. Yuan, D. D. Kitts, and D. V. Godin, *J. Nutr.*, **128**, 1620 (1998).
22. Y. V. Yuan and D. D. Kitts, *J. Nutr.*, **133**, 679 (2003).
23. P. Khosla and K. C. Hayes, *Biochem. Biophys. Acta.*, **1210**, 13 (1993).
24. A. Pronczuk, P. Khosla, and K. C. Hayes, *FASEB J.*, **8**, 1191 (1994).
25. F. Hu, R. van Dam, and S. Liu, *Diabetologia*, **44**, 805 (2001).
26. J. Lovejoy, *Current Atheroscler. Rep.*, **1**, 215 (1999).
27. M. Manco, G. Mingrone, A. Greco, E. Capristo, D. Gniuli, A. De Gaetano, and G. Gasbarrini, *Metabolism*, **49**, 220 (2000).
28. B. Vessby, M. Uusitupa, K. Hermansen, G. Riccardi, A. Rivellesse, L. Tapsell, C. Nalsen, L. Berglund, A. Louheranta, B. Rasmussen, G. Calvert, A. Maffetone, E. Pederen, I. Gustafsson, and L. Storlien, *Diabetologia*, **44**, 312 (2001).
29. F. Perez-Jimenez, J. Lopez-Miranda, and P. Mata, *Atherosclerosis*, **163**, 385 (2002).
30. D. O'Bryne, S. O'Keefe, and R. Shireman, *Lipids*, **33**, 149 (1998).
31. B. Halvorsen, A. Rustan, L. Madsen, J. Reseland, R. Berge, P. Sletnes, and E. Christiansen, *Annals Nutr. Metab.*, **45**, 30 (2001).
32. D. O'Bryne, K. Knauff, and R. Shireman, *Lipids*, **32**, 687 (1997).
33. E. Sarkkinen, U. Schwab, L. Niskanen, M. Hannuksela, M. Savolainen, K. Kervinen, A. Kesaniemi, and M. Uusitupa, *European J. Clin. Nutr.*, **50**, 592 (1996).
34. P. Kris-Etherton, *J. Nutr.*, **129**, 2280 (1999).
35. P. Kris-Etherton, T. Pearson, Y. Wan, R. Hargrove, K. Moriarty, V. Fishell, and T. Etherton, *Amer. J. Clin. Nutr.*, **70**, 1009 (1999).
36. C. Aguilera, M. Ramirez-Tortosa, M. Mesa, and A. Gil, *Recent Res. Develop. Lipids Res.*, **4**, 369 (2000).
37. P. Yaqoob, *Eur. J. Clin. Nutr.*, **56**, S3:S9 (2002).
38. K. Ando, K. Nagata, R. Yoshida, K. Kikugawa, and M. Suzuki, *Lipids*, **35**, 401 (2000).
39. M. Fukushima, T. Matsuda, K. Yamagishi, and M. Nakano, *Lipids*, **32**, 1069 (1997).
40. O. A. Al-Shabanah, *Food Chem. Toxicol.*, **35**, 769 (1997).
41. M. Wettasinghe and F. Shahidi, *Food Chem.*, **70**, 17 (2000).
42. M. M. Engler and M. B. Engler, *Nutr. Res.*, **18**, 1533 (1998).
43. Y. Naito, K. Kasama, H. Yoshida, and N. Ohara, *Food Chem. Toxicol.*, **38**, 811 (2000).
44. J. D. Palombo, S. J. DeMichele, J. W. Liu, B. R. Bistran, and Y. S. Huang, *Lipids*, **35**, 975 (2000).
45. T. Kumamoto and T. Ide, *Lipids*, **33**, 647 (1998).
46. M. Fukushima, T. Ohhashi, S. Ohno, H. Saitoh, K. Sonoyama, K. Shimada, M. Sekikawa, and M. Nakano, *Lipids*, **36**, 261 (2001).

47. J. B. Hansen, R. K. Berge, A. Nordoy, and K. H. Bonna, *Lipids*, **33**, 1123 (1998).
48. P. Weber, *Lipids*, **34**, S269 (1999).
49. M. Noguchi, *Food Chem. Toxicol.*, **35**, 633 (1997).
50. A. Nordoy, R. Marchioli, H. Arnesen, and J. Videback, *Lipids*, **36**, S127–S129 (2001).
51. C. J. Huang and M. L. Fwu, *J. Nutr.*, **122**, 1182 (1992).
52. S. D. Clark and D. B. Jump, *Prog. Lipid Res.*, **32**, 139 (1993).
53. L. Demaison and D. Moreau, *Cell Mol. Life Sci.*, **59**, 463 (2002).
54. Y. Y. Fan, K. S. Ramos, R. S. Chapkin, in 91st AOCS annual Meeting and Expo, San Diego, California, 2001.
55. Y. Y. Fan, K. S. Ramos, and R. S. Chapkin, *J. Nutr.*, **131**, 1675 (2001).
56. S. Hrelia, A. Bordoni, P. Biagi, C. Rossi, L. Bernardi, D. Horrobin, and A. Pession, *Biochem. Biophys. Res. Commun.*, **225**, 441 (1996).
57. R. K. Furse, R. G. Rossetti, and R. B. Zurier, *J. Immunol.*, **167**, 490 (2001).
58. S. Kaku, K. Ohkura, S. Yunoki, M. Nonak, H. Tachibana, M. Sugano, and K. Yamada, *Prostaglandins Leuko. Essential Fatty Acids*, **65**, 205 (2001).
59. W. Jiang, S. Hiscox, D. Horrobin, R. Bryce, and R. Mansei, *Biochem. Biophys. Res. Commun.*, **237**, 639 (1997).
60. J. Simon, J. Fong, J. Bernert, and W. Browner, *Amer. J. Epidemiol.*, **148**, 854 (1998).
61. T. Coste, M. Pierlovisi, J. Leonardi, D. Dufayet, A. Gerbi, H. Lafont, P. Vague, and D. Raccach, *J. Nut. Biochem.*, **10**, 411 (1999).
62. V. A. Ziboh, M. Yun, D. M. Hyde, and S. N. Giri, *Lipids*, **32**, 759 (1997).
63. J. Ritskes-Hoitinga, P. Verscuren, G. Meijer, A. Wiersma, A. van de Kooij, W. Timmer, C. Blonk, and J. Weststrate, *Food Chem. Toxicol.*, **36**, 663 (1998).
64. Y. Ko, C. Lij, C. Ou, J. Liu, W. Lin, and H. Chen, *J. Agric. Food Chem.*, **48**, 4144 (2000).
65. A. Demoz, N. Willunsen, and R. K. Berge, *Lipids*, **27**, 968 (1992).
66. R. Hempenius, B. Lna, and R. Hagitt, *Food Chem. Toxicol.*, **38**, 127 (2000).
67. E. Koskelo, K. Boswell, L. Carl, S. Lanoue, C. Kelly, and D. Kyle, *Lipids*, **32**, 397 (1997).
68. L. Arterburn, K. Boswell, T. Lawlor, M. Cifone, H. Murli, and D. Kyle, *Food Chem. Toxicol.*, **38**, 971 (2000).
69. Y. V. Yuan, D. D. Kitts, and D. V. Godin, *Br. J. Nutr.*, **80**, 89 (1998).
70. G. E. Mott, E. M. Jackson, C. A. McMahan, and H. C. McGill, *J. Nutr.*, **122**, 1397 (1992).
71. Lipid Research Clinic Program, *J. Amer. Med. Assoc.*, **251**, 351 (1984).
72. J. Masuda and R. Ross, *Arteriosclerosis*, **10**, 164 (1990).
73. S. K. Peng, C. B. Taylor, J. C. Hill, and R. J. Morin, *Atherosclerosis*, **54**, 121 (1985).
74. U. P. Steinbrecher and M. Loughheed, *Arterio. Thromb.*, **12**, 608 (1992).
75. R. J. Morin and S. K. Peng, in *Biological Effects of Cholesterol Oxides*, CRC Press, Inc., Boca Raton, Florida, 1992, pp. 89–101.
76. P. B. Addis, *Food Nutr. News*, **62**, 1 (1990).
77. M. D. Stringer, P. G. Gorog, A. Freeman, and V. V. Kakkar, *Brit. Med. J.*, **298**, 281 (1989).
78. M. E. Rosenfeld, W. Palinski, S. Yia-Herttuala, S. Butler, and J. L. Witztum, *Arteriosclerosis*, **10**, 336 (1990).



79. W. Palinski, M. E. Rosenfeld, S. Yla-Herttuala, G. C. Gurtner, S. S. Socher, S. W. Butler, S. Parthasarathy, T. E. Carew, and D. Steinberg, *Proc. Natl. Acad. Sci. USA*, **86**, 1372 (1989).
80. S. Dzeletovic, O. Breuer, E. Lund, and U. Diczfalusy, *Anal. Biochem.*, **225**, 73 (1995).
81. M. T. Quinn, S. Parthasarathy, L. G. Fong, and D. Steinberg, *Proc. Natl. Acad. Sci. USA*, **84**, 2995 (1987).
82. U. P. Steinbrecher, *Can. J. Cardiol.*, **9**, 3B (1993).
83. P. Debruyne, E. Bruyneel, X. Li, A. Zimber, C. Gespach, and M. Mareel, *Mut. Res.*, **480**, 359 (2001).
84. N. Caillol, E. Pasqualini, E. Mas, A. Valette, A. Verine, and D. Lombardo, *Lipids*, **32**, 1147 (1997).
85. A. Benedetti, D. Alvaro, C. Bassotti, A. Gigliozzi, G. Feretti, T. La Rosa, A. Di Sario, L. Baiocchi, and A. Jezequel, *Hepatology*, **26**, 9 (1997).
86. K. Phillips, M. Tarrago-Trani, and K. Stewart, *Food Chem.*, **64**, 415 (1999).
87. V. Piinoren, D. G. Lindsay, T. A. Meittinen, J. Toivo, and A. M. Lampi, *J. Sci. Food Agric.*, **80**, 939 (2000).
88. F. Shahidi and V. K. S. Shukla, *Inform.*, **7**, 1227 (1996).
89. T. A. Miettinen and H. Gylling, *Curr. Opin. Lipidol.*, **10**, 9 (1999).
90. P. Jones and M. Raeini-Sarjaz, *Nutr. Rev.*, **59**, 21 (2001).
91. H. Gylling, P. Puska, E. Vartiainen, and T. A. Miettinen, *J. Lipid Res.*, **40**, 593 (1999).
92. T. A. Niettinen and H. C. Gylling, *Curr. Opin. Lipidol.*, **10**, 9 (1999).
93. T. T. Nguyen, *J. Nutr. Sci.*, **129**, 2109 (1999).
94. V. Baker, P. Hepburn, S. Kennedy, P. Jones, L. Lea, J. Sumpter, and J. Ashby, *Food Chem. Toxicol.*, **37**, 13 (1999).
95. P. Hepburn, S. Horner, and M. Smith, *Food Chem. Toxicol.*, **37**, 521 (1999).
96. D. Waalkens-Berendsen, A. Wolterbeek, M. Wijnands, M. Richold, and P. Hepburn, *Food Chem. Toxicol.*, **37**, 683 (1999).
97. J. Weststrate, R. Ayesh, C. Bauer-Plank, and P. Drewitt, *Food Chem. Toxicol.*, **37**, 1063 (1999).
98. R. Ayesh, J. Weststrate, P. Drewitt, and P. Hepburn, *Food Chem. Toxicol.*, **37**, 1127 (1999).
99. A. Wolfreys and P. Hepburn, *Food Chem. Toxicol.*, **40**, 461 (2002).
100. D. Sanders, H. Minter, D. Howes, and P. Hepburn, *Food Chem. Toxicol.*, **38**, 485 (2000).
101. M. Moghadasian, *Food Chem. Toxicol.*, **35**, 528 (1997).
102. M. Moghadasian, H. Mohammed, and J. Frohlich, *J. Medicine*, **107**, 588 (1999).
103. M. Davidson, K. Maki, D. Umporowicz, K. Ingram, M. Dicklin, E. Schaefer, R. Lane, J. McNamara, J. Ribay-Mercado, G. Perrone, S. Robins, and W. Franke, *J. Amer. College Nutr.*, **20**, 307 (2001).
104. P. J. H. Jones, F. Y. Nianios, M. Raeini-Sarjaz, and C. A. Vanstone, *Amer. J. Clin. Nutr.*, **69**, 1140 (1999).
105. H. Gylling, P. Puska, E. Vartiainen, and T. Miettinen, *Atheroscler.*, **145**, 279 (1999).
106. H. Relas, H. Gylling, and T. Miettinen, *Br J. Nutr.*, **85**, 141 (2001).

107. J. Plat, E. van Onselen, M. van Heugten, and R. Mensink, *Eur. J. Clin. Nutr.*, **54**, 671 (2000).
108. J. Plat and R. Mensink, *Metabolism: Clinical Experimental*, **50**, 520 (2001).
109. M. Noakes, P. Clifton, F. Ntanos, W. Shrapnel, I. Record, and J. McInerney, *Amer. J. Clin. Nutr.*, **75**, 79 (2002).
110. M. Mora, C. Tourne-Peteilh, M. Charveron, B. Fabre, A. Milon, and I. Muller, *Chem. Phys. Lipids*, **101**, 255 (1999).
111. A. Awad and C. Fink, *J. Nutr.*, **130**, 2127 (2000).
112. A. Awad, C. Fink, H. Williams, and U. Kim, *Eur. J. Cancer Prevention*, **10**, 507 (2001).
113. R. Russell, *Amer. J. Clin. Nutr.*, **71**, 878 (2000).
114. J. Erickson and A. Mawson, *J. Theoretical Biol.*, **206**, 47 (2000).
115. B. Bray, M. Goodin, R. Inder, and R. Rosengren, *Food Chem. Toxicol.*, **39**, 1 (2001).
116. G. Mulder, N. Manley, J. Grant, K. Schmidt, W. Zeng, C. Eckhoff, and L. Maggio-Price, *Teratology*, **62**, 214 (2000).
117. B. Benoist, J. de Martines, and T. Goodman, *Food Nutr. Bull.*, **22**, 320 (2001).
118. A. Hendrickx, P. Peterson, D. Hartmann, and H. Hummler, *Reproductive Toxicol.*, **15**, 445 (2001).
119. U. Wiegand, S. Hartmann, and H. Hummler, *Intern. J. Vit. Nutr. Res.*, **68**, 411 (1998).
120. G. Mao, M. Collins, and F. Derguini, *Toxicol. Appl. Pharmacol.*, **163**, 38 (2000).
121. M. Murata and S. Kawanishi, *J. Biol. Chem.*, **275**, 2003 (2000).
122. F. Dal-Pizzol, F. Klamt, M. Frota, Jr., L. Moraes, J. Moreira, and M. Benfato, *Free Rad. Res.*, **33**, 677 (2000).
123. A. Woodall, S. Lee, R. Weesie, M. Jackson, and G. Britton, *Biochim. Biophys. Acta*, **1336**, 33 (1997).
124. K. Scott and D. Rodriguez-Amaya, *Food Chem.*, **69**, 125 (2000).
125. H. Nakamura, F. Furukawa, A. Nishikawa, M. Miyauchi, H. Son, T. Imazawa, and M. Hirose, *Food Chem. Toxicol.*, **39**, 799 (2001).
126. N. Ymashita, M. Murata, S. Inoue, M. Burkitt, L. Milne, and S. Kawanishi, *Chem. Res. Toxicol.*, **11**, 855, 1998).
127. D. Liebler and J. Burr, *Lipids*, **35**, 1045 (2000).
128. P. Poon, P. Y. Y. Wong, P. Dubeski, T. D. Durance, and D. D. Kitts, *J. Sci. Food Agric.*, **83**, 542 (2003).
129. E. Makinen and A. Hopia, *Lipids*, **35**, 1215 (2000).
130. C. Faustman, D. Liebler, and J. Burr, *J. Agri. Food Chem.*, **47**, 1396 (1999).
131. D. Cornwell, K. Jones, Z. Jiang, L. Lantry, P. Southwell-Keely, I. Kohar, and D. Thornton, *Lipids*, **33**, 295 (1998).
132. A. Carr, B. Zhu, and B. Frei, *Circ. Res.*, **87**, 349 (2000).
133. B. McIntyre, K. Briski, M. Tirmenstein, M. Fariss, A. Gapor, and P. Sylvester, *Lipids*, **35**, 171 (2000).
134. H. Shige, T. Ishikawa, M. Suzukawa, M. Nishiwaki, T. Yamashita, K. Nakajima, T. Ito, K. Higashi, M. Ayaori, A. Yonemura, P. Nestel, and H. Nakamura, *Lipids*, **33**, 1169 (1998).
135. R. Vieth, *Amer. J. Clin. Nutr.*, **69**, 842 (1999).

136. M. Terry, M. Lanenga, J. McNaughton, and L. Stark, *Vet. Human Toxicol.*, **41**, 312 (1999).
137. Y. Guven, S. Yildirim, and C. Kasapoglu, *Biol. Trace Element Res.*, **37**, 85 (1993).
138. J. Wimsatt, S. Marks, T. Campbell, J. Johnson, and R. Nachreiner, *J. Vet. Internal Medicine*, **12**, 42 (1998).
139. D. Li, H. Liu, P. Xi, Y. Chen, and Y. Li, *J. China Agric. Univ.*, **6**, 87 (2001).
140. S. Rajasree, P. Umashankar, A. Lal, P. Sarma, and C. Kartha, *Life Sci.*, **70**, 1777 (2002).
141. E. Gimeno, E. Costa, M. Gomar, A. Massone, and E. Portiansky, *J. Vet. Medicine Physiol. Pathol. Clin. Med.*, **47**, 201 (2000).
142. S. Blank, K. Scanlon, T. Sinks, S. Lett, and H. Falk, *Amer. J. Public Health*, **85**, 656 (1995).
143. X. Lexun, M. Lipkin, H. Newmark, and W. Jiarmin, *J. National Cancer Inst.*, **91**, 176 (1999).
144. P. Price, J. Buckley, and M. Williamson, *J. Nutr.*, **131**, 2910 (2001).
145. E. Kallay, P. Bareis, E. Bajna, S. Kriwanek, E. Bonner, S. Toyokuni, and H. Cross, *Food Chem. Toxicol.*, **40**, 1191 (2002).
146. C. Redaelli, M. Wagner, D. Gunter-Duwe, Y. Tian, P. Stahel, L. Mazzuchelli, R. Schmid, and M. Schilling, *Kidney Intern.*, **61**, 288 (2002).
147. S. Booth, J. Pennington, and J. Sadowski, *Lipids*, **31**, 715 (1996).
148. S. Booth, A. Lichtenstein, M. O'Brien-Morse, N. McKeown, R. Wood, E. Satzman, and C. Gundberg, *Amer. J. Clin. Nutr.*, **74**, 783 (2001).
149. T. Koivu-Tikkanen, V. Ollilainen, and V. Piironen, *J. Agric. Food Chem.*, **48**, 6325 (2000).
150. S. Booth, K. Tucker, C. Honglei, M. Hannan, D. Gagnon, L. Cupples, P. Wilson, J. Ordovas, E. Shaefer, B. Dawson-Hughes, and D. Kiel, *Amer. J. Clin. Nutr.*, **71**, 1201 (2000).
151. M. Valko, H. Morris, M. Mazur, P. Rapta, and R. Bilton, *Biochim. Biophys. Acta General Subjects*, **1527**, 161 (2001).
152. J. Kramer, F. Sauer, E. Farnsworth, D. Stevenson, and G. Rock, *Lipids*, **33**, 1 (1998).
153. T. Kickler, W. Zinkham, A. Moser, J. Shankroff, J. Borel, and H. Moser, *Biochem. Mol. Med.*, **57**, 125 (1996).
154. T. Green and S. Innis, *Lipids*, **35**, 607 (2000).
155. A. Marzo, S. Curti, P. Marzo, and R. Lisciani, *Bollettino Chimico Farmaceutico*, **136**, 651 (1997).
156. H. Reyes, J. Ribalta, I. Hernandez, M. Arrese, N. Pak, M. Wells, and R. Kirsch, *Hepatology*, **21**, 1373 (1995).
157. A. Cicero and A. Gadd, *Phytotherapy Res.*, **15**, 277 (2001).
158. Z. Xu and J. Godber, *J. Agri. Food Chem.*, **47**, 2724 (1999).
159. J. Kim, J. Godber, J. King, and W. Prinyawiwatkul, *J. Am. Oil Chem. Soc.*, **78**, 685 (2001).
160. N. Rong, L. Ausman, and R. Nicolosi, *Lipids*, **32**, 303 (1997).
161. T. Sunitha, R. Manorama, and C. Rukmini, *Plant Foods Hum. Nutr.*, **51**, 219 (1997).
162. M. Sugano and E. Tsuji, *J. Nutr.*, **127**, 521S (1997).

163. M. Sugano, K. Koba, and E. Tsuji, *Anticancer Res.*, **19**, 3651 (1999).
164. I. Igwe and O. Ogbobe, *J. Appl. Polym. Sci.*, **78**, 1826 (2000).
165. F. Okieimen, O. Bakare, and C. Okieimen, *Indust. Crops Prod.*, **15**, 139 (2002).
166. T. Gaginella, F. Capasso, N. Mascolo, and S. Perilli, *Phytotherapy Res.*, **12**, S128 (1998).
167. N. Mascolo, F. Barbato, L. Grumetto, F. Quaglia, and F. Capasso, *Intern. J. Pharmacology*, **35**, 364 (1997).
168. C. Vieira, S. Fetzter, S. Sauer, S. Evangelista, B. Averbeck, M. Kress, P. Reeh, R. Cirillo, A. Lippi, C. Maggi, and S. Manzini, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **364**, 87 (2001).
169. C. Vieira, S. Evangelista, R. Cirillo, A. Lippi, C. Maggi, and S. Manzini, *Mediators Inflamm.*, **9**, 223 (2000).
170. A. Andrianaivo-Rafehivola, M. Siess, and E. Gaydou, *Food Chem. Toxicol.*, **33**, 377 (1995).
171. Anonymous, *Intern. J. Toxicol.*, **20**, Suppl 2:21 (2001).
172. M. Christensen, S. Lund, L. Simonsen, U. Hass, S. Simonsen, and C. Hoy, *J. Nutr.*, **128**, 1011 (1998).
173. M. Christensen and C. Hoy, *Lipids*, **32**, 185 (1997).
174. H. Mu and C. Hoy, *J. Lipid Res.*, **42**, 792 (2001).
175. M. Nonaka, Y. Nagamori, K. Yamada, and M. Sugano, *J. Jap. Soc. Food Sci. Technol.*, **46**, 638 (1999).
176. S. Terada, M. Iwami, H. Tanizawa, and T. Shimazu, *J. Jap. Soc. Nutri. Food Sci.*, **54**, 339 (2001).
177. D. Zhou, X. Xu, H. Mu, C. Hoy, and J. Adler-Nissen, *J. Agric. Food Chem.*, **49**, 5771 (2001).
178. J. Snook, S. Park, G. Wardlaw, R. Jandacek, D. Palmquist, L. Myoung-Sook, and J. Hoover, *Nutr. Res.*, **16**, 925 (1996).
179. K. W. Miller, K. D. Lawson, D. H. Tallmadge, B. L. Madison, J. R. Okenfuss, P. Hudson, S. Wilson, J. Thorstenson, and P. Vanderploeg, *Fund. Appl. Toxicol.*, **24**, 229 (1995).
180. W. M. Lafranconi, P. H. Long, J. E. Atkinson, A. L. Knezevich, and W. L. Wooding, *Food Chem. Toxicol.*, **32**, 789 (1994).
181. E. Kirk, P. Sutherland, S. Wang, A. Chait, and R. LeBoeuf, *J. Nutr.*, **128**, 954 (1998).
182. M. Tikkanen and H. Adlercreutz, *Biochem. Pharmacol.*, **60**, 1 (2000).
183. C. Sirtori, *Food Chem. Toxicol.*, **35**, 428 (1997).
184. S. Kullig and M. Metzler, *Food Chem. Toxicol.*, **35**, 605 (1997).
185. C. Lamartiniere, J. Want, M. Smith-Johnson, and I. Eltoun, *Toxicol Sci.*, **65**, 228 (2002).
186. K. Mizutani, K. Ikeda, T. Nishikata, and Y. Yamori, *J. Hypertension*, **18**, 1833 (2000).
187. O. Domon, L. McGarrity, M. Bishop, M. Yoshioka, J. Chen, and S. Morris, *Mutation Research – Fundamental Molecular Mechanisms of Mutagenesis*, **474**, 129 (2001).
188. C. Rao, *Food Chem. Toxicol.*, **35**, 1228 (1997).
189. R. Newbold, E. Banks, B. Bullock, and W. Jefferson, *Cancer Res.*, **61**, 4325 (2001).
190. L. Strauss, S. Makela, S. Joshi, I. Huhtaniemi, and R. Santii, *Mol. Cell. Endocrinol.*, **144**, 83 (1998).

191. L. Hilakivi-Clarke, E. Cho, I. Onojafe, M. Raygada, and R. Clarke, *Oncology Reports*, **6**, 1089 (1999).
192. S.A. El-Sawi and M. Mohamed, *Food Chem.*, **77**, 75 (2002).
193. G. Ruberto and M. Baratta, *Food Chem.*, **69**, 167 (2000).
194. P. Crowell, *Breast Cancer Res. Treat.*, **46**, 191 (1997).
195. S. Richheimer, M. Bernart, G. King, M. Kent, and D. Bailey, *J. Amer. Oil Chem. Soc.*, **73**, 507 (1996).
196. P. Debersac, M. Vernevaut, M. Amiot, M. Suschetet, and H. Siess, *Food Chem. Toxicol.*, **39**, 109 (2001).
197. P. Debersac, J. Heydel, M. Amiot, H. Goudonnet, Y. Artur, M. Suschetet, and M. Siess, *Food Chem. Toxicol.*, **39**, 907 (2001).
198. J. Whysner and G. Williams, *Pharmacol. Therapeut.*, **71**, 127 (1996).
199. F. Araujo, *Food Chem. Toxicol.*, **34**, 1021 (1996).
200. F. Paumgarten, R. De-Carvalho, C. Souza, K. Madi, and I. Chahoud, *Brazilian J. Med. Biol. Res.*, **31**, 955 (1998).
201. V. Rolseth, R. Djurhuus, and A. Svardal, *Toxicol.*, **170**, 75 (2002).
202. A. Bysted, G. Holmer, and P. Lund, *J. Amer. Oil Chem. Soc.*, **75**, 225 (1998).
203. N. Morgado, J. Sanhueza, A. Galleguillos, A. Garrido, S. Nieto, and A. Valenzuela, *Annals Nutr. Metab.*, **43**, 310 (1999).
204. L. Granlund, L. Larsen, E. Christiansen, and J. Pedersen, *Br. J. Nutr.*, **84**, 681 (2000).
205. N. Matthan, L. Ausman, A. Lichtenstein, and P. Jones, *J. Lipid Res.*, **41**, 834 (2000).
206. N. Matthan, K. Cianflone, A. Lichtenstein, L. Ausman, M. Jauhiainen, and P. Jones, *J. Lipid Res.*, **42**, 1841 (2001).
207. N. Morgado, A. Galleguillos, J. Sanhueza, A. Garrido, S. Nieto, and A. Valenzuela, *Lipids*, **33**, 669 (1998).
208. M. Miyazake, M. Huang, S. Watanabe, T. Kobayashi, and H. Okuyama, *Nutr. Res.*, **18**, 1049 (1998).
209. C. Loi, J. Chardigny, C. Cordelet, L. Leclere, M. Genty, C. Ginies, J. Noel, and J. Sebedio, *Lipids*, **35**, 911 (2000).
210. L. Whigham, M. Cook, and R. Atkinson, *Pharmacol. Res.*, **42**, 503 (2000).
211. J. Palombo, A. Ganguly, B. Bistrián, and M. Menard, *Cancer Lett.*, **177**, 163 (2002).
212. R. Devery, A. Miller, and C. Stanton, *Biochem. Soc. Trans.*, **29**, 341 (2001).
213. H. Park, J. Ryu, Y. Ha, and J. Park, *Br. J. Nutr.*, **86**, 549 (2001).
214. H. Cantwell, R. Devery, M. OShea, and C. Stanton, *Lipids*, **34**, 833 (1999).
215. P. Jones, L. Lea, and R. Pendlington, *Food Chem. Toxicol.*, **37**, 1119 (1999).
216. J. Scimeca, *Food Chem. Toxicol.*, **36**, 391 (1998).
217. A. Smedment and B. Vessby, *Lipids*, **36**, 773 (2001).
218. P. Benito, G. Nelson, D. Kelley, G. Bartolini, P. Schmidt, and V. Simon, *Lipids*, **36**, 221 (2001).
219. P. Benito, G. Nelson, D. Kelley, G. Bartolinit, P. Schmidt, and V. Simon, *Lipids*, **36**, 229 (2001).
220. S. Livisay, S. Zhou, C. Ip, and E. Decker, *J. Agri. Food Chem.*, **48**, 4162 (2000).
221. L. Kohlmeier, *Food Chem. Toxicol.*, **35**, 1228 (1997).

222. W. McKelvey, S. Greenland, and R. Sandler, *Epidemiol.*, **11**, 469 (2000).
223. P. Pientinen, *Food Chem. Toxicol.*, **35**, 854 (1997).
224. N. de Roos, M. Bots, and M. Katan, *Arterio. Thomb. Vascular Biol.*, **21**, 1233 (2001).
225. N. de Roos, Trans fatty acids, HDL-cholesterol, and cardiovascular disease risk: Effects of dietary changes on vascular reactivity, Wageningen Universiteit, Wageningen, the Netherlands, 2001.
226. M. Craig-Schmidt, *Lipids*, **36**, 997 (2001).
227. S. Elias and S. Innis, *Amer. J. Clin. Nutr.*, **73**, 807 (2001).
228. S. Carlson, M. Clandinin, H. Cook, E. Emken, and L. Filer, Jr., *Amer. J. Clin. Nutr.*, **66**, 715S (1997).
229. I. Wauben, H. Xing, D. McCutcheon, and P. Wainwright, *J. Nutr.*, **131**, 1568 (2001).
230. K. Skog, *Food Chem. Toxicol.*, **40**, 1197 (2002).
231. E. Muckel, H. Frandsen, and H. Glatt, *Food Chem. Toxicol.*, **40**, 1063 (2002).
232. H. Frandsen, H. Frederiksen, and J. Alexander, *Food Chem. Toxicol.*, **40**, 1125 (2002).
233. K. Turteltaub, R. Mauthe, K. Dingley, J. Vogel, C. Frantz, R. G. Garner, and N. Shen, *Mut. Res.*, **376**, 243 (1997).
234. B. Stavric, B. Lau, T. Matula, R. Klassen, D. Lewis, and R. Downie, *Food Chem. Toxicol.*, **35**, 199 (1997).
235. G. Keating and K. Bogen, *Food Chem. Toxicol.*, **39**, 29 (2001).
236. R. Sinha, M. Knize, C. Salmon, E. Brown, D. Rhodes, J. Felton, O. Levander, and N. Rothman, *Food Chem. Toxicol.*, **36**, 289 (1998).
237. A. Solyakov and K. Skog, *Food Chem. Toxicol.*, **40**, 1205 (2002).
238. N. Tran, C. Salmon, M. Knize, and M. Colvin, *Food Chem. Toxicol.*, **40**, 673 (2002).
239. M. Knize, R. Sinha, N. Rothman, E. Brown, C. Salmon, O. Levander, P. Cunningham, and J. Felton, *Food Chem. Toxicol.*, **33**, 545 (1995).
240. E. Snyderwine, N. Sadrieh, R. King, and H. Schut, *Food Chem. Toxicol.*, **36**, 1033 (1998).
241. J. Breneman, J. Briner, M. Ramsey, A. Kirector, and J. Tucker, *Food Chem. Toxicol.*, **34**, 717 (1996).
242. M. Ward, *Food Chem. Toxicol.*, **35**, 634 (1997).
243. N. Probst-Hensch, *Food Chem. Toxicol.*, **35**, 633 (1997).
244. J. Felton, M. Malfatti, M. Knize, C. Salmon, E. Hopmans, and R. Wu, *Mut. Res.*, **376**, 37 (1997).
245. M. Miyauchi, A. Nishikawa, F. Furukawa, K. Kasahara, H. Nakamura, M. Takahashi, and M. Hirose, *Cancer Letts.*, **142**, 75 (1999).
246. M. Nagao, T. Ushijima, N. Watanabe, E. Okochi, M. Ochiai, H. Nakagama, and T. Sugimura, *Environ. Mol. Mutagen.*, **39**, 158 (2002).
247. P. Pais, C. Salmon, M. Knize, and J. Felton, *J. Agric. Food Chem.*, **47**, 1098 (1999).
248. C. Tai, K. Lee, and B. Chen, *Food Chem.*, **75**, 309 (2001).
249. C. Chiu and B. Chen, *Food Chem.*, **68**, 267 (2000).
250. A. Brash, *Lipids*, **35**, 947 (2000).
251. K. Ando, K. Nagata, R. Yoshida, K. Kikugawa, and M. Suzuki, *Lipids*, **35**, 401 (2000).
252. T. Kaneko and S. Tahara, *Lipids*, **35**, 961 (2000).

253. M. Shimonov, I. Pinchuk, A. Bor, I. Beiel, M. Fainaru, M. Rubin, and D. Lichtenberg, *Lipids*, **34**, 255 (1999).
254. K. Eder, *Lipids*, **34**, 717 (1999).
255. M. Kawakami, L. Ward, and H. Doi, *Lipids*, **35**, 205 (2000).
256. B. Nwanguma, A. Achebe, L. Ezeanyika, and L. Eze, *Food Chem. Toxicol.*, **37**, 413 (1999).
257. S. Gorelik and J. Kanner, *J. Agric. Food Chem.*, **49**, 5945 (2001).
258. M. Lynch and C. Faustmann, *J. Agric. Food Chem.*, **48**, 600 (2000).
259. C. Faustmann, D. Liebler, T. McClure, and Q. Sun, *J. Agric. Food Chem.*, **47**, 3140 (1999).
260. S. Zhou and E. Decker, *J. Agric. Food Chem.*, **47**, 1932 (1999).
261. A. Kanazawa, T. Sawa, T. Akaike, S. Morimur, K. Kida, and H. Maeda, *Biofactors*, **13**, 187 (2000).
262. G. Leonarduzzi, M. Arkan, H. Basaga, E. Chiarpotto, A. Sevenaian, and G. Poli, *Free Rad. Biol. Med.*, **28**, 1370 (2000).
263. P. Eaton, D. Hearse, and M. Shattock, *Cardiovascular Res.*, **51**, 294 (2001).
264. F. Ursini, A. Zamburlinii, G. Cazzolato, M. Maiorino, G. Bon, and A. Sevanian, *Free Rad. Biol. Med.*, **25**, 250 (1998).
265. D. Pratico, *Trends Cardiovascular Med.*, **11**, 112 (2001).
266. K. Uchida, *Free Rad. Biol. Med.*, **28**, 1685 (2000).
267. L. Wood, D. Fitzgerald, P. Gibson, D. Cooper, and M. Garg, *Lipids*, **35**, 967 (2000).
268. R. Noding, S. Schonberg, H. Krokan, and K. Bjerve, *Lipids*, **33**, 285 (1998).
269. C. Silwood and M. Grootveld, *Lipids*, **34**, 741 (1999).
270. C. Bauer-Plank and L. Steenhorst-Slikkerveer, *J. Amer. Oil Chem. Soc.*, **77**, 477 (2000).
271. L. Steenhorst-Slikkerveer, A. Louter, H. Janssen, and C. Bauer-Plank, *J. Amer. Oil Chem. Soc.*, **77**, 837 (2000).
272. F. Guardiola, R. Coedony, P. Addis, M. Rafecas, and J. Boatella, *Food Chem. Toxicol.*, **34**, 193 (1996).
273. C. Adcox, L. Boyd, L. Oehrl, J. Allen, and G. Fenner, *J. Agri. Food Chem.*, **49**, 2090 (2001).
274. D. Vine, K. Croft, L. Beilin, and J. Mamo, *Lipids*, **32**, 887 (1997).
275. M. Lyons and A. Brown, *Lipids*, **36**, 701 (2001).
276. A. Brown and W. Jessup, *Atherosclerosis*, **142**, 1 (1999).
277. J. Rong, S. Rangaswamy, L. Shen, R. Dave, Y. Chang, H. Peterson, H. Hodis, G. Chisolm, and A. Sevanian, *Arterio. Thromb. Vas. Biol.*, **18**, 1885 (1998).
278. S. K. Peng, C. B. Taylor, P. Tham, N. T. Werthessen, and B. Mikkelsen, *Arch. Pathol. Lab. Med.*, **102**, 57 (1978).
279. W. E. Donaldson, *Poultry Sci.*, **61**, 2097 (1982).
280. R. L. Smith and B. H. Johnson, *Free Rad. Biol. Med.*, **7**, 285 (1989).
281. S. K. Peng, A. Savanian, and R. J. Morin, in S. K. Peng and R. J. Morin, eds., *Biological Effects of Cholesterol Oxides*, CRC Press, Inc., Boca Raton, Florida, 1992, pp. 147–166.
282. Y. O'Callaghan, J. Woods, and N. O'Brien, *Cell Biol. Toxicol.*, **17**, 127 (2001).
283. J. Woods and N. O'Brien, *Nutr. Cancer*, **31**, 192 (1998).

284. M. Ares, M. Porn-Ares, J. Thyberg, L. Juntti-Berggren, P. Berggren, U. Diczfalusy, B. Kallin, I. Bjourkhem, S. Orrenius, and J. Nilsson, *J. Lipid Res.*, **38**, 2049 (1997).
285. E. Isong, E. Essien, O. Eka, and I. Umoh, *Food Chem. Toxicol.*, **38**, 997 (2000).
286. E. Isong, P. Ebong, E. Ifon, I. Umoh, and O. Eka, *Plant Foods Hum. Nutr.*, **51**, 159 (1997).
287. C. Yang, C. Kendall, D. Stamp, A. Medline, M. Archer, and W. Bruce, *Nutr. Cancer*, **30**, 69 (1998).
288. G. Billek, *Eur. J. Lipid Sci. Technol.*, **102**, 587 (2000).
289. K. Eder, *J. Anim. Physiol. Anim. Nutr.*, **82**, 271 (1999).
290. K. Narasimhamurthy and P. Raina, *Mol. Cell. Biochem.*, **195**, 143 (1999).
291. M. Williams, W. Sutherland, M. McCormick, S. Jong, R. Walker, and G. Wilkins, *J. Amer. College Cardiol.*, **33**, 1050 (1999).
292. J. Kamsiah, S. Tan, and I. Syed Zahir, *Cutting Edge Technologies for Sustained Competitiveness: Proceedings of the 2001 PIPOC International Palm Oil Congress, Food Technology and Nutrition Conference, Hotel Istana, Kuala Lumpur*, Malaysian Palm Oil Board, Kuala Lumpur, 2001, p. 336.
293. K. Ammu, M. Raghunath, T. Sankar, K. Lalitha, and K. Devadasan, *Nahrung Food*, **44**, 368 (2000).
294. J. Martin, F. Joffre, M. Siess, M. Vernevaute, P. Collenot, M. Genty, and J. Sebedio, *J. Nutr.*, **130**, 1524 (2000).
295. W. Christie and G. Dobson, *Eur. J. Lipid Sci. Technol.*, **102**, 515 (2000).
296. T. Chiang, P. Wu, S. Liao, L. Wang, and Y. Ko, *Food Chem. Toxicol.*, **37**, 125 (1999).
297. T. Chiang, P. Wu, and Y. Ko, *Environmental Res.*, **81**, 18 (1999).
298. S. Lopez-Varela, F. Sanchez-Muniz, and C. Cuesta, *Food Chem. Toxicol.*, **33**, 181 (1995).
299. J. McSavage and S. Trevisan, *Food Serv. Technol.*, **1**, 85 (2001).
300. N. Vahcic and M. Hruskar, *Food Technol. Biotechnol.*, **37**, 107 (1999).
301. A. Romero, F. Sanchez-Muniz, and C. Cuesta, *J. Sci. Food Agric.*, **80**, 2135 (2000).
302. N. Kazerouni, R. Sinha, C. Hsu, A. Greenberg, and N. Rothman, *Food Chem. Toxicol.*, **39**, 423 (2001).
303. B. Chen and Y. Chen, *J. Agric. Food Chem.*, **49**, 5238 (2001).
304. W. Rauter, *Ernaehrung*, **21**, 447 (1997).
305. P. Simko, *J. Chromatogr.*, **770**, 3 (2002).
306. J. Kjallstrand and G. Petersson, *Food Chem.*, **74**, 85 (2001).
307. M. Guillen, P. Sopelana, and M. Partearroyo, *J. Agri. Food Chem.*, **48**, 5083 (2000).
308. P. Simko, P. Simon, and V. Khunova, *Food Chem.*, **64**, 157 (1999).
309. M. Espe, R. Nortvedt, O. Lie, and H. Hafsteinsson, *Food Chem.*, **77**, 41 (2002).
310. K. Kangsadalampai, C. Butryee, and K. Manoonphol, *Food Chem. Toxicol.*, **35**, 213 (1997).
311. A. Hakura, J. Sonoda, Y. Tsutsui, T. Mikami, T. Imade, M. Shimada, S. Yaguchi, M. Yamanaka, M. Tomimatsu, and K. Tsukidate, *Reg. Toxicol. Pharmacol.*, **27**, 273 (1998).
312. H. Wang, T. Chen, P. Yang, Y. Ma, C. Yu, and T. Ueng, *Food Chem. Toxicol.*, **40**, 653 (2002).



313. P. White, *Mutat. Res. Genetic Toxicol. Environ. Mutagenesis*, **515**, 85 (2002).
314. G. Williams, M. Iatropoulos, and J. Whysner, *Food Chem. Toxicol.*, **37**, 1027 (1999).
315. A. Botterweck, H. Verhagen, R. Goldbohm, J. Kleinjans, and P. van Den Brandt, *Food Chem. Toxicol.*, **38**, 599 (2000).
316. S. Tamano, M. Hirose, H. Tanaka, A. Hagiwara, and T. Shirai, *Food Chem. Toxicol.*, **36**, 299 (1998).
317. H. Tryphonas, F. Lacroix, E. Lok, P. Jee, D. Clayson, S. Hayward, D. Miller, and R. Mehta, *Food Chem. Toxicol.*, **37**, 671 (1999).
318. A. Safer and A. al-Nughamish, *Histol. Histopathol.*, **14**, 391 (1999).
319. L. Dwyer-Nield, J. Thompson, G. Peljak, M. Squier, T. Barkder, A. Parkinson, J. Cohen, D. Dinsdale, and A. Malkinson, *Toxicol.*, **130**, 115 (1998).
320. G. Rao, K. Parthasarathi, and A. Sundararaj, *Cheiron*, **29**, 91 (2000).
321. C. Yeh, J. Chung, H. Wu, Y. Li, Y. Lee, and C. Hung, *Food Chem. Toxicol.*, **38**, 977 (2000).
322. G. Maziero, C. Baunwart, and M. Toledo, *Food Add. Contam.*, **18**, 365 (2001).
323. S. Oikawa, K. Nishino, S. Oilawa, S. Inoue, T. Mizutani, and S. Kawanishi, *Biochem. Pharmacol.*, **56**, 361 (1998).
324. T. Guentherer and G. Luo, *Toxicology*, **160**, 47 (2001).
325. J. Lazutka, J. Mierauskiene, G. Slapsyte, and V. Dedonyte, *Food Chem. Toxicol.*, **39**, 485 (2001).
326. G. Hasheminejad and J. Caldwell, *Food Chem. Toxicol.*, **32**, 223 (1994).
327. V. Chan and J. Caldwell, *Food Chem. Toxicol.*, **30**, 831 (1992).
328. S. Abraham, *Food Chem. Toxicol.*, **39**, 493 (2001).
329. N. Gorelick, *Mutat. Res.*, **326**, 199 (1995).
330. P. Newberne, R. Smith, J. Doull, J. Goodman, I. Munro, P. Portoghese, B. Wagner, C. Weil, L. Woods, T. Adams, C. Lucas, and R. Ford, *Food Chem. Toxicol.*, **37**, 789 (1999).
331. T. Liu, C. C. Chen, C. L. Chen, and C. Chi, *Food Chem. Toxicol.*, **37**, 697 (1999).
332. H. Daimon, S. Sawada, S. Asakura, and F. Sagami, *Carcinogenesis*, **19**, 141 (1998).
333. H. Tseng, *Food Science Taiwan*, **25**, 799 (1998).
334. M. Tawfik and A. Huyghebaert, *Food Chem.*, **64**, 451 (1999).
335. F. Arcadi, C. Costa, C. Imperatore, A. Marchese, A. Rapisarda, M. Salemi, G. Trimarchi, and G. Costa, *Food Chem. Toxicol.*, **36**, 963 (1998).
336. R. Ppon, P. Lecavalier, R. Mueller, V. Valli, B. Procter, and I. Chu, *Food Chem. Toxicol.*, **35**, 225 (1997).
337. J. Peteresn, E. Naamansen, and P. Nielsen, *Food Add. Contam.*, **12**, 245 (1995).
338. V. Komolprasert and A. Lawson, *J. Agric. Food Chem.*, **45**, 444 (1997).
339. J. Hotchkiss, *Food Add. Contam.*, **14**, 601 (1997).
340. M. Tawfik and A. Huyghebaert, *Food Add. Contam.*, **15**, 592 (1998).
341. K. Date, K. Ohno, Y. Azuma, S. Hirano, K. Kobayashi, T. Sakurai, Y. Nobuhara, and T. Yamada, *Food Chem. Toxicol.*, **40**, 65 (2002).
342. P. Laurienzo, M. Malinconcino, M. Volpe, D. Luongo, V. Ranieri, and M. Scoponi, *Packaging Technol. Sci.*, **14**, 109 (2001).

343. G. Hilbert, L. Lillemardk, S. Balchen, and C. Hojskov, *Chemosphere*, **37**, 1241 (1998).
344. M. Zabik, A. Booren, M. Zabik, R. Welch, and H. Humphrey, *Food Chem.*, **55**, 231 (1996).
345. A. Smith and S. Gangolli, *Food Chem. Toxicol.*, **40**, 767 (2002).
346. E. Elijarrat, A. Monjonell, J. Caixach, and J. Rivera, *J. Agric. Food Chem.*, **50**, 1161 (2002).
347. N. van Larebeke, L. Hens, P. Schepens, A. Covaci, J. Baeyens, K. Everaert, J. Bernheim, R. Vlietinck, and G. de Poorter, *Environ. Health Perspec.*, **109**, 265 (2001).
348. D. Soong and Y. Ling, *Chemosphere*, **34**, 1579 (1997).
349. J. Jacobson and S. Jacobson, *Neurotoxicol.*, **18**, 415 (1997).
350. D. Gilroy, *J. Toxicol. Environ. Health*, **60**, 317 (2000).
351. M. de la Paz, R. Philen, I. Borda, A. de la Camara, J. Socieas, and E. Kilbourne, *Food Chem. Toxicol.*, **34**, 251 (1996).
352. G. Diggle, *Intern. J. Clin. Practice*, **55**, 371 (2001).
353. H. Schurz, R. Hill, M. de la Paz, R. Philen, I. Borda, S. Bailey, and L. Needham, *Chem. Res. Toxicol.*, **9**, 1001 (1996).
354. E. Gelpi, M. de la Paz, B. Terracini, I. Abaitua, A. de la Camara, E. Kilbourne, C. Lahoz, B. Nemery, R. Philen, L. Soldevilla, and S. Tarkowski, *Environ. Health Perspect.*, **110**, 457 (2002).
355. M. Ruiz-Mendez, M. de la Paz, J. Abian, R. Calaf, B. Blount, N. Castro-Molero, R. Philen, and E. Gelpi, *Food Chem. Toxicol.*, **39**, 91 (2001).
356. D. Closa, E. Golch, R. Calaf, J. Abian, J. Rosello-Catafau, and E. Gelpi, *Lipids*, **36**, 1125 (2001).
357. M. Alvarez, P. Cano, E. de Cabo, G. de Aguinaga, I. Martinez, and P. de la Paz, *Rev. Clin. Esp.*, **200**, 305 (2000).
358. J. Arevalo, M. Perez-Gonzalez, C. Sanchez-Bernal, and M. Llanillo, *Comp. Biochem. Physiol.*, **120**, 111 (1998).
359. S. Bell, I. Kuntze, A. Caputo, and R. Chatelain, *Food Chem. Toxicol.*, **40**, 19 (2002).

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## *Quality Assurance of Fats and Oils*

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### **1. INTRODUCTION**

The quality of fats and oils is dictated by several physical and chemical parameters that are dependent on the source of oil; geographic, climatic, and agronomic variables of growth in the case of plant oils as well as processing and storage conditions. Thus, quality assurance criteria may depend partly on the type of oil under investigation as well as on other factors that may vary depending on the intended use and regulations that vary from country to country (1-3).

Edible oils may originate from animals both land-based and aquatic, higher plants, and algal sources. Regardless of the source, the extraneous matters such as large pieces of wood, metal pieces, soil, and so on should be eliminated. For oilseeds, these are usually passed through a magnetized sieve. However, this process does not eliminate environmental pollutants that might exist endogenously or have been introduced into the raw material. The physical state of food lipids, mainly their crystallinity and whether they exist in the liquid or solid form, is dictated primarily by the degree of saturation/unsaturation of the oil. As a result,

the approximate composition of the source material must be determined. For example, solid fat content may be estimated by low-resolution nuclear magnetic resonance (NMR) spectroscopy. Near-infrared (NIR) spectroscopy allows determination of fat content and other components of oilseeds. The color of the oil, which is dependent on several factors, may be determined visually or with a Lovibond tintometer or other handheld color measuring devices (4). The color may be from carotenoids, chlorophylls, or other components. When the harvested seeds are immature, often chlorophyll content of the resultant oil is high and this affects the stability of products (5). Chlorophylls are photosensitizers; hence, their presence leads to enhanced photo-oxidation of the oil. Obviously, agronomic conditions, season of the harvest, and many other related factors affect the quality of the resultant oil. In this regard, soil may affect the content of certain unwanted minerals, such as cadmium, in the seeds, but these do not usually end up in the oil. Furthermore, as explained earlier, other contaminants may be introduced into the raw material during harvest, processing, and transport. Thus, fats and oils and their source material have to be tested for the presence and level of contaminants. In addition, diseases and pests always lead to decreased quality of oils, as reflected in their high acid values (6). In the case of animal fat, every effort should be made to process the raw material in the fresh state or use fresh-frozen material to ensure premium quality of the product.

In the extraction of edible oil from seeds, cleaning and subsequent conditioning of the seeds followed by heating during or immediately after crushing are needed as these deactivate the endogenous enzymes and help in releasing of the oil. After expelling part the oil, the resultant leftover material may be flaked and then extracted with hexanes. The expelled and solvent-extracted oils are then combined and desolventized to afford crude oil.

The edible oils, after rendering or extraction from source material, may be subjected to degumming, refining, bleaching, deodorization, and possibly winterization and blending and hydrogenation and/or addition of stabilizers/antioxidants. Discussion of these steps in any detail is beyond the scope of this overview. However, each processing step carries with it many advantages and some disadvantages. To explain these briefly, it is essential to first examine the constituents of fats and oils in a cursory manner.

Edible oils are composed of triacylglycerols (triglycerides) as their main components. The phospholipids are minor components that are generally removed during the degumming process (7). The recovered phospholipids, often called lecithin, may possibly be dietary supplements. Free fatty acids are then eliminated during the refining process, and bleaching of the oil leads to the removal of colored materials as well as decomposition of hydroperoxides to secondary oxidation products. The deodorization step is then designed to remove the odorous secondary oxidation products from the oil. However, many useful minor components present in the oils are also removed during the deodorization process. The deodorizer-distillate is often high in the content of tocopherols and tocotrienols that can be removed, purified, and sold as dietary supplements or used in specialty applications. The final oil after refining, bleaching, and deodorizing (RBD) may further be subjected to

winterization, a cooling process that allows the removal of more saturated fats as well as possible blending. Some oils may also be subjected to hydrogenation to enhance their oxidative stability. However, hydrogenation often leads to the production of 30–50% *trans*-fats that are a health concern because of their potential harmful effect on the cardiovascular system (8). Therefore, novel formulations with more saturated oils in the mix has become popular (9).

Among the parameters often checked or evaluated for quality assurance of edible oils are those related to the makeup of the oil or their properties. Table 1 summarizes a list of parameters usually employed to assess quality of edible fats and oil. However, not all parameters listed may be evaluated for each oil.

In addition to parameters listed in Table 1 that dictate the quality of fats and oils, storage and transport conditions are of considerable importance as they determine the final quality of the oil. Obviously, of the above factors, fatty acid composition and oxidative stability are of utmost importance, both from nutritional and sensory quality viewpoints. In general, intake of omega-3 fatty acids in the western world is much less than desired. Nutritionally, one would like to have a ratio of 1:2–1:5 for omega-3 to omega-6 fatty acids in the diet. However, a high content of omega-3 fatty acids in edible oils is responsible for their rapid quality deterioration. Hence, much effort has been made to eliminate the omega-3 fatty acids, mainly linolenic acid, from vegetable oils. However, recent trends have reflected the concern about low intake of omega-3 fatty acids and its deleterious effects.

Adulteration of fats and oils is another matter of concern, which might occur accidentally or deliberately. Rendering of pork fat and beef tallow in the same equipment without proper washing is an example of accidental and unintended contamination/adulteration. However, often cheaper oils have been sold in place of, or mixed with, more expensive oils. Thus, before to the recognition of health benefits of hazelnut oil, this oil was an adulterant in olive oil (10). As mentioned earlier, different oils have considerably different sterol compositions. Thus, sterols could be a means of identifying adulterants because often fatty acid compositions of the adulterant and the original oils are similar (11-13).

In addition, depending on the intended use, the quality of oil during storage and use must be monitored. The oils may undergo hydrolytic rancidity, autoxidation, photo-oxidation, and thermal oxidation. The latter type of oxidation is observed primarily in the frying oil and causes quality deterioration that must be monitored with different parameters such as color, viscosity, polar components and polymers, among others (14,15). Obviously, oils that are highly unsaturated are not suitable for frying purposes. On the contrary, autoxidation is a process that proceeds slowly for properly stored oils. However, if the oil is kept in clear bottles, photo-oxidation may occur, especially when photosensitizer chlorophyll is present. Thus, parameters of interest for quality assurance of fats and oils begin at the farm gate and continue up to the dinner table, which includes proper holding and use of oil at home after purchase that, despite its importance, is often ignored by most consumers.

The following sections provide some further details about determination of quality of fats and oils. Other specifics may be found in several chapters in this series and in several other publications.

TABLE 1. Quality Parameters of Fats and Oils.

Parameter	Details
Fatty acid composition and distribution	Percentage of total; depends on the type of material
Relative density	At 20°C or 40°C relative to water at 20°C (<1)
Refractive index	At 40°C
Viscosity	At 20°C
Color	Visual, Lovibond or Colormet
Turbidity	Visual or instrumental
Solidification point, titer, solid fat content, and cooling curve	For water-insoluble fatty acids
Odor and taste	Sensory evaluation
Saponification value	mg KOH/g
Iodine value (IV)	g iodine/100-g sample (WIJS method)
Unsaponifiable matter	g/kg
Acid value (AV)	mg KOH/g
Smoke, flash and fire points	°C
Oxidative state	
Peroxide value (PV)	meq oxygen/100-g sample
Thiobarbituric acid reactive substances (TBARS)	μmol/g
para-Anisidine value (p-Anv)	mg/kg
TOTOX	2PV + p-Anv
OSI, Rancimat and AOM value	—
Polar Lipids	Percentage
Polymers	Percentage
Volatile mater (%)	At 105°C
Phosphorus	mg/kg
Iron, copper, lead, arsenic	mg/kg
Cadmium	μg/kg
<i>Trans</i> -fatty acids	Percentage; measured at ~10 μ
Cholesterol content	Percentage, mainly for animal fat
Contaminants and foreign matter, including plasticizers (%)	—
Carotenoids and chlorophylls	mg/kg
Squalene	C <sub>30</sub> H <sub>50</sub>
Sterols	GC determination
Tocols	HPLC determination
Synthetic antioxidants	BHA, BHT, TBHQ, PG
Antifoaming agents	Dimethyl polysiloxane, singly or with silicon dioxide
Metal chelators	Citric acid or citrates, phosphoric acid
Crystallization inhibitor	Oxystearin
Adulterants	Fingerprinting using sterols or other minor components

Abbreviations: BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; TBHQ, *tert*-butylhydroquinone; and PG, propyl gallate.

## 2. OIL COMPOSITION

Fats and oils contain various classes of compounds (16). These compounds are primarily neutral lipids that include triacylglycerols (triglycerides) with lower amounts of diacylglycerols (diglycerides), monoacylglycerols (monoglycerides),

and free fatty acids. Partial acylglycerols are produced by hydrolysis of triacylglycerols. Some oils such as cottonseed oil contain about 10% diacylglycerols. The amount of free fatty acids should be less than 0.1%, preferably less than 0.05% in freshly refined oils. In addition, polar lipids, mainly phospholipids, and to a lesser extent, glycolipids are present. The content of phosphorus in crude oils may reach 500 ppm, and in refined oils, which from phospholipids, the content is generally less than 5 ppm, and may be below 2 ppm. In addition, fats and oils, in general, contain a small amount of unsaponifiable matter, generally at 0.3–2.0% mainly tocopherols, tocotrienols, phytosterols, hydrocarbons (e.g., squalene and carotenes), among others. Phenolics such as hydroxytyrosol and oleuropein might also be present (17). Trace metals, mainly iron and copper, and other components often exist. The content of iron and copper in freshly refined oils should be less than 0.1 and 0.01 ppm, respectively. Crude palm oil was 0.6 ppm for iron, 6.05 ppm for copper, 0.6 ppm for magnesium, 1.2 ppm for chromium, and 2.2 ppm for nickel (18).

The triacylglycerols of fats and oils contain a range of fatty acids, and their arrangements on the glycerol backbone may vary, depending on the source material. High-performance liquid chromatography as well as gas liquid chromatography may be used for separation and tentative identification of individual triacylglycerols based on their carbon number.

In neutral oils and fats, the fatty acids are not usually randomly distributed among different positions on the glycerol backbone and are associated in particular patterns. As an example, saturated fatty acids such as palmitic and stearic acids are associated with the *sn*-1 and *sn*-3 positions of soybean oil, albeit at higher proportions in the *sn*-1 position. However, the reverse is observed at high content of saturated fatty acids. Linoleic acid is preferably in the *sn*-2 position, whereas oleic acid is randomly distributed among the three positions. Linolenic acid is primarily at *sn*-2 followed by *sn*-1 and *sn*-3 positions. The stereospecific distribution of fatty acids has a marked effect on the oxidative stability of the resultant oils, and their presence at the *sn*-2 position helps their stability (19).

The fatty acids present in fats and oils may be analyzed after their hydrolysis and subsequent conversion by methylation to volatile methyl esters. In this Process, different methylating agents may be used, and these are methanol/sulfuric acid (20) or methanol-BF<sub>3</sub> (21). The methyl esters so produced are then identified with gas chromatography. Standard fatty acids methyl esters are often used for tentative identification purposes. For determination of fatty acid isomers, including *trans*-fatty acids, it is necessary to use appropriate columns and conditions for analysis.

Other parameters that are indirectly related to the composition of edible oils include iodine value and saponification value. The iodine value is a simple chemical constant for a fat or oil. It measures unsaturated or the average number of double bonds in fats and oils. Iodine value is defined as the number of grams of iodine that could be added to 100 g of oil, which is measured with the AOCS Method cd 1-25 (22). Meanwhile, saponification value is a measure of the alkali-reactive groups in fats and oils and is defined as the mg of KOH needed to saponify 1 g of oil. Shorter chain fatty acids give higher saponification values than do longer chain fatty acids.

### 3. MINOR COMPONENTS

*Polar lipids.* Polar lipids, mainly phospholipids, are present in fats and oils, and these originate primarily as components of cell membranes and serve biological functions in the cells. Among phospholipids present are phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI). In general, saturated fatty acids are present at the *sn*-1 and unsaturated fatty acids at the *sn*-2 positions of phospholipid molecules.

Sphingolipids are also important bioactive components of all membranes. Their hydrolysis products participate in regulation of growth, differentiation, and apoptosis by cells. They may also participate in reducing the cancer risk in humans; colon and skin cancers are particularly inhibited.

The content of polar lipids is reduced during oil refining. Degumming removes most polar lipids. However, refining, bleaching, and deodorization would also bring about a reduction in the content of polar lipids.

### 4. UNSAPONIFIABLES MATTER

In general, unsaponifiable matters are present in edible oils at less than 2% (23,24), which include tocopherols/tocotrienols, other phenolics, phytosterols, hydrocarbons, among others. The content of these unsaponifiable matters is varied in different oils and depending on the extent of oil refining. Although tocopherols and other phenolics as well as phytosterols are removed during different stages of oil refining, their main reduction occurs during deodorization of oils. Thus, deodorizer distillates rich in tocopherols and sterols may be used for production of these components, which may ultimately be used as nutraceuticals or for other food applications.

The dominance of tocopherols, namely, alpha-, beta-, gamma-, and delta- and the corresponding tocotrienols, depends on the type of oil under investigation. Thus, tocotrienols occur primarily in palm and rice bran oils. Meanwhile, tocopherols are more widely present in different oils. However, their proportions in different oils is dependent on the source material. As an example, sunflower oil contains mainly alpha-tocopherol and very small amounts of other tocopherols, whereas soybean oil contains mainly gamma-tocopherol with decreasing amounts of delta-, alpha-, and beta-tocopherols as determined by high-performance liquid chromatography.

Another group of unsaponifiable matter is phytosterols, fatty acid esters of phytosterols, and sterol glycosides. Again, their amount is reduced during processing. The presence of high amounts of phytosterols in soybean germ oil has been documented, which include beta-sitosterol, campesterol, stigmasterol, and  $\Delta^5$ -avenasterol. Phytosterols are recognized for their cholesterol-lowering properties (25). Phytosterols are usually analyzed with gas chromatography.

The hydrocarbons present in oils are composed mainly of squalene and carotenoids such as beta-carotene, among other carotenes. In addition, oxygenated



derivatives of carotenoids may be present. Palm oil serves as a rich source of carotenoids at 500–700 ppm.

## 5. CHARACTERISTICS OF FATS AND OILS

Fats and oils pass through a series of crystallization phases at cooling (26). Therefore, when melting such crystals, the melting points of fats and oils provides an estimate of their degree of saturation/unsaturation that parallels the saturation/unsaturation pattern dictated by their fatty acid constituents. *Trans*-fats, when present, have a higher melting point than do their *cis*-counterparts because of better packing of *trans*-fatty acids when compared with their *cis* counterparts. The melting behavior and crystal structures are major factors that are important when using such products in different applications such as in confectionary products.

Fats and oils often show multiple melting points. As an example, tristearin has three melting points at 52°C, 64°C and 70°C, because fats and oils solidify in more than one crystal form; this property is known as polymorphism. Crystallization of fats and oils occurs in two stages of nucleation and growth.

Titer is another variable often recorded for fats and oils (16). It measures the solidification point of the fatty acids as per AOCS Method ce 12-59 (22).

The density of liquid oils is dependent on their fatty acid composition, minor components, and temperature. An equation taking these into account was developed by Pantzaris (27) using iodine value, saponification value, and temperature. The density of liquid oils is in the range of 0.909–0.921 and for solid fats varies between 0.858 and 0.893. The lower values are for more solid fats such as lard and tallow. In a similar way, the viscosity of various vegetable oils depends on their fatty acids. Generalized methods have been developed that allow calculation of density and viscosity of different oils. Coupland and McClements (28) and Fisher (29) have related viscosity and density, refraction, surface tension, and other physical properties. Viscosity of fats and oils also depends on the temperature.

The refractive index of oils depends on their molecular weight, fatty acid chain length, degree of unsaturation, and degree of conjugation. Triacylglycerols have higher refractive indices than do their constituent free acids. Values of refractive index for different oils generally vary between 1.447 and 1.482.

Smoke point is another characteristic that is important if oils are used for frying. The temperature at which smoking is observed with actual frying or heating is measured with AOCS Method Ca 9a-48 (22). Smoke point depends primarily on the content of free fatty acids as they are more volatile than their corresponding triacylglycerols.

## 6. COLOR AND APPEARANCE

Most oils are yellow-red or amber liquids. The color is from the presence of chlorophylls and carotenoids. The colored bodies are often removed during the bleaching

process. Often lighter color has been associated with better quality oils, especially for salad oils and shortenings.

The presence of chlorophylls not only renders a green color to products, but also they act as sensitizers for fats and oils oxidation. However, unrefined olive oils contain 1–20 ppm of chlorophylls that are considered important as extra virgin quality indicators for this oil.

Carotenoids are present in edible oils at different levels. These are powerful antioxidants against both autoxidation and photo-oxidation. Therefore, attempts have been made to retain them or recover them, as in the case of palm oil. However, carotenoids may be degraded to colorless products at high temperatures exceeding 150°C.

The color of edible oils is measured by the so-called Wesson method that is described in the AOCS Method Ce 136-45 (22) by comparison with red and yellow Lovibond glasses of known characteristics. The oil is placed in Lovibond containers that are 1 or 5.25 inches, and the color superimposes a mixture of red and yellow standards to adjust to the color of the sample. Although color is three-dimensional, the brightness factor is not considered. Yellow is needed to allow the color to look similar, but yellow is considered unimportant in this method and only the redness is measured. This method is the one used by the U.S. edible oil industry. The British standard, however, uses Lovibond tintometer. The geometry and color scales for these two methods are different, as in the tintometric method, a series of permanently colored glass standards of red, yellow, and blue are used. Each standard color is numbered. The addition of the blue color field provides a greater degree of brightness and greenness than in the Wesson method (30).

## 7. OXIDATIVE QUALITY AND STABILITY TESTS

Oxidative stability of edible oils depends primarily on their fatty acid composition and, to a lesser extent, in the stereospecific distribution of fatty acids in the triacylglycerol molecules. The presence of minor components in the oils also affects their oxidative stability. A detailed discussion of oxidative processes in fats and oils is provided elsewhere in this series. Oxidation may occur via different routes and includes autoxidation, photo-oxidation, thermal oxidation, and hydrolytic processes, all of which lead to production of undesirable flavor and products harmful to health. Flavor and odor defects may be detected by sensory analysis or by chemical and instrumental methods. However, chemical and instrumental procedures are often employed in the processing and during usage of edible oils. Indicators of oxidation are those that measure the primary or secondary products of oxidation as well as those from hydrolytic processes or from thermal oxidation, including polymers and polar components (15).

*Peroxide value.* Peroxide value (PV) is the most common measurement of lipid oxidation. Hydroperoxides have no flavor or odor of their own, but they are unstable and break down rapidly to other products such as aldehydes that have a strong, disagreeable flavor and odor. Peroxide value measures the milliequivalents of oxygen

(hydroperoxides) per gram of oil. The iodometric AOCS Method Cd 8-53 (22) is used. PV is most widely used for determination of edible oil quality. The maximum PV of 0.1 and preferably less than 0.05 is expected for freshly refined oils. A peroxide value of higher than 10 meq/kg is considered unacceptable. Conjugated dienes and trienes absorbing at 234 and 268 nm, respectively, are directly related to hydroperoxides and are often used in addition or in place of PV.

## 8. CARBONYL COMPOUNDS

Carbonyl compounds in oxidized fats and oils are the secondary oxidation products that originate from decomposition of hydroperoxides. They usually have low threshold values and hence are responsible for off-flavor development in oxidized oils. Therefore, content of carbonyl compounds corresponds with sensory data.

*Anisidine value.* The *p*-anisidine value (*p*-AnV) measures the amount of unsaturated aldehydes in fats and oils. In this method, *p*-anisidine reacts with aldehydes in acetic acid to afford a yellowish color that is measured at 350 nm. The color intensity depends on the amount of aldehydes as well as on their structure. The AOCS Method Cd 18-90 (22) has been standardized for anisidine value analysis. The Totox value, which is  $2 \text{ PV} + p\text{-AnV}$ , provides information about the current status of oxidation as well as its history and is used by the industry.

*Thiobarbituric Acid Value.* The 2-thiobarbituric acid (TBA) test is a popular method for measuring sensory oxidation products. It is based on the formation of a colored complex between two molecules of TBA reagent with one molecule of malonaldehyde or TBA reactive substances (TBARS). This intensity of the pink chromogram is measured at 532 nm.

*Gas Chromatographic Methods.* Gas chromatographic methods may be used for measuring volatile oxidation products. Static headspace, dynamic headspace, or direct injection methods may be employed. Specific aldehydes may be measured as indicators for oxidative stability of oils and fats. Thus, propanal is an and as indicator for stability of omega-3 fatty acids, whereas hexanal is best for following the oxidative stability of omega-6 fatty acids.

*Free Fatty Acid/Acid Value.* Hydrolytic processes lead to the formation of free fatty acids by splitting of acylglycerols that can affect flavor. The Standard AOCS Method Ca 5a-40 and Cd 3a-63 (22) for acid value are commonplace. Free fatty acids are normally calculated as free oleic acids on a percentage bases. Free fatty acids are important quality indicators during processing and storage of fats and oils. They are also found during frying of fats and oils. The amount of moisture from foods fried and the frying temperature are important.

## 9. POLYMERS AND POLAR COMPONENTS

The content of polymers and polar components in oils increases during frying process. Size exclusion chromatography and HPLC may be used for the analysis of such components. The content of polar lipids should not exceed about 20%.

## 10. ANTIOXIDANTS

Antioxidants are used widely in fats and oils products to delay oxidative processes. Synthetic antioxidants, namely, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and propyl gallate (PG), are permitted antioxidants that are frequently used in products. Their presence and concentration may be determined with HPLC and GC methods. Meanwhile, metal chelators such as citric acid may be determined by HPLC analysis.

## 11. ADULTERATION

Adulteration of fats and oils is an old problem. Many older tests involved determination of physical properties such as refractive index, melting point, and viscosity. However, color tests were later used for this purpose. Thus, Baudouin reaction for sesame oil and the Halpben test for cottonseed oil have been noted. In both cases, a compound characteristic to an oil determines the presence of the oil. However, today such detections and quantitations are carried out with GC and HPLC procedures. Thus, cholesterol and phytosterols may be determined by gas chromatography for fingerprinting purposes; however, fatty acid analysis might also be used for higher levels of contamination (31). Detailed discussion of issues related to oil authentication and adulteration has taken place (11).

## 12. POLLUTANTS

Environmental pollutants such as pesticides and herbicides may be present in fats and oils. In this connection, special attention should also be paid to the presence of polychlorinated biphenyls (PCB) as well as dioxin as well as polycyclic aromatic hydrocarbon in the oil. The presence of high levels of such unwanted matters in the oil may render them unfit for edible purposes.

## REFERENCES

1. FAO/WHO. *Recommended International Standard for Edible Sunflower Seed Oil*, FAO and WHO, Rome, Italy, 1970.
2. H. W. Lawson, *Standards for Fats and Oils*, AVI Publishing Company, Westport, Connecticut, 1985.
3. J. B. Rossell, in J. H. P. Tyman and M. H. Gordon, eds. *Developments in the Analysis of Lipids*, Royal Society of Chemistry, London, U.K., 1994, pp. 179–202.
4. R. J. Hamilton, and J. Cast, eds. *Spectral Properties of Lipids*. CRC Press, Boca Raton, Florida, 1999.
5. M. S. O. Gomes, P. Sinnecker, R. T. Tanaka, and U. L. Lanfer-Marquez, *J. Agric. Food Chem.*, **51**, 1634–1639 (2003).
6. B. Muik, B. Lendl, A. Molina-Diaz, B. Ortega-Calderon, and M. J. Ayora-Canada, *J. Agric. Food Chem.*, **52**, 6055–6060 (2004).

7. R. Zamora, C. Olmo, J. L. Navarro, and F. J. Hidalgo, *J. Agric. Food Chem.*, **52**, 4166–4171 (2001).
8. G. L. Johnson, R. M. Machado, K. G. Freidl, M. L. Achenbach, P. J. Clark, and S. K. Reidy, *Org. Process Res. Develop.*, **6**, 637–644 (2002).
9. G. R. List, F. Orthoefer, T. Pelloso, K. Warner, and E. Neff, in *Physical Properties of Fats and Oils and Emulsifiers*, N. Willak, ed., AOCS Press, Champaign, Illinois, 2000, pp. 226–237.
10. E. C. Lopez-Diez, G. Bianchi, and R. Goodacre, *J. Agric. Food Chem.*, **51**, 6145–6150 (2003).
11. M. Jee, in M. Jee, ed., *Oils and Fats Authentication*, Blackwell Publishing (CRC Press), Boca Raton, Florida 2002, pp. 1–24.
12. M. H. Gordon, in M. Jee, ed., *Oils and Fats Authentication*, Blackwell Publishing (CRC Press), Boca Raton, Florida, 2002, pp. 143–155.
13. F. Shahidi, in K. R. Cadwallader and H. Weenan, eds., *Freshness and Shelf Life of Foods*, ACS Symposium Series 836, American Chemical Society, Washington, D.C., 2002, pp. 201–211.
14. J. Benedito, A. Mulet, J. Velasco, and M. C. Dobarganes, *J. Agric. Food Chem.*, **50**, 4531–4536 (2002).
15. E. G. Perkins, in A. J. St. Angelo, ed., *Lipid Oxidation in Food*, ACS Symposium Series 500, American Chemical Society, Washington, D.C., 1992, pp. 310–321.
16. R. D. O'Brien, in *Fats and Oils: Formulating and Processing for Applications*, Technomic Publishing Co., Lancaster, Pennsylvania, 1998, pp. 181–250.
17. D. Bosku, in F. D. Guston, ed., *Vegetable Oils in Food Technology: Composition, Properties and Uses*. CRC Press, Boca Raton, Florida, 2002, pp. 244–277.
18. K. G. Berger, and R. J. Hamilton, in R. J. Hamilton, ed., *Developments in Oils and Fats*, Blackie Academic & Professional, Glasgo, U.K., 1995, pp. 192–203.
19. F. D. Gustone, *Vegetable Oils in Food Technology: Composition, Properties and Uses*. CRC Press, Boca Raton, Florida, 2002.
20. M. A. Khan, and F. Shahidi, *J. Food Lipids*, **6**, 331–339 (2000).
21. S. M. Budge, and C. C. Parish, *Lipids*, **38**, 781–791 (2003).
22. AOCS. *Official Methods and Recommended Practices of the American Oil Chemists' Society*. 4th ed., AOCS Press, Champaign, Illinois, 1990.
23. F. Shahidi, and V. J. Shukla, *Inform* **7**, 1227–1232 (1996).
24. F. Shahidi, in K. R. Cadwallader, and H. Weenan, *Freshness and Shelf Life of Foods*, ACS Symposium Series 836, American Chemical Society, Washington, D.C., 2002, pp. 201–211.
25. P. C. Dutta, *Phytosterols as Functional Food Components and Nutraceuticals*, Marcel Dekker, New York, 2004.
26. N. Widlak, *Physical Properties of Fats, Oils and Emulsifiers*, AOCS Press, Champaign, Illinois, 1999.
27. T. P. Pantzaris, *Fat Sci. Technol.*, **97**, 397–402 (1985).
28. J. N. Coupland, and D. J. McClements, *J. Amer Oil Chem. Soc.*, **74**, 1559–1564 (1997).
29. C. H. Fisher, *J. Amer. Oil Chem. Soc.*, **75**, 1229–1232 (1998).
30. A. A. Belbin, *Inform*, **4**, 648–654 (1993).
31. J. M. N. Marikkar, H. M. Ghazali, Y. B. Che Man, T. S. G. Peiris, and O. M. Lai, *Food Chem.*, **90**, 23–30 (2005).

## *Dietary Lipids and Health*

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### 1. INTRODUCTION

Lipids support multiple biological functions in the body. They serve as the structural building material of all membranes of cells and organelles. Lipids are the most efficient fuel for living organisms containing more than twice the energy content compared with carbohydrates and proteins on a weight basis. Lipids and their derivatives also serve as signaling molecules that facilitate a variety of physiological functions. In addition, lipids are recognized as important biomarkers of disease and are involved in several pathological conditions. The cellular activities in tissues and organs are to some extent a result of biological actions of fatty acids mediated by changes in the membrane bilayer structure to impact the processes of membrane-associated receptors and signal transduction systems and ion channels. Recent literature also demonstrates a specific role of fatty acids in gene modulation and protein expression to influence risk of chronic disease.

In contrast to the shorter chain and more saturated fatty acids, the essential fatty acids (EFAs), linoleic acid (LA, an omega-6 fatty acid, 18:2n-6), and  $\alpha$ -linolenic acid (LNA, an omega-3 fatty acid, 18:3n-3) serve as substrates for the production

of polyunsaturated fatty acids (PUFAs) used in cellular structures and as precursors for the biosynthesis of many of the body's regulatory biochemicals [glycerolipids, long-chain (LC) PUFAs, and eicosanoids] (1). The eicosanoids are powerful, short-lived, hormone-like compounds synthesized from specific PUFA. In addition, formation and dietary sources of the LC-PUFA, arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) balance prostanoid production. An absolute requirement of DHA is necessary for normal neural and retinal development in the infant and young child. Biochemical and clinical studies indicate that sources of LNA and stearidonic acid (SDA, 18:4n-3) alter the balance of their respective LC-PUFA, principally EPA. A short-term human study indicated that vegetable oils containing SDA could be a dietary source of n-3 fatty acids that would be more effective in increasing tissue EPA concentrations than with LNA-containing seed oils (2). However, the concentration of DHA in tissues was not affected by dietary SDA supplementation, and this should be a concern when DHA is intended to be enhanced, for example, in infant nutrition.

This chapter introduces the contemporary understanding of food lipids in nutrition and health. The health aspects of dietary lipids and the underlying cellular and molecular mechanisms of PUFA actions are also discussed. A primary focus is placed on the role of dietary fat in cardiovascular disease and atherosclerosis.

### 1.1. The Dietary Reference Intakes

New dietary reference intakes (DRIs) for healthy individuals and populations were recently published by the Institute of Medicine of the National Academies in 2002. Along with setting the Adequate Intake (AI) of LA levels at 17 g/day for young men and 12 g/day for young women, the new report provides the first comprehensive recommendations for n-3 PUFA in the United States (3, 4). The DRIs for n-3 PUFAs place a primary emphasis on adequate consumption of LNA to satisfy the principle requirement for all ages and both genders. To a lesser extent, provisions for modest recommended intakes are made for the long-chain n-3 PUFAs, EPA, and DHAs. The daily AI for LNA are 1.6 and 1.1 g/d for adult men and women, respectively. The acceptable macronutrient distribution range (AMDR) for LNA is 0.6% to 1.2% of daily energy intake (4). Both EPA and DHA can satisfy 10% of the AMDR (0.06% to 0.12% energy) for n-3 PUFAs, and an optimal ratio of LA/LNA (n-6/n-3 fatty acids) is proposed to range from 5 to 10. The AMDR for total fat is set at 20% to 35% of energy. No DRIs are set for saturated fat and *trans*-fatty acids because of their perceived adverse effects on health and the tolerable upper intake levels (ULs) were not set for these fatty acids because of practical issues. In light of these DRIs for fatty acids, the food supply may provide a reasonable way for all healthy individuals and at-risk groups to achieve these intakes.

### 1.2. General Nutrition and Health of Lipids

Lipids (fatty acids, glycerol lipids, and cholesterol) are vital nutrients that serve as an energy source, structural components of the living organisms, and essential to

biological activities of homeostasis. Beyond their traditional role as nutrients, lipids or fatty acid molecules also facilitate critical biochemical and physiological functions as modulators of cell actions and genes. The n-6 and n-3 PUFAs have been recognized as ligands for peroxisome proliferator-activated receptors (PPARs). The PPARs regulate gene expression in lipid and carbohydrate metabolism (5, 6), but emerging evidence indicates that different PPARs are involved in a much broader capacity as biological regulators (7).

Long-chain PUFAs and their derivatives, such as prostaglandins and leukotrienes, are PPAR activators. The n-6 and n-3 PUFAs are the most potent PPAR ligand fatty acids. These natural ligands work on all three types of PPARs (PPAR $\alpha$ , PPAR $\gamma$ , and PPAR $\delta$ ) by binding to PPARs, however, the affinity to bind and expression of PPARs vary depending on the type of tissue and cell. The PUFA ligands (LA, AA, EPA, and DHA) are known agonists or antagonists of COX-2 expression through the activation of PPARs (8).

### 1.3. n-3 PUFA

The biological effects of dietary lipids on human health remain a primary focus of nutrition research as consumption recommendations are continually updated in response to new information obtained through epidemiological, clinical, and animal investigations. The role of n-3 PUFAs (DHA) in the development of the infant nervous system and retina is clearly established (9). Moreover, implications of a therapeutic effect (10, 11) on reducing cardiovascular disease and cancer risk and actions of their derivatives as biological effectors of human pathologies further drive biochemical and molecular investigations to elucidate the health benefits of dietary fatty acids (12). In addition to their beneficial impact on cardiovascular pathologies and cancers, n-3 fatty acids are also known to lessen the severity and minimize symptoms of chronic inflammatory diseases (13, 14), including rheumatoid arthritis (15) and inflammatory bowel disease (16), and may even benefit in correcting psychological disorders (17).

There are three major n-3 PUFA species present in food. These are LNA in vegetables, oilseeds, and nuts, and EPA and DHA in cold water fishes and algae. Another n-3 PUFA receiving greater attention recently is SDA, which is high in some plant oils (such as hempseed oil and black currant seed oil) but can be isolated and concentrated from marine fish. SDA may function as an important human dietary component for people with deficits in  $\Delta 6$ -desaturase activity. In human and other mammals, fatty acids of the n-3 series longer than 18 carbons cannot be synthesized from common carbon sources. Thus, n-3 PUFAs in the human body are either ingested directly or formed from LNA, which renders LNA as the essential fatty acid of the n-3 series PUFA. Although it is known that the human can make LC n-3 PUFAs starting with LNA, some evidence suggests that a supplement of preformed LC-PUFA is beneficial, especially in early infancy (formula-feeding) and under certain metabolic disease conditions.

James et al. (2) reported recently that consuming encapsulated SDA oil (ethyl ester) increased EPA and DPA<sub>(n-3)</sub> (22:5n-3) in healthy male and postmenopausal



female subjects ( $n = 15/\text{group}$ ) in a double-blind, parallel-group design study. The results clearly showed that SDA was more efficiently converted to biologically active 20 and 22 carbons  $n-3$  PUFAs and implies that vegetable oils containing SDA could be a dietary source of  $n-3$  fatty acids that would be more effective in increasing tissue EPA concentrations than LNA-containing oils. The introduction of SDA-containing oils in food manufacturing could provide a wide range of dietary alternatives for increasing tissue  $n-3$  PUFA concentrations to fulfill the benefits proposed for a variety of chronic health problems.

#### 1.4. Conjugated Linoleic Acid

Conjugated linoleic acid (CLA) is the name given to describe a group of positional and geometric fatty acid isomers of octadecadienoic acid. The CLA isomers are reported to have antioxidant capacity, reduce carcinogen-DNA adduct formation, induce apoptosis, modulate tissue fatty acid composition and prostaglandin  $E_2$  ( $PGE_2$ ) formation, and alter the expression and action of cytokines and growth factors (18). Though numerous biological actions of CLA have been reported, the most consistent findings include anticancer effects in rodents and cancer cells, and reduction of body fat in growing animals. In some cases, the biological responses observed from CLA isomers were influenced by the amounts of dietary  $n-6$  and  $n-3$  PUFAs (19–21).

CLA isomers have been recognized as effective anticarcinogenic agents for several types of cancers. The cytotoxic effects of CLA isomers on growth of various human and animal-derived cancer cells seem to be mediated by lowering the expression of the gene transcription factor Bcl-2 family members that inhibit apoptotic cell death or induce caspase-dependent apoptosis (22–26). CLA also prevented basic fibroblast growth factor-induced angiogenesis (27), a critical process for growth and metastasis of cancers. Evidence for the anticarcinogenic effects of CLA isomers indicates a modifying role in  $PPAR\alpha$  action (28).

Research demonstrated that CLA isomers reduce body fat in growing animals (29–31) and its actions on fat and energy metabolism may, in part, be directed through changes in both  $PPAR\alpha$  and  $PPAR\gamma$  (32, 33). In addition, specific effects of CLA isomers on activity and expression of enzymes associated with anabolic pathways of lipid metabolism are reported (34). For example, CLA was observed to decrease the mRNA level of the  $\Delta 9$ -desaturase enzyme in both liver tissue and hepatocyte cultures (35).

CLA may also modulate immune function by diminishing the production of an array of pro-inflammatory products in macrophages through activation of  $PPAR\gamma$  (32) and lowering basal- and lipopolysaccharide-stimulated IL-6 and basal tumor necrosis factor (TNF) production in rat resident peritoneal macrophages (19). Through activation of  $PPAR\gamma$ , CLA decreased interferon- $\gamma$ -induced mRNA expression of cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), TNF $\alpha$ , and pro-inflammatory cytokines [interleukin (IL)-1 $\beta$  and IL-6] in RAW macrophage cell cultures (32). Dietary CLA isomers also reduced ex vivo  $PGE_2$  production in rat bone organ cultures (20). Similar effects of CLA on  $PGE_2$

production in various biological systems have been demonstrated (36–38). Based on these aforementioned actions of CLA isomers, the likely biochemical and molecular targets that integrate their potential impact on biology include PPARs, COX enzymes, and other transcription factors.

### 1.5. Food Fortification

Although significant strides have been directed at reducing fat content in food products, certain lipid ingredients and sources of fatty acids are used to enhance the health and nutritional quality of foods. For example, CLA isomers were enriched in both dairy and nondairy products to convey its anticancer and antiobesity effects that were reported repeatedly in animal studies (39). Sources of n-3 PUFAs are also added directly to infant formula to provide sufficient DHA for normal development of the nervous system during early infancy. In the United States, DHA was approved by the FDA in 2001 to be added into infant formula (40, 41).

Biological enrichment of n-3 PUFAs has been reported in lamb muscle (42), chicken meat (43–46), eggs (43, 47), ewe milk (48–50), and pork (43). Sources of n-3 PUFA in the above mentioned food products included fish meal, fish oil, vegetable oils rich in n-3 PUFA (linseed oil and canola oil), algae and algal oil, and oilseeds. Clinical trials clearly showed improvement in visual function in infants fed LC-PUFA (DHA and AA)-enriched formula matching that of breast-fed infants (9). In most cases, addition of LC-PUFA did not dramatically affect the physical and sensory quality of the food products. However, enhancing the level of LC-PUFA resulted in elevated susceptibility to lipid peroxidation both in the food system and in the human body. To counter this problem, increased amounts of Vitamin E were tested and found to be satisfactory in reducing lipid peroxidation (47, 51). Several human studies indicated that n-3 PUFA fortification provides an effective means to increase n-3 PUFA intakes to satisfy the new DRIs for this group of health-enhancing fatty acids (52, 53).

Numerous studies have been conducted to enrich dairy products with CLA and n-3 PUFAs by providing sources containing high amounts of LC n-3 PUFAs to the ration of dairy cows. Feeding fish oil (200 ml and 400 ml/head/d) to dairy cows consistently increased milk CLA yield by as much as three-fold compared with the control group (54). Milk from multiparous Holstein cows supplemented with 2% added menhaden oil contained higher concentrations of CLA, transvaccenic acid, and total unsaturated fatty acids (0.68 and 2.51; 1.42 and 6.28; and 30.47 and 41.71 g/100 g of fat, respectively) compared with the milk from controls that consisted of a 50:50 ratio of forage to concentrate. Butter made from milk enriched with CLA inherently had higher concentrations of CLA (55). Similar findings were also reported by Donovan et al. (56) that by feeding lactating cows menhaden oil at 2% dry-matter basis, the CLA content in milkfat increased 356% (to 2.2 g/100 g fatty acids) compared with the milkfat of control cows. Aside from changes in CLA content, the n-3 PUFA also increased from a trace amount to over 1 g/100 g of milk fatty acids (LNA 0.22, EPA 0.40, and DHA 0.20 g/100g) when a diet with 3% fish oil was fed. In another study, concentrations of n-3 PUFAs

in milkfat were increased proportionally to the fishmeal amount in the diet and the CLA concentrations were higher with the 100% fishmeal diet than with the 100% soybean meal diet (57).

## 2. PUFA BIOCHEMISTRY

### 2.1. PUFA Formation

In human and most of the mammalian species, n-6 and n-3 series of PUFAs are derived either from the diet or via *in vivo* synthesis from precursor EFAs, i.e., LA and LNA because of the lack of enzymes needed to form these 18-carbon PUFAs from *de novo* biosynthesis. The formation of LC-PUFAs occurs in two locations within the cell. In the endoplasmic reticulum, LA or LNA is first desaturated by a  $\Delta 6$  desaturase (rate-limiting reaction step) and then elongated to form 20:3n-6 and 20:4n-3, followed by another desaturation step catalyzed by a  $\Delta 5$  desaturase to give rise to AA and EPA, respectively. The steps of elongation yield 22:4n-6 or 22:5n-3 from n-6 and n-3 EFA. The biosynthesis of 22:5n-6 and DHA is more complicated and involves the Sprecher pathway. In the formation of 22:5n-6 and DHA, as well as 22:4n-6 and 22:5n-3, the PUFAs are elongated in microsomes to 24:4n-6 or 24:5n-3, then transported to peroxisomes where another double bond is introduced by  $\Delta 6$  desaturase. Following the desaturation step, 2 carbons are removed (retroconversion) by  $\beta$ -oxidation and the resulting 22:5n-6 or DHA are transported to various sites to fulfill their cellular functions (58, 59). Recent studies with a selective  $\Delta 6$  desaturase inhibitor, SC-26196, further confirmed this pathway in human cell cultures (60). Leonard et al. (61) also identified a new elongation enzyme and its gene that is specific to LC-PUFA, which provided further support that this biosynthetic pathway for LC-PUFA occurs in the human.

The primary site for PUFA formation is in the liver, although other tissues appear to have the capacity to generate PUFA. Brain and retina are two special tissues, in which cellular phospholipids accumulate large amounts of AA and DHA. Dietary AA and DHA supplementation significantly (50–70%) reduces the conversion of LA and LNA in adults (62, 63). Age is also a factor that affects one's ability to form LC-PUFA from precursors. Carnielli et al. (64) showed that 30-day-old low-birthweight premature infants are capable of synthesizing DHA from LNA that was contained in the formula. It appears that, in aging, there is a gradual decline in the rate-limiting step of  $\Delta 6$  desaturation supporting LC-PUFA formation, and the decrease is greater in women than in men (65).

### 2.2. Prostanoids and Cyclooxygenases

The biosynthesis of prostanoids involves three important enzymatic reactions. In the case of PGE<sub>2</sub>, formation begins with the release of AA from phospholipids by phospholipase A<sub>2</sub> (PLA<sub>2</sub>), followed by the synthesis of PGH<sub>2</sub> by COX, and conversion of PGH<sub>2</sub> to specific prostanoids (e.g., PGE<sub>2</sub>) by terminal PG synthases (66).

The resulting PGE<sub>2</sub> is then released from the cell and exerts its effects via specific receptors (EP1, EP2, EP3, and EP4) to regulate various biological events (66).

COX plays a central role in prostanoid biosynthesis. Two main isoenzymes of COX have been identified to mediate the initial reactions of PG biosynthesis: COX-1 and COX-2 (67, 68). COX-1 is constitutively expressed in most tissues and is localized predominantly in the endoplasmic reticulum. COX-2 is typically expressed following stimulation with growth factors or cytokines and found, principally, in the nuclear and perinuclear membranes of the cell (69). COX-1 and COX-2 play central roles in PGE<sub>2</sub> production. Bone cells contain both inducible and constitutive cyclooxygenases (COX-1 and COX-2) and they are differentially regulated (70). Two genes have been identified to be responsible for the two isoforms of cyclooxygenase (67, 68). The regulation of PGE<sub>2</sub> production is predominantly through the regulation of COX-2, rather than COX-1 (71). PGE<sub>2</sub> was also shown to amplify its own production through stimulation of COX-2 in the same study (71). Recently, COX-3, a variant of COX-1 that is made from the same COX-1 gene but retains intron-1, was discovered in cerebral cortex and heart and seems to play a role in pain and fever development (72).

Nonsteroidal anti-inflammatory drugs (NSAIDs) nonselectively inhibit the activities of both COX-1 and COX-2. Out of concern for the gastrointestinal side effects of NSAID, scientists have discovered and developed many specific COX-2 inhibitors that facilitate a safer choice for controlling inflammatory diseases (73). For example, NS-398, a selective COX-2 inhibitor, has been shown to reduce tension (mechanical stress)-induced PGE<sub>2</sub> production from periodontal ligament cell cultures (74).

Dietary fat has also been shown to regulate the expression of COX-2. Singh et al. (75) reported that a high-fat corn oil diet (high in n-6 PUFA) may promote colon tumorigenesis by up-regulating COX-2 expression, whereas a high-fat diet with fish oil (high in n-3 PUFA) may exert its antitumor effect by inhibiting COX-2 expression. Moreover, dietary saturated fatty acids down-regulated COX-2 in rat liver with alcohol-induced disease (76). The nuclear receptor PPAR $\gamma$  has been shown to either down-regulate (77) or up-regulate (8) the expression of COX-2, depending on the model system. It is reasonable to speculate that dietary fat may exert a regulatory role in the expression and function of COX-2 to affect many physiological and pathological processes.

### 3. MOLECULAR ACTIONS

#### 3.1. Signal Transduction

Signal transduction is the sequential events that are initiated when a signal molecule binds with the cell surface receptor and ultimately causes changes in target genes. Fatty acids or their derivatives could affect the signal transduction system at each step of the cascade. Many signaling molecules that play critical roles in transmitting extracellular signals are known to be acylated to facilitate cross-membrane

translocation. These lipid molecules can also be signal mediators by themselves and even modulate the action of transcriptional factors, such as PPARs (78).

Certain PUFAs are known for their inhibitory effects on inflammation and autoimmune responses through regulation of signal transduction pathways. Zeyda et al. (79) showed that, when treated with PUFA, c-Jun NH<sub>2</sub>-terminal kinase, one member of the mitogen-activated protein kinase families, is inhibited. PUFAs have also been shown to modulate mammary cancer cell growth through regulation of the epidermal, growth-factor receptor/mitogen-activated protein kinase signal transduction cascade (80).

### 3.2. Transcription Factors

Transcription factors are protein molecules that convey metabolic parameters into nuclear transcriptional regulatory events and are fundamental components of the gene expression modulation mechanism. These transcription factors can be induced by endogenous and exogenous chemicals via either the signal transduction pathways or directly by their ligands and can directly regulate gene expression in response to these inducer molecules to affect a number of physiological functions such as fatty acid and glucose metabolism. Study of these signal factors will facilitate the elucidation of the molecular mechanisms underlying the regulation of metabolism and its possible disorders.

### 3.3. Peroxisome Proliferators Activated Receptors

PPARs are gene regulators belonging to the steroid/thyroid nuclear receptor superfamily. The PPARs heterodimerize with retinoid X receptors (RXRs) to form a PPAR/RXR heterodimer prior to interacting with target genes through their PPAR response elements (PPREs) in the target gene promoter region. Three isoforms of PPARs are identified, each demonstrating different tissue prevalence and regulatory functions. PPAR $\alpha$  is highly expressed in brown adipose tissue and is found in great levels in liver, kidney, heart, mucosa of digestive system, and skeletal muscle (81). PPAR $\alpha$  plays an important role in fatty acid oxidation in liver. PPAR $\gamma$  has two subtypes: PPAR $\gamma$ 1 and PPAR $\gamma$ 2. They both are expressed in adipocytes while PPAR $\gamma$ 1 is also found in other tissue and cell types, such as colon, kidney, macrophages, and skeletal muscle. The main function of PPAR $\gamma$  is regulation of adipogenesis and adipocyte differentiation as well as the control of insulin sensitivity.

Unsaturated or saturated fatty acids, LC-PUFA and their derivatives, such as prostaglandins and leukotrienes, are PPAR activators. The PUFAs are natural ligands that work on all three types of PPARs by binding to them with low affinity. The PUFA ligands (LA, AA, EPA, and DHA) are known agonists or antagonists of COX-2 expression through the activation of PPARs (8). EPA has been shown to increase PPAR $\gamma$ 1 gene expression in human adipocyte cultures (82). CLA has been shown to be a potent ligand and activator of PPAR $\alpha$  (33, 83, 84).

Among the genes that are involved in fatty acid metabolism, some are mediated by PPARs while others are not (85). PUFAs have been shown to suppress hepatic lipogenic gene expression through another group of transcription factors termed sterol regulatory element-binding proteins (SREBPs) (86). LC-PUFAs were also shown to directly modulate the transcriptional activity of the hepatocyte nuclear factor (HNF-4 $\alpha$ ), which plays an important role in regulation of hepatocyte differentiation, ureagenesis, and lipid metabolism (87–89). Other nuclear factors that are regulated by fatty acids include liver X receptors (LXRs) and retinoid X receptors (RXRs) (90).

### 3.4. Polymorphisms and Single Nucleotide Polymorphisms

Gene polymorphisms are variations in DNA sequences between individuals that happen more frequently than mutations. In the human, many chronic disease risks are defined by dual influences of the genetic makeup and the environment. As most human diseases, especially those that are chronic in nature, have complex inheritance patterns and variable genetic traits, it is believed that the genetic component that predetermines the risk and outcome of these diseases are sophisticated and heterogeneous. Therefore, with many genes contributing to disease risk, the polymorphisms have a modest independent and interactive influence overall to impact the phenotype of disease. Among the genetic variations, genetic polymorphisms, common variants as obtained from large population and family-based association studies, are recognized in recent years to play a role in common and complex chronic diseases. By this concept, each of the susceptible alleles only modestly affects the risk of the disease in an individual but can account for a large proportion of the population-attributable risk. Among the genetic variants, single nucleotide polymorphism (SNP, a single nucleotide substitution) is the most frequently occurring gene mutations in human genomes. The search for SNPs that are associated with disease risks is currently under wide investigation in the biotechnology and pharmaceutical industry.

By identifying genetic susceptibility factors, it is believed that prevention of or delay in the onset of chronic disease might be possible by understanding the role of dietary components that modify genes or SNPs. Couture et al. (91) reported that a reduction effect of 18.5% in low-density lipoproteins (LDL)-cholesterol from a high-carbohydrate diet in men was attributable to apolipoprotein E (apoE) polymorphism E2 allele. It was also shown that human subjects carrying scavenger receptor class B, type I (SRB-I) gene exon-1 polymorphism homozygous for allele 1 are less susceptible to the presence of saturated fatty acids in the diet than subjects that were heterozygous for allele 2 (92). Vincent et al. (93) reported in a human dietary intervention study that several gene polymorphisms of key proteins have been identified and linked to variable responses of diet. Some interactions found in this study include apoE and LDL-cholesterol and triacylglycerols, apoA-IV and LDL-cholesterol, microsomal transfer protein and LDL-cholesterol, as well as intestinal fatty-acid-binding proteins and triacylglycerols. Rantala et al. (94) showed that the activity of paraoxonase-1 (PON1), an enzyme that may help prevent

lipoprotein oxidation of high-density lipoproteins (HDLs), was affected by dietary vegetable content with high levels of vegetables in the diet resulting in a lower amount of the enzyme activity as well as reduced cholesterol levels. This response was modulated by the genetic variance of PON1. Therefore, future research on dietary fats and their components may demonstrate an important association between modifying chronic disease risk via genes and SNPs by PUFA in the individual.

## 4. FAT AND CHRONIC DISEASES

### 4.1. Association of Fat and Chronic Diseases

The food we eat plays a critical role in our overall health. It is believed that a substantial amount of chronic disease risk is diet-related and could be significantly reduced through improvements in dietary habits, e.g., up to 70% of all cancers in the United States are attributable to diet (95). Indeed, a recent report by the USDA indicates that poor diet and diet-related chronic diseases contribute to five of the ten leading causes of death (heart disease, cancer, stroke, diabetes, and arteriosclerosis) costing the United States economy an estimated \$250 billion annually (96).

Dietary fat intake has remained a key focus of nutrition research in recent years and dietary guidelines for fat consumption have been continually updated in response to new information gained through epidemiological and clinical studies. Links to cardiovascular disease (CVD), degenerative and inflammatory arthritis, cancer and osteoporosis, and the recognition of fats or their derivatives as biological effectors of human pathologies have fueled efforts to characterize the behavior of lipids *in vivo*. Initially, the association of cholesterol and saturated dietary fat with increased risk of CVD spurred dietary recommendations to reduce the intake of animal fat and to increase the intake of plant oils. However, mounting evidence is now showing that with increased dietary intake of plant oils, such as corn, safflower, and soybean (especially the partially hydrogenated form), which are high in LA, the dietary ratio of n-6/n-3 fatty acids has increased significantly during the past years (97). The high intake of n-6 with an inadequate amount of n-3 fatty acids in the diet could contribute to the development of some cancers and other chronic diseases.

Intake of dietary sources of n-3 fatty acids is associated with reduced incidence and severity of inflammatory disorders, cardiovascular diseases, and some cancers in humans (12, 98–103). Populations consuming fish that are rich in n-3 fatty acids are known to have a low incidence of atherosclerotic disorders (104). Dietary fish oil, which is high in EPA and DHA, also was shown to reduce myocardial ischemic damage (105) and ventricular fibrillation (106). The antitumorigenic effect of n-3 fatty acids was demonstrated in breast cancer (107), colon cancer (108–110), and pancreatic neoplasm (111). In addition to their beneficial influence on cardiovascular disorders and cancers, n-3 fatty acids are also known to decrease the severity and minimize symptoms of inflammatory diseases, including rheumatoid arthritis (15) and inflammatory bowel disease (16), and may be of benefit in correcting psychological disorders (17).

Tissue fatty acid composition correlates well with dietary intake of foods rich in n-3 and n-6 PUFA; therefore, it is possible to examine the consequences (positive or negative) of major changes in dietary habits between and within ethnic populations. For example, in Japan, the intake of n-6 fatty acids has markedly increased over the past 40 years while the level of n-3 fatty acids consumed has remained constant. This may be explained by a decreased popularity of fish and fresh vegetables among younger Japanese and their adoption of American dietary habits. As a result, fatty acid intakes for this group mirror the trends observed in average Americans (112). Currently, the average Japanese consumes over 14 g/day of LA and 2 g/day and 1.6 g/day, respectively, of LNA and EPA plus DHA (113). As a consequence, a dietary increase in n-6 intake has led to an increase in the ratio of n-6/n-3 fatty acids from 2.8 in 1955 to over 4 by 1985.

Interestingly, the incidence of breast cancer for native Japanese and Chinese is lower compared with Japanese and Chinese who have immigrated to the United States. Furthermore, the incidence of breast cancer in Japanese and Chinese residing in the United States, and presumably consuming a Western diet, is similar to the incidence in American women. These results confirm the influence of environmental factors, especially diets high in fat, on the incidence of breast cancer (114). Cancer incidence of epithelial origin in Japan is increasing, and includes lung, stomach, colorectal, mammary, and uterine. These cancers, that are termed Western-type cancers, are rising with the subsequent increase in the dietary ratio of n-6/n-3 fatty acids. Mortality rates of these cancers are approaching precedent levels based on epidemiological data in the United States. In addition, the incidence of rheumatoid arthritis in Japan is much less than predicted (115) in keeping with the expected effect of dietary n-3 fatty acids on prostanoid formation (116).

The development of these chronic, Western-type diseases is associated with an excessive formation and function of eicosanoids derived from n-6 fatty acids. As balance can be restored to eicosanoid biosynthesis by dietary n-3 fatty acids, an effective strategy to diminish cardio-cerebrovascular mortality (in addition to several other serious disorders) may be to decrease the intake of n-6 fatty acids and replace them with n-3 fatty acids (116). Such a strategy is supported by studies that show an increased incidence of cardiovascular diseases, specifically ischemic heart disease, in Japanese whose diet has increasingly become more Westernized (113, 117).

#### **4.2. Mode of Action of n-3 PUFA Against Chronic Diseases**

To advance understanding of the dynamic influence of dietary lipids, research efforts are focusing on the importance of the balance between n-6 and n-3 fatty acids in the human diet. What is emerging is recognition that these PUFAs modulate eicosanoid biosynthesis in numerous tissues and cell types, alter signal transduction, and influence gene expression (87, 118). The effect of n-6 and n-3 PUFA on CVD, cancer and bone/joint health is related to the newer discoveries of how dietary PUFA impact health.



In the human and other mammals, eicosanoids derivatives of 20 carbon n-3 or n-6 PUFA, such as prostaglandins, leukotrienes, and thromboxanes, act and lead to the activation of various signaling mechanisms that have effects on numerous cellular functions that may be either beneficial or detrimental. Thus, modulation of PUFA formation and eicosanoid biosynthesis could be a feasible way to reduce the risks of certain chronic illnesses like arthritis, diabetes, inflammation, cancer, and cardiovascular disease (119). Therefore, although numerous mechanisms are involved in the biological activities of the n-3 fatty acids, a unifying attribute is the down-regulating effect on eicosanoid production from n-6 fatty acid precursors. Eicosanoid biosynthesis requires activation of phospholipases and the availability of the free fatty acid substrate, AA and EPA. The primary eicosanoid precursor is AA. Unesterified AA is transformed to eicosanoids by cyclooxygenase (two isoforms, COX-1 and COX-2) and lipoxygenase enzymes. Several lines of evidence indicate that the amount of eicosanoids produced from AA correlates with the severity of inflammation in rheumatic diseases (120).

Eicosanoids derived from the n-3 PUFAs are up to 100-fold less biologically potent for inducing pro-inflammatory cellular responses than those derived from AA (121, 122). It is proposed that dietary n-3 PUFAs act by two different mechanisms to control the amount of n-6 eicosanoids maintained in tissues: first, by competing for incorporation into tissue lipid esters, thus reducing the rate of tissue formation of active n-6 eicosanoids; and second, by forming weaker n-3 eicosanoids (123, 124) that compete at cellular receptor sites and diminish signaling by eicosanoids derived from n-6 PUFAs. As a result, diets with n-3 PUFAs create conditions that reduce the formation and constrain the function of active n-6 eicosanoids in stimulated cells (116).

Dietary supplementation of n-3 PUFA-rich fish oil significantly decreased the ability of bone tissue to produce PGE<sub>2</sub> in animals compared with those given high-n-6 fatty acid diets (20, 125). It is presumed that prostaglandin E<sub>3</sub> (PGE<sub>3</sub>), an eicosanoid derivative of EPA, could have increased in these animals given the high-n-3 diet. Although PGE<sub>3</sub> is as potent as PGE<sub>2</sub> in mediating bone resorption, EPA is a less effective substrate for cyclooxygenase than AA (126). Hence, supplementation with oils high in EPA typically reduces the 2-series PG derived from AA with a small increase in 3-series PG derived from EPA (127). In addition, PGE<sub>3</sub> is less inflammatory than PGE<sub>2</sub> (128), which partly explains why n-3 PUFA is beneficial for controlling inflammatory bone/joint diseases.

The modulatory effect of n-3 PUFAs on eicosanoid production could also be achieved at the enzyme level. The action of n-3 fatty acids that decreases the production of PGE<sub>2</sub> could be an effect of down-regulation of COX-2 activity in local tissues (129, 130). In a study with rats, dietary n-6 PUFA up-regulated COX-2 and, to some extent, COX-1 expression leading to a concomitant increase in COX enzyme activity and prostaglandin synthesis, but fats containing added menhaden oil (high in n-3 PUFAs) had an opposite effect (131).

Experiments by Curtis et al. (129) gave evidence that the observed effects of n-3 fatty acids were not always mediated by the alterations in eicosanoid metabolism. In that study, supplementation of IL-1-stimulated cultured chondrocytes

with LNA resulted in gene suppression of IL-1, TNF $\alpha$ , and COX-2, and one of the cartilage-degrading enzymes, aggrecanase. The supplemented LNA was not converted to the longer chain n-3 fatty acids, i.e., EPA and DHA, however, supplementation of chondrocytes with EPA has produced effects similar to those enriched with LNA.

The LC-PUFAs are believed to possess the ability to covalently attach to a variety of proteins, thus dramatically affecting translocation, cell-to-cell signaling, and protein function (118). Dietary fatty acids may regulate cell signaling pathways via cell surface receptors, proximal/accessory components of receptor-mediated pathways, at intermediate signaling steps, and via nuclear receptors. They act as cellular second messengers and modulators during cellular transduction of external signals. They also modify the activities of enzymatic processes, such as those catalyzed by phospholipases, protein kinases, G-proteins, adenylate and guanylate cyclases, as well as ion channels and other biochemical events involved in stimulus-response coupling mechanisms (132).

The n-3 PUFA may also alter gene expression via direct interaction with proteins involved in gene transcription (87). Investigations suggest that a variety of PUFA (EPA, DHA, etc.) influence transcriptional regulatory mechanisms including the previously described PPARs, and nuclear factor kappa B (NF- $\kappa$ B), the SREBP, and a PUFA response element (133). Camandola et al. (134) showed that AA supplementation of human monocytes strongly stimulated nuclear translocation of NF- $\kappa$ B, a transcription factor that is believed to regulate the expression of gene products involved in inflammatory reactions. Activation of NF- $\kappa$ B could also be achieved by stimulation with PGE<sub>2</sub>, but not with the n-3 PUFA, EPA. Therefore, a low dietary ratio of n-6/n-3 fatty acids may down-regulate NF- $\kappa$ B-mediated gene expression of pro-inflammatory mediators. The PUFAs could also modify the expression of oncogene *in vivo*. Rats given safflower oil (high in LA) had elevated levels of p21<sup>ras</sup> protein compared with those offered menhaden oil (high in n-3 PUFA). The Ha-*ras* mRNA was also increased in rats given a diet high in n-6 PUFA and high in fat content (21% of diet) compared with those given a high-fat diet rich in n-3 PUFA, implying a tumor promoting potential of n-6 fatty acids (131).

## 5. ROLE OF DIETARY FAT IN CARDIOVASCULAR DISEASE AND ATHEROSCLEROSIS

### 5.1. Introduction

Even though the mortality from coronary heart disease has declined recently, atherosclerosis and related vascular disorders still are the leading cause of death in the Western world. The etiology of this disease is multifactorial, with hyperlipidemia, smoking, diabetes mellitus, hypertension, and obesity being well-established risk factors for the development of atherosclerosis. Dietary fat affects plasma lipids, lipoproteins, and vascular inflammation and, thus, is linked to atherosclerosis.

Injury to or abnormal mechanisms of the vascular endothelium may be initiating events in the etiology of atherosclerosis.

Although epidemiological studies suggest that dietary cholesterol and saturated fatty acids increase serum cholesterol, recent evidence suggests that high intakes of polyunsaturated fats, and, especially, fats high in n-6 PUFAs, may be equally atherogenic because of their ability to convert easily to cytotoxic lipid peroxidation products, thus contributing to a cellular imbalance in oxidative stress antioxidant status and to an inflammatory response. Most of the data available in the literature are from studies where high-fat diets were used. Conversely, low-fat diets, independent of the fat source, may be the prudent choice in prevention and treatment of atherosclerosis. Low-fat diets usually are high in dietary fiber, antioxidants, and other undefined materials, all of which may protect against atherosclerosis.

## 5.2. Pathogenesis of Atherosclerosis

*Theories:* There are numerous theories for the pathogenesis of atherosclerosis. Despite considerable research, the etiology of this disease is not well understood. The current trend is to consider atherosclerosis as a response of the vascular wall to a variety of initiating agents and multiple pathogenic mechanisms (e.g., hyperlipidemia) contributing to the development of atheromatous plaques. In fact, recent research suggests that atherosclerosis is a chronic inflammatory disorder accompanied by risk factors such as oxidized LDL, reactive oxygen species, diabetes, and infection. It appears that the major participants in the atherosclerotic disease process include an active vascular endothelium, smooth muscle cells, blood-borne cells such as monocytes and macrophages, and circulating lipoproteins. The result is a multifactorial sequence of events involving endothelial cell injury/dysfunction, uptake of circulating blood monocytes and their differentiation into macrophages, coupled with smooth muscle cell migration and proliferation.

The most intensely studied current hypothesis of atherosclerosis is the response to injury hypothesis (135). The hypothesis takes into account the cellular interactions that occur during the different phases of lesion initiation, development, and progression. The initiating event appears to be injury to or dysfunction of the endothelium via lipids or lipoprotein derivatives or via mechanical, chemical, toxic, viral, or immunologic agents. These events may induce growth-factor secretion and changes in endothelial cell surface adhesive glycoproteins. Monocytes are attracted and attach to endothelial cells that will contribute directly or indirectly to continued secretion of growth factors and other biologically active molecules. Subsequent subendothelial migration of monocytes may lead to fatty-streak formation and release of cytokines and growth factors. Monocytes and macrophages are major cellular components of lesions and, thus, are likely to play a role in their initiation and evolution. These events provide three possible sources of cytokines and growth factors, namely from platelets, macrophages, and the endothelium. Some of the smooth muscle cells in the proliferative lesion themselves may form and secrete cytokines and growth factors, such as platelet-derived growth factor. It is not clear what role dietary fat plays in the above stated events. However, hyperlipidemia, or

some component(s) of hyperlipidemic serum, as well as other risk factors, are thought to cause endothelial injury/dysfunction, resulting in endothelial cell activation, adhesion of platelets or monocytes, increase in cytokine activity, and transmigration of monocytes into the arterial intima. Once in the subendothelial space, monocytes transform into macrophages, take up substantial amounts of lipid, and become foam cells. These foamy macrophages, as well as other cells, also can produce cytokines and growth factors that cause migration of smooth muscle cells from the media into the intima. These interactions then lead to fibrous plaque formation and further lesion progression. In other words, once smooth muscle cells proliferate in the intima, there is further lipid accumulation as well as elaboration of the extracellular components of the atheromatous plaque.

Even though numerous risk factors, including hyperlipidemia, smoking, and hypertension, seem to contribute to the development of atherosclerosis, to date it has not been possible to link these risk factors into a common pathogenic mechanism. There is evidence, however, that modulations in the level of activity of a select set of endothelial transcription factors (e.g., endothelial NF- $\kappa$ B) may provide a mechanism for linking these seemingly diverse processes with the generation of dysfunctional endothelium and the onset of atherosclerotic lesion formation (136, 137). Stimuli known to activate the NF- $\kappa$ B complex include inflammatory cytokines, with the common denominator apparently being reactive oxygen species. One may speculate that oxidized lipids, when present in inappropriate levels, may induce endothelial oxidative stress and generate excess reactive oxygen species, which activate NF- $\kappa$ B and modulate endothelial gene expression. Antioxidants and related compounds may protect against atherosclerosis by inhibiting the activation of endothelial transcription factors such as NF- $\kappa$ B.

*Dietary Fat:* There is ample evidence demonstrating that serum cholesterol is a predictor of atherosclerosis and that serum cholesterol concentrations can be modified by varying the composition of dietary fat. Keys et al. (138), in 1957, and Hegsted (139), in 1965, provided the first quantitative estimates of the relative effects of the various classes of fatty acids on serum cholesterol concentrations. Both studies indicated that saturated fatty acids increased, whereas PUFA decreased, serum cholesterol. Also, monounsaturated fatty acids had no specific effect on cholesterol concentrations. These conclusions were based on combining results from numerous studies and then describing mathematically the relationship between dietary fatty acid composition and serum total cholesterol concentrations. However, it is now known that saturated fatty acids are not equally hypercholesterolemic. For example, stearic acid (18:0) and saturated fatty acids with less than 12 carbon atoms seem to have little or no effect on raising serum cholesterol levels. This suggests that the cholesterol-raising properties of saturated fatty acids should be attributed solely to lauric (12:0), myristic (14:0), and palmitic acid (16:0). However, these three saturated fatty acids appear to have different effects on serum total-cholesterol concentrations as well. Numerous studies suggest that lauric acid is less, and myristic acid probably more, hypercholesterolemic than palmitic acid (139, 140). Furthermore, human studies suggest that lauric acid raises total serum cholesterol and LDL cholesterol concentrations compared with oleic acid (18:1n-9). However, it is not as

potent in increasing cholesterol concentrations as is palmitic acid. Although in normocholesterolemic men and women, dietary palmitic and oleic acids seemed to exert similar effects on serum cholesterol and lipoprotein profiles. Much still needs to be learned about the effects of dietary fat on serum cholesterol levels and metabolism (141–143). It is clear from these and other studies that extremes in types of dietary fat should be avoided and that moderation in dietary fat is advisable.

Even though serum cholesterol appears to be a risk factor for atherosclerosis, i.e., each 1% rise in serum cholesterol is predicted to increase the risk of coronary disease by about 2%, the effect of dietary cholesterol on serum cholesterol concentration is not clear and far from being understood. It appears, however, that the average baseline consumption of cholesterol-containing foods can modulate the magnitude of a mathematically predicted change in serum cholesterol due to changes in dietary cholesterol (144). An increase in dietary cholesterol is expected to have the greatest effect on serum cholesterol level when the past baseline amount of dietary cholesterol was near zero. However, if the baseline cholesterol consumption is greater than 500 mg/d, additional dietary cholesterol will contribute to little measurable change in serum cholesterol. This suggests that people who desire maximal reduction of serum cholesterol by dietary means may have to reduce their dietary cholesterol to minimal levels in order to observe significant serum cholesterol reductions. Furthermore, the responsiveness to dietary cholesterol can be extremely variable, with some individuals being much more responsive (hyperresponders) than others. Thus, the need to limit cholesterol intake should apply more strictly to diet-sensitive hypercholesterolemic individuals rather than to the population in general. Individual variations in the response to dietary cholesterol may be mediated by differences in fat absorption efficiency, neutral sterol excretion, conversion of hepatic cholesterol to bile acids, or modulation of key enzymes involved in intracellular cholesterol metabolism, such as HMG-CoA reductase.

Although regression analysis of numerous human studies suggests that cholesterol and saturated fatty acid intake are primary determinants of serum cholesterol, the role of dietary fat in the development of atherosclerosis remains controversial and not well understood. The question arises whether or not dietary saturated fats should be replaced by unsaturated fats. Unsaturated fats, especially n-3 fatty acids, may be beneficial to human health (145, 146). Some populations, such as the Greenland Eskimos who consume high levels of n-3 fatty acids from fish and sea mammals, have lower incidence of coronary heart disease (147). In patients with hyperlipidemia, n-3 fatty acids only at high doses result in a decrease of LDL cholesterol. However, these fatty acids consistently lower serum triacylglycerols in normal subjects and in patients with hypertriglyceridemia. There is clear evidence that hypertriglyceridemia is an independent risk factor of cardiovascular diseases such as atherosclerosis (148, 149). Increasing the intake of foods rich in n-3 PUFA may be most critical in decreasing the risk of cardiovascular diseases, especially in light of a recent human-derived study, suggesting that consumption of total 18:3n-3 is inversely related to plasma triacylglycerol concentrations (150).

Diets high in n-6 and n-3 fatty acids may lead to a decrease in serum cholesterol but replacing saturated with unsaturated lipids may not be desirable because of their tendency to be oxidized. As mentioned earlier, mounting evidence is now showing that with increased dietary intake of plant oils, such as corn, safflower, and soybean oil, which are high in LA, the dietary ratio of n-6/n-3 fatty acids has increased significantly during the past years (97). The high intake of LA-rich fats will lead to serum hypertriglyceridemia and an increase in cellular oxidative stress. There is evidence that most age-related diseases are initiated by elevated cellular oxidative stress or an imbalance in the body's oxidative stress/ antioxidant status, as well as a state of chronic low-level inflammation.

Dietary antioxidants, such as Vitamin E, might act as antiatherogenic agents by suppressing oxidative modification of LDL and the recruitment of monocytes into the arterial subendothelium by smooth muscle cells (151). In fact, data from subjects with varying degrees of coronary atherosclerosis support the hypothesis that high-serum PUFA levels, when insufficiently protected by antioxidants, may indicate a higher risk of atherosclerosis (152). In particular, a positive relationship between LA intake and coronary artery disease has been observed in animal and human studies (153, 154). In fact, the potential detrimental health effect of high intakes of LA has been termed the "linoleic acid paradox" (155), in which a supposedly healthy fatty acid (i.e., one that lowers total cholesterol) is associated with increasing rates of cancer and inflammatory and cardiovascular diseases. Moreover, a low intake of LNA and other n-3 (fish) oils may further compound this paradox. All these studies lead one to conclude that the type of fat becomes a less significant component in the pathogenesis of atherosclerosis, when one consumes a low-fat diet, rich in soluble fibers and natural antioxidants.

### 5.3. Lipoprotein Metabolism

Plasma lipoproteins are units of complex lipid and protein compositions. Lipoproteins function primarily as carriers of lipids in the blood. The apoprotein fractions of the different lipoproteins play an important role in the regulation of the metabolic fate of the different plasma lipoproteins via their role as enzymatic cofactors and their interactions with specific receptors in cell membranes. Lipoproteins can be separated by density into four major different classes: chylomicrons, very low-density lipoproteins (VLDLs), LDLs, and HDLs. Both chylomicrons and VLDLs are triacylglycerol-rich particles. Chylomicrons are of mucosal cell origin and function mainly as carriers of lipids of exogenous dietary origin to the liver and peripheral tissues. VLDLs, on the other hand, are of hepatic origin and transport endogenous lipids. LDLs are generated primarily by the metabolism of VLDL and are high in cholesterol and cholesteryl esters. HDLs are the smallest particles and contain the highest relative amount of protein. These units are relatively rich in cholesterol and phospholipids. High levels of LDL are associated with atherosclerosis and coronary heart disease, whereas high levels of HDL provide protection from these diseases. In fact, HDL particles appear to play an important role in the efflux of cholesterol from the extrahepatic tissues.

## 5.4. Dietary Fat and Lipoprotein Metabolism

There is substantial evidence that indicates that dietary fat can influence significantly not only serum levels of cholesterol and triacylglycerols but also the lipid composition and content of lipoproteins (156–159). Much attention has been placed on the effects of diet on LDL levels, and saturated fatty acid and cholesterol itself have been identified as the major nutritional factors that can raise serum LDL-cholesterol levels. However, LDL cholesterol is only one of the many risk factors for atherosclerosis, and it is not known if oxidative modification of LDL is an equally or more important factor in the pathogenesis of atherosclerosis than total LDL cholesterol *per se*. More longitudinal studies are needed to answer these questions. If lipid peroxidation is a major risk factor for atherosclerosis, then excess consumption of highly unsaturated fats may not be advisable.

The quantitative relationship between cholesterol intake and cholesterol levels is still controversial, especially because in humans, there appears to be a high individual variability in processing of dietary cholesterol. However, numerous animal and human studies support the concept that dietary cholesterol can raise LDL-cholesterol levels and change the size and composition of these particles as well. LDL particles become larger in size and enriched in cholesterol esters. Mechanisms contributing to these events include an increase in hepatic synthesis of apoB-containing lipoproteins, increased conversion of VLDL remnants to LDL, or a decrease in the fractional catabolic rate for LDL. Reduced LDL receptor activity due to an increase in hepatic cholesterol content, secondary to excess dietary cholesterol, may lead to a decreased uptake of both LDL and VLDL remnants.

In addition to dietary cholesterol, saturated fatty acids also are thought to raise serum LDL-cholesterol levels as well as total cholesterol concentrations. The major effect of saturated fatty acids on serum cholesterol appears to be due to a reduction in LDL-receptor activity. It is likely that saturated fatty acids may contribute to a cellular redistribution of cholesterol and cholesterol oxidation derivatives, leading to a favorable environment by these lipid particles to suppress LDL-receptor synthesis. In addition to their effects (directly or indirectly) on LDL-receptor activity, saturated fatty acids also may promote the synthesis of apoB-containing lipoproteins.

It is now known that not all saturated fatty acids are equally hypercholesterolemic. For example, medium-chain saturated fatty acids of carbon length 8–10, as well as stearic acid (18:0), have little or no effect on serum cholesterol concentrations. In contrast, evidence indicates that palmitic acid (16:0), the principle fatty acid in most diets, can increase serum cholesterol concentrations in humans. However, in normocholesterolemic humans, dietary palmitic and oleic acids have been shown to exert similar effects on serum cholesterol, suggesting that only humans or animal species sensitive to dietary cholesterol and selected fats (“hyperresponders”) may exhibit significant changes in serum cholesterol in response to dietary fat intake. Myristic acid (14:0) and, to a lesser extent, lauric acid (12:0), which are relatively high in coconut oil, both can raise serum cholesterol and LDL-cholesterol levels. Overall, it is not clear why humans respond so differently to cholesterol or

saturated fatty acids. Variations may exist at the level of fatty acid catabolism and regulation of LDL-receptor activity.

In contrast to saturated fatty acids, unsaturated fatty acids may not be cholesterol-emic. However, because of their ability to become oxidized and thus to contribute to oxidative stress within a cell, some unsaturated fats could indirectly be highly atherogenic. Oleic acid, the major monounsaturated fatty acid in the diet, often is called a "neutral" fatty acid because it has a neutral or cholesterol-lowering effect on serum cholesterol. The main classes of unsaturated fatty acids in the diet can be divided into n-6 and n-3 PUFA. LA is the predominant n-6 fatty acid, and the parent n-3 fatty acid is LNA. Both occur in plant oils. Fish oils contain large amounts of LC-PUFA, e.g., 20:5n-3, which have their origins of plant sources. With regard to cholesterol metabolism, LA may lower serum cholesterol levels by up-regulating LDL-receptor activity or by inhibiting hepatic synthesis of apoB-containing lipoproteins. LC n-3 PUFA appear to have a greater influence on triacylglycerol than on cholesterol metabolism. High intake of fish-oil-derived n-3 fatty acids reduces triacylglycerol levels, especially when fed to individuals with hypertriglyceridemia.

The role of dietary fats on HDL metabolism is not as well understood. In general, saturated fatty acids do not reduce HDL cholesterol, and dietary monounsaturated fatty acids, when substituted for saturated fatty acids, contribute to a favorable modification of the lipoprotein ratios, i.e., a decrease in the ratio of LDL/HDL. In contrast to monounsaturated fatty acids, a high intake of n-6 PUFA (e.g., LA) reduces HDL cholesterol concentrations, possibly by reducing the synthesis of apoA-I, a major HDL apoprotein. The actions of n-3 fatty acids on HDL-cholesterol levels are similar to those of LA. Even though unsaturated fatty acids do not appear to be hypercholesterolemic in general, their HDL cholesterol-lowering capacity might be of concern, as HDL is directly protective against atherosclerosis. Decreased serum HDL levels would indicate reduced removal of lipids from the arterial wall.

## 5.5. Lipids and Endothelial Cell Dysfunction

As the knowledge of the pathogenesis of atherosclerosis rapidly increases, it appears that an active vascular endothelium, smooth muscle cells, and blood-borne cells such as monocytes and macrophages all play active roles in the atherosclerotic disease process. Risk factors, such as elevated plasma levels of certain lipids, prooxidants, and cytokines, may contribute to the chronic activation/stimulation as well as to the damage of the endothelium and other vascular tissues (160). There is evidence that supports the hypothesis that it is not only pure cholesterol and saturated fats but rather oxidation products of cholesterol and unsaturated fats (and possibly certain pure unsaturated fats) that are atherogenic, possibly by causing endothelial cell injury/dysfunction. Lipid-mediated endothelial cell dysfunction may lead to adhesion of monocytes, increased permeability of the endothelium to macromolecules, i.e., a decrease in endothelial barrier function, and disturbances in growth control of the vessel wall.



## 5.6. Fatty Acids

Although many mechanisms for the etiology of atherosclerosis have been proposed, endothelial injury/dysfunction clearly plays a role in the atherosclerotic disease process. There is evidence suggesting that diet-derived lipids metabolically interact with the vascular endothelium and may be responsible for abnormal regulatory mechanisms and a subsequent alteration of endothelial integrity. For example, high levels of circulating triacylglycerol-rich lipoproteins (chylomicrons and VLDLs) have been implicated in the injury process of the endothelium. Plasma chylomicron levels are elevated in humans after consuming a high-fat meal, and hepatic synthesis of VLDL is increased when caloric intake is in excess of body needs. When plasma triacylglycerol-rich lipoproteins are elevated, hydrolysis of triacylglycerols by lipoprotein lipase, and, thus, elevated concentrations of fatty acid anions, occurs in proximity to the endothelial surface. Such high levels of diet-derived fatty acids can cause endothelial injury or dysfunction and, thus, disrupt the ability of the endothelium to function as a selective barrier. This would result in lipid deposition by allowing increased penetration of cholesteryl ester-rich remnant lipoproteins into the arterial wall. Thus, when exposing cultured endothelial cells to selected fatty acids, LA (the parent n-6 fatty acid) most markedly activated endothelial cells and disrupted endothelial barrier function (161). Furthermore, the disruption in endothelial barrier function was exacerbated greatly in the presence of small amounts of oxidation derivatives of unsaturated fatty acids. This suggests that, in general, fatty acid oxidation derivatives, but not pure lipids, are extremely cytotoxic.

It is not clear why LA and none of the saturated fatty acids that were studied disrupted endothelial barrier function. The injurious effects of LA on cultured endothelial cells may be mediated, in part, by the induction of peroxisomes and, thus, by excessive hydrogen peroxide formation. In addition, enrichment of endothelial lipids with selective fatty acids can modify specific cellular lipid pools and alter the morphology of cultured cell monolayers. Such fatty acid-mediated compositional changes may be sufficient to alter membrane properties, e.g., fluidity and activities of membrane-bound enzymes. One may speculate from these and other data that high dietary intakes of certain unsaturated fatty acids, such as LA, might not be entirely safe.

## 5.7. Cholesterol

There is experimental evidence that suggests that some oxysterols, but not pure cholesterol, are the prime cause of atherosclerotic lesion formation (162). Upon cholesterol feeding, a strong relationship was seen between plasma oxysterols and aortic wall oxysterols. One may speculate that the deposition of pure lipids, such as cholesterol and its esters, may be merely a secondary process in response to oxysterol-induced endothelial cell injury. Cell injury/dysfunction and the subsequent disruption of endothelial barrier function by oxysterols (163, 164) could initiate the early events in atherosclerosis. Such injury could allow increased uptake

of cholesterol-rich lipoproteins into the arterial wall by decreasing endothelial cell prostacyclin production, thereby enhancing platelet adhesion and aggregation and by increasing monocyte adhesion and infiltration. These studies suggest that oxysterols, and not pure cholesterol, cause dysfunction of vascular endothelial cells. Not all oxysterols, however, are equally cytotoxic, and different mechanisms of endothelial cell injury by different types of cholesterol oxidation derivatives may exist. As relatively high concentrations of oxysterols are formed in certain processed foods, and because they are easily absorbed and transported in the blood, cholesterol oxidation derivatives may be an important dietary risk factor in cardiovascular disease.

### 5.8. Lipids, C-Reactive Protein, Homocysteine, and PPARs

In addition to various plasma lipids and lipoprotein species, other markers of cardiovascular disease and atherosclerosis are now considered as potent screening tools to predict these diseases. These markers are mostly related to the discoveries that a low-level chronic inflammation and related disorders of the immune system are as much, and probably more, of a clinical predictor of cardiovascular pathology than dietary fat and associated lipoproteins (165).

*C-reactive protein:* Evidence suggests that C-reactive protein (CRP) is a better predictor of the risk of cardiovascular events than LDL cholesterol. Such significant clinical data are supported by laboratory and experimental evidence that demonstrate that atherosclerosis, in addition to being a disease of lipid accumulation, also represents a chronic inflammatory process. CRP is a hepatically derived pentraxin that plays a key role in the innate immune response. The proatherogenic properties of CRP include activation of endothelial cells to express adhesion molecules, secretion of inflammatory cytokines such as IL-6, and decreased expression and bioavailability of endothelial nitric oxide synthase (166). Using high-sensitivity assays, CRP levels of <1, 1 to 3, and >3 mg/L correspond to low-, moderate-, and high-risk groups, respectively, for future cardiovascular events (165). Effects of dietary fats on CRP levels is not clear, but may be related to the overall inflammatory potential of a particular type of dietary fat. For example, since n-6 rich oils, and especially oils rich in LA, are pro-inflammatory, one might predict elevated CRP levels in populations consuming diets high in the ratio of n-6 to n-3 fatty acids.

*Homocysteine:* Another clinical marker of inflammation and risk factor of atherosclerosis is elevated plasma levels of homocysteine (167). Homocysteine, a sulfur-containing amino acid, is an intermediate formed during the metabolism of the essential amino acid methionine (168). In the general U.S. population, some hyperhomocysteinemia is quite common and often due to mild nutritional deficiencies in mostly folic acid, but also in Vitamins B<sub>12</sub> and B<sub>6</sub>. In fact, folic acid (or folate) deficiency in adults can increase the risk of coronary artery disease, stroke, several types of cancer, and possibly Alzheimer's and Parkinson's diseases (169). Even though a causal role of homocysteine in cardiovascular disease remains to be established (170), the emerging data strongly suggest that elevated plasma homocysteine levels increase the risk of multiple age-related diseases, and that adequate

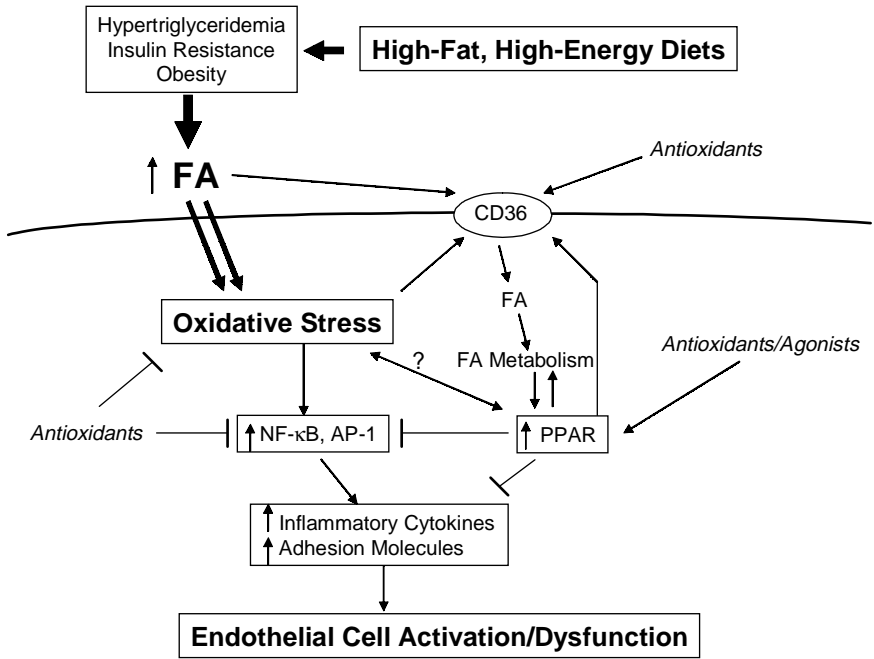
dietary or supplemental folate can be a primary means of normalizing homocysteine levels and of increasing health span.

*Peroxisome proliferators-activated receptors:* PPARs appear to possess potent anti-inflammatory signaling properties (171). PPARs are transcription factors, which regulate gene expression by binding with the retinoid receptor RXR as a heterodimeric partner to specific DNA sequence elements termed PPAR-responsive elements (171). The PPARs comprise three subtypes, PPAR $\alpha$ , PPAR $\delta$  (or  $\beta$ ), and PPAR $\gamma$ , with distinct expression patterns and biological functions (172, 173). PPAR $\alpha$  is predominantly expressed in liver, heart, muscle, and kidney, where it regulates fatty acid catabolism. Molecular and genetic studies have established roles for PPAR $\gamma$  in adipocyte differentiation, lipid storage, and glucose homeostasis. PPAR $\delta$  is expressed in most tissues and is implicated in lipid homeostasis and wound healing. All three PPARs regulate macrophage cholesterol homeostasis by enhancing cholesterol efflux. PPARs control plasma levels of cholesterol and triacylglycerols and regulate expression of key proteins involved in all stages of atherogenesis by exerting antiatherogenic actions at the level of the vascular wall (172). Dietary fatty acids and AA metabolites are natural activators of PPARs. For example, PPAR $\alpha$  and PPAR $\gamma$  are activated by eicosanoids derived from AA via the lipoxygenase and cyclooxygenase pathways. Similar to PPAR $\alpha$  and PPAR $\gamma$ , PPAR $\beta/\delta$  is a receptor for unsaturated fatty acids. PPAR $\alpha$  and PPAR $\gamma$  are also expressed in both endothelial and smooth muscle cells *in vitro* and *in vivo* in the human atherosclerotic plaque. There is general agreement in the literature that PPARs have anti-inflammatory actions (172), but mechanisms and the importance of selected vascular cells and tissues in this process are not clear.

In addition to regulating gene transcription via PPAR responsive elements, PPARs have recently been shown to modulate gene expression by interfering with other transcription factor pathways. PPARs have been shown to down-regulate inflammatory response genes by negatively interfering with the NF- $\kappa$ B, AP-1, and STAT transcriptional pathways (172). Furthermore, by regulating antioxidant enzyme activities, such as catalase, PPAR activators may inhibit NF- $\kappa$ B activation by reducing oxidative stress. PPAR activators also may antagonize NF- $\kappa$ B activation through the expression of the inhibitory protein I $\kappa$ B $\alpha$ . Such repression mechanisms via protein-protein interactions and cofactor competition may explain, in part, the anti-inflammatory actions of PPARs. It needs to be determined how individual types of fatty acids, e.g., n-6 and n-3 fatty acids and their biological metabolites, regulate PPAR signaling to direct antiatherogenic actions.

## 5.9. Summary and Dietary Advice

Numerous animal and human studies suggest that dietary cholesterol and certain saturated fatty acids increase serum as well as LDL-cholesterol concentrations. Even though humans with elevated serum cholesterol levels may be at risk, evidence also is mounting to suggest that the complicated processes that occur during atherosclerosis involve not only the participation of modified lipoproteins, but also low level and chronic inflammation and related disorders of the immune



**Figure 1.** Proposed role of dietary fat (in particular unsaturated fat), excess calories and hypertriglyceridemia, in the etiology of atherosclerosis. Dietary fats, rich in certain unsaturated lipids, are atherogenic by contributing to increased cellular oxidative stress, leading to activation of oxidative stress-sensitive transcription factors (e.g., NF-κB), which, in turn, promote cytokine production, adhesion molecule expression, and, ultimately, endothelial barrier dysfunction and atherosclerosis. The ability of membrane- or intracellular-mediated receptors or transcription factors, like CD36 or PPARs, in modulating these events is still under investigation. Certain nutrients, chemicals, or agonists, which have antioxidant properties, may protect against atherosclerosis by acting at any one of the progressive steps of these signaling pathways.

system. Modified lipoprotein particles (e.g., oxidatively modified LDL) cause secretion of inflammatory cytokines from blood-borne and arterial wall cells, which will lead to endothelial cell activation. The resulting disturbances in endothelial integrity possibly allow increased penetration of cholesterol-rich lipoprotein remnants into the arterial wall, a critical event in the etiology of atherosclerosis. Modulations in the level of activity of a select set of oxidative stress-responsive transcription factors (e.g., endothelial NF-κB) may provide a common mechanism for linking these diverse processes (Figure 1). Reactive oxygen species appear to be the common denominator in the many stimuli known to activate the NF-κB complex and subsequent inflammatory events. One may speculate, then, that high levels of dietary n-6 PUFA, which are easily oxidizable, can activate oxidative stress-responsive transcription factors, which, in turn, may promote cytokine production, adhesion molecule expression, endothelial barrier dysfunction, and, ultimately,

accelerated atherosclerosis. Interestingly, the activation of NF- $\kappa$ B can be inhibited by a variety of antioxidants (Figure 1), which suggests that certain nutrients that have antioxidant properties may protect against atherosclerosis by interfering with the proposed mechanisms of endothelial cell dysfunction. In summary, the research on atherosclerosis is complex, but the advice for patients at risk is simple: eat less fat or calories, independent of its source, and eat only enough food to match energy needs.

## REFERENCES

1. D. Spielmann, U. Bracco, H. Traitler, G. Crozier, R. Holman, M. Ward, and R. Cotter, *J. Parenter. Enteral Nutr.*, **12**, 111S (1988).
2. M. J. James, V. M. Ursin and L. G. Cleland, *Am. J. Clin. Nutr.*, **77**, 1140 (2003).
3. *Dietary reference intakes — Energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*, The National Academies Press, Washington, D.C., 2002.
4. *Dietary reference intakes—Energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*, The National Academies Press, Washington, D.C., 2002.
5. S. D. Clarke, *J. Nutr.*, **131**, 1129 (2001).
6. S. D. Clarke, *Br. J. Nutr.*, **83**, S59-S66 (2000).
7. E. D. Rosen and B. M. Spiegelman, *J. Biol. Chem.*, **276**, 37731 (2001).
8. E. A. Meade, T. M. McIntyre, G. A. Zimmerman, and S. M. Prescott, *J. Biol. Chem.*, **274**, 8328 (1999).
9. R. Uauy, D. R. Hoffman, P. Peirano, D. G. Birch, and E. E. Birch, *Lipids*, **36**, 885 (2001).
10. S. H. Werkman and S. E. Carlson, *Lipids*, **31**, 91 (1996).
11. E. E. Birch, D. R. Hoffman, R. Uauy, D. G. Birch, and C. Prestidge, *Pediatr. Res.*, **44**, 201 (1998).
12. U. Suchner and U. Senfleben, *Infusionsther. Transfusionsmed.*, **21**, 167 (1994).
13. P. C. Calder, *Proc. Nutr. Soc.*, **61**, 345 (2002).
14. A. Gil, *Biomed. Pharmacother.*, **56**, 388 (2002).
15. M. J. James and L. G. Cleland, *Semin. Arthritis Rheum.*, **27**, 85 (1997).
16. S. Endres, R. Lorenz, and K. Loeschke, *Curr. Opin. Clin. Nutr. Metab. Care*, **2**, 117 (1999).
17. J. R. Hibbeln and N. Salem, Jr., *Am. J. Clin. Nutr.*, **62**, 1 (1995).
18. B. A. Watkins and Y. Li, in R. E. C. Wildman, ed., *Hand Book of Nutraceuticals and Functional Foods*, CRC Press, Boca Raton, Florida, 2001.
19. J. J. Turek, Y. Li, I. A. Schoenlein, K. G. D. Allen, and B. A. Watkins, *J. Nutr. Biochem.*, **9**, 258 (1998).
20. Y. Li and B. A. Watkins, *Lipids*, **33**, 417 (1998).
21. Y. Li, M. F. Seifert, D. M. Ney, M. Grahn, A. L. Grant, K. G. Allen, and B. A. Watkins, *J. Bone Miner. Res.*, **14**, 1153 (1999).
22. J. R. Liu, B. Q. Chen, Y. M. Yang, X. L. Wang, Y. B. Xue, Y. M. Zheng, and R. H. Liu, *World J. Gastroenterol.*, **8**, 999 (2002).
23. A. Miller, C. Stanton, and R. Devery, *Anticancer Res.*, **22**, 3879 (2002).

24. E. J. Kim, J. G. Jun, H. S. Park, S. M. Kim, Y. L. Ha, and J. H. Park, *Anticancer Res.*, **22**, 2193 (2002).
25. J. D. Palombo, A. Ganguly, B. R. Bistran, and M. P. Menard, *Cancer Lett.*, **177**, 163 (2002).
26. M. Yamasaki, H. Chujo, Y. Koga, A. Oishi, T. Rikimaru, M. Shimada, K. Sugimachi, H. Tachibana, and K. Yamada, *Cancer Lett.*, **188**, 171 (2002).
27. E. J. Moon, Y. M. Lee, and K. W. Kim, *Oncol. Rep.*, **10**, 617 (2003).
28. P. Thuillier, G. J. Anchiraco, K. P. Nickel, R. E. Maldve, I. Gimenez-Conti, Muga, SJ, K. L. Liu, S. M. Fischer, and M. A. Belury, *Mol. Carcinog.*, **29**, 134 (2000).
29. K. M. Hargrave, C. L. Li, B. J. Meyer, S. D. Kachman, D. L. Hartzell, M. A. Della-Fera, J. L. Miner, and C. A. Baile, *Obes. Res.*, **10**, 1284 (2002).
30. N. Tsuboyama-Kasaoka, M. Takahashi, K. Tanemura, H. J. Kim, T. Tange, Okuyama, H, M. Kasai, S. Ikemoto, and O. Ezaki, *Diabetes*, **49**, 1534 (2000).
31. A. Akahoshi, Y. Goto, K. Murao, T. Miyazaki, M. Yamasaki, M. Nonaka, K. Yamada, and M. Sugano, *Biosci. Biotechnol. Biochem.*, **66**, 916 (2002).
32. Y. Yu, P. H. Correll, and J. P. Vanden Heuvel, *Biochim Biophys Acta*, **1581**, 89 (2002).
33. J. M. Peters, Y. Park, F. J. Gonzalez, and M. W. Pariza, *Biochim. Biophys. Acta*, **1533**, 233 (2001).
34. M. A. Belury, S. Y. Moya-Camarena, K. L. Liu, and J. P. Vanden Heuvel, *J. Nutr. Biochem.*, **8**, 579 (1997).
35. K. N. Lee, M. W. Pariza, and J. M. Ntambi, *Biochem. Biophys. Res. Commun.*, **248**, 817 (1998).
36. M. Sugano, A. Tsujita, M. Yamasaki, K. Yamada, I. Ikeda, and D. Kritchevsky, *J. Nutr. Biochem.*, **8**, 38 (1997).
37. J. A. Bulgarella, D. Patton, and A. W. Bull, *Lipids*, **36**, 407 (2001).
38. K. L. Liu and M. A. Belury, *Cancer Lett.*, **127**, 15 (1998).
39. B. A. Watkins and Y. Li, *Adv. Conjugated Linoleic Acid Res.*, **2**, 174 (2003).
40. Agency Response Letter GRAS Notice No. GRN 000041, 5-17-2001, U. S. Food and Drug Administration, Washington, D.C.
41. Agency Response Letter GRAS Notice No. GRN 000080, 12-11-2001, U. S. Food and Drug Administration, Washington, D.C.
42. E. N. Ponnampalam, G. R. Trout, A. J. Sinclair, A. R. Egan, and B. J. Leury, *Meat Sci.*, **58**, 151 (2001).
43. P. R. C. Howe, J. A. Downing, B. F. S. Grenyer, E. M. Grigonis-Deane, and W. L. Bryden, *Lipids*, **37**, 1067 (2002).
44. S. Lopez-Ferrer, M. D. Baucells, A. C. Barroeta, and M. A. Grashorn, *Poult. Sci.*, **78**, 356 (1999).
45. S. Lopez-Ferrer, M. D. Baucells, A. C. Barroeta, and M. A. Grashorn, *Poult. Sci.*, **80**, 741 (2001).
46. S. Lopez-Ferrer, M. D. Baucells, A. C. Barroeta, J. Galobart, and M. A. Grashorn, *Poult. Sci.*, **80**, 753 (2001).
47. T. Grune, K. Kramer, P. P. Hoppe, and W. Siems, *Lipids*, **36**, 833 (2001).
48. S. M. Kitessa, D. Peake, R. Bencini, and A. J. Williams, *Anim. Feed Sci. Tech.*, **108**, 1 (2003).

49. M. Mozzon, N. G. Frega, B. Fronte, and M. Tocchini, *Food Technol. Biotech.*, **40**, 213 (2002).
50. G. Papadopoulos, C. Goulas, E. Apostolaki, and R. Abril, *J. Dairy Res.*, **69**, 357 (2002).
51. A. Bendich and P. E. Brock, *Int. J. Vitam. Nutr. Res.*, **67**, 213 (1997).
52. J. A. Lovegrove, C. N. Brooks, M. C. Murphy, B. J. Gould, and C. M. Williams, *Br. J. Nutr.*, **78**, 223 (1997).
53. J. M. W. Wallace, A. J. McCabe, P. J. Robson, M. K. Keogh, C. A. Murray, G. Marquez-Ruiz, H. McGlynn, W. S. Gilmore, and J. J. Strain, *Ann. Nutr. Metab.*, **44**, 157 (2000).
54. P. Y. Chouinard, L. Corneau, W. R. Butler, Y. Chilliard, J. K. Drackley, and D. E. Bauman, *J. Dairy Sci.*, **84**, 680 (2001).
55. R. J. Baer, J. Ryali, D. J. Schingoethe, K. M. Kasperson, D. C. Donovan, A. R. Hippen, and S. T. Franklin, *J. Dairy Sci.*, **84**, 345 (2001).
56. D. C. Donovan, D. J. Schingoethe, R. J. Baer, J. Ryali, A. R. Hippen, and S. T. Franklin, *J. Dairy Sci.*, **83**, 2620 (2000).
57. A. A. Abu-Ghazaleh, D. J. Schingoethe, and A. R. Hippen, *J. Dairy Sci.*, **84**, 1845 (2001).
58. S. M. Innis, H. Sprecher, D. Hachey, J. Edmond, and R. E. Anderson, *Lipids*, **34**, 139 (1999).
59. A. E. Leonard, S. Pereira, H. Sprecher, and Y. Huang, *Prog. Lipid Res.*, **43**, 36 (2004).
60. S. D. Harmon, T. L. Kaduce, T. D. Manuel, and A. A. Spector, *Lipids*, **38**, 469 (2003).
61. A. E. Leonard, B. Kelder, E. G. Bobik, L. T. Chuang, C. J. Lewis, J. J. Kopchick, P. Mukerji, and Y. S. Huang, *Lipids*, **37**, 733 (2002).
62. E. A. Emken, R. O. Adlof, S. M. Duval, and G. J. Nelson, *Lipids*, **34**, 785 (1999).
63. E. A. Emken, R. O. Adlof, S. M. Duval, and G. J. Nelson, *Lipids*, **33**, 471 (1998).
64. V. P. Carnielli, D. J. Wattimena, I. H. Luijendijk, A. Boerlage, H. J. Degenhart, and P. J. Sauer, *Pediatr. Res.*, **40**, 169 (1996).
65. C. Bolton-Smith, M. Woodward, and R. Tavendale, *Eur. J. Clin. Nutr.*, **51**, 619 (1997).
66. M. Murakami and I. Kuda, *Prog. Lipid Res.*, **43**, 3 (2004).
67. W. L. Xie, J. G. Chipman, D. L. Robertson, R. L. Erikson, and D. L. Simmons, *Proc. Natl. Acad. Sci. U. S. A.*, **88**, 2692 (1991).
68. D. A. Kujubu, B. S. Fletcher, B. C. Varnum, R. W. Lim, and H. R. Herschman, *J. Biol. Chem.*, **266**, 12866 (1991).
69. I. Morita, M. Schindler, M. K. Regier, J. C. Otto, T. Hori, D. L. DeWitt, and W. L. Smith, *J. Biol. Chem.*, **270**, 10902 (1995).
70. C. C. Pilbeam, P. M. Fall, C. B. Alander, and L. G. Raisz, *J. Bone Miner. Res.*, **12**, 1198 (1997).
71. H. Kawaguchi, L. G. Raisz, O. S. Voznesensky, C. B. Alander, Y. Hakeda, and C. C. Pilbeam, *Endocrinology*, **135**, 1157 (1994).
72. N. V. Chandrasekharan, H. Dai, K. L. Roos, N. K. Evanson, J. Tomsik, T. S. Elton, and D. L. Simmons, *Proc. Natl. Acad. Sci. U. S. A.*, **99**, 13926 (2002).
73. M. T. Donnelly and C. J. Hawkey, *Aliment. Pharm. Therap.*, **11**, 227 (1997).
74. N. Shimizu, Y. Ozawa, M. Yamaguchi, T. Goseki, K. Ohzeki, and Y. Abiko, *J. Periodontol.*, **69**, 670 (1998).
75. J. Singh, R. Hamid, and B. S. Reddy, *Cancer Res.*, **57**, 3465 (1997).

76. A. A. Nanji, D. Zakim, A. Rahemtulla, T. Daly, L. Miao, S. Zhao, S. Khwaja, S. R. Tahan, and A. J. Dannenberg, *Hepatology*, **26**, 1538 (1997).
77. H. Inoue, T. Tanabe, and K. Umesono, *J. Biol. Chem.*, **275**, 28028 (2000).
78. D. Hwang, *Annu. Rev. Nutr.*, **20**, 431 (2000).
79. M. Zeyda, A. B. Szekeres, M. D. Saemann, R. Geyeregger, H. Stockinger, G. J. Zlabinger, W. Waldhausl, and T. M. Stulnig, *J. Immunol.*, **170**, 6033 (2003).
80. B. E. Cowing and K. E. Saker, *J. Nutr.*, **131**, 1125 (2001).
81. T. Lemberger, B. Desvergne, and W. Wahli, *Annu. Rev. Cell Dev. Biol.*, **12**, 335 (1996).
82. C. Chambrier, J. P. Bastard, J. Rieusset, E. Chevillotte, D. Bonnefont-Rousselot, P. Therond, B. Hainque, J. P. Riou, M. Laville, and H. Vidal, *Obes. Res.*, **10**, 518 (2002).
83. S. Y. Moya-Camarena, J. P. Vanden Heuvel, S. G. Blanchard, L. A. Leesnitzer, and M. A. Belury, *J. Lipid Res.*, **40**, 1426 (1999).
84. Y. Takahashi, M. Kushiro, K. Shinohara, and T. Ide, *Biochim. Biophys. Acta* **1631**, 265 (2003).
85. E. Duplus and C. Forest, *Biochem. Pharmacol.*, **64**, 893 (2002).
86. J. M. Ntambi and H. Bene, *J. Mol. Neurosci.*, **16**, 273 (2001).
87. D. B. Jump and S. D. Clarke, *Annu. Rev. Nutr.*, **19**, 63 (1999).
88. G. P. Hayhurst, Y. H. Lee, G. Lambert, J. M. Ward, and F. J. Gonzalez, *Mol. Cell. Biol.*, **21**, 1393 (2001).
89. Y. Inoue, G. P. Hayhurst, J. Inoue, M. Mori, and F. J. Gonzalez, *J. Biol. Chem.*, **277**, 25257 (2002).
90. S. A. Khan and J. P. V. Heuvel, *J. Nutr. Biochem.*, **14**, 554 (2003).
91. P. Couture, W. R. Archer, B. Lamarche, N. Landry, O. Deriaz, L. Corneau, J. Bergeron, and N. Bergeron, *Metabolism*, **52**, 1454 (2003).
92. P. Perez-Martinez, J. M. Ordovas, J. Lopez-Miranda, P. Gomez, C. Marin, J. Moreno, F. Fuentes, R. A. F. de la Puebla, and F. Perez-Jimenez, *Am. J. Clin. Nutr.*, **77**, 809 (2003).
93. S. Vincent, R. Planells, C. Defoort, M. C. Bernard, M. Gerber, J. Prudhomme, P. Vague, and D. Lairon, *Proc. Nutr. Soc.*, **61**, 427 (2002).
94. M. Rantala, M. L. Silaste, A. Tuominen, J. Kaikkonen, J. T. Salonen, G. Alfthan, A. Aro, and Y. A. Kesaniemi, *J. Nutr.*, **132**, 3012 (2002).
95. R. Doll and R. Peto, *J. Natl. Cancer Inst.*, **66**, 1191 (1981).
96. Healthy, well nourished population—Interaction of diet and health, 1999. The United States Department of Agriculture. Science and Education Impact.
97. A. P. Simopoulos, *Biomed. Pharmacother.*, **56**, 365 (2002).
98. M. de Lorgeril, P. Salen, J. L. Martin, I. Monjaud, J. Delaye, and N. Marnier, *Circulation*, **99**, 779 (1999).
99. W. S. Harris, *Am. J. Clin. Nutr.*, **65**, 1645S (1997).
100. A. Leaf and P. C. Weber, *N. Engl. J. Med.*, **318**, 549 (1988).
101. D. P. Rose, *Proc. Soc. Exp. Biol. Med.*, **216**, 224 (1997).
102. D. P. Rose and J. M. Connolly, *Pharmacol. Therap.*, **83**, 217 (1999).
103. A. P. Simopoulos, *Can. J. Physiol. Pharm.*, **75**, 234 (1997).
104. Y. Goto, H. Tamachi, and E. H. Moriguchi, *Prostaglandins Leukot. Essent. Fatty Acids*, **48**, 337 (1993).



105. C. E. Hock, M. A. Holahan, and D. K. Reibel, *Am. J. Physiol.*, **252**, H554–H560 (1987).
106. G. E. Billman, J. X. Kang, and A. Leaf, *Lipids*, **32**, 1161 (1997).
107. L. M. Braden and K. K. Carroll, *Lipids*, **21**, 285 (1986).
108. T. Minoura, T. Takata, M. Sakaguchi, H. Takada, M. Yamamura, K. Hioki, and M. Yamamoto, *Cancer Res.*, **48**, 4790 (1988).
109. B. S. Reddy and H. Maruyama, *Cancer Res.*, **46**, 3367 (1986).
110. B. S. Reddy and S. Sugie, *Cancer Res.*, **48**, 6642 (1988).
111. T.P.O'Connor, B. D. Roebuck, F. J. Peterson, B. Lokesh, J. E. Kinsella, and T. C. Campbell, *J. Natl. Cancer Inst.*, **81**, 858 (1989).
112. W. E. Lands, T. Hamazaki, K. Yamazaki, H. Okuyama, K. Sakai, Y. Goto, and V. S. Hubbard, *Am. J. Clin. Nutr.*, **51**, 991 (1990).
113. H. Okuyama, T. Kobayashi, and S. Watanabe, *Prog. Lipid Res.*, **35**, 409 (1996).
114. T. Kuroishi and S. Tominaga, *Jpn. J. Cancer Chemoth.*, **28**, 168 (2001).
115. K. Shichikawa, Y. Takenaka, A. Maeda, R. Yoshino, M. Tsujimoto, H. Ota, T. Kashiwade, and I. Hongo, *Ryumachi*, **21**, 35 (1981).
116. W. E. Lands, *Ann. N. Y. Acad. Sci.*, **676**, 46 (1993).
117. Y. Goto, *Nutr. Rev.*, **50**, 398 (1992).
118. D. Hwang and S. H. Rhee, *Am. J. Clin. Nutr.*, **70**, 545 (1999).
119. D. B. Jump, *J. Biol. Chem.*, **277**, 8755 (2002).
120. O. Adam, *Eur. J. Clin. Nutr.*, **49**, 703 (1995).
121. J. W. Alexander, *Nutrition*, **14**, 627 (1998).
122. D. R. Robinson, S. Tateno, B. Patel, and A. Hirai, *J. Parenter. Enteral Nutr.*, **12**, 37S (1988).
123. T. H. Lee, T. Sethi, A. E. Crea, W. Peters, J. P. Arm, C. E. Horton, M. J. Walport, and B. W. Spur, *Clin. Sci.*, **74**, 467 (1988).
124. P. Needleman, A. Raz, M. S. Minkes, J. A. Ferrendelli, and H. Sprecher, *Proc. Natl. Acad. Sci., U. S. A.*, **76**, 944 (1979).
125. B. A. Watkins, Y. Li, K. G. D. Allen, W. E. Hoffmann, and M. F. Seifert, *J. Nutr.*, **130**, 2274 (2000).
126. L. G. Raisz, C. B. Alander, and H. A. Simmons, *Prostaglandins*, **37**, 615 (1989).
127. H. R. Knapp, I. A. Reilly, P. Alessandrini, and G. A. FitzGerald, *N. Engl. J. Med.*, **314**, 937 (1986).
128. J. M. Kremer, *Lipids*, **31**, S243–S247 (1996).
129. C. L. Curtis, C. E. Hughes, C. R. Flannery, C. B. Little, J. L. Harwood, and B. Caterson, *J. Biol. Chem.*, **275**, 721 (2000).
130. L. C. Hamilton, J. A. Mitchell, A. M. Tomlinson, and T. D. Warner, *FASEB J.*, **13**, 245 (1999).
131. A. F. Badawi, A. El Sohemy, L. L. Stephen, A. K. Ghoshal, and M. C. Archer, *Carcinogenesis*, **19**, 905 (1998).
132. R. Graber, C. Sumida, and E. A. Nunez, *J. Lipid Mediat. Cell Signal*, **9**, 91 (1994).
133. J. A. Ross, A. G. Moses, and K. C. Fearon, *Curr. Opin. Clin. Nutr. Metab. Care*, **2**, 219 (1999).

134. S. Camandola, G. Leonarduzzi, T. Musso, L. Varesio, R. Carini, A. Scavazza, E. Chiarpotto, P. A. Baeuerle, and G. Poli, *Biochem. Biophys. Res. Commun.*, **229**, 643 (1996).
135. R. Ross, *N. Engl. J. Med.*, **340**, 115 (1999).
136. T. Collins, *Lab. Invest.*, **68**, 499 (1993).
137. F. De Nigris, L. O. Lerman, M. Condorelli, A. Lerman, and C. Napoli, *Antioxid. Redox Sign.*, **3**, 1119 (2001).
138. A. Keys, J. T. Anderson, and F. Grande, *Lancet*, **2**, 959 (1957).
139. D. M. Hegsted, R. B. McGandy, M. L. Myers, and F. J. Stare, *Am. J. Clin. Nutr.*, **17**, 281 (1965).
140. R. P. Mensink, *Am. J. Clin. Nutr.*, **57**, 711S (1993).
141. P. Kris-Etherton, R. H. Eckel, B. V. Howard, S. St Jeor, and T. L. Bazzarre, Nutrition Committee Population Science Committee and Clinical Science and Committee of the American Heart Association, *Circulation*, **103**, 1823 (2001).
142. F. Perez-Jimenez, J. Lopez-Miranda, and P. Mata, *Atherosclerosis*, **163**, 385 (2002).
143. F. M. Sacks and M. Katan, *Am. J. Med.*, **113**, 13S (2002).
144. P. N. Hopkins, *Am. J. Clin. Nutr.*, **55**, 1060 (1992).
145. M. Laidlaw and B. J. Holub, *Am. J. Clin. Nutr.*, **77**, 37 (2003).
146. R. N. Lemaitre, I. B. King, D. Mozaffarian, L. H. Kuller, R. P. Tracy, and D. S. Siscovick, *Am. J. Clin. Nutr.*, **77**, 319 (2003).
147. W. E. Connor and S. L. Connor, *Adv. Internal Med.*, **35**, 139 (1990).
148. M. J. Malloy and J. P. Kane, *Adv. Internal Med.*, **47**, 111 (2001).
149. M. A. Austin, B. McKnight, K. L. Edwards, C. M. Bradley, M. J. McNeely, B. M. Psaty, J. D. Brunzell, and A. G. Motulsky, *Circulation*, **101**, 2777 (2000).
150. L. Djousse, S. C. Hunt, D. K. Arnett, M. A. Province, J. H. Eckfeldt, and R. C. Ellison, *Am. J. Clin. Nutr.*, **78**, 1098 (2003).
151. D. R. Janero, *Free Radical Biol. Med.*, **11**, 129 (1991).
152. K. Esposito, F. Nappo, F. Giugliano, G. Giugliano, R. Marfella, and D. Giugliano, *Am. J. Clin. Nutr.*, **77**, 139 (2003).
153. J. M. Hodgson, M. L. Wahlqvist, J. A. Boxall, and N. D. Balazs, *Am. J. Clin. Nutr.*, **58**, 228 (1993).
154. R. J. Nicolosi, T. A. Wilson, G. Handelman, T. Foxall, and J. F. J. Keaney, *J. Nutr. Biochem.*, **13**, 392 (2002).
155. E. Vos and S. C. Cunnane, *Am. J. Clin. Nutr.*, **77**, 521 (2003).
156. S. M. Grundy and M. A. Denke, *J. Lipid Res.*, **31**, 1149 (1990).
157. S. M. Grundy, N. Abate, and M. Chandalia, *Am. J. Med.*, **113**, 25S (2002).
158. F. B. Hu and W. C. Willett, *J. Am. Med. Assoc.*, **288**, 2569 (2002).
159. P. M. Kris-Etherton, P. M. Kris-Etherton, A. E. Binkoski, G. Zhao, S. M. Coval, K. F. Clemmer, K. D. Hecker, H. Jacques, and T. D. Etherton, *Proc. Nutr. Soc.*, **61**, 287 (2002).
160. P. O. Bonetti, L. O. Lerman, and A. Lerman, *Arterioscl. Throm. Vas. Biol.*, **23**, 168 (2003).
161. M. Toborek, Y. W. Lee, R. Garrido, S. Kaiser, and B. Hennig, *Am. J. Clin. Nutr.*, **75**, 119 (2002).

162. S. K. Peng, C. B. Taylor, J. C. Hill, and R. J. Morin, *Atherosclerosis*, **54**, 121 (1985).
163. G. A. Boissonneault, B. Hennig, and C. M. Ouyang, *Proc. Soc. Exp. Biol. Med.*, **196**, 338 (1991).
164. B. Hennig and G. A. Boissonneault, *Atherosclerosis*, **68**, 255 (1987).
165. P. M. Ridker, *Circulation*, **107**, 363 (2003).
166. E. T. H. Yeh and J. T. Willerson, *Circulation*, **107**, 370 (2003).
167. J. A. Farmer and G. Torre-Amione, *Curr. Atheroscl. Rep.*, **4**, 92 (2002).
168. B. K. Nallamothu, A. M. Fendrick, and G. S. Omenn, *Pharmacoeconomics*, **20**, 429 (2002).
169. M. P. Mattson, I. I. Kruman, and W. Duan, *Ageing Res. Rev.*, **1**, 95 (2002).
170. E. Falk, J. Zhou, and J. Moller, *Lipids*, **36**, S3 (2001).
171. A. Tedgui and Z. Mallat, *Circ. Res.*, **88**, 877 (2001).
172. O. Barbier, I. P. Torra, Y. Duguay, C. Blanquart, J. C. Fruchart, C. Glineur, and B. Staels, *Arterioscl. Throm. Vas. Biol.*, **22**, 717 (2002).
173. C. H. Lee and R. M. Evans, *Trends Endocrinol. Metabol.*, **13**, 331 (2002).

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# 1

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## *Butter*

David Hettinga

### 1. INTRODUCTION

Buttermaking is one of the oldest forms of preserving the fat component of milk. Its manufacture dates back to some of the earliest historical records, and reference has been made to the use of butter in sacrificial worship, for medicinal and cosmetic purposes, and as a human food long before the Christian era. Documents indicate that, at least in the Old World, the taming and domestication of animals constituted the earliest beginnings of human civilization and culture. There is good reason to believe, therefore, that the milking of animals and the origin of buttermaking predate the beginning of organized and permanent recording of human activities.

The evolution of the art of buttermaking has been intimately associated with the development and use of equipment. With the close of the eighteenth century, the construction and use of creaming and buttermaking equipment (other than that made of wood) began to receive consideration, and the barrel churn made its appearance.

By the middle of the nineteenth century, attention was given to improvement in methods of creaming. These efforts gave birth to the deep-setting system. Up to that time, creaming was done by a method called shallow pan. The deep-setting system shortened the time for creaming and produced a better quality cream. An inventive Bavarian brewer, in 1864, conceived the idea of adapting the principle of the laboratory centrifuge. In 1877, a German engineer succeeded in designing a machine that, although primitive, was usable as a batch-type apparatus. In 1879,

engineers in Sweden, Denmark, and Germany succeeded in the construction of cream separators for fully continuous operation (1).

In 1870, the year before the introduction of factory buttermaking, butter production in the United States totaled 514 million lbs, practically all farm made. Authentic records concerning the beginning of factory buttermaking are meager. It appears that the first butter factory was built in Iowa in 1871. This also introduced the pooling system of milk for creamery operation (1).

Other inventions that assisted in the development of the butter industry included the Babcock test (1890), which accurately determines the percentage of fat in milk and cream; the use of pasteurization to maintain milk and cream quality; the use of pure cultures of lactic acid bacteria; and refrigeration to help preserve cream quality.

Multiple butter fat products, including butter oils, anhydrous butter fat, butter fat-vegetable oil blends, and fractionated butter fats, are manufactured around the world today. In the past, butter fat in the form of butter was the primary preservation technique. Today, the preferred preservation method involves the processing of butter fat to the anhydrous butter oil state, then hermetically packaging under nitrogen to substantially increase the shelf life and reduce the incidence of degradation.

Historically, milkfat has been held in the highest esteem, whether in liquid milk, as cream, or as butter. Its consumption was associated with a higher standard of living. In recent times, with the prosperity of the Western world, per capita consumption has been decreasing. Ironically, this phenomenon contradicts all historical patterns for butter fat consumption and use. Several reasons exist for this decline. This chapter explores the chemical composition, marketing, technology, processing, quality, legal restrictions, and uses for butter and butter fat.

## 2. CHEMICAL COMPOSITION

Some of the information in this chapter comes directly from the fourth edition of Bailey's (2). Jensen and Clark (3) have provided a complete review of the lipid composition, and data have been selected for inclusion in this review.

The composition of milkfat is somewhat complex. Although dominated by triglycerides, which constitute some 98% of milkfat (with small amounts of diglycerides, monoglycerides, and free fatty acids), various other lipid classes are also present in measurable amounts. It is estimated that about 500 separate fatty acids have been detected in milk lipids; it is probable that additional fatty acids remain to be identified. Of these, about 20 are major components; the remainder are minor and occur in small or trace quantities (4, 5). The other components include phospholipids, cerebrosides, and sterols (cholesterol and cholesterol esters). Small amounts of fat-soluble vitamins (mainly A, D, and E), antioxidants (tocopherol), pigments (carotene), and flavor components (lactones, aldehydes, and ketones) are also present.

The composition of the lipids of whole bovine milk is given in Table 1 (4, 5). The structure and composition of the typical milkfat globule is exceedingly

**TABLE 1. Composition of Lipids in Whole Bovine Milk (4, 5).**

Lipid	Weight Percent
Hydrocarbons	Trace
Sterol esters	Trace
Triglycerides	97–98
Diglycerides	0.28–0.59
Monoglycerides	0.016–0.038
Free fatty acids	0.10–0.44
Free sterols	0.22–0.41
Phospholipids	0.2–1.0

complex. The globule is probably 2–3  $\mu$  in diameter with a 90-Å-thick membrane surrounding a 98–99% triglyceride core. The composition of the milkfat membrane is quite different from milkfat itself in that approximately 60% triglycerides are present, much less than in the parent milkfat (Table 2) (6, 7).

It has been generally recognized that butter fat consists of about 15 major fatty acids, with perhaps 12 or so minor (trace quantity) acids. Triglycerides are normally defined with respect to their carbon number (CN), i.e., the number of fatty acid carbon atoms present in the molecule; the three carbon atoms of the glycerol moiety are ignored. As the fatty acid spectrum of milkfat is dominated by acids containing an even number of carbon atoms, so is the triglyceride spectrum. However, the proportion of triglycerides with an odd carbon number is about three times greater than the proportion of odd-numbered fatty acids.

Although obvious correlations exist between fatty acid composition and triglyceride distribution, detailed information is lacking that would enable the triglyceride distribution to be predicted from the fatty acid composition. Much more needs to be understood of the strategy used in the bovine mammary gland in assembling a

**TABLE 2. Composition of Lipids from Milkfat Globule Membrane (6, 7).**

Lipid Component	Percent of Membrane Lipids
Carotenoids (pigment)	0.45
Squalene	0.61
Cholesterol esters	0.79
Triglycerides	53.4
Free fatty acids	6.3 <sup>a</sup>
Cholesterol	5.2
Diglycerides	8.1
Monoglycerides	4.7
Phospholipids	20.4

<sup>a</sup>Contained some triglycerides.

**TABLE 3. Characteristics and Composition of Butter Fats.**

Characteristic	Value <sup>a</sup>	Range of Values <sup>b</sup>	GLC <sup>c</sup>
Iodine number	32.9	—	—
Saponification equivalent	236.3	—	—
Reichert-Meissle value	32.5	—	—
Polenske value	—	—	—
Kirschner value	—	—	—
Fatty acid, wt. %			
Butyric	3.5	2.8–4.0	3
Caproic	1.4	1.4–3.0	1
Caprylic	1.7	0.5–1.7	1
Capric	2.6	1.7–3.2	3
Lauric	4.5	2.2–4.5	4
Myristic	14.6	5.4–14.6	12
Palmitic	30.2	26–41	29
Stearic	10.5	6.1–11.2	11
Above C18	1.6	—	2
Total saturated	70.6	—	66
Decenoic	0.3	0.1–0.3	—
Dodecenoic	0.2	0.1–0.6	—
Tetradecenoic	1.5	0.6–1.6	2
Hexadecenoic	5.7	2.8–5.7	4
Octadecenoic (oleic, etc.)	18.7	18.7–33.4	25
Octadecdienoic	2.1	0.9–3.7	2
C20 and C22 unsaturated	0.9	—	1
Total unsaturated	29.4	—	34

<sup>a</sup> From (8) and (9).<sup>b</sup> From (10) and (11).<sup>c</sup> From (12).

complex array of fatty acids into triglycerides. This is not an arcane study; it is necessary if processes such as fractionation are to yield products with consistent qualities throughout the year. In effect, the detailed structure of milkfat is not yet understood. Perhaps this is not surprising if we consider only the 15 major fatty acids; there are  $15^3$  (3375) possible triglyceride structures using a purely random model.

The data in Table 3 represent general characteristics and composition of butter fat as reported by several sources (8–12). Note the range in values. Precise and repeatable values are not highly correlated due to such variables as stage of lactation, feed source, cattle breed, etc. Although 16 categories of fatty acids are outlined, it was generally appreciated that many other fatty acids are present in small or trace quantities. For nutritional and dairy science purposes, these data are of value, but from a detailed scientific point of view, they afford only a vague, broad generalization of the actual state of fatty acid composition of butter fat. A more complete view of composition is provided in Table 4 (13, 14).

From 1956 to 1983 a great volume of information became available on the occurrence of many minor constituents in butter fat. Somewhat less intensity



TABLE 4. Fatty Acid Composition of Milk and Butter Fat.<sup>a</sup>

Fatty Acid <sup>b</sup>	June <sup>c</sup>	December <sup>d</sup>	Average <sup>e</sup>	Moore and Co-workers <sup>f</sup>
4:0	4.22	3.51	3.57	3.98
6:0	2.53	2.24	2.22	2.36
8:0	2.34	1.07	1.17	1.36
9:0	0.05	0.05	0.03	—
10:0	2.24	2.57	2.54	2.76
10:1	0.32	—	—	—
11:0	0.34	0.29	0.33	—
12:0	2.40	2.77	2.81	3.14
13:0 (12:1)	0.29	0.29	0.33	0.14
14 (br) <sup>g</sup>	0.23	0.14	0.17	0.12
14:0	9.01	10.58	10.06	8.39
14:1 (15 br)	1.54	1.61	1.63	1.84
15:0	1.29	1.11	1.09	1.34
16 (br)	0.42	0.39	0.38	0.35
16:0	22.05	25.98	24.97	30.05
16:1 (17 br)	2.29	2.98	2.55	2.80
17:0	0.69	1.08	0.91	1.00
17:1 (18 br)	—	—	—	0.37
18:0 (br)	0.31	0.40	0.38	—
18:0	14.27	11.58	12.07	11.74
18:1	30.41	24.75	27.09	24.93
18:8 <sup>h</sup>	0.24	1.56	1.26	—
18:2	1.23	2.75	2.39	1.78
18:3 (20:0)	2.61	2.30	2.06	1.23

<sup>a</sup> In weight percent.

<sup>b</sup> Structural assignments are not necessarily authentic, but represent, in almost all instances, the most likely structure for the fraction.

<sup>c</sup> Data from the Department of Animal Industries, Storrs (Conn.) Agricultural Experiment Station; 408 samples of milk plant production from June 1960 to June 1961.

<sup>d</sup> Data from Storrs Agriculture Experiment Station; 4–8 samples.

<sup>e</sup> For 108 samples.

<sup>f</sup> Sec (14).

<sup>g</sup> Branched chain.

<sup>h</sup> Carbon number obtained by semilog plots retention time/chain length.

of interest has prevailed since then, but further information continues to appear, and we can expect more data on butter fat as a consequence of research on the relationship between dairy cow feeding studies and resulting butter fat fatty acid composition.

The great variety of fatty acids in butter fat cannot be treated in detail here; reference will be made to only a few of the many available reports. Octadecadienoic acids are present in significant amounts; there are traces of hexadecadienoic acid, octadecatrienoic acids, and highly unsaturated C20 and C22 acids. Traces of dihydroxystearic acid and hydroxypalmitic acid have been detected (8, 9). A small proportion of the octadecenoic acid consists, not of oleic acid, but of *trans*-11,12 isomer, vaccenic acid (8, 9). One report states that about 66% of one octadecenoic

**TABLE 5. Positional and Geometric Isomers of Bovine Milk Lipid Fatty Acids (wt. %) (16).**

Position of Double Bond	<i>Cis</i> -Isomers				<i>Trans</i> -Isomers	
	14:1	16:1	17:1	18:1	16:1	18:1
5	1.0	Trace	—	—	2.2	—
6	0.8	1.3	3.4	—	7.8	1.0
7	0.9	5.6	2.1	—	6.7	0.8
8	0.6	Trace	20.1	1.7	5.0	3.2
9	96.6	88.7	71.3	95.8	32.8	10.2
10	—	Trace	Trace	Trace	1.7	10.5
11	—	2.6	2.9	2.5	10.6	35.7
12	—	Trace	Trace	—	12.9	4.1
13	—	—	—	—	10.6	10.5
14	—	—	—	—	—	9.0
15	—	—	—	—	—	6.8
16	—	—	—	—	—	7.5

acid content is normal linoleic acid, and the remainder consists of the *cis*-9, *trans*-12 or the *trans*-9, *cis*-12 isomers (15); but other positional and geometric isomers are undoubtedly also present (4). The positional and geometric isomers of bovine milk lipid fatty acids are presented in Table 5 (16).

**TABLE 6. Fatty Acid Distributions of 82 Acids in Butter Fat.<sup>a</sup>**

Saturated <sup>b</sup>		Branched <sup>c</sup>		Monoenes	
Acid	Weight Percent	Acid	Weight Percent	Acid	Weight Percent
—	—	12:0 i	0.01	10:1	0.48
8:0	0.69	13:0 i	Trace	12:1	0.05
10:0	1.88	14:0 i	0.03	13:1	0.003
11:0	0.12	15:0 i	0.14	14:1	0.75
12:0	2.96	15:0 2	0.23	15:1	0.02
13:0	0.10	16:0 i	0.2	16:1	1.84
14:0	11.2	17:0 i	0.36	17:1	0.2
15:0	1.52	18:0 i	0.02	18:1	30.3
16:0	27.8	19:0 br	0.01	19:1	0.14
17:0	0.71	20:0 br	0.01	—	—
18:0	12.1	21:0 br	0.01	—	—
19:0	0.05	22:0 br	0.02	—	—
20:0	0.02	23:0 br	0.01	—	—
21:0	0.06	24:0 br	0.02	—	—
22:0	0.04	25:0 br	0.0004	—	—
23:0	0.01	26:0 br	0.0004	—	—
24:0	0.02	20:1	0.52	—	—
25:0	0.02	21:1	0.01	—	—
26:0	0.02	22:1	0.02	—	—
27:0	0.00004	23:1	0.05	—	—
28:0	0.00004	24:1	0.0008	—	—
—	—	25:1	0.0008	—	—
—	—	26:1	0.0008	—	—

TABLE 6. Fatty Acid Distributions of 82 Acids in Butter Fat.<sup>a</sup> (Continued)

Dienes		Polyenes		Multibranch <sup>e</sup>	
Acid	Weight Percent	Acid	Weight Percent	Acid	Weight Percent
14:2	0.04	18:3	1.03	16:0 br3	0.01
16:2	0.02	18:4	0.10	17:0 br3	0.01
18:2	2.22	20:3	0.05	18:0 br3	0.16
20:2	0.12	20:4	0.07	—	—
22:2	0.14	20:5	0.02	—	—
24:2	0.02	22:3	0.03	—	—
26:2	0.0004	22:4	0.04	—	—
—	—	22:5	0.02	—	—
19:0 br4 <sup>d</sup>	0.02	—	—	—	—
20:0 br4	0.14	—	—	—	—
21:0 br4	0.02	—	—	—	—
22:0 br4	0.02	—	—	—	—
23:0 br4	0.01	—	—	—	—
24:0 br4	0.10	—	—	—	—
25:0 br4	0.10	—	—	—	—
26:0 br3	0.01	—	—	—	—
27:0 br4	0.04	—	—	—	—
28:0 br3	0.02	—	—	—	—
28:0 br4	0.12	—	—	—	—
28:0 br5	0.01	—	—	—	—

<sup>a</sup>Detected by urea fractionation and gas-liquid chromatography in 1965 (17).

<sup>b</sup>Acid below 8:0 were not determined (totally or partially lost during removal of solvent); also did not measure *trans*-isomers, conjugated dienes and trienes, and keloacids.

<sup>c</sup>*i*, iso; *br*, iso and/or anti-iso. Last number indicates number of methyl branches for multibranch acids.

<sup>d</sup>The number following *br* indicates the number of methyl branches for multibranch acids.

<sup>e</sup>Tentatively identified in appropriate urea fractions by semilogarithmic plots of GLC retention times.

Few compilations of the extensive fatty acid distributions in butter fat have been made since Iverson et al. (17) reported quantitative data on 82 fatty acids that were detected by means of urea fractionation and gas-liquid chromatography (GLC) (Table 6). Table 7 provides the fatty acid composition of bovine milk lipids.

The advent of new techniques of gas chromatography for monoglycerides, diglycerides, and triglycerides (18, 19) should assist markedly in the identification of the specific triglycerides of butter fat. It has already been possible to identify and quantify about 168 molecular species of bovine milk serum triglycerides, excluding enantiomers. Nutter and Privett (20) employed liquid-liquid and argentation thin-layer chromatography (TLC) along with pancreatic lipase hydrolysis for this purpose. As a result of their high degree of saturation, ruminant milkfats do not lend themselves readily to argentation TLC, and resolution by gas chromatography using polyester columns is a likely recourse.

There is a pronounced seasonal change in the fatty acid composition of butter fat. It is normally several iodine number units higher in the summer than in the winter, with corresponding variation in the relative proportions of unsaturated

TABLE 7. Fatty Acid Composition of Bovine Milk Lipids, August 1983 (3).

Number	Type	Identity
27	Normal saturate	2-28
25	Monobranched saturate	24; 13, 15, 17, 18 three or more positional isomers
16	Multibranched	16-28
62	Cis monoene	10-26, except for 11:1, positional isomers of 12:1, 14:1, 16:1-18:1, and 23:1-25:1
58	Trans monoene	12-14, 16-24; positional isomers of 14:1, 16:1-18:1, and 23:1-25:1
45	Diene	14-26 evens only; cis, cis; cis, trans; or trans, cis and trans; trans, geometric isomers; unconjugated and conjugated and positional isomers
10	Tripolyene	18, 20, 22; geometric positional, conjugated and unconjugated isomers
5	Tetrapolyene	18, 20, 22; positional isomers
2	Pentapolyene	20, 22
1	Hexapolyene	22
38	Keto (oxo) saturated	10, 12, 14, 15-20, 22, 24; positional isomers
21	Keto (oxo) unsaturated	14, 16, 18; positional isomers of carbonyl and double bond
16	Hydroxy, 2-position	14:0, 16:0-26:0, 16:1, 18:1, 21:1, 24:1, 25:1
	Hydroxy, 4- and 5-position	10:0-16:0, 12: $\Delta$ -6 and 12:1- $\Delta$ -9
60	Other positions	
1	Cyclic, hexyl	11; terminal cyclohexyl

and saturated fatty acids. In colder climates, the difference appears to be slightly larger. The change is usually associated with the difference in the feed of the animals in different seasons, but not completely so: cows put on green pasturage produce softer butter fat even if their feed has previously consisted of hay or silage comparable in solid composition with the green feed.

There are also differences in the butter fat of different cows on identical rations, and the age of the animal and duration of lactation have some influence on butter fat composition. Much of the dairy literature provides information relating dairy animal species and the composition of the butter fat from them.

When corn and peanut oils are protected (entrapped in formaldehyde-treated casein), significant changes in the fatty acid composition of milkfat occur (Table 8) (21).

Protected oils are hydrolyzed in the abomasum, and the fatty acids are absorbed in the small intestine, thereby avoiding hydrogenation. The 18:2 content in the milkfat was increased about five-fold, and the 14:0, 16:0, and 18:0 were decreased accordingly. Plasma and depot fats were also increased in 18:2 content by this program (21).

Results at the USDA are similar: cow's milk can be increased in 18:2 acid from 3% to 35% by feeding protected safflower oil (22, 23). However, at high 18:2 levels,

**TABLE 8. Effect of Feeding Protected Corn and Peanut Oils on Fatty Acid Composition of Bovine Milkfat (4, 21).**

Fatty Acids	Fatty Acid Composition of Milk Lipids (wt. %)		
	Corn Oil	Peanut Oil	Control
14:0	7.9	9.7	11.9
16:0	20.5	22.1	31.1
18:0	9.8	11.0	13.5
18:1	28.8	25.3	29.5
18:2	20.1	20.5	4.2
18:3	1.8	2.9	2.7
Others	11.1	8.5	7.1

milk develops an oxidized off-flavor, usually after about 24 h, and creams require a longer aging time for satisfactory churning. As expected, butter that contains more than 16% linoleic acid is soft and sticky (5).

Extensive data have been published on the Reichert-Meissl, Polenske, and Kirschner values of mixtures of butter fat, coconut, and palm kernel oils (Table 9) (24–26). Other average characteristics of butter fat are approximately as follows: density at 60°C, 0.887; melting point, 38°C; titer, 34°C; and unsaponifiable matter, 0.4%. The optical properties of butter fat are misleading and are in part contributed by the nonglyceride components.

A significant variation in milkfat composition can occur in colostrum milk. Ahren et al. (27) analyzed the content of glycerol ethers in neutral lipids and phospholipids isolated from bovine colostrum and milk (Table 10). Lactone content of butter fat has also been determined (Table 11).

Odd-numbered methyl ketones containing from 3 to 15 carbon atoms are found in small quantities in butter fat. These compounds, along with microtraces of acetone, acetaldehyde, methyl sulfide, C4–C10 free fatty acids, and the various lactones already mentioned, generally are considered to be the substances that comprise the pleasant, bland, olfactory, nonoxidative flavor and odor of milkfat. Representative concentrations of homologous methyl ketones have been well documented (30–32).

**TABLE 9. Distinctive Characteristics of Butter Fat Compared with Other Fats (23).**

Characteristic	Butter Fat	Coconut Oil	Palm Kernel Oil	Soy and Corn Fats and Oils
Saponification number	210–250	245–260	240–250	~200
Refractive index, 60°C	~1.4465	~1.4410	~1.4430	>1.4465 <sup>a</sup>
Reichert-Meissl value	22.34	6.8	5.7	<1
Polenske value	2–24	14–18	10–12	<1
Kirschner value	20–26	1–2	0.5–1	<0.5

<sup>a</sup>Unless the iodine number is very nearly zero.

**TABLE 10. Content of Glycerol Ethers in Neutral Lipids and Phospholipids Isolated from Bovine Colostrum and Milk (27).**

Characteristic	Colostrum (% wt/wt)	Milk (% wt/wt)
Total lipids	5.6	3.9
Neutral lipids in total lipids	99.0	99.3
Phospholipids in total lipids	1.0	0.7
Glycerol ethers in total lipids	0.061	0.009
Glycerol ethers in natural lipids	0.06	0.007
Glycerol ethers in phospholipids	0.16	0.25
Glycerol ethers in natural lipids of total glycerol ethers	97.4	80
Glycerol ethers in phospholipids of total glycerol ethers	2.6	20

The phospholipids of milkfat are found in the fat globule membrane in association with proteins and cerebrosides. Phospholipids are amphipolar in nature and are strongly surface active. These properties enable them to stabilize both oil-in-water and water-in-oil emulsions (Table 12) (4-6).

The sterols found in the unsaponifiable fraction of milk lipids are mostly cholesterol esters, small quantities of lanosterol, and even smaller quantities of two new constituents: dihydrolanosterol and  $\beta$ -sitosterol (33).

From the standpoint of nutritional value, the Vitamin A content of butter is important. As the source of Vitamin A in butter is  $\beta$ -carotene or other carotenoid pigments in the feed of the cows, the content of this vitamin varies considerably, being highest in the summer when the dairy herds are in pasture and lowest in winter when there are no green feedstuffs in their rations. A portion of the carotene

**TABLE 11. Amounts of  $\gamma$ - and  $\delta$ -Aliphatic Lactones Isolated from Butter Fat (ppm) (2, 28, 29).**

Carbon Number	$\delta$ -Lactones	$\gamma$ -Lactones
6	2.0	Trace
7	0.2 <sup>a</sup>	—
8	2.6	0.5
9	0.4 <sup>a</sup>	0.2
10	15.0	1.2
11	0.7	0.5
12	35.0	1.6
13	1.5	0.5
14	34.0	1.4
15	6.4	1.3
16	23.2	1.3
18	2.3	—
2,3-Dimethyl-2,4-nonadien-4-olide	—	0.5

<sup>a</sup>Semiquantitative.

**TABLE 12. Phospholipid Content of Bovine Milk (4-6).**

Phospholipid	Mole Percent
Phosphatidylcholine	34.5
Phosphatidylethanolamine	31.8
Phosphatidylserine	3.1
Phosphatidylinositol	4.7
Sphingomyelin	25.2
Lysophosphatidylcholine	Trace
Lysophosphatidylethanolamine	Trace
Total choline phospholipids	59.7
Plasmalogens	3
Diphosphatidyl glycerol	Trace
Ceramides	Trace
Cerebrosides	Trace

in the feed is transferred to the butter fat without change. The amount of carotene transferred by the cow into the butter fat varies with the feeding regimen parallel to variations in the production of Vitamin A, so that the intensity of the yellow color of butter, to some extent, serves to indicate its Vitamin A content.

The Vitamin A potency of butter is in part due to Vitamin A as such and in part to carotene, which is partially converted to the vitamin in the human body. The Vitamin A content of butter is usually within the range of 6-12 mg/g, and the carotene content is in the range of 2-10 mg/g (33); 1 IU of Vitamin A is defined as the amount possessing the biological activity of 0.6  $\mu$ g of pure  $\beta$ -carotene.

The Vitamin D content of butter is much less significant than that of Vitamin A, but it is nevertheless appreciable. It varies from about 0.1 IU/g to 1.0 IU/g, being highest in the summer and lowest in the winter (33).

The composition of milkfat is the most important factor affecting the firmness of butter and, therefore, its spreadability. The composition of milkfat changes primarily according to the feed; therefore, the entire problem is connected to the animal's diet. The fatty acid composition of milkfat produced in various countries has been rather accurately determined, as have the seasonal variations. In Europe, the amount of saturated fatty acids is generally highest in winter and lowest in summer or fall (see Table 11) (34). Green fodder decreases the amount of saturated fatty acids and correspondingly increases the amount of unsaturated fatty acids. The differences between the maximum and minimum values can be fairly large. For palmitic and oleic acids, the quantitatively most important fatty acids, a difference of more than 10% between the maximum and minimum values was found in some cases. This makes it understandable that there are also significant differences in the physical characteristics of the butter. The structure of the triglycerides in the milkfat, along with the fatty acid composition, is important in determining the physical characteristics of the fat, because the softening point of fat has been found to rise as the result of interesterification (35).

**TABLE 13. Compositional Characteristics of Summer and Winter Milkfat (36).<sup>a</sup>**

Samples	Fatty Acids				Iodine Number
	Volatile	Saturated	Monounsaturated	Polyunsaturated	
Average of total	10.98	56.50	29.81	2.50	32.2
Summer	9.49	58.82	33.53	3.14	36.8
Winter	12.45	59.15	26.15	1.86	27.7

<sup>a</sup>*N* = 140.

Textural characteristics of butter significantly depend on milkfat composition and the method of manufacture. If the chemical composition of the milkfat is known, it is possible to select the appropriate technological parameters of the buttermaking to improve its texture. To obtain butter with constant rheological characteristics and to control the parameters of the buttermaking process, it is necessary to take into account the difference in the chemical composition and the properties of the milkfat in various seasons. Table 13 shows various compositional changes of milkfat derived from summer and winter milk (36).

### 3. MODIFICATION OF MILKFAT

#### 3.1. Melting and Crystallization of Milkfat Triglycerides

The complex fatty acid composition of milkfat is reflected in its melting behavior. Melting begins at  $-30^{\circ}\text{C}$  and is complete only at  $37^{\circ}\text{C}$ . At any intermediate temperature, milkfat is a mixture of solid and liquid. To a large extent, the solid: liquid ratio determines the rheological properties of the fat. For example, at refrigeration temperature, butter has a higher solids content than does a tub margarine. Hence, the latter product is more easily spread (37).

As crystallization proceeds, the growing crystals impinge to form aggregates. A network results, in which both the solid and liquid phases may be regarded as continuous. Formation of the network greatly increases the firmness of the fat.

As a liquid fat is cooled, crystallization begins. There are two parts to the crystallization: (1) nucleation and (2) growth. In a bulk fat, nucleation occurs at the surfaces of impurities, a phenomenon described as heterogeneous nucleation. A considerable degree of supercooling is necessary to initiate nucleation. Subsequent growth of the nuclei tends to be slow in natural fats because of competitive inhibition. In materials of low molecular weight, impurities are rejected at the face of the growing crystal. In fats, however, the various triglyceride species are so closely related that the term *impurity* tends to lose its meaning (38).

#### 3.2. Hydrogenation

Hydrogenation of various fats and oils is used extensively in industry but is not generally applied to butter fat (the high cost of the raw material argues against its use as



a feedstock). The process reduces the degree of unsaturation of the fat and increases its melting point.

Given the criticism directed at milkfat because of its saturated nature, there appears to be little future in increasing the degree of saturation by means of hydrogenation. The reverse procedure, desaturation or dehydrogenation, offers more attractive prospects.

Flavor deterioration in fat-rich milk and dairy products is mainly due to autoxidative degradation of lipids. This degradation may be retarded by partial hydrogenation. The objective of partial hydrogenation or trace hydrogenation is a selective saturation of the polyunsaturated fatty acids without saturation of the monounsaturated fatty acids to improve the oxidative stability. Selective hydrogenation has been studied for years in the vegetable oil industry with some success. This process has been applied by some researchers to milkfat (Figure 1) (39).

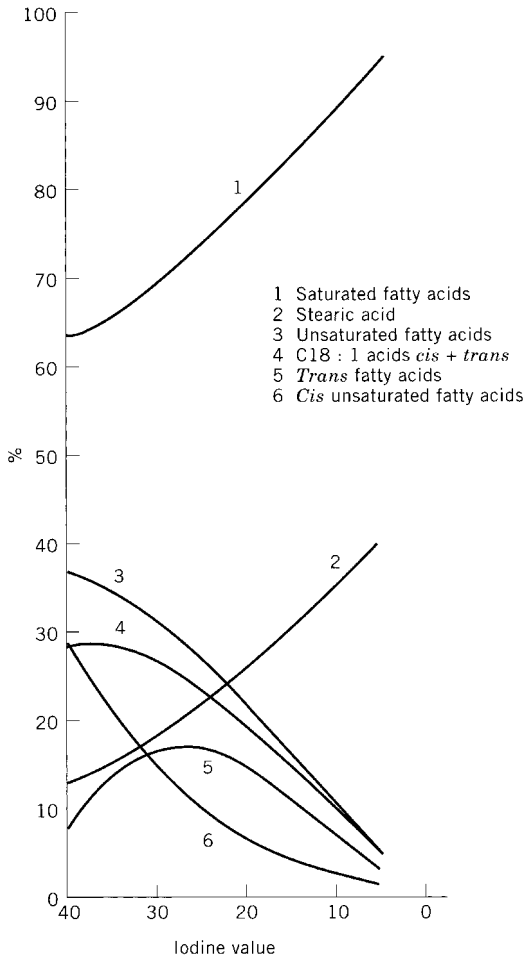


Figure 1. Changes in the composition of fatty acids during the hydrogenation of milkfat (38).

### 3.3. Interesterification

Interesterification (also called ester interchange, randomization, and *trans*-esterification) involves the exchange and redistribution of acyl groups among triglycerides. This technology was initially developed as high-temperature interesterification in Germany during 1920–1930. Since about 1950–1960, the process has been developed still further in the United States and Europe (39). The resultant product exhibits the same total fatty acid composition as the starting material, but the triglyceride composition and the physical properties are changed. Interesterification catalyzed by chemical catalysts or by lipases is used in the fat industry for the manufacture of margarines, shortenings, and confectionery fat (40).

The fatty acid composition is not modified by interesterification, but there is a significant modification of the glyceride composition (Table 14). In the untreated milkfat, the triglycerides may be divided into two groups: triglycerides with lower molecular weights (lower than C42) and the triglycerides with higher molecular weights (C44–C54) (39).

Interesterification offers opportunities for modifying the glyceride composition of milkfat and recombined butter. The technique confers some positive nutritive value to milkfat. One disadvantage of the interesterification reaction is the loss of flavor during neutralization, interesterification, and subsequent deodorization. Most manufacturers would support that little or no commercial interest will result from using this process.

**TABLE 14. Glyceride Composition of Natural and Interesterified Milkfat (mol %) (39).**

Triglyceride	Natural	Interesterified
C22	—	0.1
C24	0.3	0.8
C26	0.1	1.4
C28	0.6	1.5
C30	0.9	1.3
C32	1.9	2.0
C34	4.4	3.0
C36	9.5	5.9
C38	13.1	9.1
C40	12.1	9.9
C42	7.7	7.6
C44	6.8	8.3
C46	7.5	10.7
C48	8.8	12.8
C50	11.2	14.2
C52	10.8	10.9
C54	4.6	0.4
Ratio C38:C50	1.17	0.64

### 3.4. Reduction of Cholesterol in Milkfat

One obvious area for development is in the modification of dairy products to satisfy the changing dietary habits of consumers. The mounting health concerns are related to intake of calories, cholesterol, and saturated fats. Concern about cholesterol in the diet originates from the fact that high-serum cholesterol, especially the low-density lipoproteins, is one of the risk factors associated with atherosclerosis. Dietary intake of cholesterol may be one of the factors contributing to the elevation of serum cholesterol; other dietary factors are high total fat, high saturated fat, and low dietary fiber intake.

There have been many cholesterol-reduction technologies developed all over the world because of high interest by the dairy industry. However, there are only a few technologies available for technology transfer. Fractionation by thermal crystallization, steam stripping, short-path molecular distillation, supercritical fluid extraction, selective absorption, and crystallization using solvents or enzymatic modification can achieve fat alterations of significance to the dairy industry.

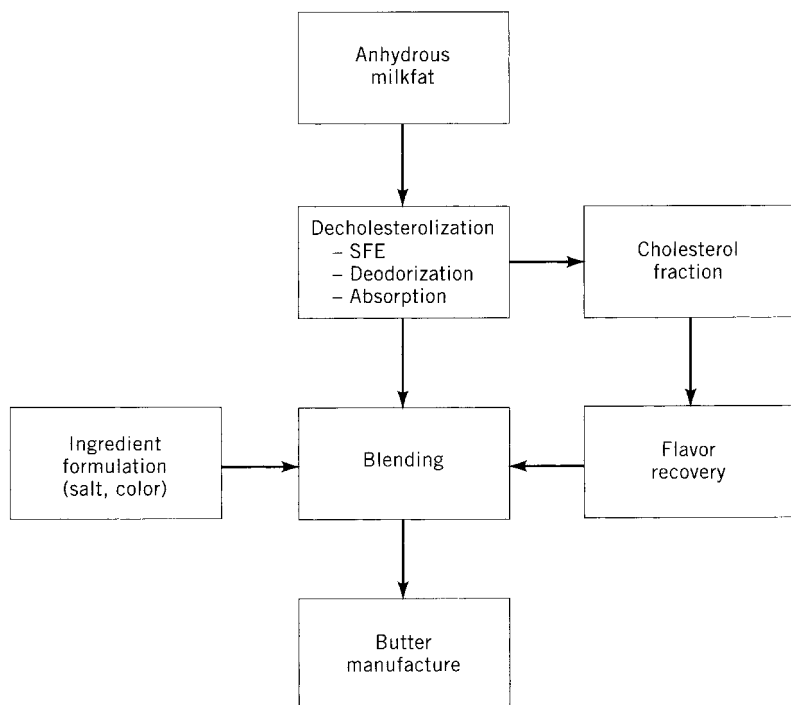
*Vacuum Steam Distillation.* There has been direct application of cholesterol removal by vacuum steam distillation, an old technology. This process is widely used in the fats and oils industry for deodorization.

Cholesterol is a low-volatile compound, but it is more volatile than the major triglycerides of milkfat. Superheated steam can be bubbled through the oil, heating it indirectly, which provides for the latent heat of vaporization of the distilling compounds and prevents steam condensation. Thus, the temperature and pressure can be varied independently. When the sum of the partial vapor pressures of water vapor and the distillates is equal to the total pressure, water vapor and the low-volatile components, such as cholesterol and free fatty acids, distill over.

The process for cholesterol removal from anhydrous milkfat was patented by General Mills (41). Fractionment Tirtiaux also disclosed the development of a vacuum steam distillation system called the LAN cylinder (38). The steam distillation process (Figure 2) was commercialized, producing a 90–95% cholesterol reduction in anhydrous milkfat with a 95% yield that was reconstituted into 2% fat fluid milk (42). The major disadvantage to the process is that it strips or removes most all volatile flavor components from the fat. These flavor components must be captured (i.e., vacuumation) before the distillation process to attempt to reproduce the delicate flavors so desired for reconstitution into a butter product.

*Short-path Molecular Distillation.* Short-path molecular distillation offered great promise for the selective removal of cholesterol from milkfat. The process achieves the simplest separation of desired substance from a mixture of components of high molecular weight.

The rate of distillation at any given temperature is a function of the ratio  $P:M^{1/2}$ , where  $P$  is the partial pressure of the compound and  $M$  its molecular weight. Owing to the temperature dependence of the rate of distillation, a fractionation of components with different molecular weights can be carried out by holding the temperature constant until the more volatile constituents are removed. Figure 3 illustrates potential capability of the process (43).

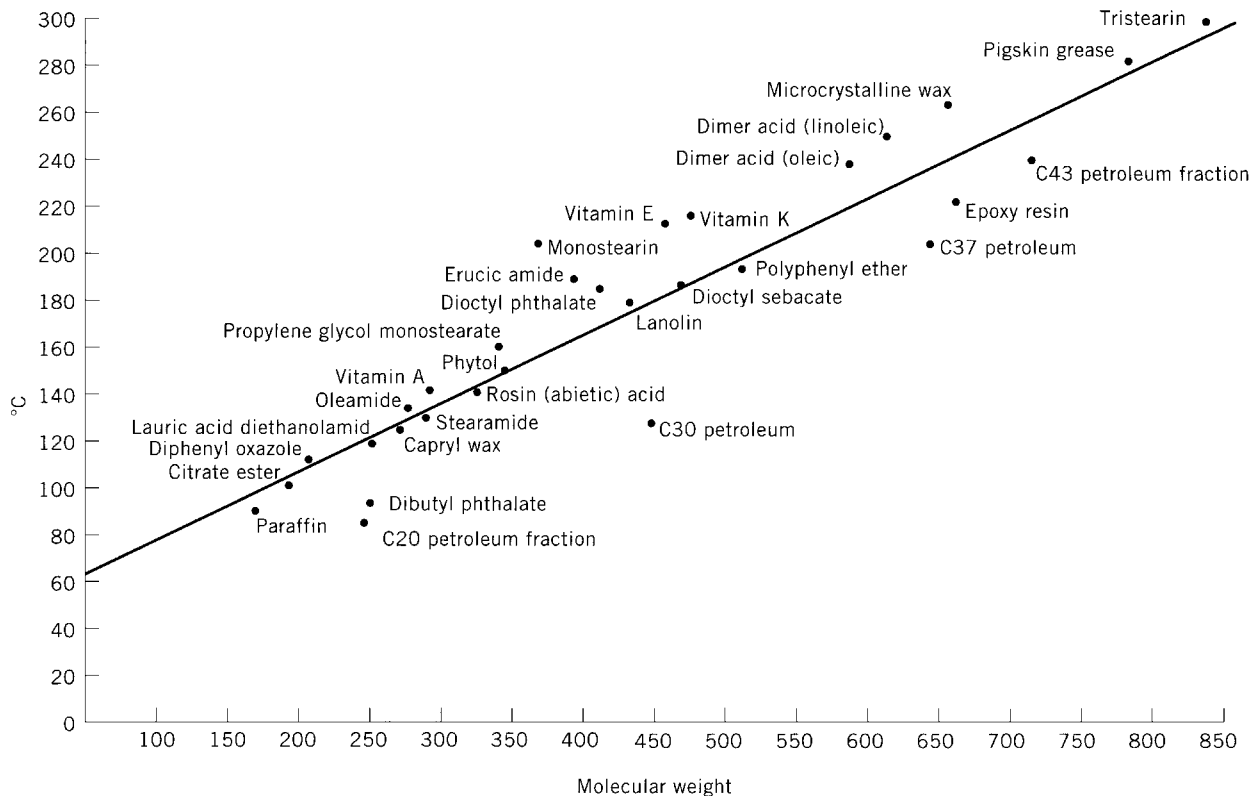


**Figure 2.** Schematic for the decholesterolization of milkfat (41).

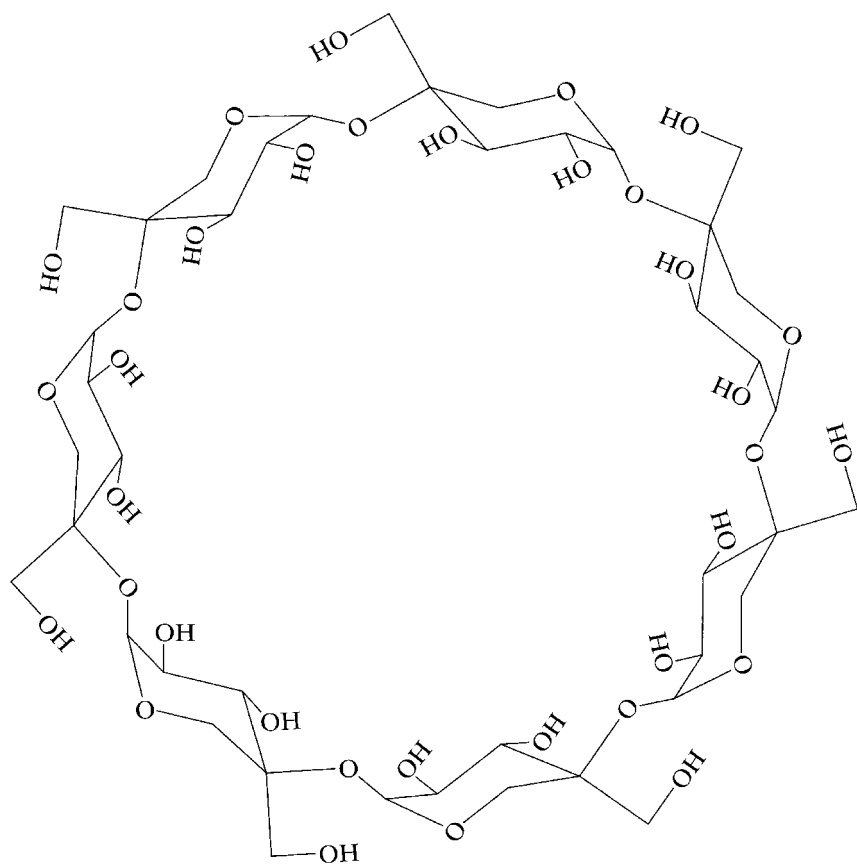
The process has been applied to strip oil-soluble vitamins, sterols, and fatty acids from fats and oils. Cholesterol has been successfully removed from anhydrous milkfat in the range of 70–90% (44, 45). Extensive studies were performed and various temperatures and pressures were used to fractionate milkfat (46). Unfortunately, the process has not proved to be economically feasible due to the low butter fat yield when significant cholesterol was removed (Land O'Lakes research).

*Absorption.* One of the most promising technologies has been the use of cyclodextrins to complex cholesterol from a mixture and then selectively separate the cholesterol–cyclodextrin complex. European researchers have pioneered almost all of the research in this area, and patents have been issued (47, 48).

The process is based on the fact that  $\beta$ -cyclodextrin specifically forms an insoluble inclusion complex with cholesterol.  $\beta$ -Cyclodextrin is a cyclic oligosaccharide of seven glucose units. It consists of 1,4-*a*-D-linked glucopyranose residues, as shown in Figure 4. As a consequence of the C1 conformation of the glucopyranose units, the secondary OH groups are located on the edge of the torus-like cyclodextrin molecule, whereas all the primary OH groups are on the other side (Figure 5) (48). The central cavity is, therefore, hydrophobic, giving the molecule its affinity for nonpolar molecules such as cholesterol. The radius of the cavity can accommodate a cholesterol molecule almost exactly, explaining the highly specific nature of  $\beta$ -cyclodextrin's ability to form an inclusion complex with cholesterol.



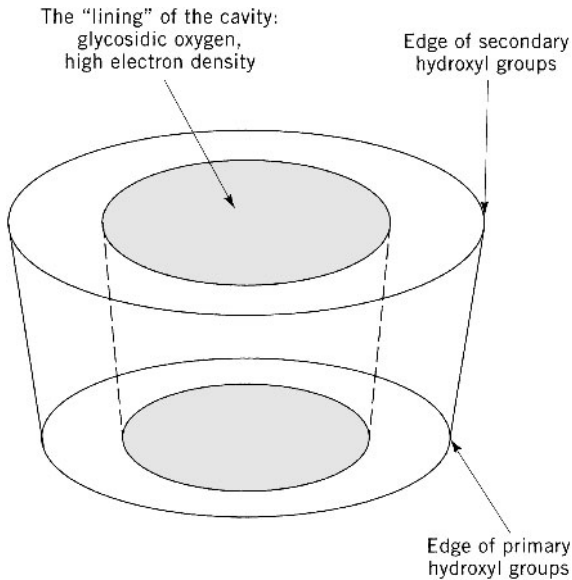
**Figure 3.** Distillation under vacuum (43).



*Figure 4.*  $\beta$ -Cyclodextrin

This process appears to provide considerable economic and practical advantages over alternative cholesterol reduction technologies, such as steam distillation and supercritical carbon dioxide fluid extraction. For instance, there is no absorption of vitamins, it is a low-temperature operation, and it has a low capital cost. The only economic concern is that the ratio of the addition of  $\beta$ -cyclodextrin to the cholesterol removed is high, creating the potential for a high-cost process. Even so, the Europeans have commercialized the process, and reduced-cholesterol butter and cheese products have been introduced into the marketplace (49).

Multiple absorbants have been researched, and they include digitonin, tomatine, sodium cholate (bile salts), and active carbon (45, 47, 50). Most will face severe food regulatory problems. New Zealand (48) performed extensive research on active carbon, carbon impregnated with different metal salts, and inert supports impregnated with selected organic compounds. This technique (active carbon) is



**Figure 5.** Schematic of the  $\beta$ -cyclodextrin molecule, showing the hydrophobic cavity (48).

not promising, because it does not retain the delicate butter flavors or the carotenoid pigments. An off-flavor develops in the milkfat. The University of California has evaluated the use of food-grade saponins as absorbents (51). This process showed promise, but most work has been discontinued due to U.S. regulatory prohibitions.

**Solvents.** The use of organic solvents, such as acetone, in the laboratory has proven to be an effective method for removal of milkfat components, including cholesterol. Unfortunately, this method creates regulatory and negative consumer perceptions due to the potential of solvent residues in the natural butter/butter fat-containing products.

**Supercritical Fluid Extraction.** The supercritical fluid extraction process created extensive excitement in the mid-1980s in the research community as a preferred process for cholesterol removal. Extensive research at various universities was initiated to evaluate its potential, and significant publicity was generated within the dairy industry (45, 46, 52–56).

Liquid-like densities of supercritical gases result in liquid-like solvent powers; this property and faster diffusion characteristics due to low-gas viscosity make supercritical fluids attractive extraction agents. Solubility of substances in supercritical gases derives from van der Waals' molecular attractive forces and increases with increasing pressure at a constant temperature. The temperature influences the solution equilibria in a more complicated way than does the pressure. Compounds can be selectively dissolved by changing the density of the gas, i.e., pressure and temperature conditions.

The extraction of cholesterol into the mobile gas phase is determined by the balance of a tripartite interaction: triglyceride- $\text{CO}_2$ , triglyceride-cholesterol, and

cholesterol-CO<sub>2</sub>. As a minor constituent of the milkfat, cholesterol is probably associated with the triglycerides for which it has higher affinity, namely, short- and medium-chain triglycerides and, to some extent, long-chain unsaturated triglycerides. As CO<sub>2</sub> affinity is low for cholesterol at 200 bar and 80°C (low-gas concentration), there would be less competition between triglycerides and CO<sub>2</sub> for cholesterol. Those cholesterol molecules associated with short- and medium-chain triglycerides would be eluted into the gas phase along with them at low-gas concentrations. However, the cholesterol molecules associated with long-chain triglycerides are not eluted at low-gas concentrations because of their larger size. Cholesterol esters, being large molecules, would not be eluted at low-gas concentrations.

By the late 1980s, technologies for the removal of cholesterol with supercritical carbon dioxide were offered by a number of companies (38). Commercialization was never attempted by any major food company for removal of cholesterol. Successful scale-up and commercialization was achieved by the General Foods Corporation for removal of caffeine from coffee (45). The primary disadvantages for the dairy industry were the low yields, low cholesterol removal, and the very high capital and operating costs of the equipment.

*Enzymatic.* Biological procedures for cholesterol removal make use of micro-organisms that produce enzymes to convert cholesterol into innocuous compounds. Several enzymatic systems are being investigated in different countries of the world. Most systems use a cholesterol reductase that converts the cholesterol into coprostanol and coprosterol (52, 57). These converted compounds are very poorly absorbed by the digestive system and pass through intact. Several investigators have isolated and characterized *Eubacteria* able to convert cholesterol into coprostanol from rat, baboon, and human feces. Leaves of cucumber, soybeans, corn, and beans are known to contain similar enzymes (45, 57, 58). *Lactobacillus acidophilus* has also been reported to metabolize cholesterol (51).

Once suitable enzyme systems are identified, the next step involves transfer of the gene that codes for the enzyme into suitable micro-organisms, such as *Lactobacillus* and *Streptomyces* species, for large-scale production and purification of the enzyme. Further steps may involve attaching the enzyme onto a solid support or adding purified enzyme in soluble form to the food systems. It is equally envisioned that cholesterol-degrading enzymes, such as cholesterol reductase, can be genetically transplanted from one group of bacteria into lactic bacteria, which are the traditional dairy starter cultures. The cholesterol-reducing cultures could then be used in cultured dairy products such as cheese.

The industrial scale-up of enzymatic technology is both highly complicated and expensive. Moreover, a primary regulatory hurdle will involve demonstrating that the end products of the cholesterol-enzyme reaction and the novel compounds formed through genetic engineering are harmless.

*Regulatory-Nutritional.* Many of the processes for cholesterol removal will meet with regulatory hurdles because of residues, unapproved additives, or byproduct formation. But the real burden for the U.S. dairy industry was the 1993 Nutritional Labeling and Education Act (54). This act created commercial prohibitions,



because it requires that cholesterol-reduction claims cannot be applied to products that contain more than 2 mg/g of saturated fat; butter fat is approximately 65% saturated. The industry has not yet developed a cost-effective means to reduce butter fat saturation.

For years, the nutritional community has claimed that the effect of dietary cholesterol intake on serum cholesterol level was much less significant than the ratio of total fat to saturated fat in the diet (59). The general public is becoming aware of this and has consequently reduced its demand for low-cholesterol foods.

## 4. QUALITY CONTROL

In the production of all butter-fat-containing foods, quality control is essential to ensure shelf life, safety, and the food's appearance, flavor, and texture. The key to the final product is the quality of the raw materials used. The handling of raw milk is of particular importance. Careful attention is paid to temperature control in raw-milk-handling systems and, naturally, to the cleaning of all equipment used for storage and transport. Raw milk is held refrigerated to less than 5°C on the farms, and although this has eliminated the growth of a number of organisms, others (notably psychrotrophs) can still multiply under these conditions. Unfortunately, some of the lipolytic and proteolytic enzymes produced by these classes of organisms are heat stable, even surviving ultrahigh temperature (UHT) treatment. Today, a shelf life of many months is expected of a number of UHT-treated products, thus the presence of lipolytic and proteolytic enzymes can be disastrous. The only way to avoid this problem is to ensure that the numbers of organisms are kept to an absolute minimum during all stages of the collection and manufacturing process.

Some quality problems can be eliminated. For example, certain cattle feeds produce undesirable volatile flavors, which tend to concentrate in the butter fat portion of the milk. Historically, the industry adopted steam stripping of cream for butter-making to reduce the intensity of these flavors. The equipment universally used is the vacreator (Figure 6), which is designed to pass as much as 0.30 kg steam to each kilogram of cream. The design of the vacreator evolved during the 1930s and 1960s, culminating in the development of the Vac 25 and 16 models (59).

Vacreation accomplishes a number of tasks in one operation. Pasteurization of the cream for buttermaking is perhaps the most important of these, followed by steam stripping of off odors. Other functions include destruction of natural milk lipases and development of a slightly "nutty" or "cooked" flavor in the resulting butter.

### 4.1. Hazard Analysis and Critical Control Points (HACCP)

The World Health Organization (WHO) recognized HACCP as an effective rational means to ensure food safety from the farm to consumer. The Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) are moving

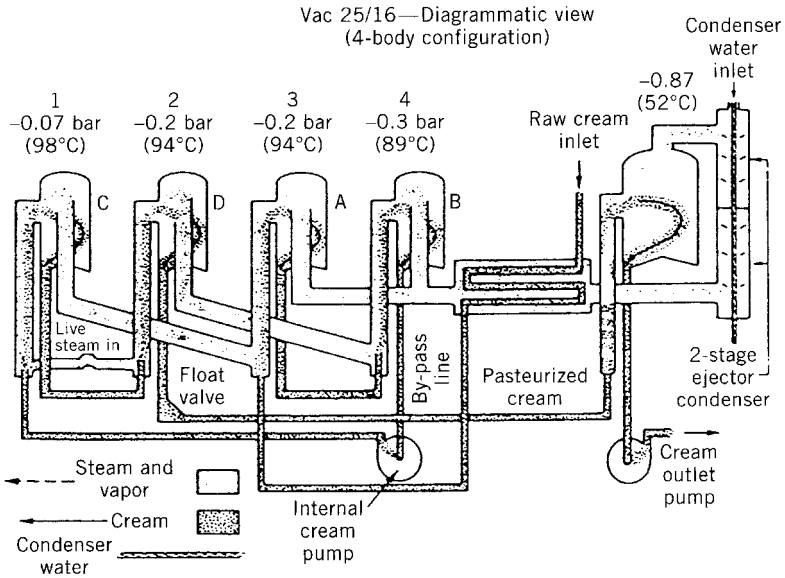


Figure 6. Diagram of the Vac 25/16 vacreator (59).

toward the adoption of HACCP systems to ensure the safety of foods sold in the United States.

The HACCP program is a management tool that provides a logical and cost-effective basis for better decision making with respect to dairy product safety. One of the key advantages of the HACCP concept is that it enables a dairy food manufacturing company to move away from a philosophy of control based on testing to a preventive approach that identifies and controls potential hazards in the manufacturing environment.

#### 4.2. Composition Control

Composition control of the butter fat content has received much attention in the ever present drive to maximize returns and create highly consistent products. As noted, multiple technologies are under development to standardize and simplify the processes.

#### 4.3. Grading, Standards, and Definition

Standards for butter in the United States were established by an act of Congress and are supported by USDA standards for grades of butter. In the revised standards, the following definitions apply: *butter* refers to the food product usually known as butter, which is made exclusively from milk, cream, or both, with or without common salt, and with or without additional coloring matter. The milkfat content of butter is not less than 80% by weight, allowing for all tolerances. *Cream* refers to the cream

separated from milk produced by healthy cows. Cream is pasteurized at a temperature of not less than 73.9°C for not less than 30 min, or it can be pasteurized at a temperature of not less than 89°C for not less than 15 s. There are other approved methods of pasteurization that give equivalent results (60).

The flavor of the cream may be enhanced by culturing, adding food-grade lactic acid bacteria, or adding natural flavors obtained by distilling a fermented milk; cream may also be added to the finished butter. In addition, color, derived from an FDA-approved source, may be used (61).

Legal requirements for butter vary considerably in different countries. For example, in Europe, butter must contain 82% fat, and in France, it may contain a maximum of 16% moisture (62). In some tropical parts of the world, milkfat is used in nearly anhydrous form, because it is less susceptible to bacterial spoilage. This product is known as ghee. In the Middle East and India, ghee is prepared from heated cow or buffalo milk.

The new nutrition labeling regulations, promulgated under the Nutrition Labeling and Education Act of 1993 (54), mandate that only strictly defined terms be used to make nutrient content claims. For example, the term *light* may only be used on products that have been specifically formulated or altered to meet one of two conditions: (1) if the product derives 50% or more of its calories from fat, reduce the fat level by 50% (as compared with a reference product), and (2) if the product derives less than 50% of its calories from fat, reduce the calorie level by one-third (compared with a reference product). Generally, butter products derive more than 50% of their calories from fat and, therefore, must achieve a minimum 50% fat reduction to use the term *light*. The term *reduced* when used as a nutrient descriptor requires a formulation alteration that achieves a minimum 25% reduction in the nutrient from a reference product (63).

When considering products to be labeled with a cholesterol claim, the following applies:

*Reduced cholesterol:* the product has an allowable maximum of 2 g saturated fat and a minimum 25% cholesterol reduction per serving.

*Low cholesterol:* maximum of 2 g saturated fat and a maximum of 20 mg cholesterol per serving are allowed.

*Cholesterol free:* maximum of 2 g saturated fat and less than 2 mg cholesterol are allowed per serving.

When considering a fat free or no fat claim, the new regulations require the product to have less than 0.5 g fat per serving and no added fat unless noted (i.e., “trivial fat”) (54).

There are three U.S. grades of butter: AA, A, and B. Butter is graded by first classifying its flavor organoleptically. In addition to the overall quality of the butter flavor itself, the standards list 17 flavor defects and the degree to which they may be present for each grade. This grade is then lowered by defects in the workmanship and the degree to which they are apparent. Deratings are characterized by negative body, flavor, or salt attributes, which are fully described in the standards. Butter

**TABLE 15. Standards for Anhydrous Milkfat, Anhydrous Butter Oil, and Butter Oil (65).<sup>a</sup>**

Composition and Quality	Anhydrous Milk Fat	Anhydrous Butter Oil	Butter Oil
Milk fat, minimum	99.8% m/m	99.8% m/m	99.6% m/m
Water	0.1% m/m	0.1% m/m	0.3% m/m
Free fatty acids, as oleic acid, maximum	0.3% m/m	0.3% m/m	0.4% m/m
Peroxide value, m Eq oxygen/kg fat, maximum	0.3	0.3	0.6
Copper, mg/kg, maximum	0.05	0.05	0.05
Iron, mg/kg, maximum	0.2	0.2	0.2

<sup>a</sup>Taste and odor at 40–45°C should be acceptable for market requirements. Texture, depending on temperature, should be smooth and fine granules to liquid.

that does not meet the requirements for U.S. Grade B is not graded. To bear the USDA seal, the finished product must fall within the following microbiological specifications:

Proteolytic count not more than 100 per gram.

Yeast and mold count not more than 20 per gram.

Coliform count not more than 10 per gram.

Butter should be stored at 4.4°C or lower or at less than –17.8°C, if it is to be held for more than 30 days (62). The International Dairy Federation (IDF) has produced specifications for milkfat (64), which include reference to the feedstock. (These specifications relate to the time of manufacture but are often used as purchase standards.) The highest grade, anhydrous milkfat (AMF), must be produced from fresh milk, cream, or butter, to which no neutralizing substances have been added. It should have a clean, bland flavor when tasted at 20–25°C and a peroxide value (PV) of less than 0.2 meq oxygen/1 kg fat. Anhydrous butter oil may be produced from butter or cream of different ages and has no pronounced, unclean, or other objectionable taste or flavor. The term *butter oil* should be used where there is no pronounced unclean or other objectionable taste or odor. The FAO/WHO Codex standard for milkfat is shown in Table 15 (65).

#### 4.4. Specialized Analytical Methods

The dairy industry produces a valuable fat that has a desirable flavor and positive consumer awareness; these attributes must be protected. Significant development activity has occurred for rapid, simple methods to detect adulteration (66, 67). As lack of spreadability was determined to be a major butter negative, a flurry of research was initiated creating the need for measurement techniques (68–70). Many procedures have been used or proposed to assess the microbiological quality of the milk or cream. Generally, microbiological tests are performed to determine the hygiene of production and storage conditions or for safety reasons. Tests include

total counts and counts for specific classes of micro-organisms, such as yeasts and molds, coliforms, psychotrophs, and pathogens such as *Salmonella*. Rapid-screening tests based on dye reduction or direct observation using a microscope or automatic total counters are also in use.

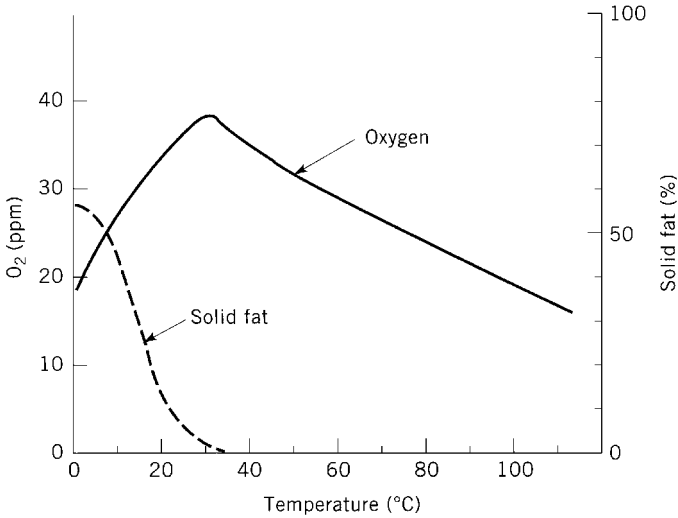
#### 4.5. Lipase Activity

An increasing problem is lipolysis in butter fat after manufacturing, which is caused by thermoresistant lipase enzymes that are created in the milk or cream by psychotrophic bacteria or by residual native lipases that survive pasteurization. Based on a determination of the lipase activity in cream, the keeping quality of manufactured butter in regard to lipolysis can be predicted with reasonable accuracy. A similar prediction for sweet cream butter can be based on lipase activity in the serum phase (71). The characteristic lipolytic flavors that can develop in milk products are primarily associated with the short- and medium-chain fatty acids that are relatively abundant in milkfat; they have lower flavor threshold values than the long-chain fatty acids. As a result of improvements in the quality of raw milk and the standards of processing, lipolytic rancidity is seldom present in the fat source before its use in recombination (72).

#### 4.6. Oxidation

The flavor of dairy products is largely determined by the fat component. Consequently, it is particularly important to restrict the development of oxidized off-flavors in the fat source before use. Oxidation is the chief mode of deterioration of fats and a major factor in determining the shelf life of fat-containing foods (72). Unsaturated fatty acid esters react with oxygen to form peroxides. Although flavorless themselves, peroxides are unstable and readily decompose to yield flavorful carbonyl compounds. The latter are the source of the characteristic oxidized flavors that are detectable at low concentrations. The rate of oxidation depends on the concentration of dissolved oxygen, the temperature, the presence of pro-oxidants such as copper and iron, the degree of unsaturation of the fat, and the presence of antioxidants that may retard the onset of oxidation. Compared with many fats, milkfat has a good oxidative stability, because it is high in total saturates, low in polyunsaturates, and contains natural antioxidants, principally  $\alpha$ -tocopherol.

The development of oxidative rancidity in milkfat is the major determinant of the stability of the fat on storage. Dissolved air in the milkfat can give dissolved oxygen levels of up to 40 ppm at 30°C. In practice, the dissolved oxygen level in the freshly processed milkfat would be about 5 ppm at 45°C, a level sufficient to permit the development of oxidative rancidity, but if the milkfat were allowed to equilibrate with the air, then this level could increase to 33 ppm with a consequent increase in the rate of development of oxidative rancidity. The solubility curve for oxygen in milkfat is a compound of the solubility curves for the liquid and solid phases (Figure 7). Though the solubility decreases with increasing temperature for both phases, the solubility of oxygen in the liquid phase is much higher than for the closely packed solid phase (73).



**Figure 7.** The effect of temperature on the solid fat content and solubility of oxygen in milkfat (73).

The oxygen level in the milkfat may be limited by either active or passive actions. For passive control, processing procedures and plant design are established to minimize air exposure. Deaeration devices (74), vacuumation (60), the use of antioxidants (72), effective destruction of lipases (75), and nitrogen spanning of container headspace are examples of active control of product quality.

## 5. BUTTER MANUFACTURE

### 5.1. Milk and Cream Separation

The most basic and oldest processing method is cream separation. Ancient people are known to have used milk freely. It is probable that they used the cream that rose to the top of milk that had been held for some time in containers, although there is little in ancient literature to suggest that such use was common. It is well established that, in early times, butter was produced by churning milk.

The principal questions concerning separation in a butter manufacturing facility are the choice of cream fat content and the choice of the separation technique, milk separation before or after pasteurization, temperature of separation, and regulation of the fat content. Separation of cream from milk is possible because of a difference in specific gravity between the fat and the liquid portion, or serum. Whether separation is accomplished by gravity or centrifugal methods, the result depends on this difference (48).

A modern dairy separator will separate virtually all fat globules larger than  $0.8 \mu\text{m}$ , and because most of the milkfat is present in the form of such globules,

it is relatively easy to separate the bulk of the milkfat. Fat globules below  $0.8\ \mu\text{m}$  are generally referred to as nonseparable globules (76).

The percentage of fat in the cream must be known and controlled. It influences fat losses during churning. Knowledge of the fat content assists in yield estimations for operational conditions in continuous manufacture. A number of satisfactory analytical procedures are available, with the Babcock test being the most common.

The chemical composition of the triglycerides, which make up milkfat, varies throughout the year, depending on the stage of lactation and the cow's diet. The seasonal variation causes a cyclic change in the melting properties of the fat. In the control of the buttermaking process and the physical properties of the finished butter, this factor must be monitored. The term *melting property* is used rather than softness or hardness, because these more correctly refer to altogether different attributes of solids. A number of procedures have been used to follow the seasonal change in the melting properties. The iodine number, refractive index, differential scanning calorimetry, or pulsed nuclear magnetic resonance spectroscopy can be used to prepare a melting curve. However, the expense and complexity of these melting curve techniques precludes this approach in most quality-control situations (77). The traditional chemical determinations for fat, saponification value, and Polenske value are of limited value. They are scarcely relevant for quality control, and the information they provide can be more usefully quantified by the determination of the fatty acid profile using gas chromatography.

Today, the use of stainless steel has essentially eliminated the exposure of the fat to copper and iron. The presence of copper and, to a lesser extent, iron can catalyze oxidative deterioration of butter during storage, particularly in the presence of salt and a low pH.

## 5.2. Crystallization

The crystal structure of fat and the resulting physical properties of butter made by both conventional and alternative processes have received considerable study. When churned conventionally or by the continuous Fritz process for butter manufacture, most of the milkfat is contained within the fat globule in cream during the cooling and crystallization process. The fat globule provides a natural limit to the growth of fat crystals. Cooling and holding of cream is normally carried out overnight, and thus, sufficient time exists for the crystallization process to approach equilibrium (78).

The principles of crystallization of plastic fats in the type of equipment used for margarine manufacture have been described (79). It is important for the butter to develop small fat crystals that remain substantially discrete and do not form a strong interlocking structure. Small crystals (e.g.,  $5\ \mu\text{m}$  diameter) have a greater total surface area than large crystals and will bind water and free liquid fat by adsorption more effectively (78). Large crystals impart a gritty texture to the product. When fats are cooled rapidly in a scraped-surface heat exchanger, fat crystallization commences, but the fat is substantially supercooled on exiting. If

crystallization is then permitted to continue under quiescent conditions, crystals will grow together and form a lattice structure. The product will thus be hard and brittle and may tend to leak moisture. If, however, crystallization is permitted to occur under agitated conditions (e.g., for 1–3 min in a pin worker), the formation of small independent crystals will be favored and the product will have a fine, smooth texture. If crystallization under agitated conditions is permitted to continue for too long, the product will be too soft for most patting or bulk filling operations, and it is likely to be too soft and greasy at warm room temperatures.

### 5.3. Neutralization

When lactic acid has developed in the raw, unpasteurized cream by microbial activity to a degree considered excessive, neutralizer may be added to return the cream acidity to a desirable level. Sodium carbonates have been found suitable in practice for batch neutralization. For continuous neutralization by pH control, sodium hydroxide is more suitable. These chemicals must be food grade.

### 5.4. Heat Treatment

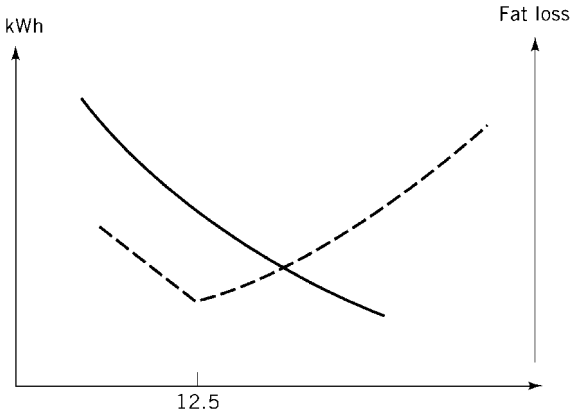
The heat treatment of cream plays a decisive role in the butter-manufacturing process and the eventual quality of the butter. It is important that milk and cream be handled in the gentlest possible way to avoid mechanical damage to the fat, a serious problem in continuous manufacture (Fritz process) of butter (80). Cream is pasteurized or heat treated for the following reasons: to destroy pathogenic micro-organisms and reduce the number of bacteria, to deactivate enzymes, to liquify the fat for subsequent control of crystallization, and to provide partial elimination of undesirable volatile flavors.

### 5.5. Batch Butter Manufacture

Today, batch processing is not used to any extent for the production of large quantities of butter. Batch systems are still encountered in small butter plants, primarily in less industrially developed countries. Continuous systems are more efficient and cost-effective for large outputs; batch systems have low capital cost.

The processing of cream by a batch churn requires filling to approximately 30–50% capacity at a cream temperature of 4.4–12.8°C. Cream temperature varies, depending on season, the butter characteristics desired, and the desired rate of fat inversion (Figure 8). Churning is accomplished by rotation of the churn at approximately 35 rpm until small butter granules appear. The process usually requires about 45 min for coalescence of the fat globules and clean separation of the buttermilk so that it can be drained (82). The granules may be washed with cold water to remove surface buttermilk. Salt is then added with water if standardization of the fat content is required. The butter is worked to ensure uniformity and desirable body and texture characteristics and the rate of fat inversion.





**Figure 8.** Energy consumption and fat loss in buttermilk in relation to churning temperature and heating temperature (81).—, Energy consumption; —, fat loss.

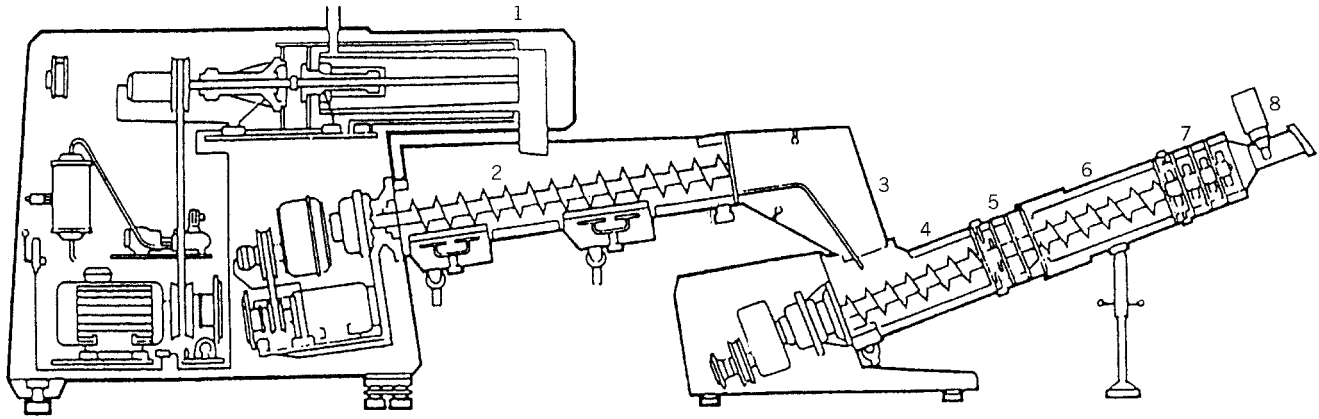
## 5.6. Continuous Butter Manufacture

Between 1930 and 1960, a number of continuous processes were developed. In the Alfa, Alfa-Laval, New Way, and Meleshin processes, phase inversion takes place by cooling and mechanical treatment of the concentrated cream. In the Cherry-Burrell Gold'n Flow and Creamery Package processes, phase inversion takes place during or immediately after concentration, producing a liquid identical to melted butter, before cooling and working. The Alfa, Alfa-Laval, and New Way processes were unsuccessful commercially. The Meleshin process, however, was adopted successfully in the former U.S.S.R. The Cherry-Burrell Gold'n Flow process appears to have been the more successful of the two American processes (78).

The Fritz continuous buttermaking process, which is based on the same principles as traditional batch churning, is now the predominant process for butter manufacture in most butter-producing countries. In the churning process, crystallization of milkfat is carried out in the cream, with phase inversion and milkfat concentration taking place during the churning and draining steps. However, because of the discovery that cream could be concentrated to a fat content equal to or greater than that of butter, methods have been sought for converting the concentrated or plastic cream directly into butter. Such methods would carry out the principal buttermaking steps essentially in reverse order, with concentration of cream in a centrifugal separator, followed by a phase inversion, cooling, and crystallizing of the milkfat (82).

Increased demands on the keeping qualities of butter require careful construction, operation, and cleaning of the milk- and cream-processing equipment, as well as research to develop machines that will ensure butter production and packing under conditions without contamination and air admixture. It has been demonstrated that butter produced under closed conditions has a better keeping quality than butter produced in open systems (83).

There are two classes of continuous processes in use: one using 40% cream, such as the Fritz process (Figure 9) (81), and the other using 80% cream, such as the



**Figure 9.** Continuous butter maker (Westfalia). 1, Churning cylinder; 2, separation section (first working section); 3, squeeze-drying section; 4, second working section; 5, injection section; 6, vacuum working section; 7, final working stage; and 8, moisture-control unit (81).

Cherry-Burrell Gold'n Flow (84). As much as 85% of the butter in France is made by the Fritz process. In this process, 40% fat cream is churned as it passes through a cylindrical beater, all in a matter of seconds. The butter granules are fed through an auger where the buttermilk is drained and the product is squeeze-dried to a low-moisture content. It then passes through a second working stage where brine and water are injected to standardize the moisture and salt content. As a result of the efficient draining of the buttermilk, this process is suitable for the addition of lactic acid bacteria cultures at this point. The process then becomes known as the NIZO method when the lactic starter is injected (78). Advantages of the NIZO method over traditional culturing are improved flavor development, improved acid values as a result of lower pH, more flexible temperature treatment of the cream because culturing and tempering often are accomplished concurrently, and most important, production of sweet cream buttermilk.

The Cherry-Burrell Gold'n Flow process is similar to margarine manufacture (84). The process starts with 18.3°C cream that is pumped through a high-speed destabilizing unit and then to a cream separator, from which a 90% fat plastic cream is discharged. It is then vacuum pasteurized and held in agitated tanks to which color, flavor, salt, and milk are added. Then this 80% fat-water emulsion, which is maintained at 48.9°C, is cooled to 4.4°C by use of scraped surface-heat exchangers. It then passes through a crystallizing tube and then a perforated plate that works the butter. Before chilling, 5% nitrogen gas is injected into the emulsion. Improvements of the processing continue to occur. It is now possible to manufacture butter from high-fat cream (>82% milkfat) on a continuous basis (85).

Although the Meleshin process continues to be in widespread use in the former U.S.S.R., the use of alternative continuous buttermaking processes based on high-fat cream has declined in Western countries during the past 20 years (78). The principal reasons for this decline appear to be economics and butter quality, particularly when compared with the Fritz process. A Fritz manufacturing process can be installed in existing batch churn factories with almost no modification to cream-handling or butter-packing equipment. The churns could be retained in case the Fritz breaks down. However, little batch plant equipment could be reused in the alternative systems (i.e., Gold'n Flow). When a completely new plant is being bought, the alternative systems still tend to be more expensive, and operational advantages over the Fritz system are not significant. Butter from the Fritz process is nearly identical in its physical and flavor characteristics to batch-churned butter, whereas butter produced by the alternative processes tends to be different (86). These differences may be perceived as defects by the consumer, and manufacturers have been reluctant to alter a traditional product.

There are a number of advantages that the alternative systems have over the modern Fritz line (87). The most attractive advantage is the flexibility to produce a wide range of products, with fat contents ranging from 30% to 95% butter-vegetable oil blends and the ability to incorporate fractionated fats (88). The alternative processes also present the possibility of a number of operational advantages. The use of an efficient centrifuge during the cream concentration stage can substantially reduce fat losses in the buttermilk. The composition of the butter can be more

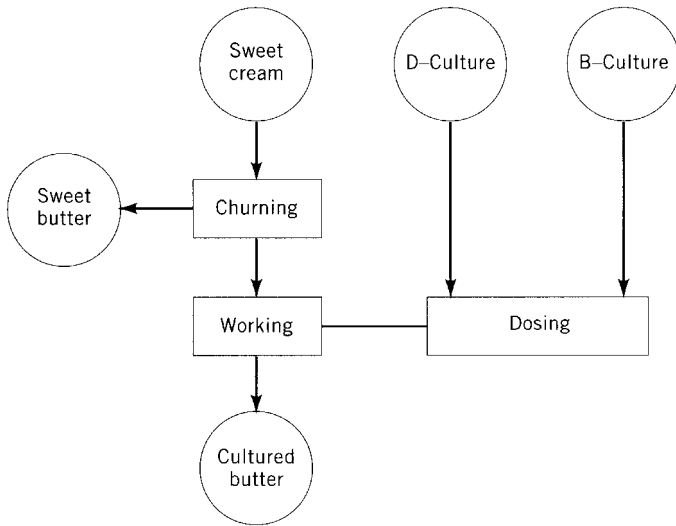


Figure 10. Schematic of the Pasilac-Danish IBC method (81).

accurately controlled, either by including a batch standardization step or by the use of accurate continuous metering systems.

## 5.7. Cultured Butter Manufacture

There are several ways of making cultured butter from sweet cream. Pasilac-Danish Turnkey Dairies, Ltd. developed the IBC method (Figure 10) (81). The main principles of the IBC method are as follows. After sweet cream churning and buttermilk drainage, a starter culture mixture is worked into the butter, which produces both the required lowering of butter pH and, because of the diacetyl content of the starter culture mixture, the required aroma. The starter mixture consists of two types of starter culture: (1) *Lactococcus lactis* and (2) *L. cremoris* and *L. lactis* ssp. *diacetylactis*. With respect to production costs, the experience with this method shows that, for the manufacture of mildly cultured butter, the direct costs are only about one-third of the costs of other methods (81).

## 5.8. Reduced Fat Butter

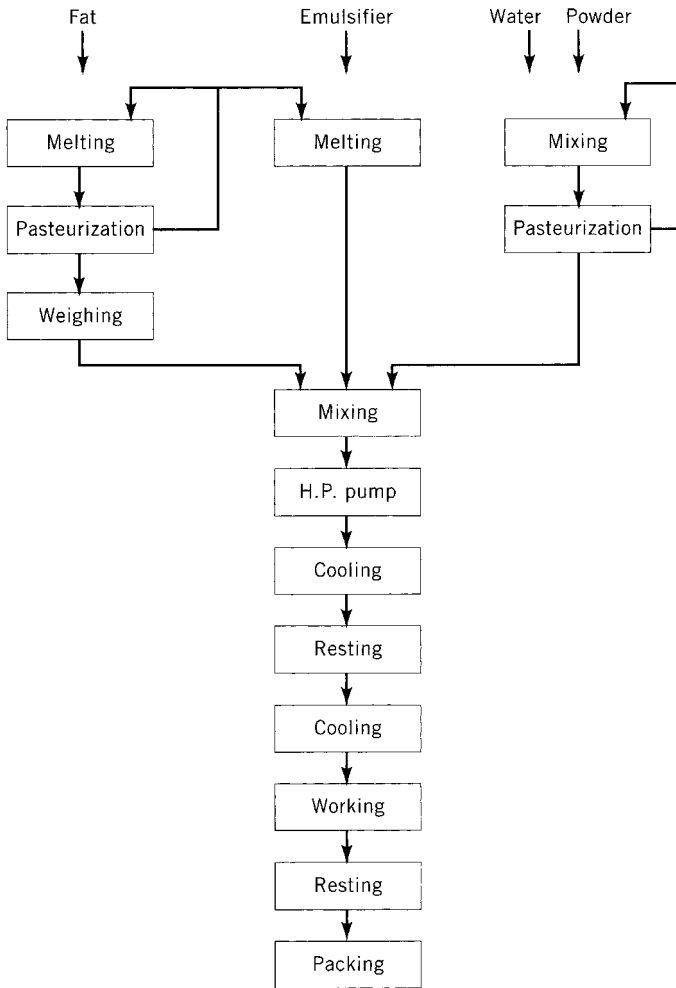
Fat shortages during World War II first stimulated interest in low-fat spreads in the United States. Oil-in-water spreads were first developed in the 1950s and 1960s. However, they had a number of limitations, including a shelf life of only 10–14 days (conventional butter shelf life is 4 months), a spongy texture, a lack of melting properties, and an inability to withstand freezing (89). The public's interest in low-calorie foods has motivated manufacturers to produce low-fat butter products. Although these products can no longer be called *butter* (according to international

standards), they are nonetheless often called low-calorie butter, half-butter, light butter, or a similar name. Many patents have been obtained for these products, because emulsifying properties are needed to deal with the water content (nearly 50%) of butter-like spreads. In addition, the emulsion must often be stabilized with additives. In some countries, a low-fat butter (40% total fat) containing vegetable oil has been designated as Minarine, but Minarine can also be prepared using only butter fat (62). Consumer interest in a reduction of additives in food products and a growing awareness of the importance of proper nutrition have created a demand for a low-fat product. It is now possible to produce a butter product based exclusively on butter fat with a fat content of 40%, without using emulsifiers. However, it is precisely this wish for a lower fat content in spreads and in other products that is forcing manufacturers to invest time and money in product development to find a use for their excess butter fat.

The first reduced-fat butter (50% fat), called Light Butter, was introduced in the United States by the Lipton Co. in the mid-1980s. The product was withdrawn due to FDA objections of not meeting Standards of Identity for nomenclature. Also, the product contained stabilizers not allowed for in the Standard of Identify for butter. In the late 1980s, Ault, Inc. introduced a reduced-fat butter (39% fat) called Pure and Simple, which contained no unusual additives (90). Unfortunately, this all-natural product had severe negatives: it had a short shelf life, experienced moisture seepage, and lacked the highly desirable butter notes. In 1990, Land O'Lakes, Inc. launched its Light Butter (52% fat), which contained emulsifiers, added Vitamin A, and preservatives. The FDA was in the process of establishing standards for reduced-fat products at this time and no objection was registered. The new standards were established in 1993 (63), which automatically required Land O'Lakes to reformulate to a 40% butter fat content; it did so and relaunched. The product was a success and has established dominance in the U.S. market.

Butter-like products with reduced-fat content are manufactured in several countries. Stabilizers, milk and soy proteins, sodium albumin or caseinate, fatty acids, and other additives are used. A product is now available on a commercial scale in the former U.S.S.R. that has the following composition: 45% milkfat, 10% nonfat solids, and 45% moisture. It has a shelf life of 10 days at 5°C (91). Each country has established its own standards for butter and butter fat products. Many are still developing standards for a reduced-fat butter product to meet the growing consumer demand.

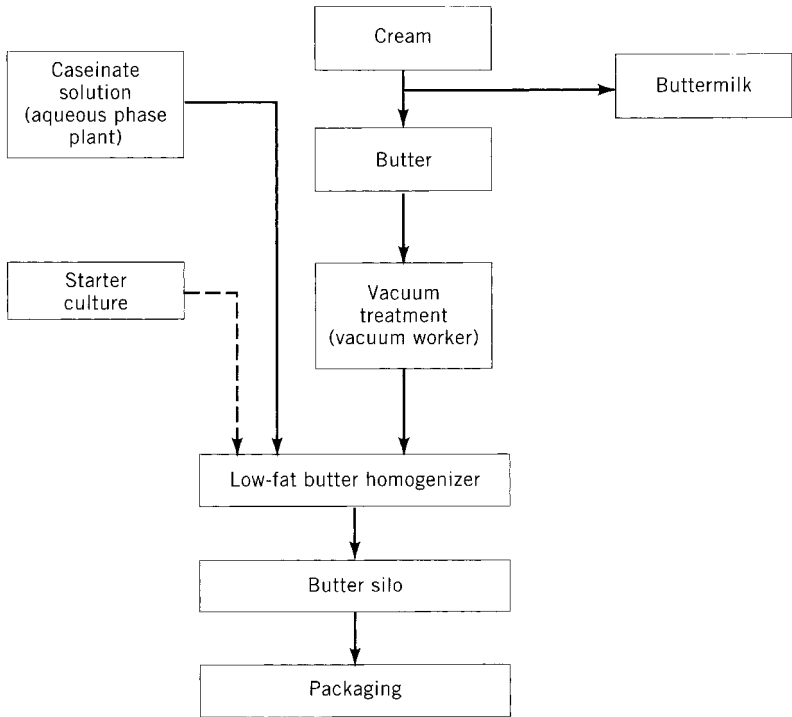
Manufacturers have experienced many problems with the production of low-fat butter (92). Low-fat butter cannot be manufactured in conventional continuous butter makers. The technology of producing low-fat butter and margarine products is similar to that of ordinary margarine production, and it has nothing in common with modern butter (Fritz process) production (Figure 11). The conditions are, of course, more critical for products that contain only 40% fat. These low-calorie water-in-fat emulsions have such a dense package of water droplets that unwanted phase inversion during processing or structural weak points in the product can occur, which may, for example, severely limit the microbiological shelf life. The scraped-surface heat exchanger type of machine is preferred for production of low-fat products.



**Figure 11.** Schematic for the production of low-fat butter and spreads (81).

As a result of the close packing of the aqueous-phase droplets, the composition of the water phase is critical. Protein concentrates, caseinate, gelling agents, and special emulsifiers have been recommended to simplify the emulsification and to stabilize the end product (93–98). For manufacture, the basic material for production is a mix that is chemically identical to the end product. This mix consists of milkfat in the form of butter, butter oil, and fractionated butter oil or cream, in many cases, it also has milk solids, milk concentrates (including dissolved milk powder and caseinates), and emulsifiers (see Figure 10) (81). The fat mix (i.e., butter, butter oil, etc.) is melted and pasteurized.

The required amount is metered into an emulsion tank. If emulsifiers are used, they are melted and mixed with a small amount of fat before being transferred to the



**Figure 12.** Flow sheet of the APV Pasilac method (99).

emulsion tank and added to the fat. The required amount of water is apportioned, nonfat milk solids are added, and the mix is pasteurized. The water–milk mix is transferred to the emulsion tank and mixed into the fat mix. The emulsion is then pumped to a specially designed scraped-surface cooler, where the emulsion, under heavy mechanical treatment and rapid cooling, is supercooled and crystallized, forming the water-in-fat emulsion.

Alternative methods have been developed. For example, the APV Pasilac method is widely used in Europe (Figure 12) (99). The continuous APV Pasilac method is quite simple. Ordinary butter with a fat content of 80–82% is mixed with an aqueous phase to the desired fat content. The mixture is then subjected to vigorous mechanical treatment in a special butter homogenizer, and the low-fat butter is ready for packaging. As Figure 12 shows, the low-fat butter equipment includes an aqueous phase plant. The production of the aqueous phase involves the following processes:

- Dissolution of one or more powders in water–milk.
- Pasteurization of the solution.
- Cooling of the solution to emulsification temperature.

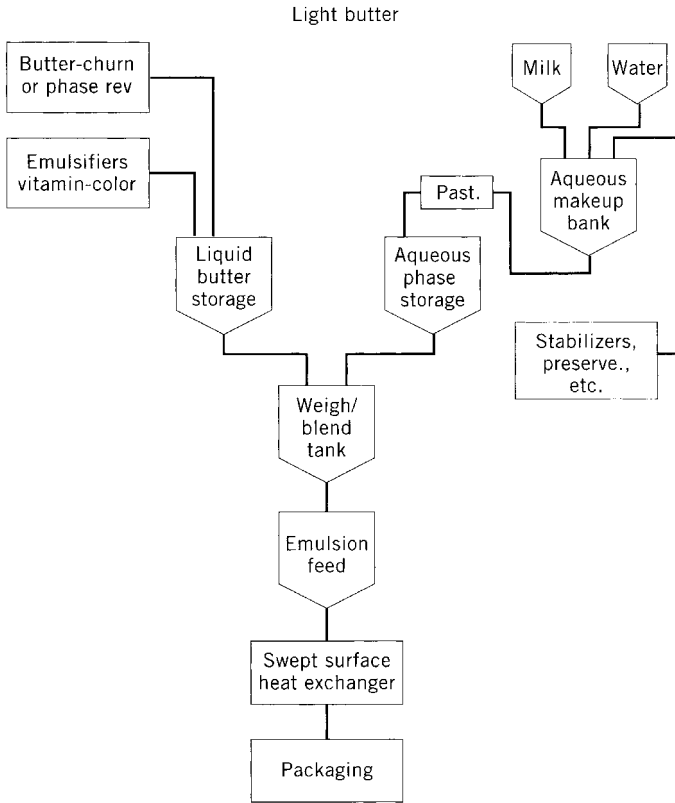


Figure 13. Schematic of Land O'Lakes's method for the manufacture of Light Butter.

The process makes it possible to manufacture a butter with a fat content as low as 28% (99).

Figure 13 shows the method used by Land O'Lakes to produce low-fat butter (40%). The method is similar to margarine manufacture.

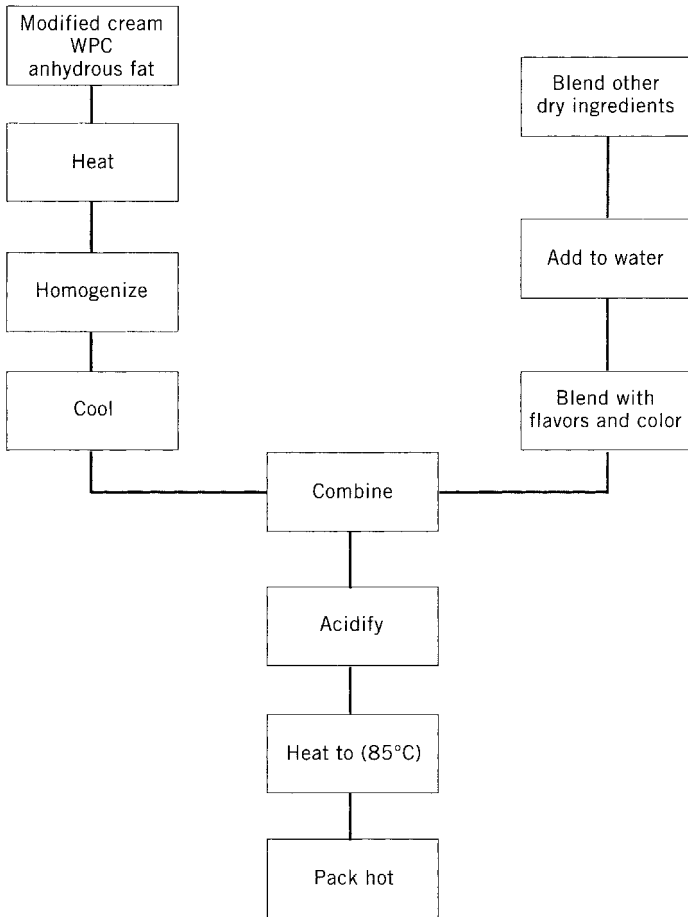
Fat substitutes and zero-calorie fats offer the potential to reduce the total fat content of foods. Nutrition and marketing experts predict that consumers will show the same enthusiasm for fat substitutes as they exhibited for alternative sweeteners (100).

Figure 14 shows a schematic of the manufacture of a no-fat spread using modified whey protein concentrate (WPC).

### 5.9. Physical and Organoleptic Characteristics

*Consistency.* Consistency has been defined as “that property of the material by which it resists permanent change of shape and is defined by the complete force flow relation” (34). This implies that the concept of consistency includes many





**Figure 14.** Low-fat spread using modified whey protein concentrate.

aspects and cannot be expressed by one parameter. Today, more importance is attached to spreadability than anything else in the evaluation of the consistency of butter. The general consensus is that butter should be spreadable at refrigerator temperatures. There is no suitable method available to measure such a subjective criterion as the spreadability of butter. For this reason, the firmness of butter, which should correlate well with spreadability, was selected as the parameter to be measured. It was recommended that the use of the cone penetrometer, along with other methods, could provide good results (101).

*Flavor.* One of the most important consumer attributes of butter is the pleasing flavor. Butter flavor is made up of many volatile and nonvolatile compounds. Researchers have identified more than 40 neutral volatiles, of which the most prominent are lactones, ethyl esters, ketones, aldehydes, and free fatty acids (102). The nonvolatiles, of which salt (sodium chloride) is the most prominent, contribute to a

balanced flavor profile. Diacetyl and dimethyl sulfide also contribute, especially in cultured butter flavor (103).

*Body and Texture.* By means of appropriate qualifications of the terms *body* and *texture*, butter graders describe the physical properties of butter that are noted by the senses. The exact meanings of these terms have not been clearly outlined. Frequently, they are used as if they had the same meaning. Certain properties such as hardness and softness refer to the body of butter, whereas properties such as openness refer to texture. However, some of the properties, such as leakiness or crumbliness, are confusing. Usually, most body and texture terms are used to describe a defect, e.g., gritty, gummy, and sticky (86). Good butter should be of fine and close texture; have a firm, waxy body; and be sufficiently plastic to be spreadable at cold temperatures.

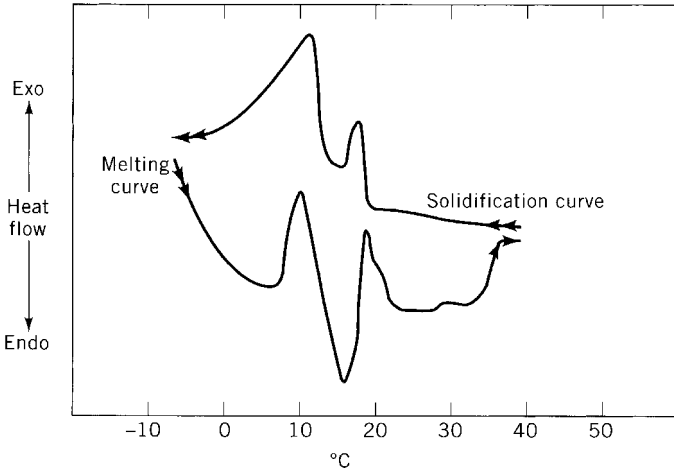
*Color.* The color of butter may vary from a light, creamy white to a dark, creamy yellow or orange yellow. Differences in butter color are the result of variations in the color of the butter fat, which is affected by the cows' feed and season of the year; variations in the size of the fat globule; presence or absence of salt; conditions of working the butter; and the type and amount of natural coloring added.

Butter colorings are oil soluble and most often are natural annatto (an extraction of the seeds of the tropical tree *Bixa orellana*) or natural carotenes (extractions from various carotene-rich plants). Because they are oil soluble, colorings are added to the cream to obtain the most uniform dispersion.

## 5.10. Texturization and Spreadability

The most common method to improve the spreadability of butter is to incorporate air or nitrogen, generally increasing the volume by 33%. The product called whipped butter is sold in a tub rather than in stick form.

The consistency of butter is determined by the percentage of solid fat present, which is directly influenced by the fat composition, the thermal treatment given to cream before churning, the mechanical treatment given to butter after manufacture, and the temperature at which the butter is held (104). The European butter market demands that butter be softer and more spreadable in winter and harder in summer. With information on the changes in fat composition from gas-liquid chromatography analysis and the use of nuclear magnetic resonance to estimate solid fat, suitable tempering procedures can be selected to modify the fat composition and to produce the most acceptable product for the consumer. A spreadable consistency of butter can be achieved by either varying the fatty acid composition or varying heat-step cream-ripening times and temperatures (105). There are a number of recognized processing options available that influence butter spreadability. Examples include mechanical treatment (texturization) (104, 106–109), temperature profiling of the cream (Alnarping) (85), blending winter and summer butters (107), fractionation of the anhydrous milkfat (37, 110–113), interesterification of the fatty acids (105, 114), the diet of the cow (38, 115–117), and cream ripening (118). When blending butters, it is important to understand milkfat melting and solidification curves (Figure 15).



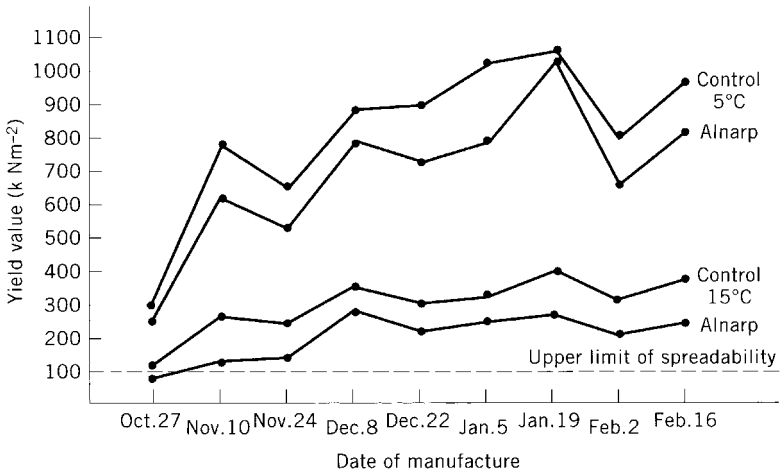
**Figure 15.** Typical milkfat melting and solidification curves obtained by differential scanning calorimetry (107).

Mechanical treatment, or working, involves physically disrupting the three-dimensional fat crystal network and breaking the bonds between the crystals. It is essential that this mechanical treatment is applied to butter in which the crystallization has been totally completed (usually 7 days after churning). The primary crystal structure is strong, and once it is destroyed by kneading it does not reform easily. A new, secondary structure is then formed. This structure is weak and reforms quickly after having been destroyed by kneading.

In the Netherlands, bulk butter is often processed in a Mikrofix homogenizer to give it more plastic structure before it is repackaged for retailing (109).

The first published cream tempering process (1937) was developed by the Alnarp Dairy Institute in Sweden, which lent its name to this and similar processes (107). In the original Alnarp process, pasteurized cream was cooled to 8°C for 2 h, warmed to 19°C for 2 h, and then cooled to 16°C and held until churning. Modifications allowed for initial cooling of the cream to initiate nucleation of the crystals at a temperature well below the main solidification temperature ranges (see Figure 14). The cream was then warmed to a temperature several degrees above that of the lower melting peak temperature to encourage a fractionation process in which high-melting glycerides crystallized and low-melting glycerides melted (Figure 16).

Another alternative to increasing butter spreadability is providing cows with specific feeds to change the fatty acid composition. The main opportunity for varying fat composition comes in the indoor feeding period, when cows receive some type of concentrate in addition to silage. If, for example, free soy oil is added to the diet, its constituent 18:2 and 18:3 fatty acids are converted in the rumen largely to 18:0 and some 11–18:1-*trans* (vaccenic acid). The gut wall and mammary gland contain a desaturase, which converts 18:0 to 11-*cis*-18:1. The milkfat resulting from such a diet may contain up to 60% of C18 acids, with 18:1 (*cis* and *trans*) assuming the



**Figure 16.** Effect of Alnarp-type treatment on firmness of winter butter (107).

dominant position. The milkfat contains a relatively low proportion of 16:0, 14:0, 12:0, 10:0, and 8:0, because of the depression of *de novo* synthesis within the gland. The weight proportion of 4:0 remains fairly constant (which implies that the molar proportion may increase slightly) and that of 6:0 decreases only slightly, both facts indicating that 4:0 and 6:0 are synthesized by a route that is relatively much less significant (38, 114, 116, 117). As a result of the high unsaturated C18 fatty acid content, this type of milkfat has a lower melting spectrum. The outcome is a butter that has reasonable spreadability at refrigeration temperatures (114).

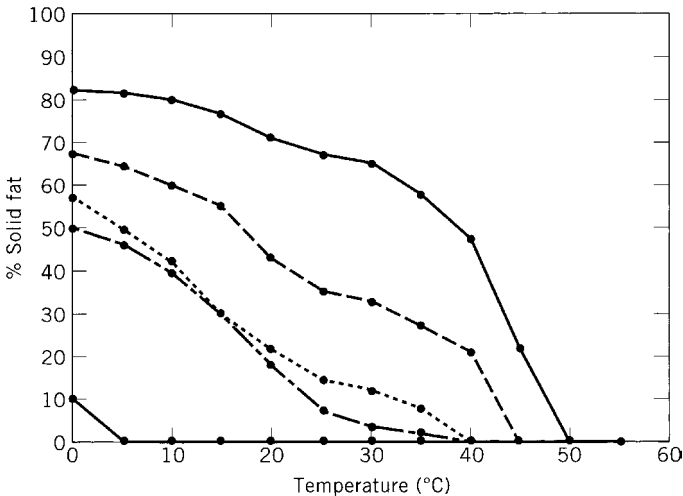
Feeding protected unsaturated fat usually leads to an increase in the proportion and yield of milkfat. However, a major cost is, in this instance, associated with the preparation of the feedstuff, involving as it does homogenization of an oil-water-protein mixture and the subsequent removal of the water by spray drying. This type of feeding practice has little commercial application at present (116). Thus, although the scientific knowledge to alter the fatty acid composition of milkfat is well established, economic considerations have prevented its exploitation.

In efforts to improve the spreading properties of butter in relation to hard butter fat, one alternative put forward is the use of softfat fractions obtained in the fractionation of anhydrous milkfat. Although several practical methods of fractionation have been presented, the use of softfat fractions in buttermaking has not become general practice. This is evidently because fractionation in all cases significantly raises the cost of the butter produced. In addition, a common problem has been to find suitable uses for the hardfat fractions. Furthermore, in fractionation methods that use solvents or additives, fractionation should be linked to fat refining, and, in this process, butter also loses its natural food classification. In studies that have used soft butter fat fractions, a substantial softening of the butter has been obtained; however, this butter, like normal butter, hardens as the temperature increases and again decreases (118). As milkfat exhibits a wide melting range from about  $-30^{\circ}\text{C}$ , it may be possible to use a dry fractionation process. Suitable sizes of crystals are

developed by controlled cooling of the melt, and the crystals are separated from the liquid phase by filtration or centrifugation. The fractionation of milkfat by melt crystallization has been extensively studied (37). The general conclusions from these investigations were as follows: the short-chain triglycerides and the short-chain and unsaturated long-chain fatty acids were enriched in the liquid fraction; the efficiency of molecular size separation in the melt crystallization process was poor; and the flavoring compounds, pigments, Vitamin A, and cholesterol were slightly concentrated in the liquid fraction.

Currently, dry fractionation of anhydrous milkfat is performed by two conventional systems—Tirtiaux and De Smet (both from Belgium)—which are bulk crystallization processes. The widely used Tirtiaux dry fractionation process enables one-step or up to five-step fractionation of anhydrous butter oil at any temperature, ranging from 50°C to 2°C (37, 110–113). The milkfat fractions thus obtained can be used as such or the fractions can be blended in various proportions for use as ingredients in various food-fat formulations. The major shortcoming inherent in this system is the long residence time (8–12 h) for nucleation and crystal growth.

Butter samples made from low-melting liquid fractions and from a combination of primarily low-melting liquid fractions and a small amount of high-melting solid fractions exhibited good spreadability at refrigerator temperature (4°C) but were almost melted at room temperature (21°C). Butters made with a high proportion of low-melting liquid fraction, a small proportion of high-melting solid, and a small proportion of very high-melting solid fractions were spreadable at refrigerator temperature and maintained their physical form at room temperature (Figure 17).



**Figure 17.** Solid fat content profiles of the control and anhydrous milkfat (---); the low-melting, high-melting, and 20% very high-melting milkfat (---); and the milkfat fractions used in the low-melting, high-melting, and 20% very high-melting butter: 30S fraction (—, bottom line), 13L fraction (—, top line), and 15S fraction (—, top line). The fraction number includes the fractionation temperature (°C) and its physical form (solid or liquid) (110).

A pourable butter, formulation, nonfractionated at room temperature has been reported in the patent literature (119).

### 5.11. Anhydrous Milkfat Manufacture

The quality assurance program for manufacture of butter oil, or anhydrous milkfat (AMF), also focuses on the quality of the raw materials. Naturally, many of the same considerations apply to handling raw cream for AMF manufacture that apply to butter, except that vacuumation is not used. As it is stored under ambient conditions, care against oxidation is essential. Oxidation is perhaps the most important mechanism by which milkfat deteriorates in quality. As the oxidation reaction is autocatalytic (i.e., the products of the reaction act as catalysts to promote further reaction), the normal quality-control tests, peroxide value and free fat acidity, could give misleading results when applied to stored butter. Methods of deaeration have been developed that could reduce potential oxidation (74).

Milkfat is present in milk or cream as part of a stable oil-in-water emulsion. The emulsion is stabilized as a result of the protein and phospholipid-rich milkfat globule membrane (MFGM) surrounding the milkfat. During AMF manufacture, the aim is to break the emulsion and to separate out all of the nonfat solids and water (Figure 18). To achieve this, the MFGM must first be disrupted mechanically or

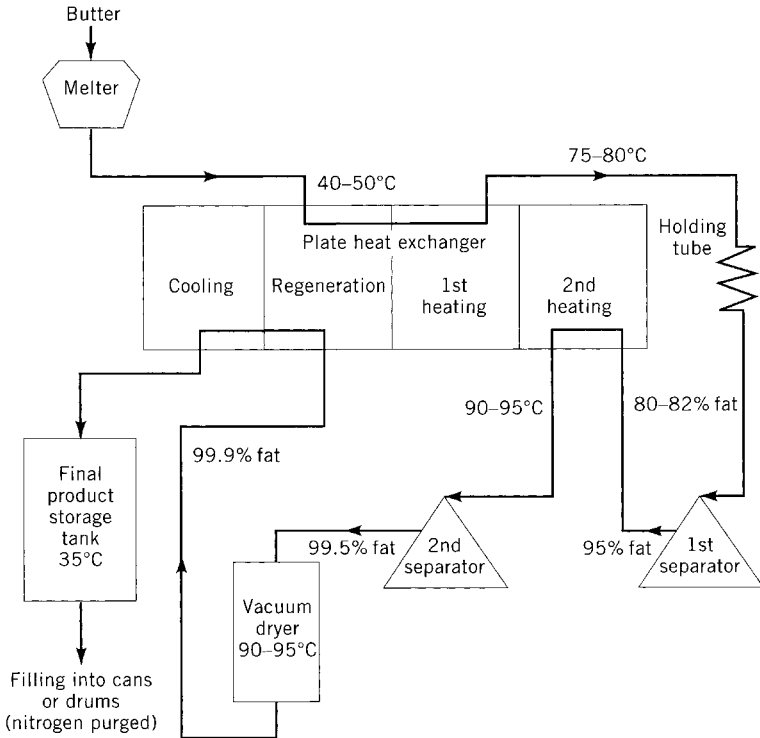


Figure 18. Anhydrous milkfat manufacture (120).

chemically. Homogenization, an example of mechanical treatment, disrupts the membrane, destroying the membrane layer. For chemical destruction of the membrane layer, an acid such as citric acid can be added to lower the pH of milk or cream to about 4.5 (120). The protein will precipitate, removing a component to maintain an intact fat globule. Direct-from-cream AMF plants usually have three separators. The first concentrates cream from 40% to about 75% fat before phase inversion in a homogenizing device. The oil separator then separates the liberated butter oil to about 99% purity. The oil is washed with water before the third (polishing) separator, and the final traces of moisture are removed in a dehydrator at 95°C and under a vacuum of 35–50 torr. The dehydrator is usually a simple vessel, and the butter oil is introduced either as a thin film on to the walls or as a spray, to maximize the surface area exposed to the vacuum. Such a device will not remove significant off-flavors, because the vapor flows, temperatures, and pressures are inappropriate for flavor stripping. When producing AMF from butter, fresh or block butter is softened just to a pumpable stage (approximately 50°C) and transferred to a plate heat exchanger to increase the temperature (70–80°C). The oil phase is concentrated through separators and dried under vacuum. Some washing is possible before the final separator removes the last traces of nonfat components (see Figure 18).

In terms of the preparation of products and their appearance and texture, AMF has several advantages over traditional butter. The latter is, in fact, subject to seasonal variations, which affect its physical properties. The advantages of AMF are linked to the possibility of standardizing its physical properties (by the selection and mixture of the raw materials used in production) and the possibility of adapting its properties using the fractionation technique. This is particularly important for its use on an industrial level where, given the automation of production and the standardization of the texturization stage (temperature), it is necessary to maintain constant physical (rheological) and organoleptic properties (121).

## 5.12. Packaging

The objective of any packaging system is to protect the product from deleterious environmental conditions. Many packaging systems have been developed to protect the milkfat from biological, chemical, and mechanical deterioration. Bulk containers, such as 5-L cans and 20- and 25-L drums, are popular for packaging industrial materials such as butter oil and AMF. These containers usually have a welded side seam and are plain internally. Unlike edible oils, milkfat is corrosive to tinplate, so an internal gold epoxy phenolic lacquer, such as International Paint's "IP 180," is required. If the containers have a welded side seam, the internal raw steel edge of the weld needs to be protected by applying a side-strip lacquer (i.e., a two-part epoxy polyamine). All lacquers used on food cans should have FDA status (122). Larger drums (i.e., 210-L nominal and 218-L maximum capacity) need more rigid walls to withstand the greater mechanical stresses in handling. When chilled storage is available, less rigid forms of packaging may be used. Concentrated butter is now being retailed for domestic cooking, aided by a European Community subsidy. This

concentrated butter is normally packaged in either parchment or a foil-parchment laminate similar to that used for butter (123).

For packaging in foil, parchment, or other flexible film, the milkfat needs to be in a plastic state, similar to that of freshly churned or reworked butter. The optimum softness of the fat depends on the packaging system being used. Traditionally, the United States has packaged consumer portion sizes of individually wrapped four 0.25-lb sticks in 1-lb units. Multiple sizes from 5 g to 25-g units of foil-wrapped “continentals” or polyethylene molded cups, have been used in the food service sector. For bulk handling, the plastic fat or butter can be packed into polyethylene-lined fiberboard boxes. In all cases, the aim is to fill the desired quantity of fat with the minimum giveaway. Aeration of the milkfat should also be avoided as oxidative rancidity is the principal factor limiting the shelf life.

### 5.13. Storage and Transport

Storage conditions depend on the end use for the product, the packaging system, and the desired storage time. Milkfat in hermetically sealed cans and drums is the least affected by its storage environment. Ambient storage is commonly used and must be to the same standards as used for other food stuffs (122). In the European community and the United States, temperature is not a major factor, but it can be in tropical countries, where the temperature may rise to 35–40°C. At temperatures in excess of 30°C, the milkfat deteriorates significantly more rapidly, and there is an increased risk that the stored fat will have a stale, oxidized off-flavor.

Transport of drums and cans may be at ambient temperature, though the storage life may benefit from refrigeration. High humidity and wet conditions should be avoided to minimize the risk of corrosion or mold growth on the packaging that would entail an additional cleanup operation. The drums should be stowed away from excessive heat and noxious chemicals. For most journeys, standard freight containers may be used.

Milkfat in polyethylene-lined fiberboard boxes is at a far higher risk from its storage environment. As the packaging is permeable to oxygen, the product is more prone to oxidation. Chilled storage (10°C, preferably 5°C) must be used both to reduce the rate of oxidation and to maintain the rigidity of the pack. The humidity of the storage area must also be controlled to prevent mold growth on the fiberboard. As the pack is also permeable to odors, the storage area should not be shared with other food stuffs with strong odors that could cause off-flavor absorption (e.g., fish, onion, garlic).

Flavor transmission and oxidation are less of a problem at temperatures below –20°C, which is preferable for long-term storage. When conserving and preserving stocks of butter for extended periods (5 years or more), a process has been developed by which butter is placed in the refrigerated chamber or warehouse, which has been sealed airtight. The air is evacuated and replaced with nitrogen or other inert gas mixture so that the pressure in the chamber is equal to the exterior atmospheric



pressure. This process allows for extended storage without mold growth and development of rancidity (124).

When butter has been frozen, textural characteristics may have been deleteriously affected. An invention to improve texture has been described in which large, deep-frozen blocks of butter with the desired moisture content are reworked by chipping them in a butter chipper while adding measured quantities of water. The butter chips are then fed through a vacuum chamber into a butter churn designed as a continuous kneading mill. The butter chips are continuously conveyed under pressure through the kneading mill by means of a high-pressure butter pump (125).

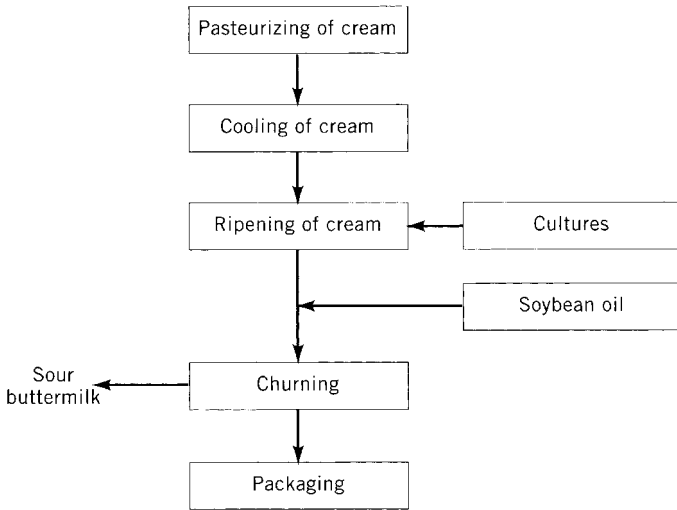
It is not possible to set rigid standards for the shelf life of milkfat. Shelf life depends primarily on the acceptance quality criteria of the user and will be affected by (1) the quality of the feedstock, (2) the packaging system, and (3) temperature. With increasing storage time, the flavor defects are more likely to become noticeable. Flavor does not correlate easily with peroxide value (126). At a peroxide value of  $<0.6$ , oxidized flavors are unlikely, but if the peroxide value is  $>1$ , then some oxidized flavor may be expected. It must be pointed out that these figures are based on the International Dairy Federation (IDF) method (127) for measuring peroxide value and that other methods are likely to give different results. Other grades of milkfat defined in the IDF standard are anhydrous butter oil and butter oil. Anhydrous butter oil is the product obtained from butter for cream; it may be of different ages and should have no pronounced, unclean, or other objectionable taste or odor. Butter oil is the product obtained from butter or cream; it may also be of different ages and should have no pronounced, unclean, or other objectionable taste or odor.

## 6. BUTTER FAT PRODUCTS

### 6.1. Butter Fat–Vegetable Oil Blends

The first commercial development of a spread made from a combination of butter fat and vegetable oil was in Sweden in 1963. The product, Bregott, contains 80% fat, of which 80% is milkfat and 20% is soybean oil (128). Bregott is a margarine according to Swedish and American food standards. Swedish scientists also developed and successfully commercialized the first reduced-fat spread in Europe in 1974. The product, Latt and Lagom, contains 39–41% fat, of which 60% is milkfat and 40% is soybean oil. It is considered to be a low-calorie margarine. Bregott is exported to Australia, and Latt and Lagom to Japan and France (89). In Finland, where Bregott is popular, oil from rapeseed is used (83).

Other products (under license from the Bregott patent) are Voimariini in Finland, Bremykt in Norway, Smjorvi in Iceland, and Dairy Soft in Australia. Similar products are Clover in the U.K.; and Dairy Gold, Kerry Gold, and Gold'n Soft in Ireland. This list is, however, not complete. The latter blends are high-fat products (75–82% fat), and the amount of butter fat of the total fat is about 50% (93). Most of these products are manufactured in a churning process in a churn or a continuous butter machine.



**Figure 19.** Flow diagram of the manufacture of butter-vegetable oil mixtures (93).

The first steps in the manufacture of Bregott are pasteurization of the cream, followed by cooling and temperature treatment. The cultures are the same as those used in buttermaking. Measured quantities of cream and soybean oil are mixed in the churn or the oil is continuously injected before churning in a continuous butter machine. The byproduct is sour buttermilk.

The most commonly used vegetable oil is soybean oil. Products with a low percentage of butter fat will contain not only vegetable oil but also hydrogenated vegetable fats to achieve a good plasticity. If the minor part of the total fat is butter fat, as in Golden Churn from the U.K., the manufacturing process is completely different from modern butter production. In this case, the technology is analogous with normal margarine manufacture, where some part of the fat is replaced with butter fat. The emulsion is cooled in scraped-surface coolers (Figure 19).

Very low-fat spreads have recently been developed. The first European commercialized product was made by St. Ivel and is called St. Ivel's Lowest. It contains 25% butter fat and has a lower saturated fat content than sunflower margarine (129).

In the early 1980s, blends of butter and vegetable oil products appeared in the U.S. market. The U.S. market leader was Country Morning Blend made by Land O'Lakes. These blends generally were 40% butter and 60% vegetable oil for a total fat content of 80%, within the margarine Standard of Identity and designation. With the increasing popularity of reduced-fat (less than 80%), spreads, starting in the mid-1980s, other blends with butter fat contents of 2–25% were introduced. The 80%-fat margarine blends are losing market shares to lower fat spreads and blends (130).

A number of processes have been developed using continuous churns (97) and alternative systems similar to the Cherry-Burrell Gold'n Flow process (98). The major disadvantage to churning, either batch or continuous, is that the resultant

buttermilk is adulterated with some vegetable fat and is less valuable than standard buttermilk. An advantage of alternative processing systems is their ability to accommodate easily the manufacture of reduced-fat spread blends. All butter fat-vegetable oil blends provided alternatives to butter for the consumer when concerns of health (e.g., fat saturation) and spreadability are desired.

## 6.2. Ghee

By definition, ghee is a product obtained exclusively from milk or fat-enriched milk products of various animal species by means of processes that result in the near total removal of water and nonfat solids (similar to anhydrous milkfat) and in the development of a characteristic flavor and texture. Even so, most ghee contains some nonfat solids to enhance the flavor.

Typically, ghee is manufactured by heating butter to temperatures well above those used during AMF manufacture. The high-temperature treatment of the nonfat milk solids and milkfat leads to the development of a strong buttery flavor. However, traditional ghee, as produced in the Middle East and Asia, has a more rancid taste due to less sophisticated methods of preparation and storage. Manufacturers in the European Community are also producing ghee by adding ethyl butyrate to anhydrous milkfat (119, 131). Alternative synthetic flavors have been developed to add ghee flavor notes to butter oil.

A synthetic mixture consists of  $\delta$ -G<sub>10</sub> lactone (3 ppm),  $\delta$ -G<sub>12</sub> lactone (15 ppm), decanoic acid (5 ppm), and kenanone-2 (10 ppm). This technique is simpler, less time-consuming, and more economical than the technique that uses powders. The shelf life of this flavored butter oil is 2.5 months. However, the addition of synthetic antioxidants, butylated hydroxyanisole (BHA) at the 0.02% level, enhances its shelf life so it can compete well with conventional ghee (131).

## 6.3. Butter Fat as an Ingredient

*Recombined Products.* For recombination of milk and dairy products, the two primary ingredients required are AMF and nonfat milk solids. A range of fat sources is available for the recombining industry, but only a few of these are in widespread use. In most countries, anhydrous milkfat is usually the sole fat source. Numerous products can be made by putting together the correct proportions of water, flavor, and other ingredients as desired (e.g., sugar, emulsifier, and stabilizers) to make sweetened condensed milk, ice cream, recombined butter, or milk (1%, 2%, or full-fat). For these applications, AMF of the highest quality should be used to avoid off-flavor development. The recombination of butter has been reported in detail (132).

In response to the problems associated with the handling of unsalted butter, a new milkfat product was developed in New Zealand to combine the superior flavor of butter with the ease of handling of AMF. Initial shipments of this product, fresh frozen milkfat for recombining (FFMR) were favorably received, and FFMR quickly became established as the preferred alternative to unsalted butter (72).

*Pastry, Cake, and Biscuit Products.* In general, fats play several essential nutritional, technological, functional, and organoleptic roles in most all-bakery applications. As a result of its physical properties, fat plays a major part in the production of the majority of items in the pastry, cake, biscuit, and chocolate confectionery sector; for example, in the preparation of pastry cream and in the desired appearance and texture of the end product. These physical properties include, above all, the rheological properties (consistency, plasticity, texture, etc.), and the properties of fusion and crystallization depend on the type of fat, the temperature, and the working conditions of the product.

The fats used in pastry and biscuit confectionery have different functions, which are determined by their rheological properties (plasticity and texture). In pastry, these principal functions are (1) an increase in the plasticity of the pastry (e.g., hard pastry with a low level of hydration) and (2) a break in the body of the pastry (i.e., the fat makes the gluten structure discontinuous, which gives the desired crumbliness in, for instance, biscuits) (121).

*Confectionery—Liquors and Liqueur.* In chocolate confectionery and for pastry creams, it is the physical properties linked to the fusion and the crystallization of the fat that are essential. For milk chocolate, for coating or in bars, AMF can be used in proportions that depend on its compatibility with cocoa butter, whose properties of hardness and rapid fusion at 35°C cannot be altered. Thus it is currently accepted that AMF with high fusion levels obtained by the fractionation technique can be used. In general, milkfat has an interesting characteristic: it inhibits the appearance of fat bloom (133).

For pastry creams, the ideal is an AMF, which causes rapid melting in the mouth. Depending on the type of pastry cream, a wider choice of AMF can also be offered, thanks to the fractionation technique.

Due to the low level of milkfat in dark chocolate, fat bloom is a problem with this product. The hard fraction of milkfat (milkfat stearin) has been reported to act as an antibloom in dark chocolate, giving the chocolate an increased shelf life. However, the use of hardened milkfat is limited in several major chocolate producing countries (133).

Regulations for the amount of milkfat, nonfat milk solids, whole milk solids, and total milk solid allowed in milk chocolate vary among countries. Fats other than milkfat are allowed in milk chocolate in some countries, although different flavors and textures may result in the chocolate.

Although the preferred source of milkfat in cream liqueurs and associated beverages is undoubtedly double cream, its use may lead to problems. In particular, cream contains calcium and other ionic materials. One solution is to wash the cream to remove all ionic materials, but this approach is cumbersome in practice. The preferred approach is to use anhydrous butter fat as the starting material.

*Other Uses.* The use of butter or anhydrous milkfat requires more added emulsifiers in ice creams and ice milks, because the naturally occurring milkfat emulsion will have been destroyed in the manufacturing process. Milkfat is also used in fresh cream, frozen cream, dry cream, and plastic cream. Ice creams contain a high level of milkfat, and its manufacture uses substantial quantities of milkfat worldwide.

## 6.4. Butter Fat Powders

The main purposes of transforming fats into powdered forms are to increase their microbial stability and to enhance their handling and functional properties. Powdered fats are available in two principal forms. The first form is feasible only if the fat contains sufficiently large amounts of high-melting compounds. If a fine jet of molten fat is sprayed into an ambient atmosphere, it will set as fine discrete particles. The second form embodies the use of a carrier. This form is necessary for oils and fats, such as milkfat, that contain appreciable amounts of low-melting triglycerides. The carrier can be added to the fat before or after spraying.

Dry creams are commonly produced as an ingredient for many applications. They consist of at least 40% butter fat, but can range up to 70% fat, 22–57% nonfat milk solids, and 0.5–5% moisture.

The Commission for Dried and Condensed Milk of the International Dairy Federation (1962) proposed to designate cream powders of 60% or more milkfat as “butter powder.” Later, it was suggested that a minimum of 80% milkfat be used for butter powder (134). Butter powder composition can vary in the amounts and types of the nonfat constituents, depending on the application. Powders can include nonfat milk solids, sodium caseinate, sodium citrate, sodium aluminum silicate, sucrose, and lactose (134). Other possible minor constituents are antioxidants, emulsifiers, flow agents, and stabilizers. The objective is to create a stable, fluffy, free-flowing, nongreasy, and loose creamy powder. Butter powder containing 80–85% fat could not possibly meet the legal and organoleptic texture requirements of butter when reconstituted with water; therefore, it is not in market competition for conventional butter use. The potential applications for high-fat powdered products include butter-like spreads, coffee or beverage whiteners, soup, sauce and dessert creamers, convenience ice cream, scrambled eggs and omelettes, pudding and pancake mixes, and aerating fats.

## 6.5. Specialty Butter Fat Products

*Flavored Butter.* Flavored butters (garlic, onion, pepper, lemon, etc.) have been successfully mass marketed in Europe (135), but this success has not translated to the U.S. market. There exists a limited specialty market in upscale stores and delicatessens and certain food service applications. Manufacture is quite simple. Creating the desired flavor blends remains an art and skill.

*Hypoallergenic Butter.* A U.S. patent was granted in 1992 for the manufacture of hypoallergenic butter (136). The patent has limited claims. The product is a sterile butter-like product made from anhydrous milkfat; it contains no nonfat solids (99.9% free of NFS).

*Butter Flavors.* Technologies for the hydrolysis of butter fat to produce and concentrate the free fatty acids to enhance the butter flavor of products have been available for decades. More recently, biotechnologists have developed methods for producing a variety of fairly pure enzymes, economically and in large quantities. The increased availability of lipases (glycerol ester hydrolases) from microbial

sources has made it possible for researchers to employ the catalytic properties of these enzymes in innovative ways. One application in which the use of lipases has become well established is the production of lipolyzed flavors from feedstocks of natural origin.

Immobilization of lipases on hydrophobic supports has the potential to (1) preserve, and in some cases enhance, the activity of lipases over their free counterparts; (2) increase their thermal stability; (3) avoid contamination of the lipase-modified product with residual activity; (4) increase system productivity per unit of lipase employed; and (5) permit the development of continuous processes. As the affinity of lipases for hydrophobic interfaces constitutes an essential element of the mechanism by which these enzymes act, a promising reactor configuration for the use of immobilized lipases consists of a bundle of hollow fibers made from a microporous hydrophobic polymer (137).

*Extended-life Creams.* Extended-life creams are produced using normal separation techniques but involve a high-temperature, single- or double-heat treatment (95–135°C). The temperatures employed render the product almost sterile. Any surviving bacteria tend to be spore forming types. Packaging is usually carried out on aseptic machines or nonaseptic machines modified with, for example, H<sub>2</sub>O<sub>2</sub> spray and ultraviolet lights (76).

*Short-life Creams.* For short-life creams, the shelf life depends on a low-bacteriological-count milk with good plant hygiene. Heat treatment tends to be in the region of 75–90°C with 3–30 s hold, followed by cooling to below 10°C. Final cooling to below 5°C is normally carried out in aging tanks or in the retail container in the cold store. Shelf life can be up to 12 days (76).

*Ultrahigh Temperature Creams.* Heat treatment for ultrahigh temperature (UHT) creams is produced by indirect or direct heating to 135–140°C with 1–4 s hold before cooling to ambient temperature. Aseptic packaging is essential. As this product is designed for long shelf life (3–4 months), formation of a cream plug or fat rise in the container must be avoided. Hence, all UHT creams, including whipping cream, must be homogenized. Homogenization can be carried out either upstream or downstream. If carried out downstream, an aseptic homogenizer must be employed (76).

*Decholesterolized Milkfat.* In the 1980s, there was significant research and market activity in developing decholesterolized milkfat. All this activity was for naught, for the hypothesis of creating a “healthier” fat (for butter or milk or other dairy product) was not sound. The nutrition community had long recognized that the link between dietary cholesterol and serum cholesterol was weak and that the ratio of total fat-saturated fat had a greater impact on health. In addition, the FDA issued new standards in 1993 (63) that effectively negated the value of decholestering milkfat. The new law required that, to be called low cholesterol, the fat must contain no more than 2 g of saturation fat per serving. Butter fat is approximately 65% saturated. As the technology to desaturate milkfat is not cost-effective, decholesterization has no economic value.

*Desaturated Milkfat.* In addition to chemical and enzymatic means of desaturation, there have been extensive studies on feeding cows specific diets to change

butter fat saturation as well as increasing the ratio of potentially desirable fatty acids (38, 115, 116). In general, good progress in the understanding of rumen physiology, digestion, and function has occurred, but economic potential remains unacceptable. The most promising technologies are the use of protected fats in a feeding regimen. These fats are protected in a way that they pass through the rumen (point of fat hydrogenation) into the remaining digestive system for absorption and subsequently into the mammary glands. Unsaturated fats that are fed to cows have a great opportunity to remain unsaturated as they are synthesized into milkfat. Biotechnology may offer alternatives in the modification of edible fats and milkfat. Research has led to new methods of lipolysis and esterification, but the developments are still at the laboratory level. Nevertheless, commercial application may emerge from these interesting areas of research.

*Nutraceuticals and Healthy Fats.* Almost all dairy products tend to be excellent carriers of specialized nutrients (vitamins, minerals, specialized cultures, and micronutrients), thus there is potential for fortification to enhance the natural nutritive properties of dairy products, creating nutraceuticals or functional foods. The use of specialized cultures such as *Lactobacillus acidophilus* and *Lactobacillus bifidus*, which have generally recognized nutritive characteristics, is ideal for cultured butter. A butter spread product using these cultures (Fittisport) has been launched in the French market; another with lower fat content has been introduced in the German market (138). In addition, it has been shown that the free fatty acids of milkfat have inhibitory effects against certain pathogens (e.g., *Listeria monocytogenes*) (139).

A structured, lipid-containing dairy fat is covered by a U.S. patent (95). The invention relates to a *trans*-esterification product of a mixture of fatty acids and triglycerides, including milkfat, in the form of cream or butter as the main component. The product has nutritional applications and may also be used as an enteral or parenteral supplement.

*Nonfood Applications.* The nonfood use of milkfat has been insignificant. Milkfat and milkfat fractions could, however, have some potential possibilities for profitable use, for example, in manufacture of pharmaceutical or technochemical products. Land O'Lakes, in association with Amerchol, has pioneered the use of milkfat fractions in cosmetics. The first product, called Cremoral, was launched into the marketplace in 1993 (140). The major factor that has stimulated renewed interest in using milkfat for technochemicals and other nonfood applications in the United States has been the significant decline in price, especially relative to alternative fats.

It is doubtful if any single market for milkfat can be found that will compensate for the decrease in butter sales. Rather, it will be necessary to look for a large number of relatively small outlets. If this is to be done, the dairy industry must understand the detailed structure of milkfat and establish the functional properties of its constituent fractions. This approach has been applied with considerable success to the protein fraction of milk. It may be just as rewarding when applied to milkfat.

7. ECONOMICS

Production and consumption of butter continues a long-declining trend. U.S. production of butter for 2001–2004 is given in Figure 20 (141). A dramatic shift occurred, starting in 1985, to the table spreads category of products (less than 80% fat) from full-fat butter, margarine, and blends (88). The spreads category encompasses all non-Standard of Identity table spreads (i.e., 0–79.9% fat).

In a 1984 survey, the most important barriers to increased butter sales were listed in the following order (88):

- Price (opinion of an overwhelming majority when butter is compared with margarine).
- Health (negative consumer attitudes toward cholesterol and saturated fats are increasing).
- Poor spreadability.
- Inadequate promotional spending.
- Product innovation in margarine and spreads.
- Legislation and regulatory restrictions.

Butter manufacture continues to serve as the safety valve for the dairy industry. It absorbs surplus milk supply above market requirements for other dairy products. Milk not required by the demand for these products overflows into the creamery, is skimmed, and the cream is converted to butter. When the milk supply for other products runs short of their demand, milk normally intended for buttermaking is diverted into the channels where needed. Even though consumption patterns have

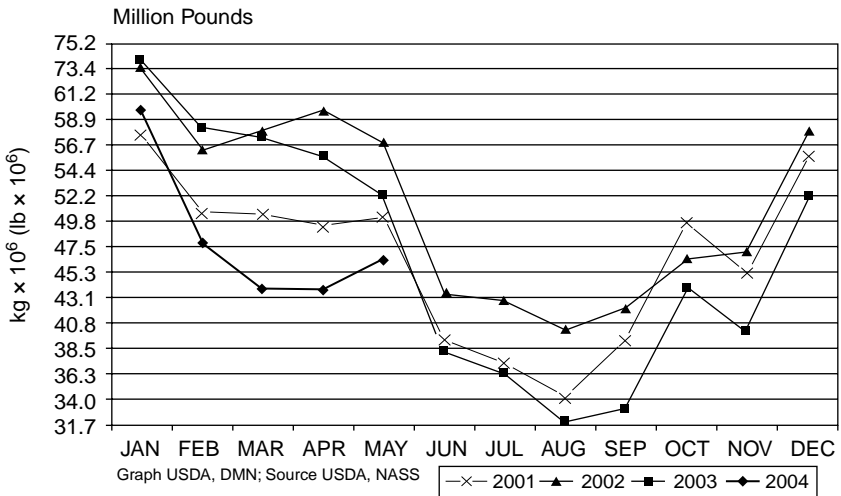


Figure 20. Per capita consumption of butter and margarine in the United States, 1968–1993.



dramatically changed over the years, the butter industry never fails to take up the slack in the relationship of supply and demand for all other dairy products.

Butter is both an intervention and a market product. To counteract growing stocks, special uses are created within the scope of the milk market organization, which contribute to not having too much butter in common storage. For some years now, between 300,000 and 400,000 tons of butter have been sold annually at reduced prices to the cheese pastry, ice cream, and chocolate industries in competition with vegetable fats (142).

Despite this and other measures, it was not possible after the milk market organization took effect to prevent an imbalance between butter production and consumption. Even the U.K.'s accession to the EC in 1973 did not bring about a turn in the overall development. Although imports from New Zealand into the new member country were cut from nearly 165,000 to 55,000 tons and although the U.K. turned mainly to France, the Netherlands, and Germany as new supplier countries, the rise of butter prices caused restricted consumption on the English market. In the course of a few years, per capita consumption dropped from 8.8 kg in 1970 to 3.3 kg in 1991 (143).

Between 1977 and 1987, there was an overall decline in the per capita consumption of butter of 16% in 14 European countries, a similar trend was noted in the United States. By 1993, the per capita butter consumption increased in the United States. Price is a strong purchase determinant, and the price of butter has significantly decreased in the United States due to USDA price support policy shifts (89).

A peak in production surplus in the EC was reached in 1986. This was due not only to increasing supplies but also to a notable drop in the consumption of milkfat. The consumer turned to products with a reduced fat content. This trend applies to almost all milk products and has substantially increased the availability of milkfat for butter production (67, 144). Table 16 gives production data for the EU-15 and other countries for butter, dairy spreads, and margarine blends (145).

U.S. prices received for butter by manufacturers, primary receivers, and others at the wholesale level are based primarily on activities on the Chicago Mercantile Exchange (CME). *The Dairy Market News* of the U.S. Department of Agriculture reports Chicago Mercantile Exchange prices, which serve as reference prices for formula pricing of butter (146).

A weekly average price for grade AA butter in July 2004, per the CME was \$1.7408 (Carlot) (147).

Spot prices on the exchange, less freight charges to Chicago, are the almost-exclusive basis for prices received at the manufacturing plants for bulk butter. In addition, a manufacturer may receive a premium for uniformity, size of shipment, a special flavor characteristic, or some other characteristic. Manufacturers who sell only bulk butter are generally pricetakers, not pricemakers.

Manufacturers who soft-print and package butter sell it to primary receivers, grocery chains, dairies, and restaurants. Such manufacturers may, depending on competitive conditions, receive a better return than those who sell only bulk.

Primary receivers buy butter from manufacturers at spot prices (plus possible premiums) and sell to several types of customers. Print butter (packaged in pound

**TABLE 16. Production of Butter, Dairy Spreads, Margarine, and Blended Spreads.**

Country	Years						
	1990	1991	1994	1995	1996	1997	1998
EU-15							
Austria <sup>a</sup>	35,283	36,131	36,609	36,700	38,600	39,416	39,901
Belgium <sup>b</sup>	55,050	55,050	55,050	33,524	26,130	29,837	30,225
Denmark	28,400	20,100	80,000	80,000	56,080	53,000	53,000
Finland <sup>c</sup>	55,700	51,800	45,300	44,700	46,628	50,000	50,000
France <sup>d</sup>	453,934	411,410	374,813	375,250	406,350	396,477	392,759
Germany	411,300	546,500	558,000	570,000	560,000	577,200	555,000
Greece <sup>d</sup>	2,400	2,400	2,140	1,826	1,514	2,587	2,975
Ireland <sup>e,f</sup>	494	249	254	5	3	3	3
Italy	79,500	80,300	80,300	80,300	80,300	80,300	80,300
Netherlands	177,794	163,304	127,991	131,954	126,094	134,500	149,039
Portugal <sup>f,g</sup>	15,000	15,986	16,300	19,400	19,400	19,400	19,400
Spain	37,500	37,500	27,000	27,000	27,000	27,000	27,000
Sweden <sup>g,h</sup>	20,649	21,211	19,700	16,400	14,500	14,500	14,500
UK	111,000	111,712	115,000	115,000	115,000	115,000	115,000
<b>Total<sup>g,h</sup> (13)</b>	<b>1,484,004</b>	<b>1,553,653</b>	<b>1,538,457</b>	<b>1,532,059</b>	<b>1,517,599</b>	<b>1,539,220</b>	<b>1,529,102</b>
Australia	116,470	118,732	142,800	131,417	NA	NA	NA
Brazil <sup>a</sup>	74	70	NA	NA	NA	NA	NA
Czech Republic	119,167	102,870	75,088	77,113	74,515	61,880	65,353
Hungary <sup>j</sup>	38,500	28,911	NA	22,000	NA	NA	NA
Israel <sup>g</sup>	4,000	4,000	NA	NA	NA	NA	NA
Japan <sup>k</sup>	90,900	85,750	NA	NA	NA	NA	NA
Morocco <sup>e</sup>	13,000	13,000	NA	NA	13,000	500	NA
Neth. Antilles	0	0	0	76	0	0	0
Norway	24,069	23,265	NA	13,791	24,433	1,971	NA
Switzerland	42,826	43,442	40,050	41,250	39,290	39,021	40,540
Turkey							

NA = not available.

<sup>a</sup>Revised figures for 1995.

<sup>b</sup>Revised figures.

<sup>c</sup>Revised figures for 1994.

<sup>d</sup>FAO estimate for 1992, 1993.

<sup>e</sup>Estimate for 1993.

<sup>f</sup>Estimate for 1997 and 1998.

<sup>g</sup>Estimate for 1990, FAO figures for 1991, 1992, 1993.

<sup>h</sup>Estimate for 1994–1995–1996.

<sup>i</sup>Estimate for 1990, 1991, 1992.

<sup>j</sup>FAO figures for 1991, 1992, 1993.

<sup>k</sup>Estimate for 1992, 1993.

cartons, usually 4 quarter pounds) is sold to grocery chains and wholesalers who supply retail food stores. Bulk butter is sold to other receivers, butter wholesalers, food processors, and cold storage firms. These sales are based on spot prices plus markup to cover handling, overhead, and profit.

Primary receivers of butter are both pricetakers and pricemakers. Prices they receive (and pay) are based on spot market prices. As many of the primary receivers

are members of the Chicago Mercantile Exchange, they can influence the spot market price by buying and selling butter there. Nonmembers can also buy and sell on the exchange through brokers.

All products sell on a combination of price, perception, and performance. Unfortunately, butter is easily the most expensive of the yellow fats. In terms of perception, all fats are under pressure because of their caloric density. Butter suffers further because it was labeled saturated and has a high-cholesterol content; both properties have been the subjects of adverse comments by the nutritional and medical community. The rise in concern for fat and cholesterol in the U.S. market has overshadowed the concern for chemicals and preservatives.

The flavor and mouth feel of butter are greatly superior to any other yellow fat, but its physical and rheological properties, particularly its poor spreadability at refrigerated temperature, make butter less attractive to many consumers. The margarine and spread industry can tailor its product in terms of spreadability. As noted earlier, many advances in the ability to alter the texture and rheology of butter have been made, but costs tend to deter manufacturers from applying the technologies for marketplace consumption. Apparently, the consumer demand for spreadability characteristics is inhibited by an unwillingness to pay for the convenience. To those who demand a natural food and appreciate its delicate flavor, butter will remain the preferred product.

## REFERENCES

1. O. F. Hunziker, *The Butter Industry*, 3rd ed., Printing Products Corp., Chicago, Illinois, 1940, pp. 1–23.
2. D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, vol. 1, 4th ed., John Wiley & Sons, Inc., New York, 1979, pp. 392–411.
3. R. G. Jensen and R. W. Clark, in N. P. Wong, ed., *Fundamentals of Dairy Chemistry*, 3rd ed., Van Nostrand Reinhold Co., Inc., New York, 1988, pp. 171–213.
4. W. R. Morrison, in F. D. Gunstone, ed., *Topics in Liquid Chemistry*, Logos Press, London, 1970, p. 51.
5. R. G. Jensen, *J. Amer. Oil Chem. Soc.*, **50**, 186–192 (1973).
6. J. R. Brunner, in E. Tria and A. M. Scann, eds., *Structural and Functional Aspects of Lipoproteins in Living Systems*, Academic Press, Inc., London, 1969, p. 545.
7. U. Bracco, J. Hidalgo, and H. Bohren, *J. Dairy Sci.*, **55**, 165 (1972).
8. J. L. Henderson and E. L. Jack, *Oil Soap*, **21**, 90–92 (1944).
9. E. L. Jack and J. L. Henderson, *J. Dairy Sci.*, **28**, 65–78 (1945).
10. E. L. Jack and L. M. Smith, *J. Dairy Sci.*, **39**, 1–25 (1956).
11. G. A. Gartan and W. R. Duncan, *J. Dairy Sci. Food Agri.*, **7**, 734–739 (1956).
12. Anonymous, *Composition and Constants of Natural Fats and Oil by Gas-Liquid Chromatography*, Archer-Daniels-Midland, Minneapolis, Minnesota, 1961.
13. R. G. Jensen, G. W. Gander, and J. Sampugna, *J. Dairy Sci.*, **45**, 329–331 (1962).
14. J. L. Moore, T. Richardson, and C. H. Amundson, *J. Amer. Oil Chem. Soc.*, **42**, 796–799 (1965).

15. M. F. White and J. B. Brown, *J. Amer. Oil Chem. Soc.*, **26**, 385–388 (1949).
16. J. D. Hay and W. R. Morrison, *Biochem. Biophys. Acta*, **202**, 237 (1970).
17. J. L. Iverson, J. Eisner, and D. Firestone, *J. Amer. Oil Chem. Soc.*, **42**, 1063–1068 (1965).
18. A. Kuksis, *Can. J. Biochem.*, **49**, 1245 (1972).
19. A. Kuksis, *J. Chromatog. Sci.*, **10**, 53 (1972).
20. L. J. Nutter and O. S. Privett, *J. Dairy Sci.*, **50**, 1194 (1967).
21. T. W. Scott, L. J. Cook, and S. C. Mills, *J. Amer. Oil Chem. Soc.*, **48**, 358–364 (1972).
22. L. F. Edmondson, R. A. Yoncoskie, N. H. Rainey, F. W. Douglas Jr., and J. Bitman, *J. Amer. Oil Chem. Soc.*, **51**, 72–76 (1974).
23. R. D. Plowman et al. *J. Dairy Sci.*, **55**, 204 (1972).
24. G. D. Elsdon and P. Smith, *Analyst*, **52**, 63–66 (1927).
25. G. D. Elsdon and P. Smith, *Oil Fat Ind.*, **4**, 103–105, 111 (1927).
26. K. A. Williams, *Analyst*, **74**, 504–510 (1949).
27. L. Ahren, L. Björck, T. Raxnikiewicz, and O. Claesson, *J. Dairy Sci.*, **63**, 741–745 (1980).
28. B. W. Tharp and S. Patton, *J. Dairy Sci.*, **43**, 475 (1960).
29. J. E. Kinsella, S. Patton, and P. S. Dimick, *J. Amer. Oil Chem. Soc.*, **44**, 202–205 (1967).
30. J. E. Kinsella, S. Patton, and P. S. Dimick, *J. Amer. Oil Chem. Soc.*, **44**, 449–454 (1967).
31. R. C. Lawrence, *J. Dairy Sci.*, **30**, 161 (1963).
32. J. E. Langler and E. A. Day, *J. Dairy Sci.*, **47**, 1291 (1964).
33. C. R. Brewington, E. A. Caress, and D. P. Schwartz, *J. Lipid Res.*, **11**, 355 (1970).
34. V. Antila, in *Proceedings of the XXI International Dairy Congress*, Moscow 2, 1982, p. 159.
35. P. W. Parodi, *J. Dairy Res.*, **48**, 131 (1981).
36. J. Kiszka, K. Batura, B. Staniewski, and W. Zatorski, *Brief Commun.*, **1**(1), 352 (1982).
37. A. Boudreau and J. Arul, *Int. Dairy Fed. Bull.*, **260**, 7–10, 13–16 (1991).
38. W. Banks, *Int. Dairy Fed. Bull.*, **260**, 3–6, 13, 22 (1991).
39. A. Huyghebaert, H. DeMoor, and J. Decatelle, in K. K. Rajah and K. J. Burgess, eds., *Milk Fat*, Society of Dairy Technology, Huntingdon, U.K., 1991, pp. 44–61.
40. E. Frede, *Int. Dairy Fed. Bull.*, **260**, 12 (1991).
41. S. S. Marschner and J. B. Fine (to General Mills, Inc.), U.S. Patent 4,804,555, 1989.
42. Anonymous, *R&D Applications, Cholesterol Reduced Fat*, Prepared Foods, Chicago, Illinois, 1989, p. 99.
43. Anonymous, *Short Path Molecular Distribution*, CVC Products, Inc., Rochester, New York, 1988.
44. U. Bracco, Brit. Patent 1,559,064, 1980.
45. D. H. Hettinga, *Altering Fat Composition of Dairy Products*, Council Agri. Sci. Technol., Ames, Iowa, 1991.
46. J. Arul, A. Boudreau, J. Makhlof, R. Tardif, and B. Grenier, *J. Dairy Res.*, **55**, 361 (1988).
47. A. R. Keen, Eur. Patent 0318326A2, 1989.
48. D. Oakenfull and G. S. Sidhu (to Sidoak), Austral. Patent Applications 54768/90 and 55112/90, 1990.

49. F. Morel, *Food Process.*, **1056**, 19–21 (1990).
50. T. J. Micich, *J. Agr. Food Chem.*, **38**, 1839 (1990).
51. Anonymous, *Research Review Special Report 2*, Wisconsin Milk Marketing Board, Madison, Wisconsin, 1989.
52. Anonymous, *Dairy Ind. Int.*, **55**(6), 37–38 (1990).
53. Anonymous, *Food Eng.*, **61**(2), 83 (1989).
54. H. Chen, S. J. Schwartz, and G. A. Spanos, *J. Dairy Sci.*, **75**(10), 2659–2669 (1992).
55. D. H. Hettinga, *Designing Foods*, National Academy Press, Washington, D.C., 1988, p. 292.
56. J. Arul, A. Boudreau, J. Makhlof, R. Tardif, and T. Bellavia, *J. Amer. Oil Chem. Soc.*, **65**, 1642 (1988).
57. S. Harlander, *J. Amer. Oil Chem. Soc.*, **65**(11), 1727 (1988).
58. D. Beitz, personal communication, 1991.
59. P. A. E. Cant, in Ref. 39, pp. 122–135.
60. *Code of Federal Regulations*, Title 7, Part, 58, Subpart P, U.S. Government Printing Office, Washington, D.C., 1983.
61. D. H. Hettinga, in Y. H. Hui, ed., *Encyclopedia of Food Technology*, vol. 1, John Wiley & Sons, Inc., New York, 1993, pp. 231–237.
62. M. M. Chrysam, in T. H. Applewhite, ed., *Bailey's Industrial Oil and Fat Products*, vol. 3, John Wiley & Sons, Inc., New York, 1985.
63. R. Wood, K. Kubena, B. O'Brien, S. Tseng, and G. Martin, *J. Lipid Res.*, **34**(1), 1–11 (1993).
64. *International Dairy Federation Standard 68A*, IDF, Brussels, Belgium, 1977.
65. *FAO/WHO Codex Standard A2*, Codex Alimentarius, Rome, Italy, 1986.
66. M. Collomb and M. Spahni, *Mitteil. Gebiete Lebensmit. Untersuch. Hyg.*, **82**(6), 615–662 (1991).
67. T. Sato, S. Kawano, and M. Iwanoto, *J. Dairy Sci.*, **73**(12), 3408–3413 (1990).
68. M. Lucisano, E. Casiraghi, and C. Pompei, *J. Texture Stud.*, **20**(3), 301–315 (1989).
69. C. J. Strugnell, *J. Food Sci. Technol.*, **14**(2), 128–129 (1990).
70. L. Forman, P. Stern, and E. Matouskova, *Milchwissenschaft*, **44**, 761–764 (1989).
71. B. Schaffer and S. Szakaly, *Brief Commun.*, **1**(1), 337 (1982).
72. R. Norris, *Int. Dairy Fed. Bull.*, **260**(5), 23–25 (1991).
73. T. Richardson and M. Korycka-Dahl, in P. F. Fox, ed., *Developments in Dairy Chemistry*, vol. 2, Elsevier Applied Science, London, 1983.
74. M. Schroder, Ger. Patent DF 36 23 313 A1, 1988.
75. F. Hulsemeyer, *Kieler Milchwirtschaft. Forschung.*, **42**(2), 115–117 (1990).
76. J. Bird, in Ref. 39, pp. 30–36.
77. B. D. Dixon, *Int. Dairy Fed. Bull.*, **204**, 3–5, (1986).
78. M. Kimenai, *Int. Dairy Fed. Bull.*, **204**, 16 (1986).
79. L. H. Wiedermann, *J. Amer. Oil Chem. Soc.*, **55**(11), 823 (1978).
80. M. Schweizer, *Int. Dairy Fed. Bull.*, **204**, 10 (1986).
81. P. Bjerre, in Ref. 39, pp. 63–73.

82. F. H. McDowall, *The Butter Maker's Manual*, New Zealand University Press, Wellington, New Zealand, 1953.
83. P. Friis, *Brief Commun.*, **1**(1), 326 (1982).
84. Anonymous, *Gold'n Flow Continuous Buttermaking Method*, Bulletin G-493, Cherry-Burrell Corp., Chicago, Illinois, 1954.
85. M. Kawanari, K. Okamoto, Y. Honda, and M. Jukushima, *J. Jpn. Soc. Food Sci. Technol.*, **39**(3), 227–232 (1992).
86. D. H. Kleyn, *Food Technol.*, **46**(1), 118–121 (1992).
87. J. Toma, J. D. Curtis, and C. Sobotor, *Food Technol.*, **42**, 93–95 (1988).
88. Anonymous, *Int. Dairy Fed. Bull.*, **170** (1984).
89. W. P. Charteris and M. K. Keogh, *J. Soc. Dairy Technol.*, **44**(1), 3–8 (1991).
90. I. Macnab, *Modern Dairy*, **68**(3), 23 (1989).
91. S. Gulyayev-Zaitsev, V. Dobronos, J. Berezansky, and N. Zhirnaya, *Brief Commun.*, **1**(1), (1982).
92. O. Gerstenberg, *Dairy Ind. Int.*, **53**(11), 28 (1988).
93. K. Anderson, *Int. Dairy Fed. Bull.*, **260**, 17–18 (1991).
94. A. Pedersen, *Scand. Dairy Inform.*, **4**(3), 74–75 (1990).
95. V. K. Babayan, G. L. Blackbrun, and B. R. Bistran (to N.E. Decaness Hospital), U.S. Patent 4,952,606, 1990.
96. G. Rosenbaum, *Dairy Foods*, **93**(7), 13 (1992).
97. F. Graves (to Land O'Lakes, Inc.), U.S. Patent 4,425,370, January 10, 1984.
98. D. Antenore, D. Schmadeke, and R. Steward (to Land O'Lakes, Inc.), U.S. Patent 4,447,463, May 8, 1984.
99. B. Schaffer and S. Szakaly, *Brief Commun.*, **1**(1), 363 (1982).
100. A. Giles, *J. Amer. Oil Chem. Soc.*, **65**, 1708–1712 (1988).
101. G. Van den Berg, in *Proceedings of the XXI International Dairy Congress*, Moscow 2, IDF, Brussels, Belgium, 1982, p. 153.
102. T. Siek and J. Lindsay, *J. Dairy Sci.*, **53**, 700 (1970).
103. J. E. Kinsella, *Food Technol.*, **29**(5), 82–98 (1975).
104. N. Cullinane and D. Eason, *Brief Commun.*, **1**(1), 349 (1982).
105. B. K. Mortensen and K. Jansen, *Brief Commun.*, **1**(1), 334 (1982).
106. S. Berntsen, Int. Patent Application WO 92/19111 A1, 1992.
107. A. M. Fearson and D. E. Johnston, *Dairy Ind. Int.*, **53**(10), 25–29 (1988).
108. A. Petersen, *Int. Dairy Fed. Bull.*, **260**, 12 (1991).
109. E. DeJong, G. Van den Berg, *Voedingsmiddelentechnologie*, **19**(5), 35–38 (1986).
110. K. E. Kaylegian and R. C. Lindsay, *J. Dairy Sci.*, **75**(12), 3307–3317 (1992).
111. J. Bumbalough (to Wisconsin Milk Marketing Board, Inc.), U.S. Patent 4,839,190, June 13, 1989.
112. F. M. Fouad, F. R. Vandervoort, W. D. Marshall, and P. G. Farrell, *J. Amer. Oil Chem. Soc.*, **67**(12), 981–988 (1990).
113. J. Makhlof, J. Arul, A. Bordreau, P. Verret, and M. R. Sahasrabudhe, *J. Can. Inst. Food Sci. Technol.*, **20**(4), 263–245 (1987).

114. J. B. Mickle, *Tech. Bull. T-83*, Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma, 1960.
115. W. Banks and W. W. Christie, *Outlook Agr.*, **19**(1), 43–47 (1990).
116. R. P. Middaugh, R. J. Baer, D. P. Casper, D. J. Schingoethe, and S. W. Seas, *J. Dairy Sci.*, **71**(12), 3179–3187 (1988).
117. G. A. Stegeman, R. J. Baer, D. J. Schingoethe, and D. P. Casper, *J. Dairy Sci.*, **75**(4), 962–970 (1992).
118. H. Rohm and S. Raaber, *J. Food Sci.*, **57**(3), 647–650 (1992).
119. T. Landon, U.S. Patent application 20040033303, February 19, 2004.
120. K. K. Rajah and K. J. Burgess, in Ref. 39, pp. 37–43.
121. R. Barts, *Int. Dairy Fed. Bull.*, **260**, 19–20 (1991).
122. R. A. Wibley, in Ref. 39, pp. 136–147.
123. Anonymous, *Technical Guide for the Packaging of Milk and Milk Products*, Document 143, 2nd ed., IDF, Brussels, Belgium, 1982.
124. R. G. Mansour, Int. Patent WO 9202 144, February 20, 1992.
125. R. Egli (to Egli AG), Int. Patent WO 89/10689 A1, 1989.
126. Proceedings of IDF Seminar, Singapore, *Int. Dairy Fed. Doc.*, **142** (1982).
127. Anonymous, IDF Study 74, Brussels, Belgium, 1974.
128. M. Zillen, *Document 116*, IDF, Brussels, Belgium, 1977, pp. 1–4.
129. W. P. Carteris and M. K. Keogh, *J. Soc. Dairy Technol.*, **44**, 3 (1991).
130. G. Behrens, *Document 224*, IDF, Brussels, Belgium, 1988, pp. 8–9.
131. B. K. Wadhwa and M. K. Jain, *Indian J. Dairy Sci.*, **44**(6), 372–374 (1992).
132. R. Spieler, *Document 149*, IDF, Brussels, Belgium, 1980, pp. 132–135.
133. M. Gordon, *Int. Dairy Fed. Bull.*, **260**, 20 (1991).
134. P. Sitaram and S. K. Gupta, *Agr. Rev.*, **9**(2), 81–104 (1988).
135. B. Guenault, *Dairy Ind. Int.*, **57**(6), 32 (1992).
136. L. S. Girsh (to Immunopath Profile, Inc.), U.S. Patent 5,112,636, May 12, 1992.
137. F. X. Malcata, C. G. Hill, and C. H. Amundson, *Biotechnol. Bioeng.*, **39**(11), 1097–1111 (1992).
138. G. Comi, S. D’Aubert, and M. Valenti, *Ind. Aliment.*, **29**(281), 358–361 (1990).
139. E. J. Mann, *Dairy Ind. Int.*, **2**, 19 (1992).
140. L. Kintish, *J. Soap Cosmetics Chem. Special.*, **69**(2), 51 (1993).
141. *Dairy Market News*, **71**, Rept 28 (July 12–16, 2004).
142. U.S. Department of Commerce, Bureau of the Census, *Statistical Abstracts of the United States*, 113th ed., U.S. Government Printing Office, Washington, D.C., 1993.
143. E. Hetzner and P. Jacknik, *Int. Dairy Fed. Bull.*, **293**, 19 (1993).
144. Anonymous, *Trends in the U.S. Consumer Attitudes and the Supermarket*, Food Marketing Institute, Washington, D.C., 1993.
145. International Margarine association of the Countries of Europe. <http://www.imace.org>.
146. Economic Research Service, USDA. (2004). *The Structure of Dairy Markets: Past, Present, Future AER-757*. Available: [USDA.gov](http://USDA.gov).
147. Chicago Mercantile Exchange, Cash Markets, July 16, 2004.

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## *Canola Oil*

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### **1. INTRODUCTION**

During the past two decades, the production of *Brassica* oilseeds has increased, making it one of the major sources of vegetable oil in the world. The development of canola oil (low erucic acid and low glucosinolate rapeseed oil) from the original high erucic acid rapeseed oil (HEAR) by Canadian plant breeders produced a premium quality oil. This improvement in the oil resulted from a reduction in erucic acid (C22:1) to levels below 2%. In fact, this acid now contributes less than 2% of the total composition of the fatty acids in canola oil varieties. In addition, the level of glucosinolates in the seed has been lowered to less than 30  $\mu\text{mol/g}$ , resulting in better meal quality. In this chapter, the origin, composition, properties, and utilization of canola and HEAR oils are discussed. In addition, utilization of both oils as edible oils, food ingredients, and industrial applications are discussed. The term “canola” is used as the name for rapeseed with substantially lowered amounts of erucic acid and glucosinolates. The term “canola” is used mainly in the American continent and in Australia, and rapeseed is used commonly in Europe and other countries.



## 2. ORIGIN

Oilseed rape species used to produce canola oil and meal are from the *Brassica* genus in the *Cruciferae* family. They were first cultivated in India almost 4000 years ago. Large-scale planting of rapeseed was first reported in Europe in the thirteenth century. The *Brassica* species probably evolved from the same common ancestor as wild mustard (*Sinapis*), radish (*Raphanus*), and arrugula (*Eruca*).

## 3. DEVELOPMENT OF CANOLA

Early rapeseed cultivars had high levels of erucic acid in the oil and glucosinolates in the meal. The presence of these components in rapeseeds was a health concern. The high levels of erucic acid were blamed for producing fatty deposits in the heart, skeletal muscles, and adrenals of rodents as well as impairing growth. Plant breeding programs initiated in Canada resulted in the identification in 1959 of Liho, a rapeseed line containing low levels of erucic acid. A program of backcrossing and selection was conducted to transfer the low erucic acid trait into agronomically adapted cultivars. This led to the first low erucic acid cultivar of *B. napus*, Oro, in 1968 and the first low erucic acid *B. rapa* cultivar, Span, in 1971. Because of the health concerns related to high levels of erucic acid, over 95% of the rapeseed grown in Canada in 1974 were low erucic acid varieties.

Glucosinolates were also considered detrimental in rapeseed meal fed to poultry, swine, and ruminants. The hydrolyzed products of glucosinolates, namely, isothiocyanates and other sulphur-containing compounds, were shown to interfere with the uptake of iodine by the thyroid gland, contribute to liver disease, and reduce growth and weight gain in animals. Consequently, plant breeders realized that if rapeseed meal was to be used in animal feed, the glucosinolate content had to be reduced. A Polish line with a low-glucosinolate trait, Bronowski, was identified by Dr. Krzymski in the late 1950s. Breeding efforts to introduce this trait into low erucic acid lines by Dr. Baldur Stefansson at the University of Manitoba resulted in the release of the world's first low erucic acid, low glucosinolate cultivar of *B. napus*, often called the double-zero rapeseed. This was followed in 1977 by the release of the first low erucic acid, low glucosinolate cultivar of *B. rapa*, Candle, by Dr. Keith Downey of the National Research Council of Canada in Saskatoon. Approximately 80% of the total Canadian rapeseed acreage in 1980 consisted of the double-zero cultivars. The detailed history of the development of canola is described in a booklet titled "The Story of Rapeseed in Western Canada" (1).

The name canola was registered by the Western Canadian Oilseed Crushers in 1978 and subsequently transferred to the Canola Council of Canada in 1980. It included those cultivars containing less than 5% erucic acid in the oil and 3 mg/g aliphatic glucosinolates in the meal. In 1986, the definition of canola was amended to *B. napus* and *B. rapa* lines with less than 2% erucic acid in the oil and less than 30  $\mu\text{mol/g}$  glucosinolates in the air-dried, oil-free meal. The oil was added to the Generally Recognized as Safe (GRAS) list of food products in the United States.

It was much more difficult to introduce the low erucic acid trait into the European rapeseed lines because they were primarily of the winter type. This extended the time required to produce each generation and crosses between spring low erucic acid rapeseed (LEAR) cultivars and winter lines resulted in undesirable segregates. Nevertheless, the development of European LEAR varieties was accomplished within 15 years. European acreage of rapeseed declined during the 1970s as result of health concerns. In 1977, the low erucic acid trait was made mandatory in Europe. Initially the new LEAR cultivars produced lower yields and lower oil content compared with the traditional rapeseed cultivars. Subsequent plant breeding overcame these problems with European production of LEAR increasing substantially by 1984. The other rapeseed growing areas of the world, notably, India and China, did not take part in the development and conversion to canola type rapeseed. In these areas, HEAR still predominates.

Canola oil produced in Canada is obtained from genetically modified seeds of *Brassica napus* and *Brassica rapa (campestris)*. These cultivars, low in erucic acid and glucosinolates, are different in chemical, physical, and nutritional characteristics from high erucic acid rapeseed oil. Current Canadian plant breeding programs continue to focus on the development of oils with specific characteristics to meet the consumer demands and food manufacturing practices, such as lowering the content of saturated fatty acids and designer oils for specific applications.

## 4. COMPOSITION

### 4.1. Nature of Edible Oils and Fats

Edible oils and fats are composed primarily of triacylglycerols (TAG), ester of one molecule of glycerol, and three molecules of fatty acids. Analysis of canola oils showed that TAGs constituted 94.4% to 99.1% of the total lipid (2). The typical composition of canola, rapeseed, and soybean oils is presented in Table 1.

**TABLE 1. Constituents of Canola, Rapeseed, and Soybean Oils.**

Component	Canola	Rapeseed	Soybean
Triacylglycerols (%)	94.4–99.1	91.8–99.0	93.0–99.2
Phospholipids (%)			
Crude Oil	up to 2.5	up to 3.5	up to 4.0
Water-degummed	up to 0.6	up to 0.8	up to 0.4
Acid-degummed	up to 0.1	—	up to 0.2
Free Fatty Acids (%)	0.4–1.2	0.5–1.8	0.3–1.0
Unsaponifiables (%)	0.5–1.2	0.5–1.2	0.5–1.6
Tocopherols (mg/kg)	700–1200	700–1000	1700–2200
Chlorophylls (mg/kg)	5–50	5–55	Trace
Sulfur (mg/kg)	3–25	5–35	Nil
Iron (mg/kg)	<2	<2	<2

Adapted from Mag (2) and Ying et al. (3).

## 4.2. Fatty Acid Composition of Canola Oil

The stigma of the erucic acid (C22:1n - 9) in rapeseed oil has lingered despite firm evidence that this fatty acid was more of a threat to rats than to humans. It is sufficient to say that the discovery of chain shortening of erucic acid to oleic acid by peroxisomes was one of the most fundamental breakthroughs in understanding fatty acid metabolism in the last few decades. Once in the oleic acid form, the erucic acid residue is as readily catabolized by mitochondria, as are palmitic and other fatty acids (4). The reduction of erucic acid in rapeseed oil resulted in a marked increase in octadecanoic acids, and their contribution in canola oil is around 95% of all fatty acids present (Table 2).

Plant breeders have also developed canola oil with the linolenic acid content reduced to 2% (5) (Table 2). The storage stability of this oil showed improvement as compared with regular canola oil (7). Frying performance of this oil was improved together with better storage stability of fried products such as french fries and potato chips (8, 9). Canola has been further genetically modified to produce oil with oleic acid content ranging from 60% to 85% (10). Field production of this oil showed that the very high content of oleic acid was hard to reproduce. The fatty acid composition of the field-produced oil is presented in Table 2. High oleic

**TABLE 2. Comparison of Major Fatty Acids in Some Vegetable Oils (w/w %).**

Fatty Acid	Canola	HEAR	LLCAN	HOCAN	LTCAN	GLCO	LLFlax	Soybean	SUN
C10:0	—	—	—	—	0.1	—	—	—	—
C12:0	—	—	—	—	38.8	—	—	—	—
C14:0	0.1	—	0.1	0.1	4.1	0.1	0.1	0.1	—
C16:0	3.6	4.0	3.9	3.4	2.7	4.2	6.4	10.8	6.2
C18:0	1.5	1.0	1.3	2.5	1.6	3.7	4.1	4.0	4.7
C20:0	0.6	1.0	0.6	0.9	0.4	1.0	0.1	—	0.2
C22:0	0.3	0.8	0.4	0.5	0.2	0.5	0.1	—	0.1
C24:0	0.2	0.3	0.3	0.3	0.2	0.2	0.1	—	0.1
<b>Saturated</b>	<b>6.3</b>	<b>7.1</b>	<b>6.6</b>	<b>7.7</b>	<b>48.1</b>	<b>9.9</b>	<b>10.9</b>	<b>14.9</b>	<b>11.3</b>
C16:1	0.2	0.3	0.2	0.2	0.2	0.2	0.1	0.3	0.2
C18:1	61.6	14.8	61.4	77.8	32.8	24.4	16.9	23.8	20.4
C20:1	1.4	10.0	1.5	1.6	0.8	0.8	0.1	0.2	—
C22:1	0.2	45.1	0.1	0.1	0.5	0.1	—	—	—
<b>MUFA</b>	<b>62.4</b>	<b>69.7</b>	<b>63.1</b>	<b>79.9</b>	<b>34.3</b>	<b>25.5</b>	<b>17.2</b>	<b>24.3</b>	<b>20.2</b>
C18:2n-6	21.7	14.1	28.1	9.8	11.3	26.1	70.1	53.3	68.8
C18:3n-3	9.6	9.1	2.1	2.6	6.3	1.3	1.8	7.6	—
C18:3n-6	—	1.0	—	—	—	37.2	—	—	—
<b>PUFA</b>	<b>31.3</b>	<b>23.2</b>	<b>30.2</b>	<b>12.4</b>	<b>17.6</b>	<b>64.6</b>	<b>71.9</b>	<b>60.8</b>	<b>68.8</b>

Abbreviations: LLCAN—Low linolenic acid canola oil; HOCAN—High oleic acid canola oil; GLCO—Canola oil with gamma linolenic acid; LLFlax—Flaxseed oil with reduced content of linolenic acid; LTCAN—Canola oil with high content of lauric acid; SUN—Sunflower oil; MUFA—Monounsaturated fatty acids; PUFA—Polyunsaturated fatty acids.

Adapted from Ackman (4), Vecchia (6), and Tso et al. (11).

acid canola oil resembles the composition of olive oil more closely than that of the regular canola oil. This oil showed improved frying stability and produced better quality fried potato chips than regular canola oil (8). Warner and Mounts (9) found that up to 2% of linolenic acid is required in frying oils to produce positive characteristic flavour in fried foods. This is caused by the formation of oxidation products from linolenic acid, which are the main contributors to the fried food flavour.

Recently, canola oil with a high content of lauric acid (39%) was developed to be used in confectionery coatings, coffee whiteners, whipped toppings, and center filling fats (Table 2). Further, canola oil with a content of stearic acid as high as 40% is available to be used as a replacement for hydrogenated fats in bread and bakery markets (6). Another canola oil containing approximately 10% of palmitic acid with better crystallization properties was developed. Canola oil for the health food market containing up to 40% of gamma linolenic acid is also now available (11).

### 4.3. Minor Fatty Acids

Minor fatty acids present in oils often differ from their acid family members by the location of the double bond. Most of these acids are present in canola oil in the 0.01–0.1% range, except for C16:1n-7, which is around 0.3%. Most of these minor fatty acids are from the n-7 series, in varying proportions to the n-9 isomers (4). A similar series of minor fatty acids were found in *B. rapa* variety Candle (12). Conjugated C18:2 fatty acids were also found in canola oils. A number of these acids are artifacts of refining and deodorization, although some were also observed as natural components in some oil seeds. The refining process is a source of artifact fatty acids caused by the isomerization of one or more of the double bonds of *cis* linolenic acid (13). These *trans*-isomers can be found in any oil containing linolenic acid after refining and deodorization accounting for 1% or more of the parent acid (4).

Canola oil is the only known edible oil containing one or more fatty acid, with sulphur atom as the integral part of the molecule. The structure of the proposed molecule of this fatty acid suggests the possible formation or presence of many isomers (14).

In the sediment from industrial winterization, additional minor fatty acids and alcohols with 26 to 32 carbon atoms in the chain have been found in waxes and triacylglycerols (15). Most of these compounds are extracted from the seed coat and can initiate sediment formation in canola oil (16).

### 4.4. Triacylglycerols

Triacylglycerols are the most abundant lipid class found in canola oil. The combination of fatty acids on the glycerol moiety represent the most complex mixture with  $n^3$  possible molecular species, where  $n$  is the number of fatty acids present in the oil.

**TABLE 3. Composition of Major Triglycerides of Canola Oils (%).**

Triacylglyceride	Canola (CO)	LLCO	HOCO
LnLO	7.6	1.7	1.5
LLO	8.6	11.0	1.1
LnOO	10.4	2.6	8.6
LnOP	2.1	0.5	1.1
LOO	22.5	28.4	12.7
LOP	5.7	4.2	2.2
OOO	22.4	32.8	49.5
POO	4.6	4.8	7.7
SOO	2.6	2.4	5.0
PPP	0.1	1.4	2.8
LLP	1.4	1.1	0.8
LOS	1.6	1.9	1.0
LLL	1.3	1.6	0.2
LnLL	1.4	0.0	0.3
LnLnO	1.7	0.4	0.1
Others	6.0	5.2	5.4

**Abbreviations:** LLCO—Low linolenic canola oil; HOCO—High oleic canola oil; Ln—Linolenic; L—Linoleic; O—Oleic; S—Stearic; P—Palmitic; Others—Group of 15 triacylglycerols with contribution below 1% each.  
Adapted from Kallio and Currie (18).

The TAG molecular species profile represents a key to understanding the physical characteristics of the oil and is a unique method of identification (17). The position of fatty acids on the glycerol molecule was originally found for HEAR oil to be based on saturation. Long-chain (C20:0 -C24:0) saturated fatty acids are found mostly in the *sn*-1 and *sn*-3 positions, whereas the octadecenoic (C18) acids, especially linoleic and linolenic, are primarily in the *sn*-2 position (18, 19). The composition of triacylglycerols in modified canola oils is presented in Table 3. As can easily be predicted, in high oleic acid canola oil, the most abundant triacylglycerols was triolein, whereas in regular canola oil, four triacylglycerols were detected in almost equal amounts, namely, olein-dilinolein, linolenin-dilinolein, triolein, and linoleic-diolein.

Jáky and Kurnik (20) investigated the concentration of linoleic acid in the *sn*-1,3 and *sn*-2 positions. They found that in HEAR oil, at least 95% of linoleic acid was concentrated in the *sn*-2 position, whereas in canola oil, only 54% was in this position. The increased amounts of linoleic acid in canola oil were incorporated into the *sn*-1,3 position to replace erucic acid. Kallio and Currie (18) found that triacylglycerols with acyl carbon number (ACN) 54 and two double bonds consisted of acylglycerols where stearic acid was present predominantly in the *sn*-2 position. Acylglycerols with saturated acids in this position usually have a higher melting point, poor solubility, and can cause problems with digestibility. Additionally, high melting acylglycerols can stimulate/initiate sediment formation and affect clarity of the oil (21).

**TABLE 4. Composition of Phospholipids in Canola Oil During Processing (%).**

Oil Sample	Phosphorus (mg/kg)	PC	PE	PI	PA	PS
Solvent	529.0	31.2	18.8	19.8	21.6	3.1
Expeller	242.3	34.3	16.1	18.7	20.3	4.5
Degummed	12.2	2.8	10.8	28.9	38.4	14.6

**Abbreviations:** PC—Phosphatidyl choline; PE—Phosphatidyl ethanolamine; PI—Phosphatidyl inositol; PA—Phosphatidic acid; PS—Phosphatidyl serine.

Adapted from Przybylski and Eskin (23).

#### 4.5. Polar Lipids

Sosulski et al. (22) examined the polar lipids (PL), phospholipids, and glycolipids in several rapeseed cultivars, including a low erucic acid winter cultivar grown in Poland and found that phospholipids accounted for the major portion (3.6%) of total polar lipids, whereas glycolipids contributed only 0.9%. A more recent study by Przybylski and Eskin (23) reported changes in phospholipids during canola oil processing, as shown in Table 4.

Significant amounts of phosphatidic acid (PA) formed during processing indicate hydrolysis of other phospholipids. This can be attributed to the effect of phospholipases and hydrothermal treatment during the conditioning of flaked seeds. Cmolik et al. (24) observed an increase in phospholipid amounts from 0.5% to 15% during conditioning of seed flakes. It was reported that hydratable phospholipids such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE) assist the removal of nonhydratable phospholipids. Phosphatidylinositol (PI) and PA are considered nonhydratable phospholipids and are difficult to remove during degumming. Phosphoric acid was found to be the most effective degumming agent for reducing the levels of lysophosphatidylethanolamine. Application of acids for degumming also removes the majority of iron from the oil. In practice, canola oil phosphatides are removed to concentrations below 0.1% using an aqueous solution of citric acid and water (25). Lecithins obtained by degumming of canola oil using only water formed more stable oil in water emulsions compared with lecithins obtained from acid degumming (26).

Sosulski et al. (22) and Smiles et al. (27) examined the fatty acid composition of the individual phospholipids in the LEAR variety from winter rapeseed cultivars (Table 5).

**TABLE 5. Fatty Acid Composition of Phospholipids (w/w%).**

Phospholipid	16:0	16:1	18:0	18:1	18:2	18:3	20:1
Phosphatidyl Choline	8.7	0.8	1.2	55.8	30.9	1.9	0.5
Phosphatidyl Inositol	21.8	0.8	1.9	33.6	38.1	3.6	—
Phosphatidyl Ethanolamine	17.7	1.8	2.0	47.7	27.3	2.7	0.5

Adapted from Sosulski et al. (22) and Smiles et al. (27).

Phosphatidylcholine contained the highest amount of unsaturated fatty acids, mostly oleic and linoleic acids. The other two principal phospholipids were rich in palmitic, linoleic, and linolenic acids. The presence of highly unsaturated fatty acids in phospholipids is important as they are prone to oxidation and can cause accelerated deterioration of the oil. It was also reported that phospholipids have a tendency to complex heavy metals, and these complexes are a stable form of catalyst, which can initiate and stimulate oxidation (28).

#### 4.6. Tocopherols

The main components of unsaponifiables in vegetable oils are tocopherols and sterols, present in different amounts. Tocopherols are recognized as very efficient natural antioxidants and their amount in the plant is probably governed by the content of unsaturated fatty acids. Canola oil contains relatively high amounts of tocopherols. Isomers of tocopherols have different antioxidative activity *in vitro* and *in vivo*. In the food system, the antioxidant activity of the tocopherol isomers decreases in the following order:  $\gamma > \delta > \beta > \alpha$  (29). Tocopherols are about 250 times more effective than butylated hydroxytoluene (BHT) (30). Lipid peroxy radicals react with tocopherols several magnitudes faster than with other lipids. A single molecule of tocopherol can protect about  $10^3$  to  $10^6$  molecules of polyunsaturated fatty acids in the plant and animal cells. This explains why the ratio of tocopherols to PUFA in the cells is usually 1:500 to provide sufficient protection against oxidation (31). These components are also effective as singlet oxygen quenchers, but they are less potent than carotenoids. A single molecule of tocopherol can react with up to 120 molecules of singlet oxygen (32). The high potency of tocopherols as antioxidants and quenchers of singlet oxygen is based on their ability to be transformed back from the oxidized form into the active structure by other molecules such as ascorbic acid and glutathione (33). Meanwhile, tocotrienols are not present in canola oil. However, plastochromanol-8, which is a derivative of  $\gamma$ -tocotrienol,

**TABLE 6. Tocopherols Content in Selected Vegetable Oils (mg/kg).**

Oil	$\alpha$	$\beta$	$\gamma$	$\delta$	P-8	Total
HEAR	268	—	426	—	97	790
Canola	272	—	423	—	75	770
LLCanola	150	—	314	7	47	517
HOCanola	226	—	202	3	42	473
HOLLCanola	286	—	607	8	83	983
Soybean	116	34	737	275	—	1162
Sunflower	613	17	19	—	—	649
Corn	134	18	412	39	—	603
LLFlax	26	—	213	9	130	377

Abbreviations: HEAR—High erucic acid rapeseed; LLCAnola—Canola oil with low content of linolenic acid; HOCAnola—Canola oil with high content of oleic acid; LLFlax—Flax oil with low content of linolenic acid; P-8—Plastochromanol-8.

Adapted from Zambiazzi (34) and Normand et al. (35).

but having a longer side chain, was detected in canola and flax oils, and its anti-oxidative activity was found to be similar to  $\alpha$ -tocopherol (34).

The composition of tocopherols in some common vegetable oils compared with canola oil is summarized in Table 6. Canola oil contains mostly  $\alpha$ - and  $\gamma$ -tocopherols, usually in a 1:2 ratio. The content of tocopherols in refined, bleached, and deodorized (RBD) oils is affected by processing, mainly extraction, refining, and deodorization. The lowest content of tocopherols was found in cold-pressed canola oil. However, when the temperature of pressing was increased, the amount of tocopherols in oil doubled. Solvent-extracted oils contain higher amounts of tocopherols than cold-pressed oils, and the content of tocopherols was similar to oils from hot pressing. The largest portion of these compounds is removed during the deodorization process (36). However, tocopherols in the distillate may be recovered and used in nutraceutical applications.

#### 4.7. Sterols

Sterols are present in canola oil in two forms in equal amounts, free sterols and esterified sterols (19, 37). The fatty acid composition of the esterified sterol fraction in canola oil is shown in Table 7.

The fatty acid distribution in esterified sterols differs from that found for canola oil. In the sterol esters, higher levels of palmitic and stearic acids were observed. All three major sterols were equally distributed in esterified and free sterol fractions in canola oil. Twice the amount of brassicasterol was found in free sterols than in esterified sterols. The total amount of sterols in rapeseed and canola oils ranges from 0.7% to 1.0%. The composition of major sterols in common vegetable oils is presented in Table 8.

Brassicasterol is the major and unique sterol present in rapeseed and canola oils. This sterol is often used to determine adulteration of other oils with rapeseed/canola oil (4, 39). Sterols are also affected by processing, with about 40% of these

**TABLE 7. Fatty Acid Composition of Esterified Sterols in Canola Oil.**

Fatty Acid	Contribution (%)	
	Sterol Esters	Canola Oil
C14:0	3.1	0.1
C16:0	17.5	3.6
C18:0	18.4	1.5
C18:1	30.9	60.2
C18:2	20.5	21.6
C18:3	7.6	9.6
C20:0	0.8	0.4
C22:1	1.2	0.2

Adapted from Gordon and Miller (38).



**TABLE 8. Proportions of Major Sterols in Selected Vegetable Oils (%).**

Sterol	HEAR	CAN	LLCAN	HOCAN	HOLLCAN	SOY	SUN	Corn
Cholesterol	0.4	0.1	0.1	0.1	0.1	0.3	0.1	0.1
Brassicasterol	13.2	13.8	12.2	10.8	16.2	—	—	—
Campesterol	34.4	27.6	31.2	33.9	28.8	18.1	7.5	17.2
Stigmasterol	0.3	0.5	0.2	0.8	0.1	15.2	7.5	6.3
$\beta$ -Sitosterol	47.9	52.3	51.3	48.7	50.9	54.1	58.2	60.3
$\Delta$ 5-Avenasterol	2.1	1.9	1.9	1.8	2.1	2.5	4.0	10.5
$\Delta$ 7-Avenasterol	1.6	1.1	1.1	1.9	0.8	2.0	4.0	1.1
$\Delta$ 7-Stigmasterol	2.1	2.3	2.1	2.1	2.3	1.4	7.1	1.8
<b>Total (g/kg)</b>	8.8	6.9	6.3	7.1	6.9	4.6	4.1	9.7
<b>Esterified (g/kg)</b>	4.5	4.2	4.0	4.4	4.2	5.8	2.1	5.6

**Abbreviations:** HEAR—High erucic rapeseed oil; CAN—Canola oil; LLCAN—Low linolenic canola oil; HOCAN—High oleic canola oil; HOLLCAN—High oleic low linolenic canola oil; SOY—Soybean oil; SUN—Sunflower oil.

Adapted from Ackman (4), Strocchi (39), Zambiasi (34), and Gordon and Miller (38).

components removed from the oil during deodorization. Refining changes the content of sterols where isomerization of these compounds is one of the processes (40, 41).

The chemical structure of phytosterols is similar to that of cholesterol so that these compounds may be involved in oxidative reactions. Przybylski and Eskin (42) found some oxidation products formed from plant sterols during storage of fried food products. Similar oxidation products were found in soybean oil and wheat flour (43). In light of health concerns associated with cholesterol oxidation products, potential health risks of phytosterol oxidation products are now receiving serious attention.

#### 4.8. Pigments

Pigments present in canola impart undesirable color to the oil. They also promote photo-oxidation as well as inhibit catalysts used for hydrogenation. Chlorophylls without phytol such as chlorophyll derivatives and pheophorbides may have health effects because of their photo-toxicity, which may be followed by photosensitive dermatitis (44). A bleaching step is necessary during oil processing to remove chlorophyll, chlorophyll derivatives, and other color bodies. Changes in chlorophylls during canola oil processing are summarized in Table 9.

During oil processing, chlorophyll completely decomposes to derivatives, which are more difficult to remove during bleaching. This necessitates much higher amounts of activated bleaching earth to be used to achieve complete removal of all chlorophyll derivatives (45).

In addition to chlorophyll pigments, carotenoids are also present in canola oil. In crude canola oil, carotenoids were present at around 130 mg/kg, composed mainly of xanthophylls (90%) and carotenes (10%). During refining and bleaching, the amount of carotenoids was reduced to 10 mg/kg (46).

**TABLE 9. Chlorophyll Pigments in Canola Oil During Processing (mg/kg).**

Oil After	Chlor <i>a</i>	Pheo <i>a</i>	Pheo <i>b</i>	Pyro <i>a</i>	Pyro <i>b</i>
Expeller	6.27	4.48	1.79	5.37	0.67
Extraction	1.88	3.31	1.34	16.57	3.13
Expeller + Extraction	1.79	5.55	1.34	9.76	1.43
Degumming	0.27	7.16	1.07	9.40	1.84
Alkaline Refining	0.22	6.27	1.12	9.13	1.79
Bleaching	—	0.56	0.32	0.21	0.25

Abbreviations: Chlor *a*—Chlorophyll *a*; Pheo *a*—Pheophytin *a*; Pheo *b*—Pheophytin *b*; Pyro *a*—Pyropheophytin *a*; Pyro *b*—Pyropheophytin *b*.

Adapted from Suzuki and Nishioka (45).

The type and content of chlorophylls and their derivatives in the seed define the quality of extracted and processed canola oil, which has an impact on the quality of the processed oil. Composition and content of these pigments is related to the maturity of the seed (Table 10).

In the fully matured seed, only 4 mg/kg of chlorophylls was observed, whereas in physiologically matured seed, 35 days before maturity, 1239 mg/kg was found. At maturity, only chlorophyll *a* and *b* were present, and all possible isomers/derivatives were observed at other stages of maturation (Table 10).

#### 4.9. Trace Elements

The proposed Codex standard for edible low erucic acid rapeseed gives the maximum levels permitted for iron, copper, lead, and arsenic. Although these metals are found in other edible oils and are present naturally in the seed, nevertheless, they can also be introduced during handling and processing. Diosady *et al.* (48) and Elson *et al.* (49) examined the effect of processing on trace elements in canola

**TABLE 10. Changes in Composition and Content of Chlorophylls During Canola Seed Maturation (mg/kg).**

Chlorophylls	Time to Maturity (Days)					
	35	27	20	14	6	0
Chlorophyll <i>a</i>	19.5	23.4	27.2	58.7	41.9	82.4
Chlorophyll <i>b</i>	22.2	22.1	15.8	27.3	54.1	17.1
Pheophytin <i>a</i>	43.1	39.8	40.9	10.1	1.1	0.0
Pheophytin <i>b</i>	8.5	7.4	11.3	2.0	0.0	0.0
Pheophorbides <i>a</i>	2.2	1.7	0.6	1.5	0.0	0.0
Pyropheophytin	1.2	2.3	2.5	0.0	0.0	0.0
Methylpheophorbide	3.2	3.5	1.8	0.5	3.0	0.5
<b>Total Amount (mg/kg)</b>	<b>1239</b>	<b>906</b>	<b>463</b>	<b>48</b>	<b>8</b>	<b>4</b>

Adapted from Ward *et al.* (47).

**TABLE 11. Mineral Elements Content in Canola Oils (mg/kg).**

Oil Sample	Phosphorus	Iron	Calcium	Sulfur	Zinc	Lead
Crude Oil	1190.0	3.52	296.0	6.5	2.4	0.24
<b>Degummed with</b>						
Water (WDG)	222.0	1.32	169.0	1.2	2.1	—
Phosphoric Acid (PDG)	117.2	0.63	34.8	1.5	—	—
<b>Bleached</b>						
WDG	0.21	0.23	5.6	—	—	—
PDG	0.19	0.59	4.1	0.87	—	—
<b>Deodorized</b>						
WDG	0.25	—	—	0.25	—	0.07
PDG	0.22	—	—	0.38	—	—

Adapted from Diosady et al. (48) and Elson et al. (49).

oils. It is evident from the data in Table 11 that processing reduces the amount of toxic and damaging trace elements, particularly lead, iron, and sulfur. Phosphorus and calcium form salts, which are insoluble in the oil and can be readily removed during the degumming process.

Sulfur in canola oil is in the form of organic compounds, which are decomposition products of glucosinolates. Although these sulfur components occur in trace quantities, they are known to inhibit catalysts used for hydrogenation as well as giving a characteristic odour to the oil. Recent developments in analytical methods for sulfur content evaluation revealed that soybean, sunflower, and even coconut oils all contain sulfur at a level of 2–10 mg/kg. Only *Brassica* oils contain significant quantities of divalent sulfur components. Crude canola oils may contain 15 to 35 mg/kg of sulfur, whereas in RBD canola oils, the amount of sulfur compounds is reduced to 9 mg/kg or lower (50). Sulfur components may also improve the stability of the oil. Some of these components can act as antioxidants and protect the oil from auto-oxidation by complexing hydroperoxy radicals with the sulfur to form stable compounds (51). Other positive action of these compounds consists of inactivating catalysts involved in oxidative processes, such as metals (51).

#### 4.10. Commercial Crude Oil, Refined and Deodorized Oil

A typical chemical composition of crude, refined, and deodorized canola oils is presented in Table 12. The deodorized oil data represents the oil quality used as a food ingredient.

The values for the crude oil compare closely with those of other commercial oils, such as soybean oil, when produced according to good extraction practices. Chlorophylls and sulfur compounds levels are higher in canola oil compared with most other commodity oils. The deodorized oil data reflect good refining practice and are similar to the data obtained with other deodorized commodity oils processed for food applications.

**TABLE 12. Typical Chemical Analysis Data of Crude and Refined, Bleached, and Deodorized (RBD) Canola Oil.**

Parameter	Crude oil	RBD
Free fatty acids, %	0.3–1.2	0.03
Phosphorus, mg/kg	300–500	<2
Water degummed	120–200	—
Acid-water degummed.	10–40	—
Chlorophyll, mg/kg	4–30	<0.025
Sulfur, mg/kg	2–15	<1
Iron, mg/kg	0.5–1.5	<0.2
Copper, mg/kg	<0.2	<0.02
Nickel, mg/kg	—	<0.3
Peroxide value, me/kg	0.5–3.0	0 (freshly deodorized)
Anisidine value	1–3	<2
Color, Lovibond	—	<1.5 Red/10 Yellow
Moisture, %	<0.3	—
Flavour	—	bland

Adapted from T. Mag (Unpublished data).

#### 4.11. Oxidative Stability

The stability of canola oil is limited mostly by the presence of linolenic acid, chlorophyll, and its decomposition products and other minor components with high chemical reactivity, such as trace amounts of fatty acids with more than three double bonds. These highly unsaturated fatty acids can possibly be formed during refining and bleaching (52). The presence of 7% to 11% of linolenic acid in the acylglycerols of canola oil places it in a similar category with soybean oil with respect to flavor and oxidative stability. The deterioration of flavor as the result of auto- and photo-oxidation of unsaturated fatty acids in oils and fats is referred to as oxidative rancidity.

The solubility of oxygen in oil is about 3–5 times greater than in water. The amount of oxygen present in oil, dissolved during manipulation, is sufficient to oxidize the oil to a peroxide value of around 10 (53, 54).

The rate of oxidation of fats and oils is affected by the oxygen partial pressure, access of oxygen, the degree of unsaturation of fatty acids, the presence of light, heat, antioxidants, and pro-oxidants such as copper, iron, and pigments. The optimal stability of oil was achieved when iron and copper were below 0.1 and 0.02 ppm, respectively (28).

The degradation of oils and fats by light exposure is primarily a photocatalyzed oxidation. During photooxidation, singlet oxygen is generated by transformation of energy to a sensitizer, which activates oxygen. Singlet oxygen is an extremely reactive specie of oxygen, 1500 times more reactive than normal oxygen, and reacts with double bonds of unsaturated fatty acids to form peroxides or free radicals. Typical photosensitizers include chlorophylls and their decomposition products formed during maturation of seed and processing, heme compounds, and polycyclic aromatic

hydrocarbons (28). It has been found that chlorophyll degradation products are more active as photosensitizers than chlorophyll (55).

## 5. PHYSICAL PROPERTIES

The properties of canola oil are governed by the components present in the oil and described by the general standards for vegetable fats and oils. Selected physical properties for canola oil in comparison with HEAR oil are shown in Table 13.

### 5.1. Relative Density

Typical values for the specific gravity of canola oil are shown in Table 13. Ackman and Eaton (56) indicated that a different proportion between eicosenoic (C20:1) and octadecanoic, polyunsaturated fatty acids could be a major factor in changing the relative density of canola oil. Nouredini *et al.* (57) described relationship between temperature and density of vegetable oils including canola. As for other liquids, the density for vegetable oils is temperature dependent and decreases in value when the temperature increases. The same authors also modified the Rackett equation to calculate density, which is based on the composition of fatty acids.

### 5.2. Viscosity

Viscosity values estimate the relative thickness or resistance of an oil to flow. The viscosity of RBD canola oil was higher than for soybean oil.

Lang *et al.* (58) and Nouredini *et al.* (59) found the viscosity of canola and other vegetable oils was affected by temperature and proposed an equation to calculate viscosity in the temperature range of 4–100°C. The viscosity of HEAR oil is significantly higher than that of canola oil.

**TABLE 13. Physical Properties of Canola and Rapeseed Oils.**

Parameter	Value	
	Canola	HEAR
Relative density (g/cm <sup>3</sup> ; 20°C/water at 20°C)	0.914–0.917	0.907–0.911
Refractive index (n <sub>D</sub> 40°C)	1.465–1.467	1.465–1.469
Crismer value	67–70	80–82
Viscosity (kinematic at 20°C, mm <sup>2</sup> /sec)	78.2	84.6
Cold test (15 hrs at 4°C)	Passed	Passed
Smoke point (°C)	220–230	226–234
Flash point, open cup (°C)	275–290	278–282
Specific heat (J/g at 20°C)	1.910–1.916	1.900–1.911
Saponification number	188–192	168–181
Iodine value	110–126	97–108

Abbreviations: HEAR—High erucic acid rapeseed oil.

### 5.3. Smoke and Flash Point

The smoke point is the temperature at which a fat or oil produces a continuous wisp of smoke. This provides a useful characterization of its suitability for frying, and often 200°C is specified as the minimum by regulations (Table 13).

The flash point defines the temperature at which the decomposition products formed from frying oils can be ignited. This temperature ranges from 275°C to 330°C for different oils and fats (Table 13). An increase in the content of unsaturated fatty acids usually decreases the flash and smoke points (60).

### 5.4. Cold Test

The cold test measures the resistance of oil to the formation of sediment at 0°C or 4°C (Table 13). Sediment formation is usually caused by compounds with a high melting temperature, mainly waxes and triacylglycerols with long-chain saturated fatty acids (15). The formation of haze in canola oil is not a common occurrence, but it may happen occasionally (2). It has been observed that canola oil produced from seeds grown in dry/drought conditions develop sedimentation more easily. This may be related to the higher content of saturated fatty acids formed as a response to drought stress conditions (15).

### 5.5. Crismer Value

The Crismer value (CV) measures the miscibility of an oil in a standard solvent mixture, composed of *t*-amyl alcohol, ethyl alcohol, and water in volume proportion 5:5:0.27 (Table 13). This parameter is one of the specification criteria used for international trade, mostly in Europe; however, today it is rarely used. The values obtained are characteristic within a narrow limit for each kind of oil. The miscibility of oil is related to the solubility of acylglycerols and is affected mainly by the unsaturation and chain length of the constituent fatty acids.

### 5.6. Saponification Value

The saponification value is defined as the weight of potassium hydroxide, in milligrams, needed to saponify 1 g of fat. This parameter is inversely proportional to the molecular weight of the fat. In other words, the higher the molecular weight, the lower the saponification value. Replacement of long-chain fatty acids such as erucic acid in canola oil by octadecenoic fatty acids increased the saponification numbers from 168–181 to 188–192 because of the reduction in molecular weight (Table 13).

### 5.7. Iodine Value

The iodine value (IV) is an empirical test indicating the degree of unsaturation of a fat or oil. It is defined as the number of grams of iodine absorbed by 100 g of fat.

**TABLE 14. Melting Characteristics of the Octadecanoic Fatty Acid Family.**

Fatty Acid	Melting Point (°C)
Palmitic	64.5
Stearic	69.6
Oleic ( <i>cis</i> 9-octadecenoic)	13.2
Elaidic ( <i>trans</i> 9-octadecenoic)	43.7
Octadecenoic ( <i>cis</i> 6)	28.6
Linoleic ( <i>cis</i> 9, 12)	-5.1
Linolenic ( <i>cis</i> 9, 12, 15)	-11.2

Adapted from Mag (2).

The higher value for canola oil is caused, in part, by the replacement of erucic acid with octadecenoic acids, mainly oleic acid, accompanied by a slight increase in linoleic and linolenic acids (Table 13). The iodine value can also be calculated from fatty acid composition using the specific factors for each unsaturated fatty acid (61). The calculation method provides more accurate data than the iodine absorption assessment.

### 5.8. Melting Characteristics, Polymorphism, and Crystal Properties

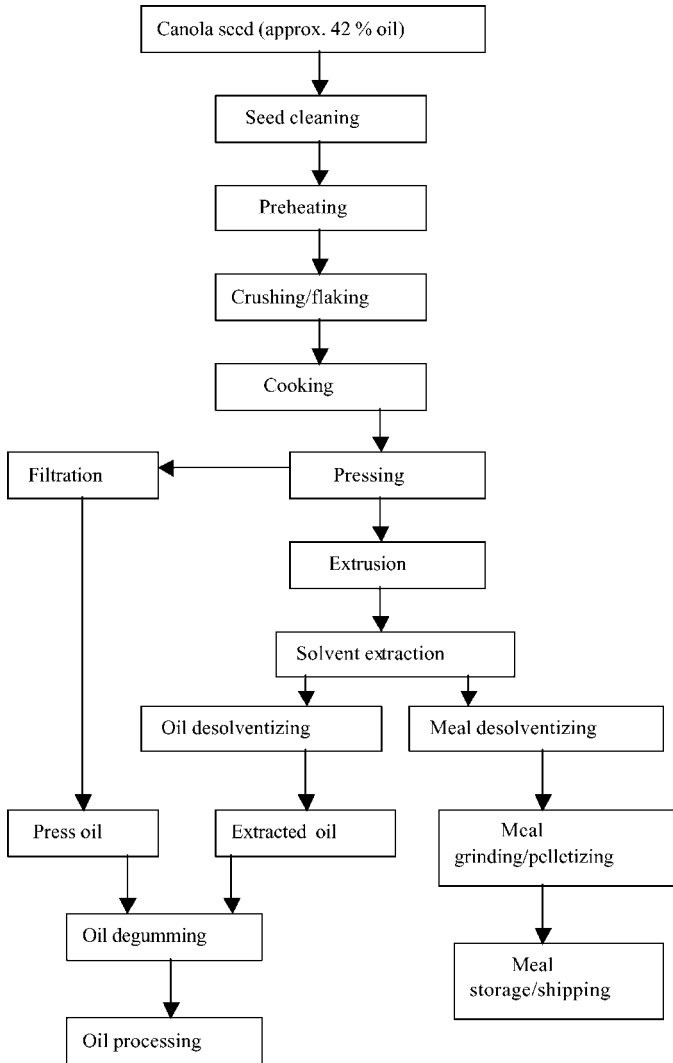
Canola oil has a homogeneous fatty acid composition, with 95% being contributed by octadecanoic fatty acids (4). Reducing the erucic acid content had a marked effect on the melting characteristics and the type of crystal formed when the oil is hydrogenated. Hydrogenation of canola oil is used to form the products such as shortenings and margarines. Increasing the degree of hydrogenation causes the fatty acid composition to be more homogeneous. This results in a tendency to form large beta crystals on solidification, which is undesirable in margarines and shortenings. *Trans*-isomers formed during hydrogenation have higher melting points than *cis* fatty acids (Table 14) (62). *Trans*-isomers introduce greater variety in the fatty acid composition of the hydrogenated oils, thereby reducing the beta crystallization tendency of the oil.

## 6. CANOLA OIL EXTRACTION AND PROCESSING

Processing methods developed over the years are designed to extract canola oil from the seeds to produce a high-quality raw oil for further processing and a high-quality protein meal as an animal feed.

### 6.1. Canola Oil Extraction

Canola seeds contain 38–44% oil (8% moisture basis) so that the most efficient method of extracting the oil is by mechanically (pressing) expelling about 60% of



**Figure 1.** Canola seed extraction.

the oil, which is followed by solvent extraction of the remainder. A similar method is applied to other seeds of high oil content, such as sunflower seeds. Figure 1 gives an overview of the process sequence most commonly applied in canola seed extraction.

**6.1.1. Seed Cleaning** Canola seed is generally received at the extraction plant directly from the farm, or from a seed gathering station. Weed seeds, grain seeds, and other foreign material must be removed from the seed before extraction of the oil. The seed cleaning process consists of three stages: aspiration to remove dust



and very light material, screening to remove oversized particles, and rescreening to remove undersized material. The equipment usually comes as one unit, and the removal of foreign material is to less than 2.5%. Disposal of the separated material is used primarily in animal feed.

**6.1.2. Preconditioning** This is essentially a preheating step to bring the seed temperature before flaking to about 30–40°C. Under these conditions, the seeds are more pliable and thus less likely to shatter during flaking seeds. Preheating equipment may be of the fluid bed type using hot air or steam, direct, or indirect heat in rotary kilns equipped with steam-heated coils.

Moisture content in the seed can be adjusted, if required, during this process step by regulating the airflow through the equipment. The desired moisture content prior to flaking is in the range of 7.0–9.5%.

**6.1.3. Flaking** The preheated canola seed is flaked on smooth-surface rolling mills. Some operators advocate flaking in two stages. In the first stage, a flake thickness of about 0.4–0.7 mm is produced. In a second set of rolls, a flake of 0.2–0.3 mm thickness is produced. The main reason for two-stage flaking is the small size and the deformability of the seed, which usually allows some whole seed to pass through in a single-stage flaking. However, many plants operate single-stage flaking with satisfactory results.

Flaking ruptures the cell walls, which allows some of the oil to be separated from the seed residue by simple pressing. Further, the oil retained in the seed residue can now be more efficiently leached out in subsequent solvent extraction of the press cake with hexane. Properly flaked material is more easily treated in the subsequent cooking operation and requires less mechanical energy in pressing. It is essential that the residual oil content in the meal be low for this operation to be economical.

**6.1.4. Cooking** The flaked material is subjected to a cooking step. In this process, the flakes are heated either indirectly on steam-heated surfaces in stack cookers or in rotary cookers equipped with steam coils. Cooking temperatures are usually held to 75–100°C. Ambient air may be admitted to the cooking equipment to adjust the moisture content of the material to 5–7% for the expeller operation.

Cooking serves several important functions. It coalesces minute oil droplets into larger ones, which can be easily separated as well as changes the properties of the protein so as to make the oil more easily extractable. But temperature and heating time must be carefully controlled to avoid negative effects on color and sulfur levels in the oil, protein degradation, and the percolation properties of the flaked material.

Cooking also inactivates two key enzymes. The first is myrosinase, which hydrolyses glucosinolates into oil-soluble sulfur-containing compounds. The second is phospholipase, which hydrolyses phospholipids into nonhydratable phosphatides. Myrosinase activity has been extensively studied and is carefully controlled during the cooking operation that minimizes hydrolysis of glucosinolates and reduces

the presence of sulfur in the oil (63, 64). The optimal temperature range for the myrosinase activity is between 50°C and 70°C with moisture content in the range of 5–7%.

Phospholipase activity as affected by cooking operations has also been a subject of interest but to a lesser extent. Dahlén and Kristofferson (65) found that as cooking temperatures were raised from 85°C to 100°C, nonhydratable phospholipids content decreased in the oil, whereas total amounts of phosphorus, color and sulfur increased. Higher color and sulfur are undesirable as increased sulfur content impairs the catalyst in hydrogenation of the oil. Unger (66) showed that not only total phosphorus, but also free fatty acid amounts, increased with higher cooking temperatures. Current cooking operations use temperatures in the range of 75–100°C. The sulfur content of canola oil is in the order of 2–15 mg/kg as determined by inductively coupled plasma (ICP) spectroscopy. It is apparent, that in practice the selection of cooking temperature is a compromise between opposing effects on oil quality and economics.

Process equipment for seed cooking other than the stack cookers and rotary cookers described above are also used in the seed extraction industry. Three processes are worth noting, even though there is no known application of these in pretreating in canola/rapeseed processing: the Alcon process by Lurgi of Germany, the Exergy process by Stork of Sweden, and the use of expanders.

All of these cooking processes have in common that they are designed to heat the material very quickly in a few minutes or even seconds. This avoids the main shortcomings of stack cookers and rotary cookers, long residence times in the range of 10–30 minutes. In these processes, rapid heating makes it possible to inactivate enzymes very quickly and to expose the seed or flakes to an increased temperature for very short time. Rapid heating depends on very good mixing of the seed material during heating, and results in very good temperature control, uniform heat treatment, and prevention of enzyme-catalyzed damage to the oil in the seed. An unexpected bonus of these heat treatments is the formation of the oilseed with high porosity, which facilitates improve oil yield during solvent extraction.

*The Alcon process.* This process is being used with soybean flakes. It was the first commercially applied rapid cooking method, and its main purpose was to inactivate phospholipases. Soybean oil from Alcon-treated soybean flakes is free of nonhydratable phosphatides, and water degumming is sufficient to completely remove phospholipids. In the treatment, soybean flakes are introduced into a tower, where direct steam with intensive agitation provides rapid heating of the seeds and the required temperature to inactivate enzymes is achieved uniformly in a few minutes. The heated flakes are discharged into a cooling chamber before the hexane extraction. To our knowledge, this process is not commercially used for canola seed processing. Details of this process were described by Penk (67).

*The Exergy process.* In this process, whole seeds or flakes are suspended in superheated steam at high pressure when they are moved turbulently through a heated pipe loop. This heat treatment requires only a few seconds to achieve the temperature needed to inactivate enzymes (68). In the case of canola, whole seed or flakes can be treated this way. Heat treatment of whole seeds prevents enzymatic

degradation of oil, which occurs after flaking when content of seed is exposed to an enzyme's action. The heated seeds are cooled before going on to flaking or extraction. The process was originally developed for efficient drying of waste sludges and is being used in Scandinavian countries to produce high-bypass protein canola meal. It has been proposed as pretreatment for canola seeds before extraction. Some commercial applications may in fact exist in Scandinavia. The cost of the equipment is an obstacle to wide application.

*Extruders.* Extruders, or expanders, are widely applied in the oilseed industry, but mostly for press-cake conditioning in canola oil extraction, and for extruding soybean flakes into expanded collets for improved extraction yield. Extruders as cookers are used in some soybean extraction plants to achieve enzyme inactivation. Lusas investigated inactivation of enzymes during extrusion of soybean (69). Extruders could be used to pretreat canola seed to assist prepressing and to inactivate the enzymes; however, commercial application of this process in canola industry is not known.

**6.1.5. Pressing/Expelling (Prepressing)** The heat-conditioned, flaked seed is conveyed to continuous screw-presses or expellers. The function of the expellers is to reduce the oil content of the seed from about 42% to 16–20%. Extraction of the remaining oil is then much more efficient and economical. Some discharge of very fine solids (7–15%) with the oil draining from the expeller is unavoidable. These fines are separated from the oil by gravity in settling tanks followed by filtration and recycled to the conditioning stage. The press-cake, which may be put through a mechanical breaker to produce uniform-sized particles, is now ready for solvent extraction. Unger (66) and especially Buhr (70) have described the operation of expellers with canola seed in detail.

**6.1.6. Extruders** In some plants, the press-cake is subjected to mechanical extrusion to improve its solvent extraction properties. Extruders (expanders) used for this purpose consist of a barrel with a rotating shaft fitted with flights. Steam is added, and heating and mixing take place along the length of the barrel. Pressure is developed, and the material is then discharged through the small openings of a die plate at the end of the barrel. The pressure release on discharge “expands” the extruded material, making it very porous. These small-diameter, porous pieces of press-cake (collets) have excellent solvent extraction properties. As a result, throughput of the solvent extraction equipment is significantly enhanced.

**6.1.7. Solvent Extraction** The press-cake from the expellers and cake breakers, or as collets from extruders, is conveyed to the solvent extraction stage. Some cooling of the 80–100°C material with ambient air is usually done during conveying to minimize vaporization of hexane when the cake enters the extractor. A variety of extractor designs are in use, often made by the same firms supplying expellers. The solvent used is extraction hexane at 50–60°C.

In the extractor, the solids, which are at about 80°C, are solvent-washed in stages, first with hexane already high in oil content (miscella) and then with progressively

leaner miscella and, finally, with pure hexane. The various extractor designs approximate countercurrent extraction to a greater or lesser extent. The oil content in the solid material (meal) is reduced down to about 1%, depending somewhat on equipment design and throughput rate and on how well the cooking, flaking, and prepress operations were carried out. The flow rate of hexane to the extractor is adjusted to provide an oil concentration of 25–30% in the oil-in-hexane solution (miscella).

**6.1.8. Desolventizing of Meal and Oil** The meal and the miscella are “stripped” of the solvent to recover solvent-free meal and oil. The solvent-saturated meal is conveyed to a desolventizer, which is a vertical tank equipped with steam-heated trays and rotating sweep arms just above each tray to agitate the solid material. Reduced pressure and some live steam sparging are used to evaporate the hexane and to dry the meal. The meal is heated and “toasted” to about 105°C with residence time of about 30–40 minutes. Residual hexane in the meal is in the order of 1500 mg/kg, and moisture in the range of 15–18%. Lower hexane concentrations are difficult to achieve. Grant et al. (71) described factors affecting canola meal desolventizing (68). Schneider and Schuette (72) have investigated the difficulties in desolventizing of canola meal in relation to fiber structure. Some removal of glucosinolates, their breakdown products, as well as protein denaturation occurs (73). To achieve the best meal quality, the process must be well controlled with respect to temperature (110°C max.) and time.

Meal cooling and drying is an integral part of the desolventizer (Desolventizer-Toaster-Dryer-Cooler) or separate rotary kilns. The temperature is reduced to 30–40°C, moisture to 8–11%, and hexane to about 800 mg/kg. This corresponds to a hexane loss of approximately 1 liter per metric ton of seed processed. Cooled meal may be ground to a uniform particle size, or pelleted, ready for storage and marketing. The hexane and moisture vapors are vented from the meal desolventizing equipment and condensed, the water and hexane are separated, and the hexane is reused. The miscella containing the oil is desolventized in three-stage evaporator equipment. The hexane vapor from this operation is condensed for reuse. A properly sized and operated extraction plant loses no more than about 1–2 liters of hexane/ton of seed processed.

**6.1.9. Quality Assurance: Seed, Oil and Meal** The first step in the quality assurance process is the grading of seed deliveries from the farm to the extraction plant. In Canada, for example, the Canadian Grain Commission as a third party is responsible for setting the grading rules by which the industry conducts trades. The rules are published in the Official Grain Grading Guide by the Office of the Chief Grain Inspector, Inspection Division, Winnipeg, Canada (74). Three grades, from No. 1 to No. 3, are distinguished, with No. 1 being best. Poorer grade seed, which may sell at a discount, produces lower quality oil. This is especially evident in higher chlorophyll and free fatty acid content. Nonhydratable phosphatide content may also be higher with poorer grades. All of these factors make for greater difficulty and higher costs in oil processing.

**TABLE 15. Canola Meal Specifications (75).**

Characteristic	Specification
Protein (weight %)	min. 36
Fat (oil) (weight %)	max. 4
Moisture (weight %)	max. 12
Crude fiber (weight %)	max. 12
Glucosinolates ( $\mu\text{mol/g}$ )	max. 30
Screen analysis	97 weight % of the meal shall pass through a 2.00-mm (US # 10) sieve, and 90 weight % shall pass through 1.70-mm (US # 12) sieve.

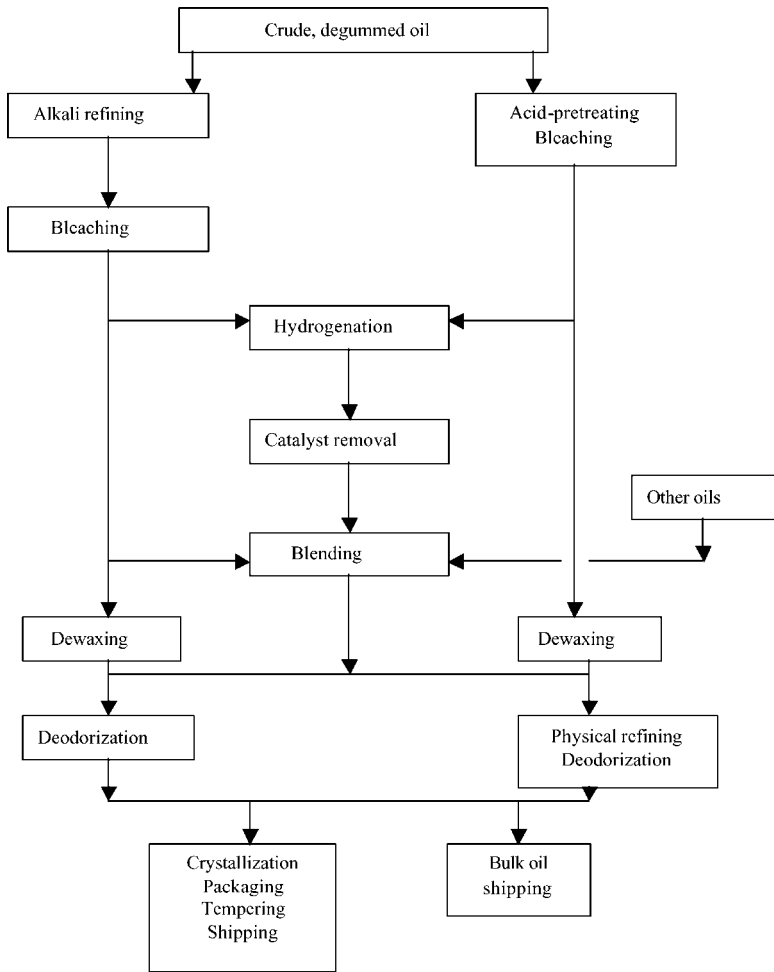
Canadian quality assurance on crude oil, degummed oil, and meal is governed by the Trading Rules of the Canadian Oilseed Processors Association (COPA) (75). Official AOCS methods are used to analyze quality parameters (76). Glucosinolate determinations on meal are not usually done. Typical properties of oil and meal are given in Table 1 and Table 15, respectively.

## 6.2. Canola Oils Processing

Processing of crude canola oil to edible oil products is very similar to that applied to other vegetable oils. Figure 2 gives an overview of the process steps that are applied in the industry.

**6.2.1. Degumming** The “crude” oil from prepressing and from solvent extraction is usually blended and then degummed before being stored for sale or further processing. Degumming removes phosphatides coextracted with the oil, as they tend to separate from the non-degummed oil as sludge during storage. The phosphatide content of crude oil varies, but it is usually in the order of 1.0–1.5% or, measured as phosphorus, 400–600 mg/kg. Two main degumming methods are in use: (1) using water to precipitate phosphatides (water degumming) and (2) using an acid such as citric, malic, or phosphoric and water (super-degumming, or acid-water degumming) to achieve nearly complete degumming. After contacting the oil with these reagents, the oil is centrifuged to separate the precipitated material. Other degumming processes for achieving nearly complete degumming are also applicable, such as enzymatic degumming, but so far has seen only very limited use.

**6.2.1.1. Degumming with Water** Degumming with water only involves contacting the oil at about 80°C with about 2% water in a gently agitated tank with a 5–30-minute contact time, to keep the precipitated phosphatides in suspension and allow time for agglomeration, and then separating in a disk centrifuge. This leaves from 100–200 mg/kg of phosphorus (0.25–0.5% phosphatides) in the oil, depending on the extent to which nonhydratable phosphatides are present. The oil lost with the separated phosphatides is in the order of 35–40% of the separated material.



**Figure 2.** Canola oil processing.

**6.2.1.2. Degumming with Acid and Water** In degumming with acid and water, the nonhydratable phosphatides (NHP) are also removed. A number of process versions, which have been developed over the years, are suitable, such as the Unilever Super-degumming (acid degumming) process (77) and various other proprietary processes. Reaction conditions are much more critical than in water degumming. Usually, the following sequence of steps, with some variations, are applied: Crude oil is brought to about 60–70°C and then contacted with about 0.1–0.5% of a 50% solution of citric or malic acid with very intensive mixing. Contact may vary from fractions of a second to about 15 minutes, depending on mixing intensity. The oil is then contacted with about 2% water, either before or after cooling to 25–45°C. About 1–3 hours of residence time is then given in a continuous, stirred reactor.

Agitation in the reactor is designed to avoid shear and keep the precipitated phosphatides uniformly suspended. The oil/phosphatide mixture is then heated very rapidly, immediately ahead of a self-desludging disk centrifuge, in which the precipitated phosphatides are separated from the oil. The oil loss in this process represents from 35–50% of the separated phosphatides, with the higher losses occurring with oils that are relatively high in nonhydratable phosphatides. The separated phosphatide phase is added to the meal in the desolventizer. This raises the residual oil content of the meal to about 2–3% and raises its energy content.

Residual phosphatide concentrations are from 5–50 mg/kg of phosphorus depending on process details and the level of nonhydratable phosphatides in the crude oil. Nonhydratable phosphatides can vary widely, but are usually in the range of 20–45% of total phosphatides. With higher concentrations of nonhydratable phosphatides, longer agglomeration time is required to achieve clean separation in the centrifuge.

In the most recent development, acid and aqueous sodium hydroxide, rather than acid and water are being used, especially with lower quality oils (acid-caustic degumming). This represents an intermediate between acid-water degumming and alkali refining. It obviates the need for cooling, similar to the practice in alkali refining. Phosphoric acid rather than an organic acid is preferred in this case. Phosphatides as well as some of the other impurities are removed, and if sufficient, alkali is applied to saponify the free fatty acids, fully refined oil is obtained.

**6.2.2. Alkali and Physical (Steam) Refining** Degummed oil is further purified in a process of refining. One of two methods are used, namely, alkali refining, especially with water-degummed oil, and physical refining with acid-water-degummed, or acid-caustic-degummed oil, that is, with the more completely degummed oil. Alkali refining is the most common process used, even with acid-water-degummed oil. Physical refining of canola oil on a plant scale is relatively rarely practiced. It requires well-degummed oil of moderate chlorophyll and free fatty acid content, but it is then economical, especially, with respect to capital investment. CanAmara Foods (now part of Bunge, Inc) in Canada has successfully operated a refinery in Western Canada based on physical refining since 1984.

**6.2.2.1. Alkali Refining** In alkali refining, the oil is first contacted with about 0.05–0.1% of concentrated phosphoric acid in a high-intensity mixer to help precipitate phosphatides. It is then contacted with an approximately 12% concentrated aqueous solution of sodium hydroxide to neutralize the free fatty acids present, any excess phosphoric acid and to precipitate phosphatides. This requires about 2–3% of the solution. Very intensive mixing is used. Temperatures and contact times may vary from about 90°C and only seconds for both acid and caustic in the “short-mix” process to about 40°C and about 15 minutes each in the “long-mix” process. The oil/soap mixture is then heated to about 90°C, if required, and centrifuged to separate the aqueous soap phase. This phase also contains the precipitated phosphatides and some triglyceride oil. Usually, self-desludging disk centrifuges are used to avoid frequent shutdowns for cleaning. The centrifuged oil must be further

contacted with about 5–10% hot, soft water to reduce soap levels from about 500 mg/kg to <50 mg/kg. Solid bowl disk centrifuges are adequate for this service.

Alkali refining reduces free fatty acids to <0.05% and phosphorus to <3 mg/kg. Iron and copper concentrations are reduced to below the detection limit. Colored compounds (chlorophyll derivatives, carotenoids) are not affected significantly, although chlorophyll derivatives can be reduced by 30–70% of the original concentration under certain process conditions. The concentration of sulfur compounds is reduced slightly. This oil is now ready for bleaching. The soap phase from this operation can be added to the meal, similar to the disposal of the phosphatides from degumming, or it can be acidulated and used as a feed fat. Mag (78) has reviewed industrial refining practice in some detail. Carlson (79) reviewed refining methods and process costs.

**6.2.2.2. Physical Refining** In physical refining, acid-water-degummed oil with phosphorus content below 50 mg/kg and preferably below 25 mg/kg is first subjected to a phosphoric acid pretreatment, as in the short-mix alkali refining process. It is then contacted with acid-activated bleaching clay in a standard bleaching process at 95–105°C. With precipitated phosphatides, chlorophyll derivatives and some carotenoids are adsorbed on the clay and removed by filtration. Together with the acid-water degumming of the oil, this is the most important stage of physical refining. It delivers bleached oil ready for physical (steam) refining/deodorizing. Except for the free fatty acids in the oil, all other minor constituents are now at the same concentrations as in alkali-refined oil, and in addition, chlorophyll derivatives are reduced to the concentration required of bleached oil, namely, <0.050 mg/kg. Usually, from 0.7% to 2% of acid-activated bleaching clay is used, depending primarily on the concentration and type of chlorophyll derivatives and the residual phosphatide concentration that was present in the degummed oil.

The removal of the free fatty acids in the bleached oil is done by steam distillation in a deodorizer. This, simultaneously, deodorizes the oil. Because deodorization is, also, the last process normally carried out on edible oils, this step may be delayed until other processes, such as hydrogenation of the oil, have been done.

**6.2.3. Adsorptive Bleaching** Alkali-refined oil requires bleaching. It usually contains traces of soap, and the concentration of chlorophylls and their derivatives is still essentially that present in the crude oil. Some of the oxidation products and traces of heavy metals, notably, iron are also removed. But, the most important effect to be achieved with canola oil is the removal of chlorophyll derivatives. These compounds accelerate oil oxidation in presence of light (80) and give an undesirable green color to the oil. Acid-activated clays are used in bleaching. Their adsorptive properties are especially effective for the removal of “green” compounds. With high chlorophyll concentrations, >30 mg/kg, “chlorophyll specific” clays have been used. One feature of these clays is that they are especially highly acid activated. As with other oils, the adsorption of carotenoids on bleaching clays is not efficient. Some reduction occurs, partly because of adsorption and partly because of heat breakdown. In usual bleaching practice, the emphasis is on



chlorophyll removal. Heat bleaching of the carotenoids during deodorization is relied on to remove most of the red color present in the oil.

**6.2.3.1. Adsorptive Clay** Adsorptive bleaching is carried out under vacuum with the oil at about 100°C. About 5–30 minutes of contact time is given, while the oil/clay slurry is progressively dried to about 0.1% moisture content. This appears to give the best adsorption efficiency. As indicated earlier, about 0.7–2% clay (10–15% moisture content) may be required to achieve chlorophyll removal to <0.050 mg/kg measured as chlorophyll *a*. This level of chlorophyll derivatives is innocuous with respect to oxidation and color of the oil. Mag (81) has given an overview of edible oil bleaching practice, with particular attention to canola oil. Brimberg (82) and Henderson (83) have investigated kinetic aspects of chlorophyll adsorption important to the process. Suzuki and Nishioka (84) investigated changes in chlorophyll derivatives during seed and oil processing up to the bleaching stage and determined the ease of adsorption of the various chlorophyll derivatives on activated clay and on active carbon. With acid-activated clay, they found the ease of adsorption to decrease in the following order: pheophytin *a* > pyropheophytin *a* ≫ pheophytin *b* > pyropheophytin *b*. Chlorophyll *a* and *b* are converted to their respective pheophytins early in seed processing. Thus, they do not play a significant role in the adsorption of chlorophyll derivatives. Bleaching processes suitable for canola oil do not differ from those used for other oils.

**6.2.3.2. Active Carbon** Active carbon is rarely used to remove chlorophyll compounds is used very little in canola oil bleaching. It presents greater difficulty in handling and it retains more oil than activated clays do and is much more expensive. In bleaching efficiency tests, active carbon has been shown to be somewhat more efficient than activated clay at high concentrations of chlorophyll compounds in canola oil, but less efficient at very low concentrations. Thus, active carbon is not a suitable adsorbent to achieve the removal of chlorophyll derivatives to the very low concentrations mentioned earlier.

**6.2.3.3. Synthetic Silicas** In recent years, the use of synthetic silicas as a pretreatment in the bleaching of oils has been advocated. In connection with canola oil bleaching, it is claimed that because of the ability of these silicas to adsorb soap and phosphatides more efficiently than acid-activated clays, adsorption of chlorophyll derivatives by the clays in subsequent bleaching is much more efficient. Presently available silicas do not adsorb chlorophyll derivatives or carotenoids. Parker (85) working for W. R. Grace & Co.-Conn., a manufacturer of silicas, is advocating a synthetic silica pretreatment followed by packed bed bleaching with acid-activated clay.

**6.2.3.4. Other Methods to Remove Chlorophyll Derivatives** Other approaches to the removal of chlorophyll derivatives compounds from canola oil than adsorption are known and are being practiced. Bergman (86) and Szemraj (87) described the use of concentrated phosphoric acid and vacuum drying of the oil to precipitate

**TABLE 16. Bleached Canola Oil After Alkali Refining or Acid Degumming.**

Characteristic		Alkali Refined/Bleached	Acid Degummed/Bleached
Free fatty acids	(%)	0.1	as for crude
Phosphorus	(mg/kg)	<3	<3
Chlorophyll	( $\mu$ g/kg)	<50	<50
Iron	(mg/kg)	<0.1	<0.1
Peroxide value	(meq/kg)	Nil	Nil
Anisidine value		1–3	1–3
Sulfur	(mg/kg)	1–13	1–13
Soap	(mg/kg)	Nil	Nil

impurities from rapeseed oil, especially chlorophyll derivatives compounds. Experience has shown that the precipitation of chlorophyll is much more efficient with super-degummed canola oil. The removal of the precipitated material from the oil can be by filtration on a suitable filter aid, or by centrifuging. When the precipitation step is followed by alkali refining of the oil, the acid-precipitated chlorophyll derivatives are simply removed as part of the heavy phase in the centrifugal soapstock separation.

Another approach is to make use of the fact that in contact with alkali, chlorophyll derivatives also react to form a precipitate (88). This can be used in the course of alkali refining. The precipitate can be removed together with the aqueous soap phase, but at this time, specific details are still proprietary. Experience so far has shown that as much as about 70% of the chlorophyll derivatives can be removed in the course of alkali refining.

Typical properties of alkali-refined, bleached canola oil and of acid-water-degummed, acid pretreated, bleached canola oil ready for hydrogenation or steam refining/deodorization are given in Table 16. With the exception of the concentration of free fatty acids, the two process routes produce the same bleached oil quality.

**6.2.4. Hydrogenation** Hydrogenation changes the melting behavior of oils and improves their oxidative stability. It is applied to many oils, including canola. Hydrogen is added to the double bonds of unsaturated fatty acids at temperatures of 160–200°C and pressures of 100–300 kPa in the presence of a nickel catalyst to facilitate the reaction. Chlorophyll (89), phosphatides, soaps, and especially sulfur compounds “poison” the catalyst, raising process costs. These are removed in refining and bleaching, except sulfur. Sulfur compounds are only reduced somewhat in these treatments. de Man et al. (90) investigated the role of sulfur compounds in nickel catalyst poisoning in canola oil hydrogenation and found that as little as 1 mg/kg of sulfur in the form of allyl isothiocyanate (AITC) had a noticeable effect on hydrogenation rate. Additions of 3 and 5 mg/kg of AITC had an even greater effect on the rate of hydrogenation, and *trans*-isomer formation was very significantly increased in the range of iodine values from 95 to 80. AITC is typical of compound formed from the breakdown of glucosinolates.

**TABLE 17. Hydrogenated Canola Oils for Margarines, Shortenings, and Frying Fats.**

Iodine	Fatty Acid Composition (%)						SFI at °C				
	C16:0	C18:0	C18:1	C18:2	C18:3	Trans	10.0	21.1	26.7	33.3	40.0
Value											
115(L)	4.5	2	60	22	10	—	—	—	—	—	—
90	4.5	3	80	9	2	20	2.5	—	—	—	—
85	4.5	5	86	2	1	42	18	5	—	—	—
80	4.5	11	83	—	—	45	30	18	7	<1	—
75	4.5	14	80	—	—	50	50	31	25	11	4

The determination of sulfur compounds in the range of concentrations, which occur in edible oils, is difficult. The literature on concentrations found in rapeseed and canola oils can be confusing. Considering relevance to hydrogenation effects and speed of analysis, ICP spectroscopy for determining sulfur in canola oil is presently the most appropriate method.

The process of hydrogenation of canola oil is usually carried out batch-wise. There are various reactor designs in use. Often, in-house designs are used, but many companies in the field of supplying the edible oil industry with process equipment are able to furnish proven hydrogenation and catalyst removal equipment.

Depending on temperature, pressure, and, to some extent, the specific catalyst properties, a different melting behavior of the hydrogenated fat can be obtained. The oxidative stability improvement, also, is modified somewhat by choice of process conditions. Usually, conditions are chosen that provide this selectivity toward the desired melting behavior and oxidative stability improvement. In canola oil, very low concentrations of linolenic and linoleic acid can be achieved with very little hardening of the oil (Table 17). Melting behavior is most usually evaluated by determining the proportion of solid fat in a sample over the temperature range of interest, usually 10–40°C. Dilatometry is still extensively used in North America for this purpose, rather than the commonly used and more modern wide-line nuclear magnetic resonance spectroscopy.

Because of the small concentration of sulfur compounds in canola oil, there is a somewhat greater tendency of sulfur poisoning of the catalyst, with the result that slightly higher *trans*-isomer concentrations may come about compared with, for example, soybean oil. Hatfield (91) has focused on this aspect of canola oil hydrogenation.

Removal of the nickel catalyst from the oil is by filtration of the oil from the reactor after cooling to about 100°C on filter aid precoated filters. Adding filter aid to the oil and then filtering is also satisfactory. Nickel concentrations of 3–15 mg/kg are achieved. This is followed by a second filtration on a filter aid precoated filter, possibly after contacting the oil with a small amount of citric acid (0.05% as a 50% aqueous solution) as a chelating agent. In stubborn cases, it is necessary to bleach the oil with acid-activated bleaching clay. Nickel must be removed to below 0.3–0.5 mg/kg, that is, to Ni-negative by the ammonium sulfide test. Synthetic silicas have also been advocated as a treatment of hydrogenated oil for nickel removal either before or after catalyst filtration (92).

**6.2.4.1. Hydrogenated Canola Oil in Edible Oil Products** Hydrogenated fats from canola oil play an important role in the production of the wide range of fat products in the countries in which canola oil is used. They often appear in blends with other oils to combine the advantages of canola oil with those of other oils. They are especially important as lightly hydrogenated oils for their exceptional stability. This is caused, in part, by the high content of monounsaturated fatty acids in canola oil. These products are very popular because of their good stability and pourability and their low saturated fatty acid content. The introduction of nonhydrogenated high-oleic acid (75%) containing canola oil in the market may eventually reduce the importance of lightly hydrogenated oil.

Table 17 shows some typical iodine values, fatty acid compositions, and solid fat indices for hydrogenated canola oils used in margarine, shortening, and frying fat formulations. The nonhydrogenated, liquid oil data are given for comparison. It should be noted that the values given might differ somewhat in practice depending on hydrogenation conditions and the catalyst used.

The above hydrogenated canola oil stocks, or similar ones, are used extensively in shortenings and as stable frying fats. Industry makes a wide variety of canola-based hydrogenated oil stocks for tailor-made shortenings. Lightly hydrogenated canola oil (IV 90), for example, has a special advantage in that even at very low levels of solid fat, it has very low polyunsaturation, which makes it an excellent pourable frying fat base of very good stability. Very highly hydrogenated canola oils are rarely used in margarine and baking shortenings, because of their tendency to form large ( $\beta$ ) crystals over time. This impairs eating properties and baking performance. deMan and deMan (93) and Naguib-Mostafa (94) studied the crystallization behavior of hydrogenated canola oil used for margarine and shortening products. D'Sousa et al. (95) stated that at least 11% of palmitic acid is required when palm oil is used in hydrogenated canola oil-based margarines and shortenings to achieve crystal stability in the beta prime ( $\beta'$ ) form for adequate shelf life of margarine. Preferably, palm oil needs to be lightly hydrogenated.

**6.2.5. Interesterification** Interesterification is another process for changing the melting properties of fats and oils. The process represents an alternative to the use of partially hydrogenated fats in manufacturing products of a variety of melting properties.

Thomas (96) and Desrosier (97) investigated chemically catalyzed interesterification of nonhydrogenated canola oil with fully hydrogenated vegetable oils. In the manufacture of zero *trans*-isomer fat products, interesterification of canola and other oils with high melting fats is of interest to take advantage of the nutritionally favorable fatty acid profile of nonhydrogenated canola oil. The process has also been proposed for use in controlling the problem with beta crystallization of hydrogenated canola oil. However, this process has not found significant use because of cost. Kurashige et al. (98) used enzyme-catalyzed interesterification of palm oil with canola oil to improve the pourability of palm oil compared with merely blending with canola oil. They used a 1,3-specific lipase. Canola oil was used because of

its low content of saturated fatty acids. Industrial scale processes to carry out enzyme-catalyzed interesterification, however, are still rare.

**6.2.6 Dewaxing** Canola oil is a natural salad oil. This means that it remains clear and liquid at refrigerator temperatures. It is used without winterization to produce bottled oil and salad dressings. However, the oil may contain a small and variable concentration of compounds (about 20–400 ppm), which may over time appear as sediment in the deodorized oil. This appears to be dependent on seed growing conditions. For the sake of simplicity, the term "waxes" is applied to these compounds, but it is known (99) that about 20–40% of these compounds are not wax esters. The crystallization behavior of the mix of compounds that can crystallize from canola oil is unpredictable in that low concentrations (<50 mg/kg) can sometimes show up in the oil as a sediment and that the sedimentation can occasionally occur in a few days, or take several months.

For some markets, it is desirable to dewax the oil to avoid any chance of a hazy appearance. The process is carried out on the bleached oil by chilling in a continuous heat exchanger to about 5°C and metering about 0.1% of a filter aid into the chilled oil stream on the way to a filter. Little or no retention time is given before filtration. This reduces the wax content to <50 mg/kg, which then usually no longer produces a visible haze. Usually, in-house dewaxing process designs are used, or processes patterned after sunflower oil dewaxing installations. Throughput rates with canola oil are much higher than with sunflower oil, primarily because of the much lower wax content.

**6.2.7. Deodorization** In edible oil processing, this is the final "refining" step. Its primary function is to remove compounds from the oil, which impart odor and taste typical of the seed from which it is derived and any odoriferous compounds formed in such processes as bleaching and hydrogenation. Further, at the temperatures required, heat bleaching of the yellow-red carotenoid compounds is an important aspect of the process. As mentioned earlier, deodorization can also serve to physically refine the oil as an alternative to the removal of free fatty acids by alkali refining. Deodorization is essentially a steam distillation of the odor and flavor compounds from the oil, as well as other relatively volatile compounds such as free fatty acids. Tocopherols and sterols are also to some extent removed. In the deodorization of canola oil, it is important that the oil be essentially free of chlorophyll and phosphatidic material and of heavy metals such as iron and nickel. The oil is heated to 225–260°C under very low pressure (0.1–0.5 kPa) to exclude air. This increases the volatility of the compounds to be removed. Steam is blown through the oil (1–3%), which increases the volatility further and allows efficient removal of the volatiles to very low levels. Heat bleaching requires a minimum of about 30 minutes at deodorizing temperatures. It is usual practice to cool the deodorized oil to about 60–80°C after the process and to sparge it with nitrogen gas before storing in tanks, or shipping in bulk. Specification for the deodorized canola oil are presented in Table 18.

**TABLE 18. Nonhydrogenated, Deodorized Canola Oil.**

Characteristic	Value
Color (Lovibond red, 5.25 inch)	0.3–1.5
Flavor	bland
Peroxide value (meq/kg)	Nil
Anisidine value	0.5–2
AOM stability, minimum	15 h/100PV

**6.2.8. Crystallization, Packaging, and Tempering** With the exception of bulk frying fats and salad oils, margarine and shortening oils are converted into semi-solid forms for final use. Margarine oils are emulsified with an appropriate water phase first. Shortenings, which do not usually involve a water phase, are crystallized directly. Products high in hydrogenated canola oil require special care to ensure that crystallization to the very small beta prime crystals is achieved. Essentially, this means very rapid and complete crystallization before the product enters the package, that is, very little or no post-packaging crystallization. Tempering of crystallized fat products refers to the process of allowing certain equilibrium processes in the crystalline/liquid fat mixture to reach completion. The properly crystallized, packaged product is held for two to four days at 25–27°C. This improves plasticity, creaming, and baking performance. It is a process that is not completely understood, but it is very important for optimum shortening properties. Margarines are usually tempered for a shorter time and then refrigerated. deMan et al. (93) studied the effect of commercial crystallization conditions and product tempering in connection with studies on the crystallization behavior of margarines and shortenings made from hydrogenated canola oil.

**6.2.9. Quality Assurance** In the processing of canola oil to edible oil products, much the same quality control procedures are applied as with other oils. A few aspects, such as for example the presence of chlorophyll derivatives in crude oil and their removal in processing, are somewhat unique. AOCS (76) or other standard methods, such as IUPAC, ISO, or DGF are commonly used.

**6.2.9.1. Degumming** Quality control in the process requires the analysis of the crude oil and the degummed oil for phosphorus.

**6.2.9.2. Alkali Refining** This involves the determination of free fatty acids, phosphorus, and, if desired, chlorophyll, before and after the process. In addition, soap concentration is determined after water washing. Soap determination can present some difficulty in establishing the endpoint of the titration with oils high in chlorophylls, because of the dark color of such oils.

**6.2.9.3. Bleaching** The main aspect in the control of the bleaching process is determination of chlorophyll. It is accepted in the industry that chlorophylls

derivatives must be reduced in the bleaching step to a concentration of  $<0.025$  mg/kg, measured as chlorophyll *a*. Lower concentrations are innocuous. Higher concentrations produce a noticeably green tinge in the oil and impair the stability of the oil when exposed to light. In addition, during deodorization, a grayish tinge may develop in the oil. The AOCS method for chlorophyll determination, Cc 13d-92 (96), is used. Lovibond red and yellow color is, of course, also measured as part of the process control in bleaching, but the colors to be achieved may vary considerably depending on the heat-bleachability of the red-yellow color compounds. When super-degummed oil is bleached to prepare it for physical refining, phosphorus determination may also routinely be done on the bleached oil.

**6.2.9.4. Hydrogenation** Process control in hydrogenation is primarily concerned with the degree of hydrogenation, determination of melting behavior, fatty acid composition, and nickel catalyst removal. Rapid analysis for control of the degree of partial hydrogenation makes use of iodine value and, more frequently, refractive index. This is followed up with determining the solid fat index (SFI), or solid fat content (SFC) and, where appropriate, the Mettler dropping point. Determination of the fatty acid composition of hydrogenated oils is by the AOCS Method Ce 1e-91 (96). Nickel traces are determined by the nickel sulfide test or by ICP spectroscopy.

**6.2.9.5. Dewaxing** The dewaxing process at present is not controlled by an analytical test that determines the content of “waxes” in the oil. This would be too complex for routine process control. Rather, the oil is subjected to the AOCS cold test, and this is supplemented by keeping samples at higher temperatures, up to room temperature (76). In this way, it is possible to gain some confidence that the dewaxing procedure is in fact performing as expected. As pointed out earlier, the crystallization behavior of the compounds implicated in sediment formation is complex. This makes these indirect tests vague as predictors of shelf stability with respect to the oil remaining clear of visible sediments.

**6.2.9.6. Deodorization/Physical Refining** Quality control is especially concerned with flavor, free fatty acid concentration, color, stability, and trace contaminants. With respect to routine flavor testing, two–three trained panelists test the flavor of small sample of the oil. The oil must be essentially bland, but very slight beany or grassy notes are tolerated. Free fatty acids, color, and stability testing is done by the AOCS official methods (76).

**6.2.9.7. Crystallization, Packaging, and Tempering** Process control of the crystallization conditions (temperature of precooling, temperature of crystallizing, temperature rise in the package) and of tempering time and temperature are especially important with margarines and shortenings containing a high proportion of hydrogenated canola oil. This is because of the greater tendency of such products to recrystallize over time from the beta prime to the beta prime form. Polarized light microscopy is sometimes used to provide an immediate assessment of the crystal state of a product. Beyond that, penetrometer readings as well as taste panels for

margarine to assess mouthfeel, and for bakery shortenings, creaming, baking, and icing performance tests, are the procedures used as a guide to determine tempering time and shelf stability.

## 7. NUTRITIONAL PROPERTIES OF CANOLA OIL

### 7.1. Nutritional Significance of Canola Oil Composition

Dietary fat serves several important nutritional functions. It is the source of essential fatty acids. Members of the n-6 and n-3 (also known as the  $\omega$ -6 and  $\omega$ -3) families of fatty acids are important constituents of cell membranes and serve as precursors of eicosanoids (biologically active compounds such as prostaglandins, thromboxanes, prostacyclins, and leukotrienes). Fat also serves as a carrier for the fat-soluble vitamins, and it is important source of energy. In addition, it has important culinary properties and contributes to the palatability of food.

The current interest in dietary fat, however, stems primarily from its implication in the origin of several chronic diseases. Interest has centered on both the amount and type of dietary fat in the development of cardiovascular disease, cancer, hypertension, and obesity. As a result, dietary recommendations in many countries call for a reduction in total fat intake, to 30% of energy, and in saturated fat intake, to less than 10% of energy. In addition, some nutrition recommendations specify recommended levels of n-6 and n-3 fatty acids in the diets. Hence, the source of fat in the diet has assumed considerable importance over the past few years. Interest in the nutritional properties of canola oil developed because of its fatty acid composition (Table 2). Canola oil is characterized by a low level of saturated fatty acids, a relatively high level of monounsaturated fatty acids, and an appreciable amount of the n-3 fatty acid  $\alpha$ -linolenic acid (18:3 n-3).

*Saturated fatty acids.* The adverse effect of saturated fat on blood cholesterol level and its implication in cardiovascular disease has stimulated concern over the level of saturated fatty acids in the diet. Canola oil contains a very low level (<7%) of saturated fatty acids: about half the level present in corn oil, olive oil, or soybean oil and about one-quarter the level present in cottonseed oil. Furthermore, canola oil contains only 4% of the saturated fatty acids (viz., lauric, myristic, and palmitic) that have been found to increase blood cholesterol level. Hence, canola oil fits well with the recommendation to reduce the amount of saturated fat in the diet.

*Monounsaturated fatty acids.* The report that monounsaturated fatty acids (viz., oleic acid) were just as effective as polyunsaturated fatty acids (viz., linoleic acid) in lowering plasma total and low-density lipoprotein (LDL) cholesterol (100) aroused interest in the nutritional properties of canola oil. Canola oil contains 60% oleic acid and is second only to olive oil, among the common vegetable oils, in oleic acid content. Although avocado oil and high-oleic sunflower oil also contain high levels of oleic acid (>70%), they are minor constituents in the average diet.



There, also, is interest in dietary monounsaturated fatty acids because of their possible protective effect against oxidation of LDL cholesterol (101). There is appreciable evidence that the uptake of LDL cholesterol and the formation of fatty streaks in the intima of large blood vessels, which is considered an early lesion of atherosclerosis, is enhanced by the oxidation of the LDL cholesterol (102, 103). LDL cholesterol was found to be appreciably more stable to oxidation when subjects were fed diets rich in oleic acid than when fed linoleic acid enriched diets (104–106).

*Polyunsaturated fatty acids.* Canola oil is intermediate among the vegetable oils in its polyunsaturated fatty acid content; it contains lower levels than corn oil, cottonseed oil, soybean oil, or sunflower oil but appreciably higher levels than olive oil or palm oil. Interest in polyunsaturated fatty acids stems from their role as essential fatty acids and their effectiveness in reducing plasma cholesterol level. Linoleic acid and arachidonic acid, members of the n-6 family of fatty acids, have long been recognized as essential fatty acids. Arachidonic acid is an important constituent of cell membranes and the precursor of eicosanoids, “hormone-like” substances that are involved in a multiplicity of physiological functions ranging from blood clotting to immune response.

$\alpha$ -Linolenic acid and other members of the n-3 polyunsaturated fatty acids likewise are essential. Docosahexaenoic acid (22:6 n-3) is a major constituent of lipids of the brain and retina of the eye, whereas eicosapentaenoic acid (20:5 n-3) is the precursor in the synthesis of an analogous but different series of eicosanoids from those formed from arachidonic acid. Canola oil contains an appreciable amount (10%) of  $\alpha$ -linolenic acid. In addition, there is a favorable balance between linolenic acid and linoleic acid (a ratio of approximately 1:2). Soybean oil is the only other major edible oil that contains a significant amount of linolenic acid (approximately 7%), but the ratio to linoleate is approximately 1:7.

*Minor constituents: tocopherols and phytosterols.* Vegetable oils are the primary source of tocopherols in the average diet. Canola oil is a relatively rich source of tocopherols (refer to Table 6); it is similar in total tocopherol content to corn oil, cottonseed oil, safflower oil, and sunflower oil (60–70 mg/100 g). Only soybean oil contains an appreciably higher level of total tocopherols (100–110 mg/100 g). However, canola oil contains a considerably higher level of  $\alpha$ -tocopherol, the main source of vitamin E biopotency among the tocopherols, than soybean oil (27 vs. 12 mg/100 g). Only cottonseed oil, safflower oil, and sunflower oil are better sources of  $\alpha$ -tocopherol than canola oil (35, 56, and 61 mg/100 g, respectively) (see Table 6).

Canola oil contains a relatively high level of phytosterols (892 mg/100 g), about twice the level in soybean oil or sunflower oil (436 and 496 mg/100 g respectively) (Table 8).  $\beta$ -Sitosterol accounts for about 50%, campesterol 35%, and brassicasterol 14% of the total phytosterols in canola oil. Canola oil is the only common vegetable oil that contains brassicasterol. Plant sterols have been reported to lower plasma cholesterol level (107) by inhibiting the absorption of dietary cholesterol and the reabsorption of biliary cholesterol (108).

## 7.2. Effect of Canola Oil on Plasma Cholesterol and Lipoproteins

Appreciable research on the effect of canola oil on plasma cholesterol and lipoproteins has been reported. The primary impetus for this research was the finding that dietary monounsaturated fatty acids were as effective as polyunsaturated fatty acids in lowering plasma total and LDL cholesterol (100, 109). These findings also provided a possible explanation for the observation that canola oil was as effective as soybean oil in lowering plasma cholesterol in normolipidemic men (110). Prevailing theory had held that saturated fatty acids raised plasma cholesterol, polyunsaturated fatty acids lowered plasma cholesterol, and monounsaturated fatty acids were neutral, they neither raised nor lowered plasma cholesterol (111, 112).

*Normolipidemic subjects.* Studies in Canada, Finland, Germany, and the United States have shown canola oil to be just as effective in lowering plasma total and LDL cholesterol levels as fat sources containing high levels of polyunsaturated fatty acids when each replaced saturated fat sources in the diet (Table 19). All diets resulted in statistically significant decreases in plasma total (mean of  $-0.47$  to  $-0.88$  mmol/L) and LDL cholesterol (mean of  $-0.43$  to  $-0.74$  mmol/L) levels. Thus, most of the decrease in total cholesterol was caused by a decrease in LDL cholesterol, which is consistent with the main objective of dietary intervention programs aimed at reducing the risk of cardiovascular disease. Furthermore, except for the study by Valstra et al. (116), decreases in total and LDL cholesterol were the same on the canola oil and the polyunsaturated fatty acid diets. Decreases in apolipoprotein B, which is the lipoprotein characteristic of the LDL fraction, also were similar on the canola and polyunsaturated diets.

None of the diets in these studies, except for the study by Kratz et al. (106), had any effect on plasma high-density lipoprotein (HDL) cholesterol levels. Kratz et al. (106) reported a decrease in plasma HDL cholesterol level on both the canola oil

**TABLE 19. Comparison of the Effect of Canola Oil and Polyunsaturated Fatty Acid Sources on Plasma Total and LDL Cholesterol of Normolipidemic Subjects.**

Dietary PUFA Source	Plasma Lipid Parameter	Baseline* (mmol/L)	% Change from Baseline		
			Canola Diet	PUFA Diet	Reference
Sunflower oil	Total cholesterol	4.42	-20	-15	
	LDL cholesterol	2.76	-25	-21	113
Safflower oil	Total cholesterol	5.39	-9	-15	
	LDL cholesterol	3.71	-12	-15	114
Soybean oil	Total cholesterol	4.40	-18	-16	
	LDL cholesterol	2.98	-25	-18	115
Sunflower oil	Total cholesterol	5.35	-15**	-12*	
	LDL cholesterol	3.17	-23*	-17*	116
Sunflower oil	Total cholesterol	4.76	-14	-17	
	LDL cholesterol	2.70	-18	-20	106

\*Plasma levels on diets typical of usual fat intake.

\*\*Significant differences ( $p < 0.01$ ) between canola and PUFA diets.

and the sunflower oil diets, whereas no decrease occurred on an olive oil diet. The levels of polyunsaturated fatty acids in the safflower oil, soybean oil, and sunflower oil diets in these studies were two to three times the levels in the average diet. Mensink and Katan (109) also found no adverse effect of a relatively high intake of polyunsaturated fatty acids with normolipidemic subjects fed diets based on customary foods. By contrast, Mattson and Grundy (100) found that very high intakes of polyunsaturated fatty acids (29% of total energy) resulted in a decrease in HDL cholesterol level of mildly hyperlipidemic subjects fed formula diets, whereas similar high intakes of monounsaturated fatty acids had no effect on plasma HDL cholesterol level.

*Hypercholesterolemic subjects.* Canola oil was also effective in reducing serum total and LDL cholesterol levels in subjects with increased blood lipid levels. Replacing 50 g of fat in the regular diet with 50 g of canola oil mayonnaise resulted in a significant decrease in serum total and LDL cholesterol levels (−9 and −10%, respectively) in a group of men with a mean serum cholesterol level of 7.1 mmol/L (117). Serum triacylglycerol and very low-density lipoprotein (VLDL) cholesterol levels also decreased, and serum HDL levels increased on the canola oil regimen. Generally, type of fat, at the level of substitution used in this study, has not affect serum HDL cholesterol levels. Bierenbaum et al. (118) also found that substituting canola oil for 30 g of the usual oils and spreads in the diets of hyperlipidemic subjects resulted in a decrease in plasma LDL cholesterol level. Plasma total cholesterol, however, did not differ from baseline levels. Substituting canola oil or canola oil margarine for butter, at much lower levels (approximately one-fifth of the total fat and 8% of total energy) than those cited above, resulted in a decrease in the plasma cholesterol level of mildly hypercholesterolemic subjects (119). Substitution of canola oil for butter resulted in a 9% decrease in total cholesterol and 13% decrease in LDL cholesterol level, and substitution of canola oil margarine for butter resulted in a 9% decrease in total cholesterol and an 8% decrease in LDL cholesterol level.

Canola oil also was effective in lowering plasma total and LDL cholesterol concentrations of mildly hyperlipidemic subjects fed a low-fat (30% of total energy) diet (120). The decline in plasma total cholesterol when canola oil or corn oil provided 20% of the total energy was −12% and −13%, respectively, which was significantly greater than the decline when olive oil (−7%) supplied the fat. However, canola oil, corn oil, and olive oil were equally effective in lowering plasma LDL cholesterol levels (−16%, −17%, and −13%, respectively); elevated plasma LDL cholesterol level is a major risk factor in coronary heart disease.

### 7.3. Effect of Canola Oil on Thrombogenesis

Cardiovascular disease is characterized by three major events: (1) the formation of atherosclerotic plaques on the intima of blood vessels, which reduce the size of the lumen of the vessel; (2) thrombosis or clot formation, which is the event leading directly to a coronary attack or stroke in many individuals; and (3) cardiac arrhythmias, uncoordinated contractions of the heart muscle resulting in irregular and ineffective

**TABLE 20. Effect of Canola Oil on Clotting Time and Factors Involved in Clot Formation.**

Parameter	Effect of Canola Oil on Parameter	Reference
n-3 Fatty acid (viz., 20:5n-3 and 22:5n-3)	Increased	124–126
Content of platelet phospholipids	No change	127, 128
Arachidonic acid content of platelet phospholipids	Decreased	124, 125, 127, 128
In vitro platelet aggregation	Reduced	124, 127
	Enhanced	128
Eicosanoid production		
Thromboxane	Decreased	113
Prostacyclin	Increased	113
Clotting time	Increased	113

heart beats that frequently results in death. Clot formation has only recently received attention from researchers even though dietary fat has been implicated for some time (121). Marked differences among Greenland Eskimos and Danes in the incidence of coronary heart disease (121) led to interest in the effect of long-chain n-3 polyunsaturated fatty acids on thrombogenesis. The long-chain n-3 fatty acids of fish oil, namely, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been found to inhibit platelet aggregation (123). A possible mechanism for the effect of long-chain fatty acids on thrombogenesis is thought to be a change in the fatty acid composition of platelet phospholipids and an accompanying change in eicosanoid formation.

Canola oil has been shown to alter several parameters linked to clot formation (Table 20). In general, phospholipid fatty acid composition reflects the fatty acid composition of the diet: Canola oil resulted in higher levels of oleic acid, whereas safflower oil, soybean oil, and sunflower oil resulted in higher levels of linoleic acid (125–129). Canola and soybean oil also resulted in higher levels of  $\alpha$ -linolenic acid, although very little linolenic acid was incorporated into phospholipids even when flaxseed oil was incorporated into the diet (126, 130). Dietary linolenic acid, however, has been found to alter the long-chain polyunsaturated fatty acid composition of platelet phospholipids. Several groups (124, 125, 127, 128) reported lower levels of arachidonic acid with canola oil diets. Canola oil diets also resulted in higher levels of eicosapentaenoic acid and docosapentaenoic acid than soybean oil, sunflower oil, or customary mixed fat diets (124–126, 129).

Not all studies, however, found higher levels of long-chain n-3 polyunsaturated fatty acids with canola oil diets (127, 128). Part of the explanation for the apparent discrepancy among studies may relate to the balance of fatty acids in the diet. Chan et al. (126) found that changes in plasma and platelet fatty acid composition varied not only with the level of linolenic acid in the diet, but also with the ratio of linolenic to linoleic acid. Canola oil was found to reduce in vitro platelet aggregation although sunflower oil and safflower oil (i.e., low level of linolenic acid and high level of linoleic acid) also reduced in vitro platelet aggregation (123, 127).

On the other hand, Mutanen et al. (128) found that canola oil and sunflower oil enhanced *in vitro* platelet aggregation. Similarly, McDonald et al. (113) found that both canola oil and sunflower oil increased prostacyclin production (an antiaggregating eicosanoid) and decreased thromboxane production (a proaggregating eicosanoid). Although the effect of canola oil on platelet activity and clot formation is not as well established as its favorable effect on plasma cholesterol and lipoprotein levels, there is evidence that it may impede clot formation.

#### 7.4. The Lyon Diet Heart Study

Coronary heart disease is a complex, multifactorial disease, and the mechanism by which diet affects its development has not been resolved fully. A clinical study, the Lyon Diet Heart Study (131), which compared the effect of a “Mediterranean-type diet” with that of a prudent postinfarction diet, found a marked reduction in coronary events (8 vs. 33) among heart patients assigned to the Mediterranean diet. Both cardiac deaths and nonfatal myocardial infarctions were reduced on the Mediterranean diet, in which olive oil and canola oil were the prescribed dietary oil sources and butter was replaced by canola oil margarine. Of particular interest was the absence of any differences between the patients on the Mediterranean diet and the prudent diet in terms of serum total, LDL, or HDL cholesterol levels. Likewise, there was no difference between the groups in platelet aggregation even though plasma levels of oleic, linolenic, and eicosapentaenoic acids were significantly higher and stearic, linoleic, and arachidonic acids were significantly lower for the patients on the Mediterranean diet. The study was terminated after a mean of 27 months follow-up of patients assigned to the diets. However, an extended follow-up of the patients in the original clinical study (mean of 46 months per patient) by de Lorgeril et al. (132) found the benefits of the Mediterranean diet were still very evident (44 coronary events on the prudent diet vs. 14 on the Mediterranean diet). Although the results of this study are very encouraging, there is need to confirm them in further clinical trials.

#### 7.5. Summary—Nutritional Properties of Canola Oil

Canola oil is characterized by a low level of saturated fatty acids (less than 4% palmitic acid) and relatively high levels of oleic acid (60%) and  $\alpha$ -linolenic acid (10%). It is second only to olive oil, among the common fats and oils, in oleic acid level and, except for soybean oil, the only common dietary fat that contains a significant amount of  $\alpha$ -linolenic acid. Furthermore, there is a favorable balance in the levels of linolenic and linoleic acids (*viz.*, 18:3/18:2 ratio of 1:2) in canola oil. Canola oil has been found equally as effective as soybean oil, safflower oil, and sunflower oil in reducing plasma total and LDL cholesterol levels in normolipidemic subjects. It also was effective in reducing plasma total and LDL cholesterol levels in hyperlipidemic subjects when it replaced saturated fat in their diets. Canola oil diets also have been shown to affect the fatty acid composition of blood

platelet phospholipids and to alter platelet activity and thrombogenesis, although the evidence supporting these observations is not as convincing as its effect on plasma cholesterol levels.

## 8. MAJOR FOOD USES

### 8.1. Standard Canola/Rapeseed Oil

In describing canola/rapeseed oil food uses, the Canadian experience is of significant interest for a number of reasons. First, canola/rapeseed was originally developed and introduced in Canada commercially so that considerable experience in using canola oil in edible oil products has been accumulated over a longer period of time. Second, canola, after its introduction, rapidly became the most important oilseed crop and the most heavily used edible oil in Canada, as documented below. Third, the Canadian edible oil products market demanded a variety of high-quality products, which led edible oil producers to develop many uses for canola oil as well as find applications especially suited for it.

With respect to the importance of canola oil in Canada, usage has grown from the early years after its introduction to about 68% of the edible vegetable oil consumed in 2000. It has been at this level for the last decade. Thus, in 1992, 1993, 1994, 1995, and 1999, the corresponding percentages were 63%, 68%, 73%, 72%, and 68%, respectively (Adapted from COPA Monthly Statistics for Feb. 1993, 1994, 1995, 1996, 2000, and 2001). Most of the oil is used as a liquid, that is, the nonhydrogenated form (probably >70%). The products using liquid oil are primarily salad oils and salad dressings, then, as the liquid oil component in margarine formulations and in household and baking shortenings.

Very lightly hydrogenated canola oil (IV about 90) and more highly hydrogenated canola oil (IV lower than 90) are used for frying and in margarine and shortenings.

#### **8.1.1. Salad Oil, Salad Dressings, Mayonnaises, and Cooking Oil Uses**

Canola oil is an "natural" salad oil. This means that it remains clear (no sedimentation) at refrigeration temperatures (3–5°C). No winterization or fractionation is required, except in some instances when, because of seed growing conditions, the oil may contain waxes and traces of other high-melting material. These compounds may crystallize over time and create appearance problems in clear bottles. Experience has shown that only for the most demanding markets it is necessary to remove these compounds. They present no health hazard and are not sufficiently concentrated to affect emulsion stability when the oil is used in mayonnaises and other emulsified salad dressings.

Canola oil is used either pure or, increasingly, in some markets, as a component in salad oil blends of several oils. Such blended salad oils are usually aimed at achieving a certain fatty acid composition for nutritional reasons. Canola oil contributes low saturated fat and some linolenic acid.

Canola oil is also used as cooking oil, including pan-frying. It is not recommended for deep-fat frying. The reason for this is that its polyunsaturated fatty acid content, which, even though only moderately high, makes it unsuitable.

In all of these applications, canola oil has gained favour in many areas of the world in the 1980s and 1990s, including North America and many European countries. This is because of its low content of saturated fatty acids compared with all other competing oils in these applications. Further, its high content of oleic acid and its moderate content of linoleic acid makes canola oil even more competitive in food applications. Linoleic acid consumption is recognized as being too high in diets that are high in fat and high in the use of soybean, sunflower, and corn oils, such as is the case in most industrially developed countries. Its linolenic acid content is recognised as a nutritional advantage over sunflower and corn oils.

The low total polyunsaturation of canola oil, about 30% versus 58% for soybean oil, along with the high content of monounsaturates, about 60% versus about 25% for soybean oil, are responsible for the good flavour stability of this oil, despite the presence of linolenic acid. Additional minor, but important reasons, for better oxidative stability of canola oil compared with soybean oil are as follows:

1. That a larger percentage of its linolenic acid content is in the *sn*-2 position in the triacylglycerols than is the case with soybean oil; this confers somewhat greater resistance on linolenic acid to oxidation.
2. The presence of some sulfur compounds, which can act as antioxidants (51).

Detailed fatty acid composition of canola, soybean, sunflower, corn (maize), and flax oils as well as some specialty canola oils and HEAR oil are given in Table 2.

**8.1.1.1. *Frying Fats*** Large amounts of canola oil are used as lightly hydrogenated (IV ~90), stable, but pourable frying fat. Canola oil is uniquely suited to combine good stability with pourability because of its fatty acid composition.

Low total polyunsaturation requires relatively little hydrogenation to reduce the levels of linolenic acid and linoleic acid to values that are low enough to confer good frying stability. During this process, very little stearic acid is formed. Coupled with the low original content of saturates of only about 6%, an oil of good stability yet still pourable at room temperature is obtained. In Table 21, the fatty acid composition and the solid fat indices (representative values) of lightly hydrogenated canola oil with 2% residual linolenic acid and IV of about 90 are given along with the fatty acid composition of the nonhydrogenated oil. The data for soybean oil are also included to show the difference in fatty acid composition, especially in the amount of saturates and polyunsaturates, as well as in the solid fat content of these two lightly hydrogenated oils. The data show the advantage of canola oil with respect to oxidative stability, which is caused by the much lower content of polyunsaturated fatty acids, and the advantage of pourability, which is caused by a much lower solid fat present at 10°C and 21.1°C (room temperature). This type of oil is very suitable and heavily used for small-scale frying in restaurants as well as in

**TABLE 21. Fatty Acid Compositions and Solid Fat Indices of Lightly Hydrogenated Canola and Soybean Oil (Residual C18:3 Content, 2 %).**

Iodine	Fatty Acid Composition (w/w %)						Solid Fat Index at °C				
	C16:0	C18:0	C18:1	C18:2	C18:3	Trans	10.0	21.1	26.7	33.3	
<i>Canola</i>											
Value											
115	4.5	2	60	22	10	—	—	—	—	—	
90	4	4	79	9	2	25	2–3	0	0	0	
<i>Soy</i>											
130	11	4	25	53	7	0	none				
95	11	7	54	25	2	15	8	4	0	0	

Adapted from T. Mag (Unpublished data).

large industrial frying operations. A negative aspect of the hydrogenated canola frying fat is the somewhat higher concentration of *trans*-isomers compared with soybean oil.

The lightly hydrogenated canola oil of IV 90, or slightly lower to produce a frying fat containing about 1% linolenic acid, has also been shown (133) to be useful as a winterized, very stable salad oil. A very significant advantage is that this “stabilized” salad oil is obtained at a yield of about 95% and a 12-hour cold test, compared with lightly hydrogenated, winterized soybean oil at a yield of 70–80% and a 6-hour cold test. In some areas of the world, this type of product, based on soybean oil, is being used, and seems to be expanding. This processing was very popular in the United States in the 1960s, but it was discontinued because of the low fractionation yields.

It is important to note that lightly hydrogenated canola oil, such as listed in Table 21, does not contribute significantly to the fat crystal matrix of fat products in which it is used. In this respect, it is similar to the use of liquid canola oil; that is, it can be used in products such as margarine and shortenings without contributing to beta crystallization problems.

Canola oil is also used in more highly hydrogenated forms to produce very stable frying fats that are essentially free of any significant amounts of polyunsaturates, but with high amounts of oleic and elaidic acids and moderate amounts of saturates. Examples of more highly hydrogenated canola oils are given in Table 22, especially

**TABLE 22. Fatty Acid Compositions and Solid Fat Indices of Highly Hydrogenated Canola Oils (%).**

Iodine	Fatty Acid Composition						Solid Fat Indices at °C				
	C16:0	C18:0	C18:1	C18:2	C18:3	Trans	10.0	21.1	26.7	33.3	40.0
82	4	5	87	2	<1	32	18	5	0	0	0
77	4	9	84	<1	<1	35	25	11	6	0	0
72	4	13	81	0	0	44	35	17	10	3	0
68	4	18	76	0	0	48	53	34	27	13	1
62	4	25	70	0	0	46	65	53	48	33	12

Adapted from T. Mag (Unpublished data).



the oils in the IV range from 82 to 72. These oils are very low in polyunsaturated fatty acids, about 2% linoleic acid, or are entirely free of this acid. They are very stable, and especially suited when a stable, but relatively firm fat is needed, such as needed in donut frying. In frying applications, the problems with beta-crystallization, formation of a grainy texture, and mouthfeel, are usually not important, unless the deep-fried foods are stored for some time. In these cases, large fat crystals may become visible at the food surface, which is undesirable. Blends with hydrogenated soybean oil, hydrogenated cottonseed oil, or with palm oil are used to control beta-crystallization problems. Additional methods that are useful for suppressing beta-crystallization are discussed below in connection with margarine products.

In Canada, only selectively hydrogenated canola oil is used. The practical reason is that the somewhat higher content of *trans*-isomers makes the more highly hydrogenated oil more resistant to beta crystallization compared with nonselectively hydrogenated canola oil. When *trans*-isomer content must be minimized in canola oil products, liquid canola oil or very lightly hydrogenated canola oil is used, such as shown in Table 21.

**8.1.1.2. Soft (Tub) Margarine** In Canada and in many other countries where margarine is consumed, soft or tub margarine is now predominant. Consumption of hard or stick margarine has decreased significantly over the last two decades. Further, a significant proportion of soft margarine in many countries is of the zero *trans*-isomer type as no hydrogenated oils are used. Palm/palm-kernel oil-based hard fat blends with a suitable solid fat content profile are used to provide the crystalline fat component. Only about 6–10% of the total fat are required to supply the necessary crystalline fat phase. The composition of the palm/palm-kernel oil-based hard fats varies depending on a variety of factors and is very often proprietary to the suppliers, which are primarily based in Malaysia. Interesterification is usually used to produce these blends. Canola oil is used only as the liquid oil component of these no *trans*-isomer products. The prime example of these products is Becel<sup>TM</sup>, which has been on the market for many years in parts of Europe and in Canada. Canola oil is often the preferred liquid oil, because it has the lowest saturated fatty acid content and it supplies some linolenic acid, and yet has good flavor stability. This oil is also used together with other liquid oils to make up the liquid oil component.

Soft margarine based on partially hydrogenated hard fat is also produced with canola oil as the sole liquid, nonhydrogenated component, or along with other liquid vegetable oils. In these margarines, canola oil can be used as the partially hydrogenated hard fat as well. One, or a combination of several, of the hydrogenated oils listed in Table 21 and 22 can be used. But, because of the tendency of canola-based hydrogenated hard fat to form beta crystals over time, partially hydrogenated soybean oil, or other, palmitic acid-containing oils, such as partially hydrogenated cottonseed oil, are preferred as the hard fat. This avoids the tendency of the margarine to have a coarse, sandy texture caused by beta crystallization. Table 23 lists typical compositions of these three types of soft margarine using canola oil.

TABLE 23. Soft (Tub) Margarine Using Canola Oil.

Type	Composition Oils Used	%	Solid Fat Indices at		
			10.0°C	21.1°C	33.3°C
1. No <i>trans</i>	a) palm/palm kernel hard fat	8 }	5-7	4-5	1-2
	b) canola oil, or other liquid vegetable oils, including blends, to achieve specific fatty acid compositions	92 }			
2. <i>Trans</i> - containing 100 % canola	a) hydrogenated canola hard fat	47 }	8-10	6-8	1-2
	b) canola, or other liquid vegetable oils, as above	53 }			
3. <i>Trans</i> - containing	a) hydrogenated soy hard fat	25 }	8-10	6-8	1-2
	b) canola, or other liquid vegetable oils, as above	75 }			

Adapted from T. Mag (Unpublished data).

In the past, margarine oils made entirely from canola oil were produced especially in Canada and Sweden. This is still of interest when the hard fat must be made from canola oil because of cost, or oil availability. Using partially hydrogenated canola hard fat requires control of beta crystals, which must be suppressed. This can be done by (1) adding about 0.3–0.5% of trisorbitan stearate to the fat blend and (2) by using several selectively hydrogenated canola hard fats of different hardness to introduce a greater variety of triacylglycerols into the fat blend. Trisorbitan stearate and the greater variety of triacylglycerols interfere with the conversion to the beta form of fat crystals and, therefore, retard the formation of a sandy margarine texture. Changing the fatty acid position in triglycerides by interesterification of some or all of the hydrogenated canola hard stock used in the oil formulation can reduce beta crystallization. It is used in Europe, but not in North America.

All of the above-mentioned measures to control beta-crystallization are costly and, in the case of trisorbitan stearate, requires a label declaration as an additive, giving the product an undesirable chemical connotation; hence, its use is limited. In today's practice, blending with another hard fat that is relatively high in palmitic acid to raise the concentration of this acid to at least 8% in the final blend is preferred. To meet this requirement, the oils used are palm at about 15% and partially hydrogenated cottonseed at about 30%. Further, when it is not required to maximize canola oil in the fat blend, partially hydrogenated soybean oil is used to supply the high melting portion of the blend instead of partially hydrogenated canola oil. Soybean oil contains 11% of palmitic acid, enough to confer acceptable crystal stability in the beta prime form.

**8.1.1.3. Hard (Stick) Margarines** Liquid and lightly hydrogenated canola oil is used to produce stick margarine. They do not contribute significantly to the crystal matrix of the product, as pointed out earlier. The tendency of the more highly

hydrogenated canola oils, such as those listed in Table 16, to form beta crystals means that these are not used to any significant extent as contributors to crystalline fat. They would have to be used in relatively large amounts, 40–50% in the fat blend, which makes it more difficult and costly to control beta crystallization. The liquid or lightly hydrogenated oil is generally used at 50% to 60% of the oil composition, the remainder being relatively highly hydrogenated soybean or cottonseed oil, or hydrogenated palm oil.

In situations where the use of canola oil must be maximized, that is the hard fat component as well as the more liquid component must be from canola oil, the same approach as outlined above for soft margarine must be used. As was already pointed out, demand for stick margarine is declining, and the need for margarine made entirely from canola oil is no longer significant even in the countries where canola oil is used as the main oil.

**8.1.1.4. Shortenings, Baking, and Pastry Margarine** Similar to the use of canola oil in making table margarine, liquid canola oil is heavily used to produce shortening and baking and pastry margarines. Lightly hydrogenated canola oil of about IV 90 is used when better oxidative stability is required than can be achieved with liquid oil. When it is desired to use canola oil even as the hard fat component in these formulations, then the considerations related to crystallization as discussed for margarine apply. Generally, highly hydrogenated canola oil is not much used.

The liquid canola oil or lightly hydrogenated canola oil is blended with hard fats, such as tallow, palm, partially hydrogenated soybean and cottonseed oils, and fully hydrogenated versions of these oils (stearine), to meet preferred specifications. Detail compositions are usually proprietary. For baking applications shortening and baking margarine, having the fat component with the beta prime crystalline form is especially important for good performance. For this reason, baking shortenings based totally on canola oil are not in use.

**8.1.1.5. Antioxidant Usage in Canola Oil Products** Present practice is to avoid the use of synthetic antioxidants in edible oil products as much as possible. This also applies to canola oil products. For many edible oil products, users now specify that chemical additives cannot be used as ingredients. This avoids having to declare on the product label that the product contains a synthetic, chemical antioxidant as an additive. Instead, there is increasing emphasis on preserving during processing the natural antioxidants present in canola oil, the tocopherols. Tocopherol losses occur primarily during deodorization of the oil, because of the high temperatures required. Processors are limiting deodorizing temperatures as much as possible when high tocopherol concentrations are required, consistent with achieving good deodorization. It is possible to retain as much as 80% of the original tocopherol content in the deodorized oil.

The tocopherols are especially important as antioxidants in frying, because of their low rate of evaporation and low rate of destruction at frying temperatures (134). The tocopherol content of standard canola oil is given in Table 6, together with the tocopherol content of some specialty canola oils and other common

vegetable oils. Alpha and gamma tocopherol are the main tocopherols present in canola oil.

It is interesting to note that canola oil is more than twice as high in alpha tocopherol (about 270 mg/kg) than soybean oil (about 116 mg/kg). Alpha tocopherol is now recognized as the main tocopherol with vitamin E function in humans (135). Canola oil is a very good source of vitamin E.

The synthetic antioxidants that are used, when there are no restrictions on using them, are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertiary butylhydroxyquinone (TBHQ). The usual added amounts are 0.02% as single or combined. This amount is the maximum allowable in Canada and the United States, for example. Regulations governing synthetic antioxidant usage can differ from one country to another.

Citric acid is used as a metal chelating agent, usually as monoacylglycerol citrate at 0.01% either by itself or along with BHA and BHT. In some countries, the United States, for example, citric acid can be used as a chelating agent without having to declare it on product labels when it is added to the oil in an aqueous solution in the cooling stage of the deodorization process. Lower amounts of 0.005% are often used.

*8.1.1.6. Canola Oil Use in Selected Areas of the World* It is interesting to review, briefly, the use of canola oil in edible oil products in various countries in the world.

The United States of America import large amounts of canola oil from Canada in addition to some domestic production. At the time of writing, about 90% of the canola oil was consumed in liquid form as salad oil and in salad dressings. This is a direct result of the emphasis on consuming oils, which are low in saturated fatty acids, and canola oil is lowest in saturated fat among vegetable oils. Canola oil is also being used in blended salad oils to achieve certain fatty acid profiles, as mentioned earlier. The relatively high use of canola oil in the United States is remarkable, because none was used before 1983.

About 10% of canola oil is hydrogenated and consumed in the form of shortening. This appears to be mostly as frying fats, also termed frying shortenings in North America, to take advantage of the low concentration of saturated fat and low polyunsaturation as discussed before. Interestingly, virtually no canola oil, liquid or hydrogenated, was used in margarine formulation at the time of this writing.

Mexico uses significant amounts of canola oil, predominantly as salad oil, salad dressings, and cooking oil. This is mostly from seed imported from Canada and the European Union.

Japan uses large amounts of canola oil. It has been an importer of Canadian canola seeds since the introduction of canola in the early 1970s, and it has for many years taken about one-half of the Canadian canola crop. The oil is predominantly used in liquid, nonhydrogenated form as cooking oil, salad oil, and salad dressings oil, pure, as well as in blends with other oils. Further, in a new development, it is used as base oil to produce dietetic cooking and salad oils made up of

about 80% diacylglycerols (DAG oil). DAG oil is more easily hydrolyzed in the digestive system and is then used as an immediate energy source rather than stored as body fat. It is worth noting that the DAG oil technology is being introduced into the United States (136).

In China, canola-type rapeseed oil products still contribute a very small proportion of total rapeseed oil products. Oil from both high erucic acid rapeseed and canola rapeseed represent the largest use of edible oil at present. The oil from these two sources is almost entirely used as cooking oil. There are very little amounts of this oil used for margarine or shortening formulations at present. Efforts are being made to widen the spectrum of edible oil products and convert from HEAR cultivation to canola cultivation.

India, similar to China, does not use significant amounts of canola-type rapeseed oil. Instead, mustard seed oil, which is high in erucic acid, similar to HEAR oil, is the most important oil used. This is used almost entirely as a liquid cooking oil. At least among the lower income segment of the population, and even in the middle class, this oil is used undeodorized and is favored for its taste. Undeodorized fresh canola/rapeseed oil cannot compete with mustard seed oil in flavor. Some blending of canola oil with mustard oil is done to lower the content of erucic acid in the mustard oil. Unless taste preferences change and there is greater attention to the health implications of the types of fat in the diet, canola oil will be used only to a limited extent in the foreseeable future.

The Middle East is beginning to use canola oil in competition with sunflower and corn oil as salad oil and salad dressings/mayonnaise. Margarine and vanaspati are in many countries based on hydrogenated soybean oil and on palm oil at present. Interest in canola oil is based on its nutritional properties, mainly its low saturated fatty acid content. There is considerable potential for using canola oil not only as a salad oil, but also, in its lightly hydrogenated form, as a vanaspati-like pourable frying fat.

Western and Eastern Europe use large amounts of canola oil, with the exception of France. Salad oils, salad dressings, mayonnaises, and table and baking margarine use large amounts of liquid canola oil. Lightly hydrogenated canola oil is heavily used for frying snacks. This is very similar to the Canadian practice. Canola oil use is driven, in part, by recognition of the positive health effects of its high oleic acid content, along with its low saturated fat, and the fact that European grown canola seeds are not genetically modified (non-GM) at present, as opposed to oil from imported soybeans. In addition, canola salad oil is considered by some to have a better shelf life than other more highly polyunsaturated vegetable oils.

Southern Europe and France use relatively little canola oil. Instead, olive, sunflower, and peanut oils predominate. In the case of France, this is somewhat surprising, because this country is a large producer of canola seeds. But France uses large amounts of canola oil for biodiesel in the form of fatty acid methyl esters.

Australia/New Zealand produces canola seed and uses the oil in much the same fashion as North America and parts of Europe.

South America uses sunflower and soybean oil and increasingly palm oil. Canola oil is not used in food in South America.

**8.2.1. Canola Rapeseed Oils with Modified Fatty Acid Composition** Since the introduction of standard canola, there has been considerable plant breeding efforts to produce canola oils with modified fatty acid compositions. These efforts were primarily to improve oxidative stability, or crystallization properties, or even produce lauric acid-containing oils and, more recently, canola oil containing gamma linolenic acid (11). The following is a list of these developments:

- Low linolenic acid canola oil (2% vs. 9%)
- High oleic acid canola oil (69–77% vs. 60%)
- High palmitic acid canola oil (10% vs. 4%)
- High stearic acid canola oil (30% vs. 2%)
- Lauric acid canola oil (about 33% C 12:0)
- Gamma linolenic acid canola oil (37% vs. about 1%).

The complete fatty acid compositions of some of these oils, namely, low linolenic and high oleic acid, lauric acid, and gamma linolenic acid oils are given in Table 2.

Low linolenic acid canola oil was developed in Canada in the 1980s to improve the oxidative stability of the oil so that light hydrogenation would not be necessary (7). The reduced linolenic acid content of this oil of about 2% compared with about 9% in the standard canola oil. This resulted in an increase in linoleic acid from 20% to 27% and the increase in oleic acid from about 60% to 61%. In Canada and the United States, this oil is available in limited quantities and is used entirely for deep-frying in place of the lightly hydrogenated standard canola oil (Table 21). Its main advantage is the much lower *trans*-isomer contents of about 1–3%, which are formed during deodorization, whereas the lightly hydrogenated oil contains 20–25%. Widespread use of this oil is, however, hampered by its price, which tends to be too high because of the low seed yields of the available varieties. Research on its frying stability and the storage stability of french fries by Warner and Mounts (9) showed that these properties were improved for low linolenic acid canola oil as compared with the standard canola. Zambiasi (34) and Normand et al. (35) showed no significant improvement in the frying stability of this oil and the storage stability of fried foods. It was found that this might be related to the lower content of tocopherols (Table 6). There are also anecdotal reports from industry that the frying stability of the oil is not sufficiently improved to warrant its higher price.

High oleic acid canola oil is another development pursued in Canada, the United States, Sweden, Australia, and elsewhere (137). As with low linolenic acid canola oil, the aim was to produce stable frying oil, which will not need hydrogenation and thus avoid *trans*-isomers formation. The oleic acid content in oil from seed developed in Canada is at about 78%, whereas linoleic and linoleic acids are lowered to approximately 8% and 3% respectively (see Table 2). Saturated fatty acid content is unchanged from the standard canola oil. There is limited commercial seed production for export to Japan. Also, there is increasing acceptance of the oil in Canada and the United States. The frying performance in tests was found to be similar to

lightly hydrogenated standard canola and mid-oleic sunflower oil. Taste tests of french fries produced with this oil showed similar consumer acceptance as typical frying fats used for making french fries (Przybylski, unpublished data). In Australia, canola oil with 69% oleic acid (Monola) is being offered for frying. In potato frying tests with ten other oils, it was rated higher in sensory and chemical tests than the other oils (138).

High palmitic acid canola oil was initially developed in Sweden (139). The purpose was to prevent the beta crystallization of hydrogenated canola oil to make it more freely useable for margarine and shortenings. The oil contains about 10–12% palmitic acid compared with only 4% in the standard oil. The increased use of canola oil in the liquid form in a large variety of edible fat products and concerns about saturated fatty acids, along with the ready availability of palmitic acid-containing oils for blending to control crystallization problems, seems to have prevented this development from gaining commercial significance.

High stearic acid canola oil containing 25–30% stearic acid was developed, but commercial scale production for food uses has been very limited (140).

High lauric acid canola oil was developed in the United States as an alternative source for coconut and palm-kernel oils for both food and nonfood uses (141). The oil contains about 35% lauric acid. Until now, this oil has not found any significant commercial use. The main reason for the lack of acceptance is said to be because of its significantly different fatty acid composition compared with coconut oil, and the consequent difference in performance in typical coconut oil applications. Some use was made of the oil in the United States as a base stock for a *trans*-isomer free margarine and in Europe as a machine oil additive (142), but there is no longer any significant seed production.

Gamma linoleic acid canola oil is of interest (11). It is an example of a development for the nutrition supplement market.

**8.2.2. High erucic acid rapeseed (HEAR) oil** In countries that grow canola, HEAR oil is used only in special food applications. Its primary use is as a fully hydrogenated oil to be added to peanut butter (143) in amounts of 1–2% to prevent oiling mainly in Canada and the United States. The HEAR oil used contains about 45–50% erucic acid, the highest erucic acid rapeseed oil available commercially at present. Because the saturated erucic acid produce behenic acid, which has a very high melting point, the completely hydrogenated HEAR oil is very effective in holding high amounts of liquid oil in its crystal matrix. The patent literature also mentions the use of fully hydrogenated HEAR oil in interesterification with palm stearin fraction to formulate a no *trans*-isomer margarine hard fat, but there appears to be no significant use.

The patent literature of the 1960s and 1970s contains a number of examples of other uses of fully hydrogenated HEAR oil. These uses were designed to exploit the beta prime crystallization properties, either as hardstock in small amounts for baking shortenings or as base stock for conversion to monoglycerides. A listing of these patents is given by Teasdale and Mag (144). However, it appears that there have been very few or no sustained commercial uses of these proposed fats.

Plant breeding work to raise the erucic acid content in the oil is being done in Canada and elsewhere. Indications are that the erucic acid content of about 80% is possible. This is of interest not only for some of the specialty food uses mentioned above, but also especially for industrial lubricants (See nonfood uses of HEAR oil).

## 9. NONFOOD USES OF STANDARD CANOLA OIL

### 9.1. Biodiesel

Triacylglycerol oils, particularly vegetable oils, are suitable as base stocks of simple ester derivatives for use as fuel for compression ignition engines, commonly known as diesel engines. The term biodiesel is applied to this type of fuel. Methanol is the main alcohol used for transesterification of the triacylglycerols to produce biodiesel. The pure methyl esters of fatty acids as well as blends of methyl esters with petroleum-based diesel fuel are used. The environmental factors are the driving force to use renewable fuel source to produce biodiesel. Fatty acid esters (including the triacylglycerols) are readily biodegradable in the presence of water and soil bacteria. They have low toxicity to plants and animals. At this time, production costs of biodiesel are higher than for petroleum-based diesel fuel. In Europe, biodiesel is taxed only at a very low rate to make it more competitive with petroleum diesel fuel. There is the tendency to use in the pure form. In North America, no tax reduction is given; blends with petroleum diesel fuel are, therefore, more popular. Blending, usually around 5–20% of biodiesel, achieves most of the benefits that biodiesel has on engine performance without raising petroleum diesel fuel costs significantly. The blends, of course, are not suitable for very sensitive environments.

The canola growing countries in Europe, notably, Germany, Austria, and France, but also others, have developed significant methyl ester production capacity and use.

Standard canola oil, mainly because of its fatty acid composition, is relatively well suited for biodiesel production. Harrington (145), and Knothe et al. (146) discussed desired properties of fatty acid ester structure for biodiesel. Knothe et al. (146) also discussed biodiesel standards in different countries, for those interested. Briefly, the desired properties of vegetable oil fatty acids for methyl ester biodiesel can be summarized as follows:

1. Long, unbranched hydrocarbon chains
2. Moderate content of saturated fatty acid chains
3. Monounsaturations, but not polyunsaturations
4. Double bond position preferably toward the end of the fatty acid carbon chain away from the methyl group.

High concentration of fatty acid methyl esters with the above properties has good combustion characteristics and good flow properties, including good low-temperature behavior.



Standard canola oil is high in C18 fatty acids, about 95%, which is higher than the other commodity vegetable oils. It is high in C18:1n - 9 oleic acid at about 60%, much higher than any other vegetable oils, and it is relatively low in polyunsaturated fatty acids, linoleic at about 21%, and linolenic at about 10%. Viscosity, cold filter plugging point, and cetane number are some of the most important biodiesel fuel properties influenced by fatty acid composition.

The n-9 oleic acid in canola oil confers good combustion properties, high cetane number, and good flow characteristics. The polyunsaturates also confer good flow properties, but they have poorer combustion characteristics and lower cetane number, because the presence of double bonds in n - 3 and n - 6 position. Canola oil saturated fatty acid content is low. Saturated fatty acids in the C16–C20 range give very high cetane numbers, but they impair the flow properties. It can be seen that the mix of canola oil fatty acids is well suited for biodiesel production, better than the other commodity vegetable oils, such as soybean, sunflower, or palm.

The desired properties of methyl esters for biodiesel are given in Table 24 (147). These properties are the German biodiesel standard Deutsche Industrie Norm (DIN) V 511605. This standard, as well as other European standards, was developed, especially with canola/rapeseed oil as the starting material in mind. The suggested

**TABLE 24. Biodiesel Standard DIN V 51606, Germany.**

Properties	Units	Test Method	Limits	
			min.	max.
Density at 15°C	g/ml	ISO 3675	0.875	0.900
Kinem. viscosity. at 4°C	mm <sup>2</sup> /s	ISO 3104	3.5	5.0
Flashpoint (Pensky-Martens)	°C	ISO 2719	100	
Cold filter plugging point	°C	DIN EN 116		
April 15–Sep. 30				0
Oct. 1–Nov. 15				–10
Nov. 16–Feb. 28				–20
March 1–Apr. 14				–10
Sulphur content	% by mass	ISO 4620		0.01
Carbon residue (10 % distill.)	% by mass	ISO 10370		0.30
Cetane Number		ISO 5165		49
Ash	% by mass	ISO 6245		0.01
Water	mg/kg	ASTM D 1744		300
Total dirt	mg/kg	DIN 51419		20
Copper corrosion (3 hr at 50°C)		ISO 2160		1
Neutralization number	mg KOH/g	DIN 51558 Part 1		0.5
Methanol	% by mass	To be agreed		0.3
Monoglycerides	% by mass	To be agreed		0.8
Diglycerides				0.1
Triglycerides				0.1
Free glycerol				0.02
Total glycerol				0.25
Iodine number	g Iodine/100g	DIN 53241 Part 1		115
Phosphorus	mg/kg	To be agreed		10

Adapted from Varese et al. (147).

USA-ASTM standard for 100% pure biodiesel is similar in many respects (146), but it is written for the use of soybean oil as the main starting material. Canola oil for methyl ester production must either be degummed ( $<20$  mg/kg of phosphorus), or in addition, must be alkali refined and bleached, depending on the methyl ester production process requirements (148).

Possibly, a designed canola oil with enhanced properties especially for biodiesel production may be developed in the future. The high oleic acid canola oil already available represents a version of such oil, because its high C18:1n - 9 content is a very desirable methyl ester component of biodiesel. The same can be said of the high oleic acid varieties of soybean and sunflower oil. It should be noted that the direct use of canola oil as diesel fuel is also being fostered, especially in some European countries, notably, Germany. The advantage is that the cost of conversion to methyl esters and marketing for glycerol, byproduct, (see below), will lower the total cost of the fuel. Farm tractors and certain stationary diesel engine and stationary heating equipment can be adapted for direct use of the oil, without transformation into esters, and the oil can easily be supplied to these users at suitable temperatures to maintain good fluidity. The same properties that make canola oil well suited as a base stock for methyl ester diesel fuel are also advantageous in using it directly. The oil must be degummed to below 50 mg/kg of phosphorus, depending somewhat on the engine manufacturer's specifications, and it must be free of any solid particles (148).

Table 25 gives a comparison of some of the basic properties of methyl esters derived from palm, soybean, and canola rapeseed oil and standard petroleum-based diesel fuel. In this table, palm oil illustrates the effect of fatty acid chain length and relatively high saturation on properties, soybean oil illustrates the effect of high unsaturation, and canola oil represents the effect of low saturation and high mono-unsaturation. It is worth noting that HEAR oil would also be a good biodiesel base stock resulting in methyl esters of higher cetane number, but higher viscosity and somewhat poorer low-temperature behavior can be expected.

It can be seen that compared with petroleum diesel fuel, methyl esters (1) have higher density, (2) fall into the lower range of viscosity, (3) have the same or higher cetane numbers, and (4) have lower heating value. Canola methyl esters are in the middle range of properties among the three oils, with low viscosity, good cetane

**TABLE 25. Some Properties of Palm, Rapeseed Canola, and Soybean Oil Methyl Esters Compared with Diesel Fuel.**

Methyl Ester	Density 15°C, g/L	Viscosity 40°C, mm <sup>2</sup> /s	Cetane Number	Iodine Value	Lower Heating Value MJ/kg
Palm oil	877-877	4.3-4.5	64.3-70.0	52	37.0
Canola oil	882	4.2	51.0-59.7	114	37.2
Soybean oil	880	4.0	45.7-56.0	131	37.1
Petroleum Diesel fuel	830-840	12.5-3.5	51	—	42.7

Adapted from Varese et al. (147).

rating, and heating value. This indicates that the standard canola fatty acid composition produces a good compromise in the balance of desirable methyl ester properties.

It is interesting to note that Varese and Varese (147) cite a highly favorable energy balance factor range of 2.5–3.5 for the production of canola seed and the conversion of the oil to canola methyl esters depending on climate, soil, and ester production method. In this context, it is also worth noting that there are quality problems with the byproduct glycerol from biodiesel production. Consequently, the price of biodiesel byproduct glycerol is very low. If these quality problems are solved, the economics of methyl ester production will become more competitive to petroleum-based diesel fuels. The vegetable oil methyl esters applied for biodiesel can also be used as biosolvents for cleaners, ink removers, and similar applications.

## 9.2. Solvents

Solvents based on vegetable oil methyl esters are finding increasing applications in many areas. Biodegradability, very low volatility, low viscosity, tolerance to low temperatures, and high flash points are the main attractions, along with good solvent properties, such as water wetting, and penetration compared with petroleum-based solvents. Especially the low volatility of methyl ester solvents is increasingly important in the manufacture of paints and coatings. Lower oxidative stability is a negative factor with esters produced from unsaturated oils, but canola oil methyl esters perform better in this respect than the other more unsaturated vegetable oils. Its low content of saturated fatty acids is an advantage in providing esters of low viscosity that are easier to work with.

## 9.3. Lubricants/Engine Oils/Heat Transfer Oils/Hydraulic Fluids/Demolding Agents/Inks

Lubricants are synthesized using a wide range of fatty acids and various alcohols, including polyols. Some of these lubricants have viscosity and lubricity properties that are not achievable with petroleum-based oils. A negative aspect is that lubricants based on oils containing a high level of unsaturated fatty acids have poor oxidative stability. Canola oil, because of its relatively low polyunsaturation compared with commodity oils such as soybean has advantages in lubrication application. As with methyl ester production, the canola oil used in these applications requires refining to various degrees, depending on the base oil specifications of the manufacturer. The same arguments also apply to the use of canola oil as a base for engine oil, heat transfer and hydraulic fluids, inks, and demolding agents.

There is considerable research and development work underway in many countries to test low-linolenic, high-oleic and high-palmitic canola oil varieties as base stocks to determine which of these oils with modified fatty acid compositions is best suited for the various products and applications.

## 9.4. Nonfood Uses of High Erucic Acid Rapeseed Oil (HEAR)

The traditional industrial use of HEAR oil was in lubrication of marine steam engines. This use was caused by the unique film-forming properties of the oil, which are particularly important in the wet steam engine environment.

Modern uses of HEAR oil are much more varied. One of the largest utilizations is as base for the production of erucamide, which is a derivative of erucic acid, used as an antiblock and slip-promoting agent in the production and functioning of plastic films. The oil is also used as a component in paints and coatings. Erucic acid can be converted by oxidative cleavage to brassylic ( $\text{HOOC}-(\text{CH}_2)-\text{COOH}$ ) and pelargonic (nonanoic acid) acids. Former bypolymerization will form nylon 1313 (this number indicates how many carbon atoms are in the acid component). This type of nylon has low moisture absorption, greater dimensional stability, and good dielectric properties, making it a good insulator. Brassylic acid is also an ingredient in the production of polyester and melamine resin coatings. Paints with these additives have balanced flexibility, low hydrolytic factor, and good hardness (149). Direct polymerization of erucic acid showed promising results in plastic formulation but will challenge plastic chemists in developing technologies and applications for it (150). Further, HEAR oil serves as base for producing high-pressure greases.

## 10. PRODUCTION OF OILSEEDS AND OILS

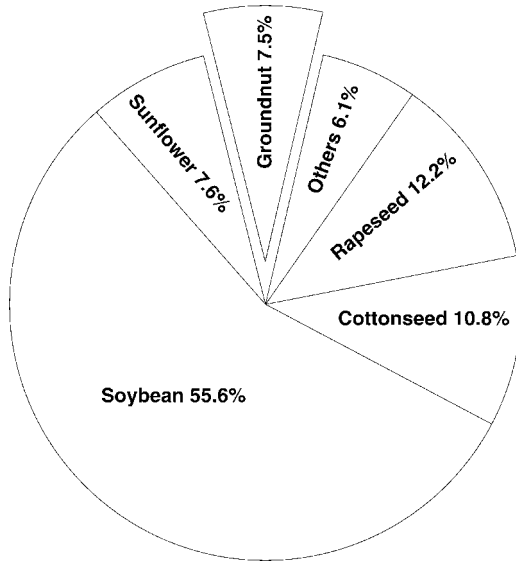
Soybean dominates world oilseed production and represents 55% of the world's total oilseed production (Figure 3). The production of canola/rapeseed seeds ranked second in the world behind soybeans in 2000–2001.

If one considers that soybean is produced for protein, it can be concluded that canola production ranks first in terms of true oilseed production. The United States, Brazil, and Argentina together produced about 75% of all soybean seeds. The world production of soybean oil is in the top position followed by palm and canola/rape-seed among major oils and fats (Figure 4) (75, 151).

The major producers of canola/rapeseed in the world are China, Canada, India, and the countries of the European Union (Figure 5).

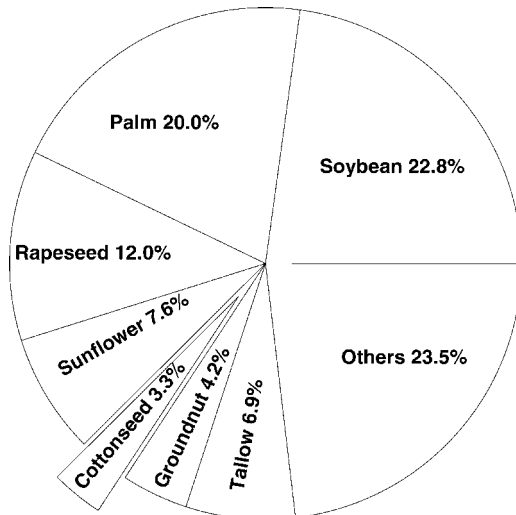
Canada, European Union countries, Poland, and Australia have been the major canola seed exporters. Production of canola seeds in Canada is presented in Figure 6, showing growing trend for the last decade.

Canola oil underwent a transition period in the 1980s as human nutritionists recognized the benefits of the low levels of saturated fats in the diet and the benefits of monounsaturates as compared with saturates and polyunsaturates. In the case of Canadian canola oil, it moved from oil that competed in the markets around the world on the basis of price to a premium priced oil. Canola is perceived as premium oil and is now virtually consumed all over the North American continent. As was discussed earlier, Canada is the world's leading consumer of canola oil on a per capita basis. The countries of the European Union and Canada dominate the export of canola oil. In Canada, canola oil represents about 70% of all vegetable oils produced. The nearest rival is soybean oil with a share close to 25%. Functional and

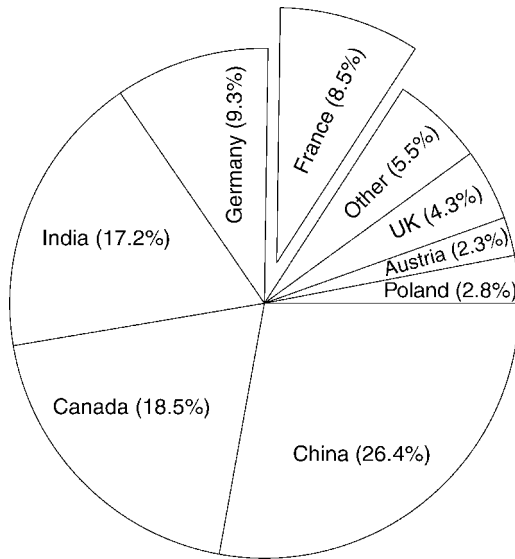


**Figure 3.** Production of oilseeds. Percentage of total world production of 306.9 million metric tons. (Source: Canadian Grains Council, Statistical Handbook 2001.)

nutritional properties of canola oil, widely recognized by consumers, has allowed canola oil to dominate the Canadian salad oil market at level of over 80% and the shortening and margarine markets at levels of 66% and 50%, respectively. Canadian exports of canola oil have become dominated by sales into the United States as



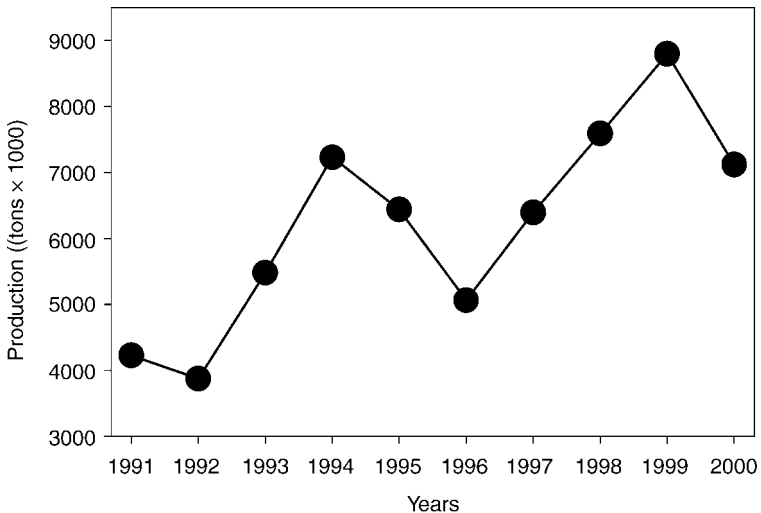
**Figure 4.** World production of oils and fats for the year 2000–2001. Total production of 117.1 million metric tons. (Source: Canadian Grains Council, Statistical Handbook 2001.)



**Figure 5.** Major world producers of canola/rapeseed seeds (ten-year average from 1990 to 2000). Production averaged 31.6 million metric tons per year. (Source: Canadian Grains Council, Statistical Handbook 2001.)

American nutrition experts, food companies, and consumers recognized the nutritional benefits of canola oil.

Oilseed products enter the world trade as either oil or protein meal or are used in a wide variety of industries. Although the products from various oilseeds may



**Figure 6.** Canola seed production in Canada. (Source: Canada Grains Council, Statistical Handbook 2001.)

appear totally interchangeable, oils vary considerably in their chemical composition, nutritional properties, and functionality. The demand for oils is determined by the basic characteristics that give them their desired functionality. This is becoming even truer in the 1990s as genetically engineered plants and oils, targeted at specific niche markets, begin to emerge and become available to food processors. Modern technology enables manufacturers to substitute one oil for another, but once the oil has been modified, absolute availability and price become important determinants of demand.

The production or import of oilseed in a particular country will be determined by whichever oilseed product is in the greatest demand. For example, in Japan, the oilseed processing industry will consider the current domestic need for oils and fats, international markets for oils and fats, and domestic and international demand for protein meals before a decision is made as to which oilseed will be imported. Basically, when domestic need for protein is high, soybean will be imported and processed. If domestic demand for oil is the driving force, Japan imports canola for processing (151).

## REFERENCES

1. Saskatchewan Wheat Pool, *The Story of Rapeseed in Western Canada*, Regina, Saskatchewan, Canada, 1974.
2. T. Mag, in F. Shahidi, ed., *Canola and Rapeseed. Production, Chemistry, Nutrition and Processing Technology*, Avi Book, Van Nostrand Reinhold, New York, 1990, p. 251.
3. C. F. Ying and J. M. deMan, *Can. Inst. Food Sci. Technol.*, **22**, 222 (1989).
4. R. G. Ackman, in F. Shahidi, ed., *Canola and Rapeseed. Production, Chemistry, Nutrition and Processing Technology*, Avi Book, Van Nostrand Reinhold, New York, 1990, p. 81.
5. R. Scarth, P. B. E. McVetty, S. R. Rimmer, and B. R. Stefansson, *Can. J. Plant Sci.*, **68**, 509 (1988).
6. A. J. Vecchio, *Inform*, **7**, 230, 235, 239, 242 (1996).
7. R. Przybylski, N. A. M. Eskin, L. Malcolmson, S. Durance-Tod, R. Carr, and M. Mickle, *Lebensm. Wiss. u. Technol.*, **26**, 205 (1993).
8. I. Petukhov, L. Malcolmson, R. Przybylski, and L. Armstrong, *J. Am. Oil Chem. Soc.*, **76**, 627 (1999).
9. K. Warner and T. L. Mounts, *J. Am. Oil Chem. Soc.*, **70**, 983 (1993).
10. R. Wong, J. D. Patel, I. Grant, J. Parker, D. Charne, M. Elhalwagy, and E. Sys, in I. McGregor ed., *Proceedings of the Rapeseed Congress, Program and Abstract*, Saskatoon, Saskatchewan, Canada, 1991, p. 53.
11. P. Tso, S. Ding, K. DeMichele, and Y. S. Huang, in Yung-Sheng Huang and Vincent A. Ziboh, eds.,  *$\gamma$ -Linolenic Acid: Recent Advances in Biotechnology and Clinical Applications*, AOCS Press, Champaign, Illinois, 2001, p. 321.
12. J. L. Sebedio and R. G. Ackman, *J. Amer. Oil Chem. Soc.*, **58**, 972 (1981).
13. R. L. Wolff, *J. Amer. Oil Chem. Soc.*, **70**, 425 (1993).

14. R. C. Wijesundera and R. G. Ackman, *J. Amer. Oil Chem. Soc.*, **65**, 959 (1988).
15. R. Przybylski, C. G. Biliaderis, and N. A. M. Eskin, *J. Amer. Oil Chem. Soc.*, **70**, 1009 (1993).
16. X. Hu, J. K. Daun, and R. Scaith, *J. Amer. Oil Chem. Soc.*, **71**, 221 (1994).
17. T. Rezanka and P. Mares, *J. Chrom.*, **542**, 145 (1991).
18. H. Kallio and G. Currie, *Lipids*, **28**, 207 (1993).
19. R. G. Ackman, in J. K. G. Kramer, F. D. Sauer, and W. J. Pigden, eds., *High and Low Erucic Acid Rapeseed Oils*, Academic Press, Toronto, Ontario, Canada, 1983, p. 85.
20. M. Jáky and E. Kurnik, *Fetten-Seifen Anstrichmittel*, **82**, 267 (1981).
21. H. Liu, C. G. Biliaderis, R. Przybylski, and N. A. M. Eskin, *J. Amer. Oil Chem. Soc.*, **70**, 441 (1993).
22. F. Sosulski, P. Zadernowski, and K. Babuchowski, *J. Amer. Oil Chem. Soc.*, **58**, 561 (1981).
23. R. Przybylski and N. A. M. Eskin, *J. Amer. Oil Chem. Soc.*, **68**, 241 (1991).
24. J. Cmolik, J. Davidek, J. Pokorny, and J. Valisek, in K. Szymanski, ed., *Proceedings of the 7th Rapeseed Congress*, Poznan, Poland, 1987, p. 1368.
25. T. K. Mag, H. G. Duff, and J. H. Ritchie, in 1987 AOCS Annual Meeting, New Orleans, Louisiana, 1987.
26. A. Smiles, Y. Kakuda, and B. E. MacDonald, *J. Amer. Oil Chem. Soc.*, **66**, 348 (1989).
27. A. Smiles, Y. Kakuda, and B. E. MacDonald, *J. Amer. Oil Chem. Soc.*, **65**, 1151 (1988).
28. T. H. Smouse, in K. Warner and N. A. M. Eskin, eds., *Methods to Assess Oil Quality and Stability*, AOCS, Champaign, Illinois, 1994, p. 12.
29. A. Kamal-Eldin and L. A. Appelqvist, *Lipids*, **31**, 671 (1996).
30. G. W. Burton and K. U. Ingold, *Ann. N. Y. Acad. Sci.*, **570**, 7 (1989).
31. L. K. Patterson, in M. A. J. Rodgers and E. L. Powers, eds., *Oxygen and Oxy-Radicals in Chemistry and Biology*, Academic Press, New York, 1981, p. 89.
32. V. W. Bowry and R. Stocker, *J. Amer. Chem. Soc.*, **115**, 6029 (1993).
33. A. L. Tapel, *Ann. N. Y. Acad. Sci.*, **203**, 12 (1968).
34. R. C. Zambiasi, Ph.D. dissertation, University of Manitoba, Winnipeg, Manitoba, Canada, 1997.
35. L. Normand, N. A. M. Eskin, and R. Przybylski, *J. Amer. Oil Chem. Soc.*, **78**, 369 (2001).
36. Th. Willner, U. Jeß, and K. Weber, *Fett/Lipid*, **99**, 138 (1997).
37. R. P. Evershed, V. L. Male, and L. J. Goad, *J. Chrom.*, **400**, 187 (1987).
38. M. H. Gordon, and L. A. D. Miller, *J. Amer. Oil Chem. Soc.*, **74**, 505 (1997).
39. A. Strocchi, *Riv. Ital. Sostanze Grasse*, **64**, 401 (1987).
40. S. B. Kochar, *Progress in Lipid Research*, **22**, 161 (1983).
41. G. Marchio, R. de Andreis, and E. Fideli, *Rivista Italiana delle Sostanze Grasse*, **64**, 185 (1987).
42. R. Przybylski and N. A. M. Eskin, in I. McGregor, ed., *Proceedings of the 8th Rapeseed Congress*, Saskatoon, Saskatchewan, Canada, 1991, p. 888.
43. J. Nourooz-Zadeh and L. A. Appelqvist, *J. Amer. Oil Chem. Soc.*, **69**, 288 (1992).
44. Y. Endo, C. T. Thorsteinson, and J. K. Daun, *J. Amer. Oil Chem. Soc.*, **69**, 564 (1992).
45. K. Suzuki and A. Nishioka, *J. Amer. Oil Chem. Soc.*, **70**, 837 (1993).



46. B. Drozdowski, I. E. Goraj-Moszara, Z. Hazuka, M. Zajac, and R. Pawlowicz, in K. Szymanski, ed., *Proceedings of the 7th International Rapeseed Congress*, Poznan, Poland, 1987, p. 1304.
47. K. Ward, R. Scarth, J. K. Daun, and T. C. Thorsteinson, *J. Amer. Oil Chem. Soc.*, **71**, 1327 (1994).
48. L. L. Diosady, P. Sleggs, and T. Kaji, in *Canola Council of Canada, 7th Progress Report Research on Canola Seed, Oil, Meal and Meal Fractions*, Winnipeg, Manitoba, Canada, 1986, p. 186.
49. C. M. Elson, D. L. Hynes, and D. L. MacNeil, *J. Amer. Oil Chem. Soc.*, **56**, 998 (1979).
50. R. C. Wijesundera, R. G. Ackman, V. Abraham, and J. M. deMan, *J. Amer. Oil Chem. Soc.*, **65**, 1526 (1988).
51. D. Barnard, L. Bateman, E. R. Cole, and J. I. Cunneen, *Chem. Ind. (London)*, July, p. 918 (1958).
52. D. M. Chapman, E. A. Pfannkoch, and R. J. Kupper, *J. Amer. Oil Chem. Soc.*, **71**, 401 (1993).
53. R. Przybylski, and N. A. M. Eskin, *J. Amer. Oil Chem. Soc.*, **65**, 629 (1988).
54. T. P. Labuza, *CRC Crit. Rev. Food Technol.*, **2**, 355 (1971).
55. R. Usuki, Y. Endo, and T. Kaneda, *Agric. Biol. Chem.*, **48**, 991 (1984).
56. R. G. Ackman, and C. A. Eaton, *J. Amer. Oil Chem. Soc.*, **54**, 435 (1977).
57. H. Noureddini, B. C. Teoh, and L. D. Clements, *J. Amer. Oil Chem. Soc.*, **69**, 1184 (1992).
58. W. Lang, S. Sokhansanj, F. W. Sosulski, *J. Amer. Oil Chem. Soc.*, **69**, 1054 (1992).
59. H. Noureddini, B. C. Teoh, and L. D. Clements, *J. Amer. Oil Chem. Soc.*, **69**, 1189 (1992).
60. V. M. Arens, G. Ghur, and J. Weibel, *Fette Seifen Anstrichm.*, **79**, 256 (1977).
61. N. B. Kyriakidis, and T. Katsiloulis, *J. Amer. Oil Chem. Soc.*, **77**, 1235 (2000).
62. L. D'Souza, J. deMan, and J. M. deMan, *J. Amer. Oil Chem. Soc.*, **68**, 153 (1991).
63. S. McCurdy, Project Report 89-326, POS Pilot Plant Corp., Saskatoon, SK, Canada, 1990.
64. R. Kruger, Project Report 91-19C, Canola Utilization Assistance Program, Canola Council of Canada, Winnipeg, Manitoba, Canada, 1992.
65. J. A. H. Dahlén and Ch. Kristofferson, in *ISF/AOCS World Congress*, New York, 1980.
66. E. H. Unger, in F. Shahidi, ed., *Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology*, van Nostrand Reinhold, New York, 1991, p. 235.
67. G. Penk, in A. R. Baldwin, ed., *Proceedings of the World Conference on Emerging Technologies in the Fats and Oils Industry*. American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 38-45.
68. Stork Friesland Scandinavia AB, Goeteborg, Sweden.
69. E. W. Lusas, L. R. Watkins, and S. S. Koseogulu, Project ASA RP-88401, Food Protein Research & Development Center, Texas A&M University, College Station, Texas, 1989.
70. N. Buhr, in D. Erickson, ed., *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, American Oil Chemists' Society, Champaign, Illinois, 1990, p. 43.
71. D. R. Grant, R. L. Eager, J. M. Pepper, and J. F. Mathews, *The 8th Progress Report*, Canola Council of Canada, Winnipeg, Manitoba, Canada, 1987, p. 141.

72. F. H. Schneider and U. Schuette, *Fett, Wissenschaft u. Technologie*, **86**, 331 (1984).
73. L. D. Campbell and B. A. Slominski, *J. Amer. Oil Chem. Soc.*, **67**, 73 (1990).
74. Canadian Grain Commission, Inspection Division, *Official Grain Grading Guide*, Winnipeg, Manitoba, Canada.
75. Canadian Oilseed Processors Association, *Trading Rules, 1993-1994*, Winnipeg, Manitoba, Canada, pp. 6, 7, 23, 33.
76. American Oil Chemists' Society, *Official Methods and Recommended Practices*, Champaign, Illinois, 2002.
77. H. J. Ringers and J. C. Segers, U.S. Patent 4,049,686, 1980, (to Unilever N.V.).
78. T. K. Mag, in F. Shahidi, ed., *Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology*, van Nostrand Reinhold, New York, 1991, p. 251.
79. K. F. Carlson, *Inform*, **4**, 272 (1993).
80. Y. Endo, R. Usuki, and T. Kaneda, *Agric. Biol. Chem.*, **48**, 985 (1984).
81. T. K. Mag, in D. Erickson, ed., *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, American Oil Chemists' Society, Champaign, Illinois, 1990, p. 107.
82. U. I. Brimberg, *Fette, Seifen, Anstrichmittel*, **83**, 184 (1981).
83. J. H. Henderson, *J. Amer. Oil Chem. Soc.*, **70**, 831 (1993).
84. K. Suzuki and A. Nishioka, *J. Amer. Oil Chem. Soc.*, **70**, 837 (1993).
85. P. M. Parker, in 84th AOCS Meeting, Anaheim, California, 1993.
86. L. O. Bergman, Canadian Patent 664,968, June 11, 1963, (to AB Pellerins Margarinfabrik).
87. H. Szemraj, *Pluszcze Jadalne*, **18**, 13 (1974).
88. P. Rothmund, in Kirk-Othmer, ed., *Encyclopedia of Chemical Technology*, Vol. 5, 2nd ed., 1964, p. 348.
89. V. Abraham, and J. deMan, *J. Amer. Oil Chem. Soc.*, **63**, 1185 (1986).
90. J. deMan, J. E. Pogorzelska, and L. deMan, *J. Amer. Oil Chem. Soc.*, **60**, 558 (1983).
91. G. G. Hatfield, MSc thesis, University of Toronto, Faculty of Applied Chemistry and Chemical Engineering, Toronto, Ontario, Canada, 1994.
92. N. Nock, in Annual Meeting of the American Oil Chemists' Society, Atlanta, Maryland, 1994.
93. J. de Man and L. deMan, *Inform*, **3**, 542 (1992).
94. A. Naguib-Mostafa, *J. Amer. Oil Chem. Soc.*, **62**, 756 (1985).
95. V. D'Souza, J. deMan, and L. deMan, *J. Amer. Oil Chem. Soc.*, **68**, 153 (1991).
96. K. C. Thomas, *J. Can. Inst. Food Sci. Technol.*, **2**, 1167 (1988).
97. W. J. Desrosier, MSc. Thesis, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1988.
98. J. Kurashige, N. Matsusaki, and H. Takahashi, *J. Amer. Oil Chem. Soc.*, **70**, 849 (1993).
99. R. Przybylski, personal communication, 1994.
100. F. H. Mattson and S. M. Grundy, *Lipids*, **26**, 194 (1985).
101. J. Westhuysen, *Ann. Clin. Lab. Sci.*, **27**, 1 (1997).
102. D. Steinberg, S. Parthasarathy, T. E. Carew, J. C. Khoo, and J. L. Witztum, *New Engl. J. Med.*, **320**, 915 (1989).

103. S. Parathasarathy and S. M. Rankin, *Prog. Lipid Res.*, **31**, 127 (1992).
104. P. Reaven et al., *Am. J. Clin. Nutr.*, **54**, 701 (1992).
105. M. Abbey, G. B. Belling, M. Noakes, F. Hirata, and P. J. Nestel, *Am. J. Clin. Nutr.*, **57**, 391 (1993).
106. M. Kratz, P. Cullen, F. Kannenberg, A. Kassner, M. Fobker, P. M. Abuja, G. Assmann, and U. Wahrburg, *Eur. J. Clin. Nutr.*, **56**, 72 (2002).
107. A. M. Lees, H. Y. I. Mok, R. S. Lees, M. A. McCluskey, and S. M. Grundy, *Atherosclerosis*, **28**, 325 (1977).
108. T. Heinemann, G.-A. Kullak-Ublick, B. Pietruck, and K. von Bergmann, *Eur. J. Clin. Pharmacol.*, **40**, S59 (1991).
109. R. P. Mensink and M. B. Katan, *New Engl. J. Med.*, **321**, 436 (1989).
110. B. E. McDonald, V. M. Bruce, E. L. LeBlanc, and D. J. King, in 4th Internal Rapeseed Congress, 1974, pp. 693–700.
111. A. Keys, J. T. Anderson, and F. Grande, *Metabolism*, **14**, 776 (1965).
112. D. M. Hegsted, R. B. McGandy, M. L. Myers, and F. J. Stare, *Am. J. Clin. Nutr.*, **17**, 281 (1965).
113. B. E. McDonald, J. M. Gerrard, V. M. Bruce, and E. J. Corner, *Am. J. Clin. Nutr.*, **50**, 1382 (1989).
114. G. M. Wardlaw, J. T. Shook, M.-C. Lin, M. A. Puangco, and J. S. Kwan, *Am. J. Clin. Nutr.*, **54**, 104 (1991).
115. J. K. Chan, V. M. Bruce, and B. E. McDonald, *Am. J. Clin. Nutr.*, **53**, 1230 (1991).
116. L. M. Valsta, M. Jauhianinen, A. Aro, M. B. Katan, and M. Mutanen, *Arterioscler. Thromb.*, **12**, 50 (1992).
117. T. A. Miettinen and H. Vanhanen, *Am. J. Clin. Nutr.*, **59**, 356 (1994).
118. M. L. Bierenbaum, R. P. Reichstein, T. R. Watkins, W. P. Maginnis, and M. Geller, *J. Am. Coll. Nutr.*, **10**, 228 (1991).
119. T. Seppanen-Lasskso, H. Vanhanen, I. Laakso, H. Kohtamaki, and J. Viikari, *Br. J. Nutr.*, **68**, 639 (1992).
120. A. H. Lichtenstein et al., *Arterioscler. Thromb.*, **13**, 1533 (1993).
121. T. L. V. Ulbricht and D. A. T. Southgate, *Lancet*, **338**, 985 (1991).
122. J. Dyerberg, *Nutr. Rev.*, **44**, 125 (1986).
123. P. M. Herold and J. E. Kinsella, *Am. J. Clin. Nutr.*, **43**, 556 (1986).
124. S. Renaud, F. Godsey, E. Dumont, C. Thevenon, E. Ortchanian, and J. L. Martin, *Am. J. Clin. Nutr.*, **43**, 136 (1986).
125. B. M. Weaver, E. J. Corner, V. M. Bruce, B. E. McDonald, and B. J. Holub, *Am. J. Clin. Nutr.*, **51**, 594 (1991).
126. J. K. Chan, B. E. McDonald, J. M. Gerrard, V. M. Bruce, B. J. Weaver, and B. J. Holub, *Lipids*, **28**, 811 (1993).
127. J.-S. Kwon, J. T. Snook, G. M. Wardlaw, and D. H. Hwang, *Am. J. Clin. Nutr.*, **54**, 351 (1991).
128. M. Mutanen, R. Freese, M. L. Valsta, I. Ahola, and A. Ahlstrom, *Thromb. Haemost.*, **67**, 352 (1992).
129. E. J. Corner, V. M. Bruce, and B. E. McDonald, *Lipids*, **25**, 598, (1990).
130. T. A. B. Sanders and K. M. Younger, *Br. J. Nutr.*, **45**, 613 (1981).

131. M. de Lorgeril et al., *Lancet*, **343**, 1454 (1994).
132. M. de Lorgeril et al., *Circulation*, **99**, 779 (1999).
133. B. F. Teasdale, Canadian Patent 726,140, 1966.
134. G. Pongracz, *Fat Sci. Technol.*, **90**, 249–258 (1988).
135. National Academy of Sciences, *Report of the Panel on Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*, Food and Nutrition Board, National Academy Press, Washington, D.C., 2000, p. 187.
136. Anonymous, *Inform*, **12**, 487 (2001).
137. Anonymous, *Inform*, **9**, 826 (1990).
138. Anonymous, *Inform*, **11**, 1061 (2000).
139. R. Scarth, P. McVetty, and S. Rimmer, in D. McGregor, ed., *The Proceedings of the Eighth Rapeseed Congress*, Saskatoon, Saskatchewan, Canada, 1991, p. 143.
140. G. Galili, S. Galili, E. Lewinsohn, and Y. Tadmor, *Critical Rev. Plant Sci.*, **21**, 167 (2002).
141. T. Voelker, T. Hayes, A. Crammer, and H. Davies, *Plant J.*, **9**, 229 (1996).
142. Anonymous, *Inform*, **12**, 1019 (2001).
143. C. H. Japiske, Canadian Patent 815,481, 1969.
144. B.F. Teasdale and T. K. Mag, in J. K. G. Kramer, F.D. Sauer, and W. J. Pigden, eds., *High and Low Erucic Acid Rapeseed Oils*, Academic Press, Toronto, Ontario, Canada, 1983, p. 197.
145. K. J. Harrington, *Biomass*, **9**, 1 (1986).
146. G. Knothe, R. O. Dunn, and M. O. Bagby, *Inform*, **7**, 827 (1996).
147. R. Varese and M. Varese, *Inform*, **7**, 816 (1996).
148. G. Boerner, private communication, OHMI GmbH, Magdeburg, Germany.
149. S. Latta, *Inform*, **1**, 438 (1990).
150. D. J. Murphy, *Trends Biotech.*, **14**, 206 (1996).
151. G. Bastin, *Vegetable Oilseeds and Oils Traders' Guide*, The International Trade Centre, UNCTAD/GATT, 1988.

# 3

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## *Coconut Oil*

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### **1. THE COCONUT PALM**

In the tropics, the coconut palm (*Cocos nucifera*, L.) is one of the most useful trees. The palm reaches 30 m or more in height, with a striped, unbranched, and relatively smooth trunk measuring approximately 25 cm in diameter and crowned with some 30 fronds, each of which is about 5–6 m long. The roots may extend up to 10 m in sandy soil, serving as anchor in addition to their primary function as nutrient absorbers and breathing organs (1).

As a perennial provider of food, beverage, shelter, animal feed, and feed-stock for the oleochemical industries, the palm is reverently described as the Tree of Life, Tree of Heaven, and other such metaphors by people of coconut-producing countries.

In some areas of the tropics, the palm grows with a minimum of attention through a lifespan of more than 50 years. Its fruit, the coconut, has been referred anticlimactically as the “lazy man’s crop”; coconuts produced throughout the year drop to the ground when mature, there to be collected at leisure. Commercial farms, however, are tended and developed to maintain and improve productivity.

#### **1.1. Geographical Distribution**

Within 20° north and south latitudes, the coconut palm is productive, especially along coastal areas (2). Palms grown beyond the limits of the Torrid Zone are

generally nonproductive. The major coconut-growing areas are located in Asia, islands of the Pacific Ocean, Africa, and Central and South America. In 1991 the world coconut hectareage was 10.9 million (3).

## 1.2. Agronomy

Coconut farms are established in a wide variety of soil types, from littoral sands to heavy clay. Palms grown in well-aerated soil are productive and tolerate soil pH ranging from 5 to 8 (4). In determining the suitability of sites for coconut farms, a good supply of soil moisture with adequate drainage are considered the foremost factors. Water supply may come from well-distributed rainfall (1300–2300 mm/year), irrigation, or from accessible water table (5). The extremes, excessive rainfall and drought, affect the nut yield per palm.

The coconut palm thrives well in plenty of sunlight. It has been observed that the palm produces more female flowers and a higher weight of copra per nut during the months of May to August when there is abundant sunshine. Furthermore, young palms grow slowly when planted under old trees in the course of rehabilitating plantations (6).

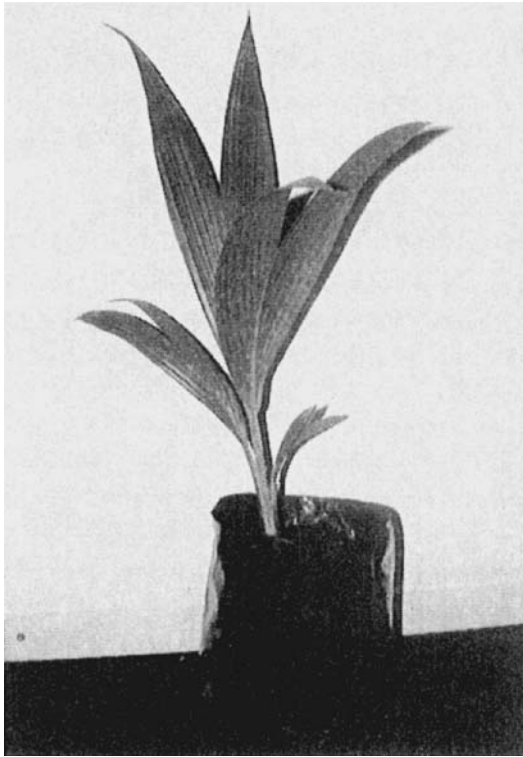
Soil, water, and foliar analyses in coconut plantations are conducted to determine the type of fertilizer and micronutrients required by the palm. Long-term studies show that the application of potassium or sodium chlorides in optimum doses as supplements to the standard nutrients for inland farms increases yields in nuts per tree and copra per nut (7, 8).

## 1.3. Propagation

The method of propagation for coconut palms is through seednuts. Due to the highly heterogeneous character of coconut palms, progenies do not necessarily exhibit all the desirable characteristics of the parent palms. Through selection of seeds from elite parents, vigorous and productive palms may be evolved. The long span of six or more years required to evaluate the performance of progenies of coconut palms has underlined the need to explore other methods of propagation; the response appears to be the current interest in the development of tissue culture techniques.

*Seedlings.* Propagation of the coconut palm starts from the collection of fully matured nuts (11–12 months old) from selected palms. The seednuts are stored in the shade for at least one month. In the nursery, seednuts are set to germinate in loose and friable soil provided with adequate moisture and drainage facilities. Some farmers transfer sprouted seednuts into polybags to allow selection of seedlings showing uniformity in growth and vigor (see Figure 1). Further, seedlings in polybags do not suffer damage and shock when transported or when transplanted in the fields.

Six-to nine-month-old seedlings with four to five leaves may be transplanted in fields and spaced to accommodate 115–160 palms per hectare. Coconut palms,



**Figure 1.** A 9-month-old seedling raised in polybag and ready for transplanting in the field.

on the average, start bearing fruits in the sixth year and continue to provide fruits perennially for more than 50 years, barring any serious damage caused by plant pests, diseases, climatic disasters, or neglect in farm management.

*Tissue Culture.* The most promising vegetative (asexual) method under development for the propagation of elite coconut palms is the tissue culture technique. In this method, the actively dividing tissue from a selected palm is removed and grown aseptically in a specially formulated culture medium containing the prescribed nutrients, hormones, and root and shoot growth-promoting factors. Under the right induction conditions, the explant develops into a callus, which is induced to differentiate into shoots and roots forming the plantlet. The plantlet is hardened in a pot kept in a greenhouse before transplanting in the field.

The progress of studies on vegetative propagation have been reported with encouraging results (9–11). Refinements of the current state of the art to improve the number of survivors from the callus stage to seedlings planted in the fields is needed to make tissue culture of coconut palms a feasible method to merit commercial application.

## 1.4. Varieties

There is a wide range of variations that may occur within a coconut palm colony with regard to phenotype characteristics such as color, size and shape of nuts, and shape and symmetry of crown, among others. In some isolated localities, colonies of coconut palm develop well-defined and uniform phenotypic characters arising from generations of natural selection.

*Makapuno*, a peculiar Philippine coconut variety, has a cavity within the kernel filled with a palatable jellylike substance. These fruits do not germinate although they contain an embryo. However, the propagation of *makapuno* has been successfully developed through *in vitro* culture of its rescued embryo and final establishment of the seedling in the field (12, 13).

The two most distinguishable varieties of coconut palm are the tall and the dwarf. The tall variety grows to a height of more than 20 m while the dwarf variety attains a height of approximately 3 m upon maturity. Tall  $\times$  dwarf hybrids and the reciprocal dwarf  $\times$  tall give progenies that have most of the superior properties of either parent. With properly selected parents, the hybrid palms are generally hardy, resistant to drought and diseases, precocious, and large fruit bearers.

## 2. THE FRUIT

The common mature coconut fruit weighs more than 1 kg and is ovoid in shape and green or yellow in color. The nut has a smooth epidermis over a fibrous mesocarp (husk) that covers the hard endocarp (shell). Within the shell is the endosperm (kernel, meat) approximately 1–2 cm thick. A thin brown layer called *testa* separates the kernel from the inner surface of the shell. The cavity within the kernel has an average volume of 300 mL and contains the endosperm liquid (coconut water) (see Figure 2).

### 2.1. Kernel

The kernel is the origin of the following products: coconut oil, desiccated coconut, coconut skim milk, coconut cream, coconut flour, protein powder, and copra meal (see Table 1).

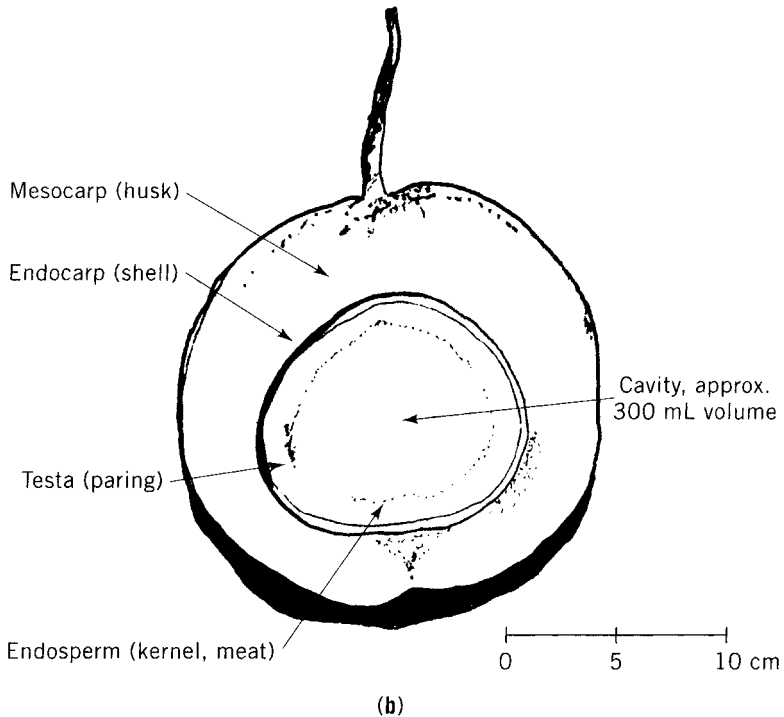
### 2.2. Coconut Water

Coconut water is a sterile liquid. From mature nuts, it has the following constituents in percent: total solids 4.7, fat 0.74, protein 0.55, ash 0.46, and reducing sugar 1. The liquid has a pH of 5.6 (14). It is a coproduct of desiccated coconut and considered a health beverage. Concentration of coconut water by reverse osmosis may further expand its use in the fermentation and food industries.

### 2.3. Testa

The thin brown layer between the kernel and shell is the testa. This layer is pared off from the kernel's outer surface to eliminate colored bodies in the production of





**Figure 2.** Mature coconut fruit and a longitudinally halved specimen.

desiccated coconut. The parings contain oil that has a fair amount of unsaturated fatty acids  $C_{18:1}$  and  $C_{18:2}$  (see Table 2).

## 2.4. Shell and Husk

The shell that encloses the kernel is a hard spherical covering 3–5 mm thick. It is used mainly for fuel in copra making. Other products derived from coconut shell are charcoal, activated carbon, filler for synthetic resin, glues, components in mosquito-repellent coils, and decorative items.

**TABLE 1. Typical Composition of Coconut Kernel (1, p. 171).**

Composition	%
Moisture	50.0
Oil	34.0
Ash	2.2
Fiber	3.0
Protein	3.5
Carbohydrates	7.3

**TABLE 2. Fatty Acid Composition of Paring (Testa) Oil (1, p. 253).**

Fatty Acid	Paring Oil (%)
Caproic C6	0.1
Caprylic C8	0.5
Capric C10	1.5
Lauric C12	22.7
Myristic C14	15.8
Palmitic C16	17.2
Stearic C18	1.3
Oleic C18 : 1	25.4
Linoleic C18/2	15.5

The husk is 5–10-cm-thick fibrous cover adhering to the coconut shell. Like the shell, it is also used mainly for fuel in farms. Products derived from the husk are coir, bristle, rubberized fiber, rope, geotextiles, and activated carbon.

### 3. COPRA

Copra is the dried kernel of coconuts. Fresh kernels contain approximately 50% moisture. Various drying methods are employed to bring down the moisture content ideally to 6–8% (see Table 3). At this level, mold growth in copra is inhibited. The conversion of kernel to copra is an essential step if the oil is to be drawn by the conventional mechanical extraction method.

#### 3.1. Sun Drying

Dehusked nuts are split into halves and drained. The halves are exposed to the sun and in due time the kernels shrink. The partially dried kernels are separated from the shells for further drying under the sun for 6–8 days. During occasional rains, the kernels are protected with adequate cover, such as plastic sheets or any other suitable material.

**TABLE 3. Calculated Yields Based on an Initial 100 g Fresh Kernel with 50% Moisture Content (1, p. 162).**

Moisture	50	15	12.5	10	7.5	5	0
Oil	34.5	58.65	60.37	62.10	63.82	65.55	69.0
Nonoils	15.5	26.35	27.13	27.90	28.68	29.45	31.0
Copra wt., g	100.0 <sup>a</sup>	58.82	57.14	55.55	54.05	52.63	50.0

<sup>a</sup>Fresh kernel.

### 3.2. Direct Fire Drying

The direct fire dryer consists of a bamboo grill platform where the split nuts are placed. Underneath is a fire hearth where coconut shells and husks are burned to provide heat for the vaporization of water from the kernels. The kernels shrink and are separated from the shells for further drying. Smoke from the burning fuel imparts a light brown color on the copra and its oil.

Small farm producers continue to use this traditional method because of the low cost of construction, simplicity of design, and ready availability of shell and husk for fuel in coconut farms.

### 3.3. Hot-Air Drying

In 1978, Lozada (15) introduced a low-cost efficient dryer that is adaptable for small farms. The dryer uses husks or shells for fuel in a specially designed burner that can operate with an even supply of heat. The dryer is provided with a steel plate bottom to isolate the kernels from the smoke generated by the fuel.

There are a number of hot-air dryer models, devices inspired by modern technology. In principle, these dryers apply hot air to expel moisture from the kernels. Since smoke does not come in contact with the kernels, the product is a cleaner and whiter copra.

The relationship of oil, non-oil, and moisture contents from fresh kernel to copra as moisture diminishes in stages is given in Table 3.

## 4. OIL EXTRACTION

On an industrial scale, the *dry process* is the traditional method of extracting oil from the coconut (16). This is done by crushing copra in an *expeller*, the trade name of the machine patented by V. D. Anderson. The meal (or cake) may be further treated with solvents to extract residual oil.

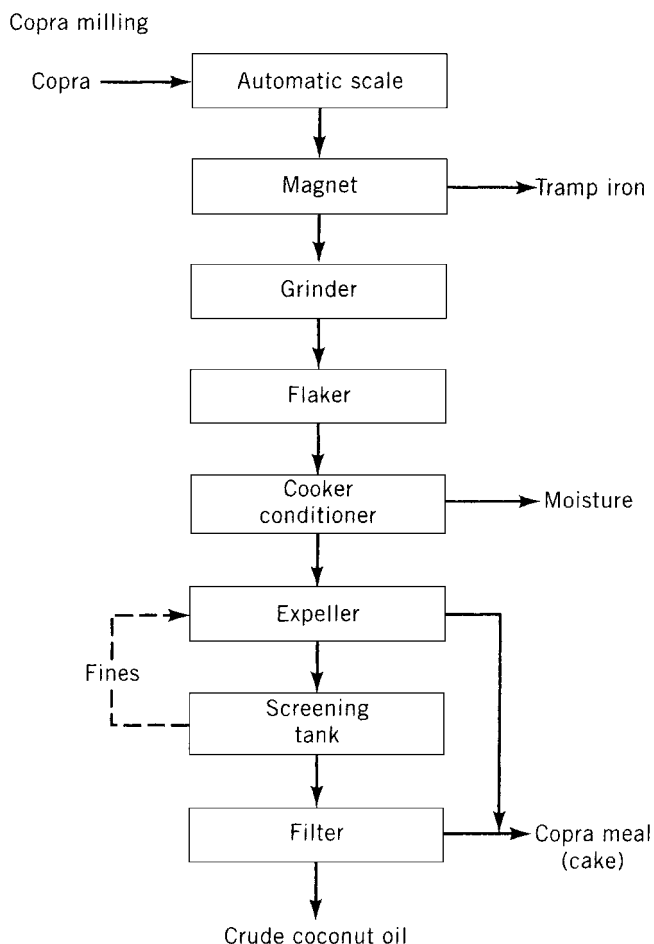
The *wet-process* feedstock is fresh kernel instead of copra. The extracted oil does not have to be refined, unlike the oil from copra. The coproducts of oil from the wet process are edible.

Sections 4.1 and 4.2 briefly describe the major steps in the extraction of oil from copra and fresh kernel, respectively.

### 4.1. Dry Process

The dry process involves mechanical extraction of oil in crushers or expellers with copra as feedstock (see Figure 3). Mechanical extraction may be supplemented with a second extraction, using solvents, to recover residual oil from the meal.

*Mechanical Extraction.* Copra with 10–12% moisture content is conveyed to an automatic scale, passes through a magnetic chamber for the removal of tramp iron, and is ground to a particle size of approximately 0.3 cm in diameter. The particles are flaked to facilitate exposure of a large surface area.



**Figure 3.** Mechanical extraction flow chart (16).

The flakes are cooked and conditioned for 20 min at 115°C in horizontal cookers. Residual moisture is brought down to 3% as fat cells of the conditioned copra flakes are ruptured and phosphatides are precipitated.

The sized, cooked, and conditioned flakes are fed to the crushers where a continuous squeezing action extracts the oil. Extraction characteristic follows a smooth diminishing curve with most of the oil drained at the inlet section of the barrel cage and tapers off toward the exit. The resulting copra meal has a typical residual oil content of 7%.

The characteristic of oil and copra meal from a well-maintained and properly operated expeller are as follows: (1) low-colored oil, 8 Red/50 Yellow in the Lovibond scale; (2) light brown copra meal with 8% maximum residual oil; and (3) copra meal with a uniform thickness of approximately 0.6 cm and 6% maximum meal fines.

A sustained optimum expeller efficiency requires cooking and conditioning of copra flakes to be controlled between 91°C and 93°C under an appropriate residence time to reduce moisture content between 3% and 4%. Flakes conditioned below 91°C with residual moisture above 4% have lower oil yield while flakes conditioned above 93°C with residual moisture below 3% would yield dark-colored oils, charred meal with the consequent disadvantage of poor oil extractability. Screened and settled fines should not exceed 10% of fresh feedstock to prevent the formation of abrasive particles during the cooking/conditioning stage.

*Post-expeller Treatment.* The oil from the expellers passes to a screening and settling tank to initiate the separation of fines, which are recycled with fresh feedstock to the system. To each ton of supernatant oil, 10 kg of bleaching earth is mixed. Passage through a polishing filter gives a clear oil ready for storage or further processing.

Copra meal (cake) from the filter press is pelletized, bagged, and despatched to animal feed millers.

*Solvent Extraction.* This operation supplements mechanical extraction and consequently minimizes residual oil in the copra meal.

Copra undergoes an accelerated preliminary extraction with a controlled residual oil content of 14–18% in the expeller meal; expeller throughput rate is almost doubled. Hexane (bp 68.7°C) is widely used as the solvent for extraction. In an extraction unit that operates on a countercurrent system, the cake is met by oil-rich miscella (hexane + oil) and leaves the extractor as it is rinsed with pure hexane. The solvent-extracted cake has a residual oil content of approximately 3.5%.

Hexane in miscella and cake is recovered for reuse in succeeding operations. Escaping hexane vapors are trapped by cold mineral oil spray to preclude hazards and maximize solvent recovery.

## 4.2. Wet Process

Feedstock in the wet process (17) is fresh kernel. In addition to oil, other edible coproducts are recovered from the kernel, namely, coconut flour, protein, carbohydrates, and vitamins.

To encourage wider commercial application of this process, the following advantages are emphasized: superior quality of the oil product and the recovery of nutrient coproducts that would otherwise be lost in copra. The process is briefly described to give a general view on the extraction of oil from fresh kernels.

Dehusked mature nuts are shelled to separate the kernels, followed by paring to remove the testa. The testa is set aside for the extraction of paring oil, a byproduct. The pared kernels are finely comminuted through a wedge and die plate mill and through a roller mill. The comminuted mass is passed through a screw press, which expels the coconut milk. The milk is filtered through a screen conveyor. The cream that is separated from the milk by centrifugation is heated to reduce its moisture content. By further centrifugation, oil is separated. Trace moisture in the oil is reduced to 0.1–0.2% level by atmospheric heating. A typical yield of 6.8 tons “natural” coconut oil is extracted from 25 tons of fresh kernel.

The skim milk is spray-dried to recover proteins and carbohydrates. Residue from the screw press and parings are milled to recover oil and coconut flour.

## 5. REFINING

Refining of crude fats and oils involves a series of steps for the removal of impurities from the glycerides to make the product suitable for human consumption and improve product shelf life. The impurities are fatty acids, phosphatides, metal ions, color bodies, oxidation products, solid particles, and volatiles that include objectionable odors. Crude coconut oil is refined by any of the following methods: (1) chemical refining (batch or continuous) and (2) physical refining. The comparative performance of both methods is summarized in Figure 4.

### 5.1. Chemical Refining (16)

The free fatty acids (FFA) of crude coconut oil are neutralized with dilute sodium hydroxide solution resulting in the formation of soap:  $\text{RCOOH} + \text{Na}^+\text{OH}^- \rightarrow \text{RCOO}^-\text{Na}^+ + \text{H}_2\text{O}$ . The soap and other impurities in the water phase are collectively called *soapstock*.

In the *batch refining* process, the separation of the water phase containing soapstock from the oil is by gravity. Some amount of neutral oil is lost by saponification and by occlusion in soapstock.

*Continuous refining* process, on the other hand, offers the following major advantages over the batch-type operation: (1) saponification of neutral oil is minimized due to a short contact time of 30–45 s between oil and sodium hydroxide; (2) the time consumed for the separation of the aqueous soapstock and wash water from the oil is reduced considerably by passage through centrifugal separators.

After refining (neutralization), the oil is bleached. Color bodies in the oil are adsorbed on the surface of the bleaching clay and activated carbon particles. Experiments cited by Brimberg (19) showed that bleaching process follows the rate formula,  $\ln(c/c_0) = -k\sqrt{t}$ , where  $t$  is the time from the addition of bleaching clay;  $c$  is the concentration of pigment at time  $t$ ,  $c_0$  is the concentration at  $t_0$ , and  $k$  is the rate constant.

Efficiency in the bleaching process may be improved for certain oils when a small amount of water is present during the mixing steps (20).

Deodorization is the last step in chemical refining. Volatile odoriferous substances, including low-molecular-weight fatty acids, are removed by stripping with steam under reduced pressure. The final product is generically called RBD (refined, bleached, and deodorized) coconut oil.

A lower grade product called *Cochin oil* is coconut oil that is chemically refined and bleached but not deodorized.

*Batch Neutralization.* Crude coconut oil from storage is fed into cylindrical tanks equipped with heating coils and stirrers. The oil is heated to 80°C while being

Crude coconut oil  
3.9% FFA 1,000 kg  
gums negl.

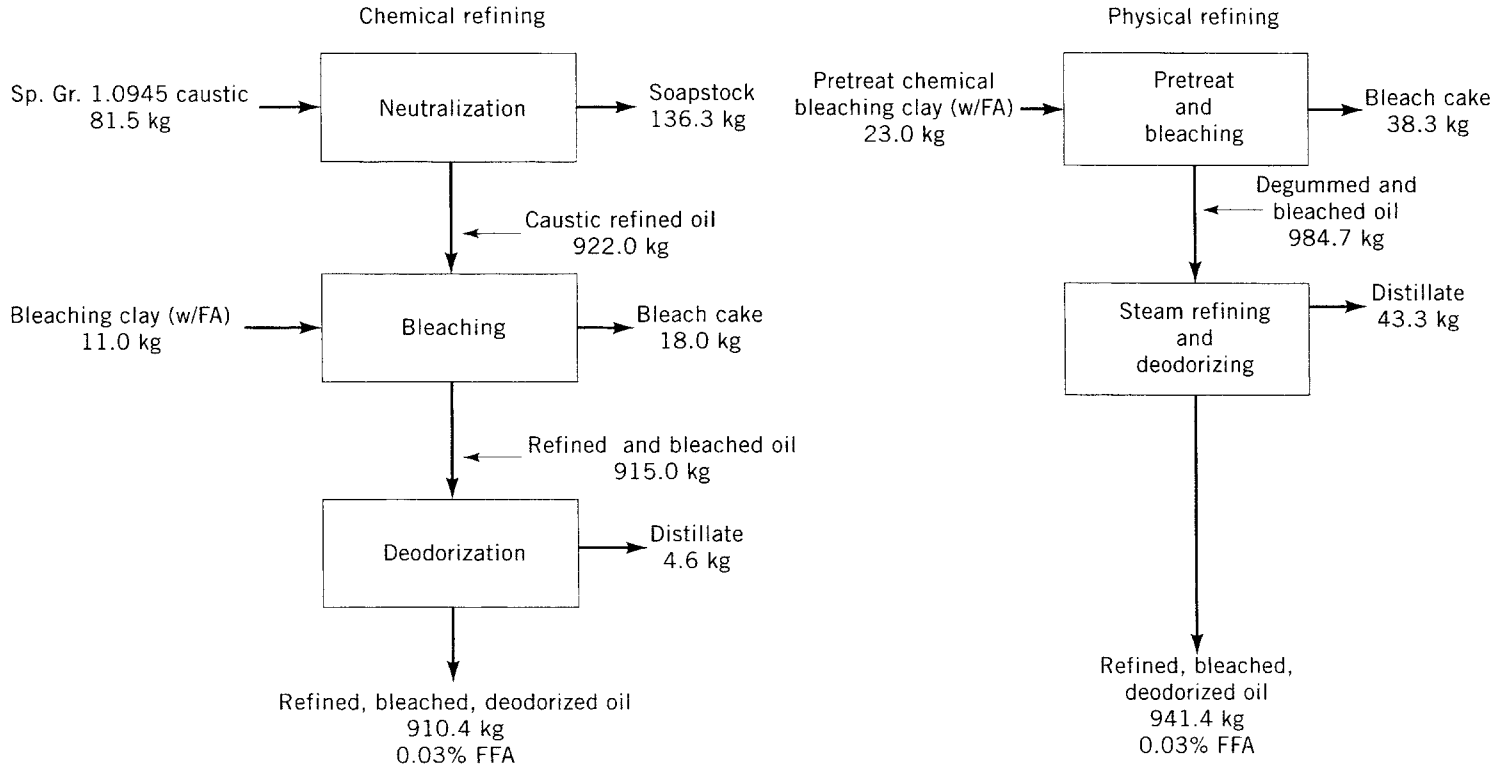


Figure 4. Material balance: chemical and physical refining of crude coconut oil. Units were converted from the original lb. and Be to kg. and sp. gr. (18).

stirred, and a measured amount of sodium hydroxide solution is introduced by spraying on the surface of the oil. An excess of 5–10% sodium hydroxide over the stoichiometric requirement is added to ensure appropriate neutralization of free fatty acids. In this stage, hydrated gums migrate to the water phase. Heating and stirring are stopped when soap “breaks” are formed. A break forms as soap coagulates with some occluded neutral oil, excess sodium hydroxide, and other impurities. The aqueous soapstock is allowed to settle and subsequently drawn off for acidulation with sulfuric acid to recover a mixture of fatty acids, occluded neutral oil, and other impurities. The mixture is called *acid oil*.

The neutral oil is washed with soft water at 10–15% dosage to remove traces of soap. The washed neutral oil is dried under vacuum and stored in buffer tanks.

*Continuous Neutralization (Short Mix)*. A regulated volume of crude coconut oil is heated through a plate-type heat exchanger to a temperature of 90–95°C, conveyed to a centrifugal mixer where a required volume of 2*N* sodium hydroxide solution is added through a metering pump. The oil-soap-stock mixture is passed through a primary centrifuge for the separation of soapstock from the neutralized oil. Neutralized oil from the primary separator is heated to 90°C and mixed with 10–15% hot water to wash off residual soap. From a second-stage separator, the washed oil cascades over a series of plates in the vacuum dryer maintained under a pressure of 3000 Pa. The dried neutral oil is stored in buffer tanks.

*Bleaching*. In a cylindrical reactor provided with heating coils and a variable agitator, the neutral oil is brought to a temperature of 90°C under a pressure of 600–1000 Pa and agitated. A predetermined amount of bleaching earth and activated carbon is added via a dosing device installed at the top of the mixer. After 30 min, the slurry is dried and later passed through a plate and filter press for the removal of spent earth and carbon. The neutralized and bleached oil is cooled down to 50°C before being transferred to storage tanks. Steam is injected through the spent earth to recover entrained oil.

*Deodorization*. The neutralized/bleached oil is pumped into a deaerator operated under a pressure of 500 Pa to evacuate entrained air. From the deaerator, the oil passes through a shell and tube economizer and is heated to a temperature of 240°C by means of a thermal oil heater. The stripper and deodorizing column operates under a pressure of 600–1000 Pa; volatile components such as low-molecular-weight fatty acids, ketones, aldehydes, and other odoriferous substances are stripped off by live steam. The rising vapors laden with volatile components pass through a cyclone scrubber where fresh fatty acid oil is sprayed on top of the vessel to recover outgoing fatty acids.

The deodorized coconut oil flows down to the drop tank and holding chamber of the distilling column. Residence time in the deodorizer ranges from 1.5 h to 2 h. The oil from the column is withdrawn by a hermetically sealed and heat-resistant pump and conveyed to the economizer, which preheats the incoming deodorizer feedstock. The oil is cooled down to 50°C, at which temperature citric acid is added. Citric acid enhances the stability of oil by immobilizing iron and copper, which are pro-oxidants. (Note: The deodorization step is omitted in the production of Cochin oil.)



## 5.2. Physical Refining

Coconut oil refiners have gained interest in the physical refining system as a substitute for chemical refining for the following reasons: (1) physical refining has lower oil losses vis-à-vis chemical refining; (2) pollution problems associated with soapstock acidulation is precluded; (3) lower installation cost; (4) lower steam, water, and power consumption; and (5) distilled fatty acids are of a higher grade than the acid oil from chemical refining (21).

Design and technological breakthroughs have improved efficiency of the system. Performance of new packed-column deodorizers for physical refining is predicted by computer calculation, for which a special program has been developed on the basis of experience with earlier columns (22).

The main feature in physical refining of crude oils is the application of steam distillation to remove the free fatty acids and volatile components from the oil. The technical feasibility of physical refining depends largely on the pretreatment stages for the removal of phosphatides, color bodies, metal ions, and nonvolatile impurities. Without an effective pretreatment, steam refining may fail to produce an oil of color and stability characteristics comparable to the classically refined product (23).

*Degumming.* Crude coconut oil is continuously heated to 80–90°C, and 85% phosphoric acid at a range of 0.05–0.10% by volume of feedstock is dispersed in the oil via an in-line static mixer. The mixture is conveyed into a coagulation tank and agitated for 20–30 min.

*Bleaching.* The oil is pumped to a slurry tank passing through a preheater at a temperature of 90–95°C. The oil, dried and deaerated under vacuum, receives a predetermined dose of bleaching earth-activated carbon (10 : 1 ratio), agitated for 20–30 min for the removal of color bodies and other adsorbable impurities. The bleached oil is filtered through a plate and frame filter press and further purified in drum-type polishing filters.

*Steam Stripping and Deodorization.* The degummed and bleached oil is deaerated and heated to 240°C as it passes through economizers and thermal oil heating units. The hot oil cascades down the stripping column operating under a reduced pressure of 600–1000 Pa pulled by four-stage steam ejectors. The oil is met by sparge steam injected at the bottom of the column. Residence time is 1–1.5 h for the removal of the odoriferous substances. Rising vapors are sprayed with fresh fatty acids through a nozzle-type sprayer to recover stripped fatty acid distillates. The deodorized coconut oil exits from the column and passes through an economizer for partial cooling by the incoming bleached oil feedstock. Final cooling takes place in a plate-type cooler where the temperature is maintained at 40–50°C. Citric acid is added before passing the product through a polishing filter.

## 6. COCONUT OIL COMPOSITION

Coconut oil belongs to unique group of vegetable oils called *lauric oils*. The most abundant fatty acid in this group is lauric acid,  $\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$ . Other sources of lauric oils are palm kernel, babassu, cohune, and cuphea.

More than 90% of the fatty acids of coconut oil are saturated. This accounts for its low iodine value ranging from 7 to 12. The saturated character of the oil imparts a strong resistance to oxidative rancidity. Assessment of the oil by active oxygen method (AOM) yielded results between 30 h and 250 h (24). Although oxidative stability is reduced in RBD oils, due to losses in the natural antioxidants of crude coconut oils, the addition of citric acid at the end of deodorization as the oil is cooled to 100°C was effective in regaining considerable oxidative stability in the oil (25).

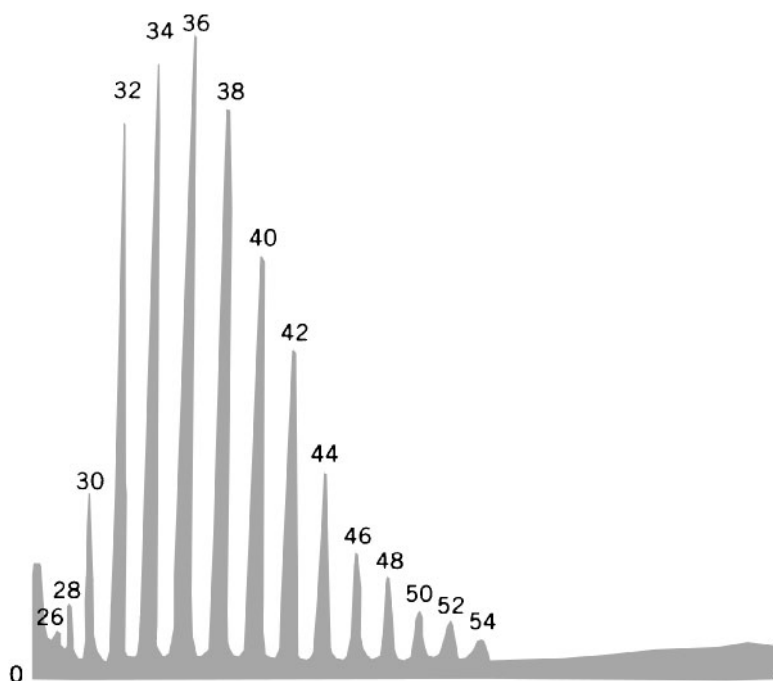
Fatty acids with less than 12 carbon atoms are classified as medium-chain fatty acids (MCFA). Esters of MCFA with glycerol, known as medium-chain triglycerides (MCT), are components in medical foods and infant food formulations. With more than 15% C6, C8, and C10 fatty acids, coconut oil is the richest source of MCFA.

Approximately 0.5% of crude coconut oil is not saponified by caustic treatment. The unsaponifiable matter consists mainly of tocopherols, sterols, squalene, color pigments, and carbohydrates. The odor and taste of coconut oil is largely due to  $\delta$ - and  $\gamma$ -lactones, which are present in trace quantities (24). Among the unsaponifiables, tocopherol contributes to the oxidative stability of crude coconut oil. A typical sample of crude coconut oil contained 55 ppm total tocopherols of which 40.7 ppm is  $\alpha$ -tocopherol (25). Most of the unsaponifiables are removed in the process of refining, bleaching, and deodorizing of crude coconut oil.

The various triacylglycerol (TAG) components of coconut oil may be separated and quantified by gas chromatography with the use of stable silicon gum stationary phase under temperature-programmed conditions and identified by reference to standard TAG solutions. The carbon number of a TAG component is the sum of carbon atoms of the fatty acids attached to the glycerol moiety. For example, the carbon numbers of trilaurin and oleodistearin are 36 and 54, respectively. The relative amounts of each TAG in a sample of fat serves to establish its identity. For coconut oil, this test may also serve to distinguish it from other lauric oils (see Table 4 and Figure 5).

**TABLE 4. Triacylglycerol Number Composition (wt %) of Coconut Oil (26, p. 228).**

TAG Carbon Number	Range (Mean)	%
C28	0.7–1.0	(0.8)
C30	2.8–4.1	(3.4)
C32	11.5–14.4	(12.9)
C34	15.6–17.6	(16.5)
C36	18.3–19.8	(18.8)
C38	15.1–17.7	(16.3)
C40	9.2–11.1	(10.2)
C42	6.5–8.0	(7.3)
C44	3.6–4.6	(4.2)
C46	2.1–3.0	(2.6)
C48	1.6–2.6	(2.3)
C50	0.8–2.0	(1.7)
C52	0.4–2.0	(1.6)
C54	0.1–1.5	(1.2)



**Figure 5.** Distribution of triacylglycerols by carbon number, coconut oil (23).

## 7. CHEMICAL AND PHYSICAL TESTS

For purposes of identifying natural fats and ascertaining their quality, a number of analytical tests are routinely employed. The test results of a sample of fat under assessment should fall within the range of established constants to confirm its identity. For coconut oil the usual tests are fatty acid composition, acid value/percent free fatty acid, saponification value, iodine value, Reichert–Meissl value, Polenske value, unsaponifiable matter, peroxide value/stability test, s.p./m.p., color, and solid fat content (see Tables 5 and 6)

The principal criteria presently being used to measure quality of coconut oil are free fatty acids content and color. In addition, sensory evaluation through taste and odor of oil serves to confirm the acceptability of the product.

Details on sampling methods and test procedures are described in various reference sources (28–32).

### 7.1. Fatty Acid Composition

The individual fatty acid composition of fats and oils are routinely assayed by gas chromatography. Samples are transesterified with methanol to convert the

**TABLE 5. Range of Fatty Acid Composition of Coconut Oil Cross Regional (26, p. 227).<sup>a</sup>**

Fatty Acid	Range Weight (Mean)	%
Caproic C6	0.4–0.6	(0.5)
Caprylic C8	6.9–9.4	(7.8)
Capric C10	6.2–7.8	(6.7)
Lauric C12	45.9–50.3	(47.5)
Myristic C14	16.8–19.2	(18.1)
Palmitic C16	7.7–9.7	(8.8)
Stearic C18	2.3–3.2	(2.6)
Oleic C18 : 1	5.4–7.4	(6.2)
Linoleic C18 : 2	1.3–2.1	(1.6)
C20	t–0.2	(0.1)
C20 : 1	t–0.2	(t)

<sup>a</sup>Five countries and a total of 21 samples.

fatty acids into relatively volatile methyl ester derivatives. The esters are volatilized and swept in a stream of inert gas through a stable inert powder support that has been treated with a liquid (stationary phase) that is not volatile under the test conditions. The esters are eluted in succession from the column in accordance with their individual retention times in the stationary phase. Each emerging fatty acid ester is recorded ideally as an individual peak; the area under the curve is a function of the quantity of eluted component.

## 7.2. Acid Value/Percent Free Fatty Acid

Acid value is the number of milligrams of potassium hydroxide required to neutralize the free acids in 1 g of oil.

A measured amount of oil is dissolved in ethyl alcohol and titrated with 0.1 *N* sodium hydroxide. For coconut oil, an equivalent term, “percent free fatty acid

**TABLE 6. Coconut Oil Product Specifications (27).**

	Crude	Cochin	RBD
Moisture and impurities % max.	1.0	0.1	0.03
Free fatty acid (as lauric) % max.	3.0	0.07	0.04
Color (5 1/4 in. cell) Lovibond R/Y max.	12/75	1/10	1/10
Saponification value		250–264	250–264
Unsaponifiable matter % max.	0.4	0.1	0.1
Iodine value		7–12	7–12
Peroxide value, max.	2.0	0.5	0.5
Slip/melting point, °C		24–26	24–26
Refractive index 40°C		1.448–1.450	1.448–1.450
Flavor/odor		Coconut flavor and odor	Bland/odorless

as lauric" (%FFA), is more commonly used. % FFA as lauric = mL 0.1 *N* NaOH  $\times$  20/g oil; acid value (mg KOH/g oil) = % FFA as lauric  $\times$  2.8.

### 7.3. Saponification Value

Saponification value is the number of milligrams of potassium hydroxide required to neutralize the free acid and saponify the esters in one gram of fat.

A slight excess of alcoholic potassium hydroxide is reacted with a measured amount of oil sample and boiled gently under reflux with an air condenser. A blank is similarly treated. Upon complete saponification, the treated sample and blank are titrated with 0.5 *N* hydrochloric acid. Saponification value =  $(B - S) \times 0.5$  *N* HCl  $\times$  56.1/g oil; where *B* = titer of blank in mL and *S* = titer of sample in mL.

### 7.4. Iodine Value

Iodine value is the number of grams of iodine absorbed in one gram of oil. The unsaturated carbon atoms of fatty acids absorb iodine according to the following reaction  $-\text{CH}=\text{CH}- + \text{I}_2 \rightarrow -\text{CHI}-\text{CHI}-$

A slight excess of either Hanus (IBr) or Wijs (ICI) solution is added to a measured amount of oil sample and shaken for 30 min. A blank is similarly treated. A specified volume of 15% potassium iodide is added to the treated sample and blank, followed by titration with 0.100 *N* sodium thiosulfate.

Iodine Value =  $(B - S) \times 0.100$  *N* thiosulfate  $\times$  12.7/g oil; where *B* = titer of blank in mL and *S* = titer of sample in mL.

### 7.5. Reichert-Meissl Value

The Reichert-Meissl value is the number of milliliters of 0.10 *N* sodium hydroxide required to neutralize volatile fatty acids (mainly C4 and C6) of 5.0 g of oil. *Polenske value* is the number of milliliters of 0.10 *N* sodium hydroxide required to neutralize insoluble fatty acids (mainly C8, C10, C12) of 5.0-g oil. Typical Reichert-Meissl and Polenske values of coconut oil are 8.4 and 11.5, respectively (33). Both values are significantly high, as expected in coconut oil, which is a rich source of MCFA.

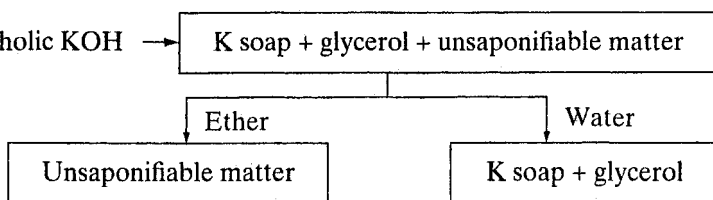
A sample of fat, weighing  $5 \pm 0.1$  g is saponified in glycerol-sodium hydroxide solution. The saponified sample is acidified with dilute sulfuric acid, followed by distillation of the volatile fatty acids. A volume of 110-mL distillate is collected, chilled to 15°C, and filtered. The filtrate is titrated with 0.1 *N* sodium hydroxide. A blank determination is carried.

Reichert-Meissl value =  $(B - S) \times 1.1$ ; where *B* = titer of blank in mL and *S* = titer of sample in mL.

The water-insoluble fatty acids on the filter paper are washed, dissolved in ethyl alcohol, and titrated with 0.10 *N* sodium hydroxide. Polenske value = *B* - *S*; where *B* = titer of blank in mL and *S* = titer of insoluble fatty acids in mL.

## 7.6. Unsaponifiable Matter

Unsaponifiable matter in fats is the ether or hexane-soluble components extracted after a fat sample is refluxed with alcoholic potassium hydroxide.



## 7.7. Peroxide Value/Stability Test

Peroxide value is expressed in milliequivalents peroxide oxygen per kilogram oil. Peroxides formed in oil during storage initiate the development of oxidative rancidity.

A measured amount of fat is dissolved in acetic acid–chloroform solvent in the presence of potassium iodide. Peroxides in the fat liberate iodine, which is titrated with 0.10 *N* sodium thiosulfate. A blank run is similarly treated. Peroxide value (meq peroxide oxygen/kg oil =  $(B - S) \times 0.10 N$  thiosulfate  $\times 1000/g$  oil; where  $B$  = titer of blank in mL and  $S$  = titer of sample in mL).

Stability test (“Swift test”/active oxygen method) gives an indication of the oil’s resistance to oxidation during storage. The test gives the time in hours required for a sample of oil to reach a peroxide value of 100 when subjected to aeration under specified conditions of temperature and airflow rate. The period of time in hours is determined by interpolation between two peroxide value determinations, which must fall between 75 meq and 175 meq.

## 7.8. Slip/Melting Points or Melting Range

The slip/melting points or melting range of fat products is the temperature at which the transition from solid to liquid state is observed. Unlike pure substances, which have sharp melting points, fats are mixtures of TAGs exhibiting a range of melting temperatures. Some methods require elaborate equipment while others, such as the capillary tube method are simple and can provide precise results for routine determinations.

By capillary action, molten fat sample is drawn into the tube, which is open at both ends. The fat is solidified by chilling and then melted in water at a regulated rate of increase in temperature. The temperatures at which the fat slips and appears clear in the liquid state are noted as its slip/melting points or melting range.

## 7.9. Refractive Index

The refractive index of a medium is the ratio of the speed of light *in vacuo* at a given wavelength to the speed of light in the medium. Measurement is done by means of a suitable refractometer at a specified temperature for a particular sample. Refractive index is useful for identification of fats and the observation of progress in reactions during hydrogenation.

**TABLE 7. Solid Fat Content of Coconut Oil (% by Pulse-NMR) (28, p. 386).**

	Fat Content (%)
SP/MP °C	24
Iodine value	8.5
% Solids	
20°C	36
30°C	0
35°C	0

## 7.10. Color

The color of coconut is measured in a Lovibond Tintometer, using a 1-inch or 5¼-inch cell for dark and light colored oils, respectively. Results are given in red and yellow units describing the combination that matches the sample color. Alternatively, the optical density of the oil can be measured with the use of a spectrophotometer in a suitable cell at a wavelength of maximum absorbance.

## 7.11. Solid Fat Content/Solid Fat Index

Nuclear magnetic resonance (NMR) is widely used for the determination of solid fat content in fats and oils. The old dilatometry method, which gives “solid fat index” values, is time consuming and inadequate as a quick means for control purposes in the processing of fats. The NMR method is quicker, more precise, and closer to the absolute solid fat content than dilatometry (see Table 7).

## 7.12. Titer

Titer is the solidifying point of the mixed fatty acids derived from a sample of fat. Fat is saponified and subsequently acidulated. The layer of mixed fatty acids is separated, washed, dried, and allowed to cool. As the solids begin to separate, the temperature rises due to the liberation of latent heat. The highest temperature reached is taken as the titer.

# 8. USES

Coconut oil is used in a wide range of food and nonfood products. It is a raw material for the production of medical foods and infant food formulations. In industry, the fatty acids of coconut oil provide a versatile feedstock for an array of products from diesel fuel substitute to hygienic products.

## 8.1. Edible Products

In coconut-producing countries, RBD coconut oil is used extensively as frying oil. Physical blends and interesterified mixtures of coconut oil and hydrogenated

palm oil are processed into margarines and shortenings. Coconut jam, a syrupy emulsion derived from coconut cream in cane sugar, is consumed as dessert, bread spread, and rice cake topping. Filled milk, in liquid or powder form, contains coconut oil (in lieu of butter fat) and a polyunsaturated oil emulsified in skim milk. As spray oil for crackers and cookies, coconut oil improves shelf life of these products because of its resistance to oxidative rancidity. Coconut oil is widely used as cream fat and as a component in biscuit cream and confectionery oil.

## 8.2. Medical and Infant Food Formulations

Medium-chain fatty acids (MCFA), mainly C8 and C10, obtained by hydrolysis of coconut oil, is reesterified with glycerol to form a mixture of randomized medium-chain triglyceride (MCT) through a method developed by Babayan (34). MCTs are absorbed and oxidized rapidly with an energy rated at 34.7 kJ/g. A number of medical and infant food formulations have MCTs as the principal source of fat supplemented with polyunsaturates (35). In a field study by Intengan et al. (36) a structured (interesterified) 75 coconut oil–25 corn oil preparation gave better weight gain and nutritional recovery, vis-à-vis a polyunsaturated vegetable oil, when given to malnourished children as supplemental fat source in their diet.

## 8.3. Nonfood Products

A commonly used nonfood product derived from coconut oil is soap. Laundry bar soaps made by boiled or cold procedures have excellent lathering property even in moderately hard water. A blend of tallow–coconut oil in ratios from 67 : 33 to 85 : 15 form an “ideal” fat charge for toilet soaps (37). Soap from such blends exhibit desirable characteristics related to lather quickness, low mechanical erosion, and absence of swelling or cracking of soap bars.

One of the major uses of acid oil from soapstock and the distillates from physical refining is in the manufacture of animal feeds. The fatty components increase the caloric density of the feed.

Derivatives of fatty acid from coconut oil are feedstock for a number of diverse nonfood products. Coconut oil fatty acids and glycerol are released by hydrolysis or alcoholysis of the fat. The fatty acids or their methyl esters, which are subsequently fractionated, constitute the starting materials for the oleochemical industry. The byproduct, glycerol, is purified by vacuum distillation. The purified product is, among others, a component of pharmaceutical preparations, an important ingredient in toothpastes, a raw material in the manufacture of nitroglycerol, and the fluid in hydraulic jacks and shock absorbers.

## 9. STORAGE

Although coconut oil has a natural resistance to oxidative rancidity, (see discussion), the bulk storage systems should take into account all factors that contribute



to the deterioration of the product during storage. These factors are light, prooxidants, air, heat, and moisture. Light may have the least effect because the oil is handled in a closed system.

From the milling of copra for the extraction of crude coconut oil through the refining steps and final storage in tanks, the oil is in continuous contact with iron and possibly copper-containing alloys, both of which are prooxidants. The addition of citric acid (25) or any other appropriate antioxidant in the last stages of deodorization of RBD oils (see discussion) affords protection to the oil from oxidative rancidity. Crude coconut oil has natural and protective antioxidants.

The introduction of air during handling procedures may be minimized by filling tanks through a subsurface entry point instead of allowing the oil to fall through air into the storage tanks.

Storage tanks are provided with mechanical agitators and heating devices, although the latter may hardly be used in most areas of the tropics. During brief spells when temperature falls below slip/melting points of coconut oil, the product is warmed with agitation to prevent localized overheating.

The effect of moisture on fat in storage is well known. In the presence of enzymes, mainly from microorganisms, hydrolysis of fat is accelerated giving rise to an unpleasant "soapy" taste peculiar to coconut oil exposed to conditions favoring hydrolytic rancidity.

## 10. ECONOMICS

World coconut oil output fell 10% in 2001/02 to 3.3 million metric tons. U.S. imports of coconut oil were 1,150 million pounds in 2001/02, up 35 million from the previous year. In anticipation of a downturn in global production lasting into 2003, importers were probably taking advantage of favorably low prices early this year to secure larger stocks. The U.S. import unit value for coconut oil declined from \$361 per metric ton in 2000/01 to \$327 in 2001/02. For palm-kernel oil, the other main lauric oil, U.S. imports in 2001/02 fell to 330 million pounds from 364 million in 2000/01. But total supplies of palm-kernel oil were higher because of an ample level of beginning stocks. A recovery in domestic disappearance from a low 2000/01 pace was possible because of the larger supplies (38).

Faced with a general deterioration of market prospects, the coconut industry continued to receive special attention in major producing countries. In **Indonesia**, support measures tended to emphasize intercropping, rehabilitation measures, and product diversification. In the **Philippines**, in 2001, coconut producers have been included in the public food distribution scheme with a view to protect farmers from the impact of declining prices for coconut products. A number of accompanying rural development programmes aim at providing alternative livelihood opportunities for small coconut farmers (39).

Table 8 gives world oilseed production data and includes copra. Table 9 gives world vegetable oils production and includes coconut oil. Copra meal production data are included in Table 10.

**TABLE 8. World Oilseed Production,  $\times 10^6$  t 1995/96 to Date (38).**

Item	Years							
	1995/96	1996/97	1997/98	1998/99	1999/2000	2000/01	2001/02	2002/03 <sup>a</sup>
Production								
Soybeans	124.90	132.22	158.07	159.82	159.90	175.10	183.78	184.49
Cottonseed	35.15	33.61	34.35	32.62	32.93	33.53	36.61	33.37
Peanuts	27.47	28.96	27.29	29.77	28.99	31.12	33.11	31.84
Sunflowerseed	25.72	23.80	23.21	26.63	27.22	23.29	21.25	23.33
Rapeseed	34.44	31.53	33.23	35.89	42.47	37.52	35.87	32.17
Copra	5.13	6.05	5.33	4.38	5.46	5.90	5.26	5.30
Palm kernel	4.87	5.21	5.05	5.62	6.41	6.91	7.24	7.40
Total	257.67	261.38	286.53	294.72	303.37	313.36	323.10	317.89

<sup>a</sup>Forecast.

Source: Foreign Agricultural Service, USDA.

**TABLE 9. World Vegetable Oils Production,  $\times 10^6$  t 1995/96 to Date (38).**

Item	Years							
	1995/96	1996/97	1997/98	1998/99	1999/2000	2000/01	2001/02	2002/03 <sup>a</sup>
Production								
Soybeans	20.17	20.53	22.57	24.65	24.74	26.80	28.72	29.85
Palm	16.26	17.64	16.97	19.25	21.80	23.93	24.88	25.37
Sunflowerseed	9.01	8.61	8.29	9.18	9.63	8.41	7.57	8.32
Rapeseed	11.24	10.52	11.43	11.81	13.64	12.96	12.20	11.41
Cottonseed	4.15	3.70	3.70	3.57	3.57	3.52	3.82	3.56
Peanut	4.15	4.38	4.18	4.44	4.15	4.30	4.75	4.51
Coconut	3.16	3.69	3.29	2.71	3.34	3.63	3.26	3.23
Olive	1.45	2.46	2.53	2.50	2.37	2.48	2.53	2.35
Palm Kernel	2.10	2.22	2.20	2.43	2.75	2.95	3.11	3.17
Total	73.08	73.76	75.16	80.54	85.97	88.98	90.85	91.79

<sup>a</sup>Forecast.

Source: Foreign Agricultural Service, USDA.

**TABLE 10. World Protein Meal Production,  $\times 10^6$  t 1995/96 to Date (38).**

Item	Years							
	1995/96	1996/97	1997/98	1998/99	1999/2000	2000/01	2001/02	2002/03 <sup>a</sup>
Soybeans	89.08	90.82	98.84	107.54	107.74	116.47	124.71	129.58
Cottonseed	13.11	11.89	11.79	11.36	11.45	11.30	12.10	11.31
Rapeseed	18.58	17.53	18.85	19.12	22.27	21.18	19.99	18.64
Sunflowerseed	10.21	10.06	9.51	10.51	10.72	9.43	8.45	9.25
Fish	6.52	6.64	5.08	5.80	6.29	5.75	5.43	5.61
Peanut	5.73	6.01	5.41	5.76	5.27	5.52	6.13	5.79
Copra	1.74	1.97	1.74	1.44	1.77	1.90	1.68	1.70
Palm Kernel	2.54	2.70	2.67	2.93	3.32	3.56	3.75	3.82
Total	147.49	147.62	153.88	164.47	168.82	175.12	182.23	185.69

<sup>a</sup>Forecast.

Source: Foreign Agricultural Service, USDA.

**TABLE 11. Edible Coconut Oil: U.S. Supply and Disappearance, ×10<sup>6</sup> lb 1991 to Date (38).**

Item	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002 <sup>a</sup>	2003 <sup>b</sup>
Stocks October	277	188	251	164	163	84	150	393	152	136	260	227	148
Imports	841	1,163	999	1,100	874	1,188	1,438	791	926	1,115	1,093	860	970
Exports	22	0	19	18	12	12	6	11	14	8	7	8	10
Domestic disappearance	910	1,084	1,067	1,083	941	1,111	1,189	1,021	927	983	1,119	930	958

<sup>a</sup>Preliminary and estimated.

<sup>b</sup>ERS and WAOB forecast.

Source: Bureau of the Census.

Table 11 gives U.S. edible coconut supply and disappearance for 1991–2003. Disappearance, as defined by the USDA-ER, means beginning food stocks, production and imports minus exports, shipments to U.S. territories, and ending stocks.

Coconut oil is generally considered an expensive oil, and it normally commands a premium over other vegetable oils.

## REFERENCES

1. J. A. Banzon and J. R. Velasco, *Coconut Production and Utilization*, Philippine Coconut Research & Development Foundation, Pasig, Metro Manila, Philippines, 1982, p. 19.
2. A. R. Diokno, *The Brown Man and the Coconut*, Manor Press, Manila, Philippines, 1962, pp. 1–121.
3. J. L. Arranza, in Proceedings of the World Conference and Exhibition on Lauric Oils, Manila, Philippines, February 20–25, 1994, p. 4.
4. S. S. Magat (Chairman), *The Philippines Recommends for Coconut*, PCARRD Phil. Recommends Series No. 2-13, Los Baños, Laguna, Philippines, 1993, p. 3.
5. Ref. 1, p. 9.
6. Ref. 1, p. 6.
7. S. S. Magat et al., *Phil. J. Coconut Studies*, **XIII**(2) (1988).
8. G. D. Padrones et al., *Response of MAWA Hybrid to Increasing Levels of NaCl*, Annual Report Agricultural Research, Philippine Coconut Authority, 1990, pp. 38–40.
9. E. P. Rillo, *Phil. J. Coconut Studies*, **XIV**(2), 16–20 (1989).
10. A. G. del Rosario et al., in Proceedings of the 5th Scientific Meeting of the Federation of Crop Science Studies of the Philippines, Iloilo City, April 26–29, 1989.
11. N. T. Thanh-Tuyen and D. Apurillo, *Phil. J. Coconut Studies*, **XVII**(1) (1992).
12. E. V. de Guzman and D. A. del Rosario, *The Philippine Agriculturist*, University of the Philippines Publication Series A, Vol. XLVIII, No. 23, (1964), pp. 82–94.
13. E. V. de Guzman and G. C. Manuel, *Phil. J. Coconut Studies*, **II**(1), 35–39 (1977).
14. J. A. Banzon et al., *Coconut as Food*, PCRDF, Pasig, Metro Manila, 1990, p. 51.
15. E. P. Lozada, *Coconuts Today*, **IX**(1), 9–23 (1992).
16. *Oil Milling Manual*, Coconut Industry Investment Fund Oil Milling Group, Metro Manila, Philippines, personal communication, 1993.
17. R. Hagenmaier, *Coconuts Today*, **I**(1), 17–23 (1983).
18. D. C. Tandy and W. J. McPherson, *JAOCs*, **61**(7), 1256(1984).
19. U. I. Brimberg, *JAOCs*, **59**(2), 74.
20. Alfa-Laval, in the Fats and Oils Industry, Fats & Oils Division, S-147 80 Tumba, Sweden, 27829-E2, Sept. 1989.
21. A. Forster and A. J. Harper, *JAOCs*, **60**(2), 265 (1983).
22. Alfa Laval Fats and Oils Packed Column for Edible Oil Refinery, Fats & Oils Division, S-147 80 Tumba, Sweden, BP 5004E1, pp. 2–7.
23. Ref. 21, p. 266.
24. F. V. K. Young, *JAOCs*, **60**(2), 376 (1983).

25. M. H. Gordon and I. A. Rahman, *JAOCS*, **68**(8), 574–576 (1991).
26. J. B. Rossell et al., *JAOCS*, **62**(2), 228 (1985).
27. *Product Specification*, Coconut Industry Fund Oil Milling Group, Metro Manila, Philippines, personal communication, 1993.
28. K. Helrich, ed., *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed., AOAC Inc., Arlington, Virginia, 1990.
29. *Food Chemicals Codex*, 3rd ed., Committee on Food Chemicals Codex, National Academy Press, Washington, D.C., 1981.
30. Ref. 28, Second Supplement to 3rd ed., 1986.
31. C. Paguot, *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 6th ed., Pergamon Press, Maxwell House, Fairview Park, Elmsford, New York.
32. W. W. Christie, *Lipid Analysis*, 2nd ed., Pergamon Press, Maxwell House, Fairview Park, Elmsford, New York, 1982.
33. N. Gopalakrishnan et al., *JAOCS*, **64**(4), 539 (1987).
34. V. K. Babayan, *JAOCS*, **45**, 23–25 (1968).
35. C. Dayrit et al., *Phil. J. Coconut Studies*, **XVII**(2), 15–17 (1992).
36. C. Intengan et al., *Phil. J. Coconut Studies*, **XVII**(2), 11–14 (1992).
37. N. O. V. Sonntag, *JAOCS*, **58**(2), 156A (1981).
38. *Oil Crop Situation and Outlook Year Book*. Economic Research Service, USDA. (Oct. 2002). Available: <http://www.usda.gov>. accessed july 2004.
39. *Review of Basic Food Policies*. Food and agriculture organization. (July 2004). Available: <http://www.fao.org>.

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## *Corn Oil*<sup>\*</sup>

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### **1. OVERVIEW**

In 2001, 2.04 MT (million tons) of corn oil was produced worldwide (representing about 2% of the total worldwide vegetable oil production), with the top three producers being the United States (57%), the EU-15 (10%), and Japan (5%) (1). Compared with the 2001 world production of other vegetable oils, corn oil ranks tenth, behind soybean (26.66 MT) > palm > canola/rapeseed > sunflower > peanut > cottonseed > coconut > palmkernel > and olive (1).

Corn oil has traditionally been considered a premium vegetable oil, and at the time of the writing of this chapter, the average U.S. wholesale price for corn oil is \$0.29/0.43 per pound (crude/refined) compared with soybean (\$0.31/0.37) and peanut (\$0.57/0.71) oils (2, 3).

Unlike most other vegetable oils that are obtained directly from seeds that contain high levels of oil, corn oil (maize oil) is obtained from seeds (kernels) that contain only 3–5% oil. Obtaining oil directly from the kernels is technically

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\*Mention of tradenames or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

**TABLE 1. Fractions Obtained by Bench-Scale Dry Milling and Wet Milling of Corn. Note: Commercial Wet Milled Fiber is a Mixture of Course and Fine Fiber.**

Fraction	Grams of Bench Scale Fraction/100 g of Kernel <sup>a</sup>	Wt % Oil in Bench Scale Fraction <sup>a</sup>	Wt % Oil in Commercial Fraction <sup>b,c</sup>
Dry Milling			
Grits	82.63	0.63 ± 0.03	
Germ	11.24	15.00 ± 0.37	20–25 <sup>b</sup>
Bran	6.13	2.11 ± 0.03	1–2 <sup>c</sup>
Wet Milling			
Starch	68.48	0.02 ± 0.01	
Gluten	8.34	0.89 ± 0.00	
Fine Fiber	8.58	1.36 ± 0.05	1–2 <sup>c</sup>
Course Fiber	5.48	2.19 ± 0.05	1–2 <sup>c</sup>
Germ	4.90	35.56 ± 0.41	44–50 <sup>b</sup>
Steepwater	3.33	0.06 ± 0.008	

<sup>a</sup>From (5).<sup>b</sup>From (6).<sup>c</sup>From (7).

possible, but “corn kernel oil” would be costly to produce (because of the low levels of oil in the kernels). Because corn kernels contain high levels of starch (60–75%), a process, “wet milling,” was developed to efficiently isolate pure starch from corn kernels. The first corn wet mill in the United States started producing cornstarch in 1842, and by 1860, several corn wet mills were in operation (4). Corn germ is generated during both wet milling and dry milling (Table 1), but the amount of germ and the composition of germ differ when produced via these two routes (Table 1). It was estimated that in 1996, about 90% of commercial corn oil in the United States was from wet milled germ and the remaining from dry milled germ (4).

During industrial wet milling, the kernel is separated into five fractions: (1) starch, (2) steepwater solubles (~7%), (3) fiber (course & fine, ~10%), (4) corn gluten meal (~6%), and (5) germ (~7%) (5). The steepwater solubles and fiber fractions are blended together to produce an animal feed called “corn gluten feed,” which contains about 21% protein and 60–70% fiber. The high fiber content restricts its use to mainly feeds for ruminants. Corn gluten meal contains about 60% protein and low fiber (<1%), and it is a premium feed for nonruminants (poultry and swine). Corn germ is rich in oil (>30%), and it is the source of all commercial corn oil, and like “wheat germ oil,” corn oil could more accurately be called “corn germ oil.” Like corn oil and wheat germ oil, rice bran oil is another edible oil that is similarly extracted from a grain processing fraction.

## 2. EXTRACTION AND REFINING

### 2.1. Conventional Corn Germ Extraction

Unlike oilseeds, where solvent extraction alone can be used to obtain oils, extraction after flaking of wet milled corn germ produces a substantial amount of “fines” that interfere with the efficiency of the extraction process. Traditionally, oil is removed from the wet milled germ using a conditioning (heating) process, followed by mechanical expelling (“prepress”) and hexane extraction (Table 2). Extrusion has also been employed as a means of germ preparation for solvent extraction, producing a crude corn oil of high quality and high yield (8). Hexane is removed from the oil-rich miscella by evaporation, heat, and vacuum, and the hexane is recycled. Hexane is also removed and recycled from the germ cake. Corn germ meal contains 23–25% protein and is usually sold as an animal feed ingredient and is often added to increase the protein content of corn gluten feed.

Among the seven major U.S. wet milling companies that are members of the Corn Refiners Association in 2003, only three companies report that they currently market crude and refined corn oil (9). The other major wet mill companies sell their corn germ, either for extraction and corn oil production, or for animal feed use, depending on seasonal markets and prices.

Oil is usually obtained from dry milled corn germ by full press (via an expeller). List et al. (10) compared some of the chemical components in corn oil from wet milled germ versus dry milled germ and reported lower levels of free fatty acids, lower levels of phosphorous, and higher levels of tocopherols in the latter. Although several “high oil” corn hybrids are available and are becoming increasingly popular, most of the crop has been used for animal feed (with the increased fat providing more calories) and little or none has been wet milled to obtain starch, corn oil, and other products.

**TABLE 2. Processes for Conventional Extraction of Corn Germ.**

Process	Equipment Used	Germ Pretreatment	Oil Remaining (%) <sup>a</sup>
Untreated Germ			44–50 (germ from wet mill) 20–25 (germ from a dry mill)
Full (Hard) Press	Expeller	Heat	6–11
Partial Press & Hexane Extraction	Expeller & Extractor	Heat	1–3
Extrusion & Hexane Extraction	Extruder (Expander) to produce collets and extractor	Heat and additional moisture	1–2

<sup>a</sup>From (6).



## 2.2. Alternative Extraction Processes

A process for the hexane extraction of oil from the flaked kernels of high-oil (>8%) corn hybrids was patented (11). An interesting aspect of the proprietary process is that it uses the same common flaking and extraction machinery that is usually employed for soybean oil extraction.

Ethanol has also been proposed as an organic solvent for the extraction of corn oil from corn germ or from whole kernels. Some advantages of ethanol over hexane are its higher flash point, it being a "food-grade" solvent, and the fact that it can be readily produced from corn via fermentation. Some disadvantages compared with hexane include its higher boiling point (requiring more energy to remove solvent from meal) and its increased polarity, which means that it extracts more polar extractants (e.g., phospholipids) that may need to be removed during refining. Hojilla-Evangelista et al. (12) developed a process, "the sequential extraction process (SEP)," which involves the ethanol extraction of whole flaked corn kernels to first extract the corn oil, and then additional steps to extract and fractionate proteins and starch. The economics of the SEP process have been rigorously evaluated, and some recent process modifications have been proposed to improve the efficiency and lower the cost (13, 14). Recently, a method was developed to remove corn oil and zein from ground corn and a proprietary process was reported to separate the corn oil and zein using membrane filters (15, 16).

Supercritical CO<sub>2</sub> extraction methods were evaluated for a number of oilseeds in the 1980s. Corn germ extraction by supercritical CO<sub>2</sub> was evaluated by ARS researchers in Peoria, IL. Methods for corn germ extraction using 100% supercritical CO<sub>2</sub> were developed (10, 17) and patented (18). Others have demonstrated that addition of ethanol modifier (0–10%) to supercritical CO<sub>2</sub> can decrease the extraction time and improve the functionality of germ proteins (19). Although there are no technical barriers, extraction of corn germ with supercritical CO<sub>2</sub> is more costly than conventional extraction methods.

Several aqueous and enzyme-assisted methods have been reported for corn oil extraction. In 1982, Stolp and Stute reported a proprietary aqueous process to obtain corn oil from corn germ (20). An aqueous enzymatic (using commercial cellulases, hemicellulases, polygalacturonases, galactomanases, and various pectinolytic enzymes) process for extracting corn oil from corn germ was also reported (21, 22). Although these aqueous and enzymatic processes have been available for a number of years, they have not been used in commercial production, mainly because of higher costs when compared with conventional extraction techniques. Recently, Verser and Eggeman received a U.S. patent for a process that uses enzymatic milling and produces ethanol via an acetic acid process that also yields corn oil (23).

## 2.3. Refining Steps

The major component of crude corn germ oil is triacylglycerols, but the crude oil also contains other minor nonpolar and polar lipid components (Table 3). Free

**TABLE 3. Polar and Nonpolar Lipid Classes in Corn (germ) Oil, Corn Kernel Oil, and Corn Fiber Oil.**

Oil	Wt% Total Lipid								Reference
	TAG	FFA	St:E	St	FPE	tocols	GL	PL	
Germ Oil (crude)	95.6	1.7	nr	1.2*	nr	0.06	nr	1.2	24
Germ Oil (crude)	96.8	0.31	0.47	0.48	0.01	0.17	nr	nr	5
Germ Oil (RBD)	98.9	0.03	nr	1.1*	nr	0.05	0	0	24
Kernel Oil (crude)	nr	Nr	0.76–3.09	0.54–1.28	0.047–0.839	0.023–0.127	nr	nr	25
Fiber Oil (crude)	84.5	2.11	5.61	1.17	4.11	0.76	nr	nr	5
Fiber Oil (crude)	nr	Nr	2.9–9.2	1.9–4.3	6.5–9.5	nr	nr	nr	26

\*Value is after saponification, meaning that it is the sum of St and St:E.

Abbreviations: nr—not reported; TAG—triacylglycerols; FFA—free fatty acids; St:—phytosterol fatty acyl esters; St—free phytosterols; FPE—phytosterol ferulate esters; tocols—tocopherols and tocotrienols; GL—glycolipids; PL—phospholipids; RBD—refined, bleached, and deodorized oil.

**TABLE 4. Steps in the Commercial Processing of Corn Oil.**

Steps	Purpose	Byproduct Produced
<b>Alkali Refining</b>		
Degumming	Remove gums	Lecithin
Alkali Treatment	Remove free fatty acids	Soapstock – a source of fatty acids
Bleaching	Remove pigments and oxidation products	Spent Clay
Dewaxing (winterizing)	Improves stability, especially at lower temperatures	Waxes and saturated triacylglycerols
Deodorization	Remove volatiles and off-flavors	Deodorizer distillate—a source of phytosterols and tocopherols
<b>Physical Refining</b>		
Degumming	Prevent precipitates at low temperature	Lecithin
Bleaching	Remove pigments and oxidation products	Spent clay
Dewaxing (winterizing)	Improves stability, especially at lower temperatures	Waxes and saturated triacylglycerols
Steam refining/ deodorization	Removes free fatty acids and other volatiles	Distillate—a source of free fatty acids, and a potential source of phytosterols and tocopherols

fatty acids, pigments, volatiles, phospholipids, and waxes are the major undesirable components in crude corn oil, and these are removed by several refining steps (Table 4). Whereas soybean oil processing usually is preceded by water degumming, during corn oil processing, degumming is often not included if corn oil is going to be processed via alkali refining (degumming is necessary if physical refining is used). In corn oil processing, most companies remove free fatty acids by alkali refining, which involves adding base and neutralizing (and sequestering) the free fatty acid soaps (and phospholipids) into a byproduct called “soapstocks” (26). A chemical comparison of the soapstocks obtained from alkali refining of oils from corn germ, peanut, and cottonseed revealed that corn oil soapstocks had a high level of phytosterols (almost 7%) and phosphorous, and an intermediate level of free fatty acids (27). Alternatively, free fatty acids can be removed by the process of “physical refining” or “steam refining,” which involves treating the oil at high temperature and vacuum to volatilize the free fatty acids. Physical refining is only advisable if the oil is of high quality—otherwise, the oil becomes dark and has poor stability (personal communication, R. Ormsbee).

Physical refining begins by removing phospholipids by water degumming (28). Failure to adequately remove the phospholipids (by either alkali refining or degumming) results in a corn oil that will form dark colors and off flavors when heated (4). After a subsequent bleaching step, the next step in physical refining is a steam distillation at high temperature and very low pressure (vacuum), which volatilizes the

free fatty acids. Leibovitz and Ruckenstein (29) reported higher yields of oil with physical refining than with alkali refining. Others have noted that oils that contain phytosteryl esters (especially ferulate-phytosteryl esters, such as those found in corn fiber oil and rice bran oil) are extensively hydrolyzed during conventional alkali refining, but they remain relatively intact during physical refining (Personal communication, R. Nicolosi). Other strategies for removing free fatty acids from crude oil include liquid–liquid extraction and a new method involving solvent extraction in a perforated rotating disk (30). Deodorization of corn oil involves treatment at high temperature ( $>200^{\circ}\text{C}$ ) and vacuum ( $\sim 2\text{--}10$  mm Hg), and it removes undesirable odors and flavor components (24). Unfortunately, the deodorization process also removes some phytosterols and tocopherols.

The byproduct of deodorization, the “deodorizer distillate,” has been used as a major industrial source of phytosterols (which are used as ingredients in phytosterol-containing functional foods and supplements or used as precursors in the synthesis of some steroid pharmaceuticals) and tocopherols (a major source of natural vitamin E). A recent study compared the levels of tocopherols and phytosterols in industrial deodorizer distillates obtained from chemical and physical refining of corn, canola, sunflower, and soybean oils (31). In another study, the effect of the type of vegetable oil refining process (physical versus chemical) on the levels of phytosterols was compared (32), and interesting differences were observed. Pigments are usually removed by treating the oil with acid-activated bleaching clay (6). Another refining step that ensures stability of oils at low temperature is dewaxing or “winterization,” which involves cooling the oil to  $5\text{--}10^{\circ}\text{C}$ , and removing precipitates via filtration (29).

### 3. COMPOSITION

#### 3.1. Comparison of Corn Germ Oil, Corn Kernel Oil, and Corn Fiber Oil

Although all of the current commercial corn oil is produced from corn germ oil, considerable research has been devoted to the study of extracting the entire corn kernel to produce “corn kernel oil” and extracting corn fiber (a byproduct of wet milling) to obtain “corn fiber oil” (Table 3). The levels of total phytosterols (the sum of free phytosterols and phytosterol fatty acyl esters) in corn germ oil (refined) averages about 1%, which is higher than the levels found in most other common vegetable oils (24). Some of these phytosterols are removed during refining, but even after refining, the levels of total phytosterols in commercial corn oil are about 1% (Table 3). Hexane-extracted corn kernel oil contains higher levels of the three phytosterol lipid classes (free phytosterols, phytosteryl fatty acyl esters, and phytosteryl ferulate esters) than those found in corn (germ) oil (30; Table 3). Moreau et al. (7) reported that a unique oil, very rich in the two phytosteryl esters (their chemical properties will be described in a later section) could be extracted from corn fiber. Corn fiber oil contains the highest levels of natural phytosterols and phytostanols of any known plant extract (33). The relevant patent for corn fiber oil covers the process to

extract the oil, the composition of matter, and the cholesterol-lowering applications (34). Recently, researchers at Eastman patented an alternative process for obtaining corn fiber oil and other products from corn fiber (35, 36).

### 3.2. Fatty Acid Composition of Corn Triacylglycerols

Edible oils are often compared by performing alkaline hydrolysis (saponification) of the triacylglycerols and by comparing the fatty acid profiles. In the 1950s and 1960s, a marketing slogan for corn oil was that it was “high in polyunsaturates,” mostly attributed to its high levels of linoleic acid, an essential fatty acid (one that is not synthesized by humans and must be obtained in the diet), abbreviated as 18:2 (Table 5). Another desirable characteristic of corn oil is that it contains relatively low levels (<15%) of saturated fatty acids and very low levels of linolenic acid, abbreviated as 18:3 (which is especially susceptible to oxidation, leading to rancidity). Although the levels of linoleic acid in U.S. corn oil average about 60%, it has been noted that its levels in corn oil produced outside of the United States are closer to 50%, with most of the difference being accounted for by higher amounts of oleic acid (26).

Several studies have reported that when the same corn hybrids are grown in multiple locations, the corn oil produced from plants grown in cooler regions contains higher levels of linoleic acid (41). It was also noted that the average levels of linoleic acid in commercial corn oil in the United States increased from 57.8% to 62.0% between 1974 and 1986 (41).

In response to the current demand for high monounsaturate-vegetable oils, efforts have been devoted to developing corn hybrids that produce corn germ oils with high levels of oleic acid. A “high oil–high oleic acid” corn hybrid has been patented (42). In addition to the extensive literature on the fatty acid composition of crude and refined corn germ oil, the fatty acid composition of crude corn kernel oil (39) and crude corn fiber oil (43) have also recently been reported. The fatty acid composition of kernel oil and fiber oil are very similar to that of corn germ oil (Table 5).

### 3.3. Triacylglycerol Molecular Species

Reversed-phase high performance liquid chromatography (HPLC) techniques have been developed to quantitatively analyze the triacylglycerols’ molecular species of fats and oils from a variety of plants and animals. Investigations of the triacylglycerols’ molecular species of refined corn oil indicated the successful identification of 19 to 27 individual molecular species, with oleate–linoleate–linoleate and linoleate–linoleate–linoleate being the two most abundant molecular species (Table 6). Silver ion HPLC was also used to quantitatively analyze corn oil triacylglycerols (46). The method separated the triacylglycerols into 11 fractions, with the largest 2 fractions having five and six double bonds, which translates to the structures oleate–linoleate–linoleate and linoleate–linoleate–linoleate (confirming the two most abundant molecular species identified in reversed-phase HPLC).

**TABLE 5. The Fatty Acid Composition of Corn (germ) Oil and Corn Fiber Oil.**

Oil	mol% of Total Fatty Acids						Ref
	16:0	18:0	20:0	18:1	18:2	18:3	
Germ oil (RBD) US	11.0 ± 0.5	1.8 ± 0.3	0.2 ± 0.2	25.3 ± 0.6	60.1 ± 1.0	1.1 ± 0.3	24
Germ oil (RBD) US	9.2–16.5	0–3.3	0.3–0.7	20–42.2	39.4–65.6	0.5–1.5	37
Germ oil (RBD) US	10.90	1.80	nr	24.2	58.0	0.70	38
Germ oil (RBD) US	11.0 ± 0.6	1.7 ± 0.3	nr	25.8 ± 0.9	59.8 ± 1.2	1.1 ± 0.4	6
Germ oil (RBD) Int	12.9 ± 1.4	2.6 ± 0.6	nr	33.1 ± 2.5	48.8 ± 2.4	1.4 ± 0.4	6
Kernel oil (crude) Int	9.2–11.8	1.1–1.7	0.3–0.5	19.5–30.4	53.0–65.3	1.2–2.1	39
Corn fiber oil (crude)	13.8 ± 0	1.7 ± 0	0.3 ± 0	23.8 ± 0.1	56.4 ± 0.1	2.6 ± 0	40

Abbreviations: nr—not reported; US—US hybrids; Int—International hybrids; 16:0—palmitic acid; 18:0—stearic acid; 20:0—arachidic acid; 18:1—oleic acid; 18:2—linoleic acid; 18:3—linolenic acid; RBD—refined, bleached, and deodorized oil.

**TABLE 6. Quantitative Analysis of Triacylglycerol Molecular Species in Refined Corn Germ Oil.**

TAG Molecular Species	Area % <sup>a</sup>	Area% <sup>b</sup>	Area % <sup>c</sup>
LLO	19.98	21.5	23.0
LLL	17.79	25.4	22.6
LLP	13.71	14.7	15.2
OOL	11.82	10.7	10.6
PLO	10.85	10.0	10.4
PPL	2.48	2.5	1.7
OOP	3.48	2.9	2.4
LLS	2.64	2.2	1.8
LOS	1.77	1.8	1.3
OOO	4.35	2.8	3.2
PPO	1.55	0.9	0.4
PLS	0.78	0.8	0.4
LLLn	0.91	1.2	0.8
LnLO	2.20	0.9	2.3
OOS	0.56	0.6	0.5
POS	0.20	0.3	0.3
PLnL	0.43	0.5	0.5
PPP	0.0	0.0	0.1
OOLn	1.09	0.1	1.0
PLnO	0.0	0.1	0.5
PPS	0.36	0.0	0.1
SSL	0.0	0.1	0.3
LnLS	0.0	0.1	0.0
SSO	0.0	0.0	0.0
PPLn	0.0	0.0	0.2
SSP	0.0	0.0	0.1
SSS	0.0	0.0	0.1

<sup>a</sup> From (44).<sup>b</sup> HPLC-Mass Spec values from (45).<sup>c</sup> HPLD-Flame Ionization Detector Values from (45).

Abbreviations; Ln—linolenic acid; L—linoleic acid; O—oleic acid; S—stearic acid; P—palmitic acid

### 3.4. Unsaponifiables and Phytosterols

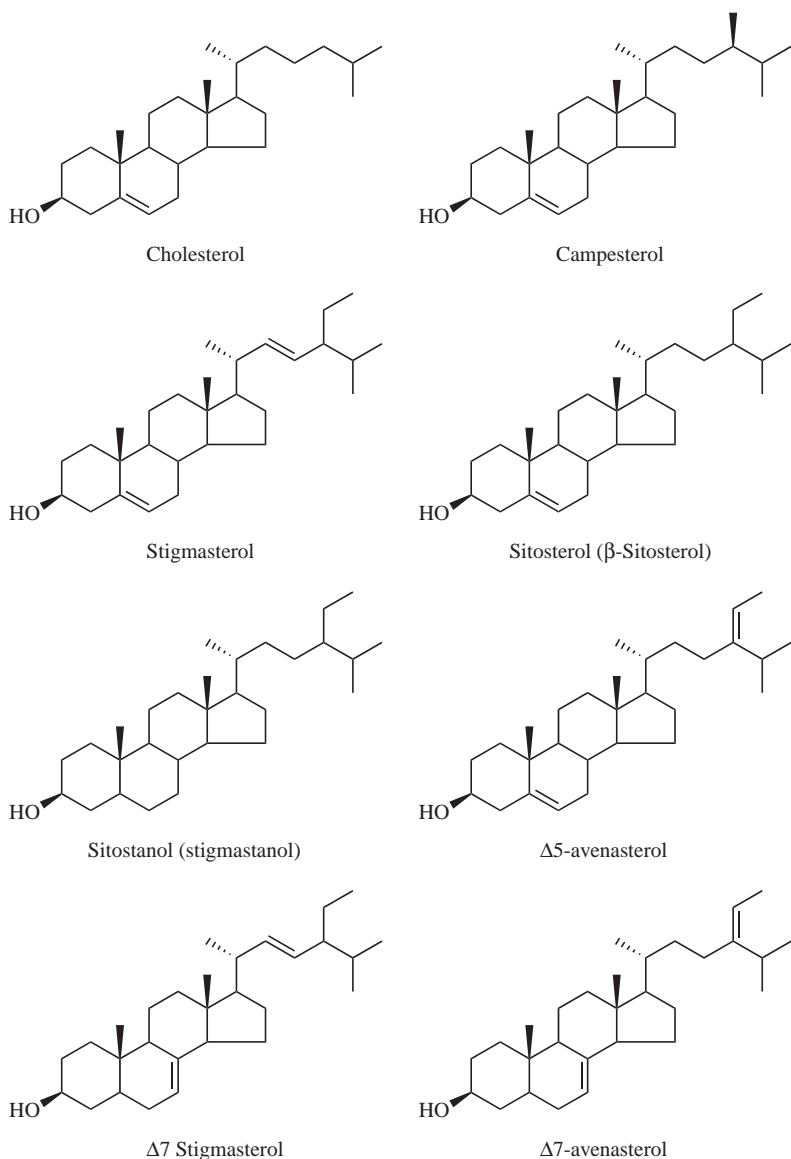
Commercial corn oil has been recognized as having the highest levels of unsaponifiables (1.3–2.3%) of all commercial vegetable oils (6). The three most abundant chemical components in the unsaponifiable fraction of corn oil are phytosterols, tocopherols, and squalene.

Corn germ oil contains two phytosterol lipid classes, free phytosterols and phytosteryl fatty acyl esters (Table 3). Phytosterols have been recognized as 1 of the 12 important classes of phytonutrients (47). Most chemical identification of phytosterols in vegetable oils has been conducted by saponifying (alkaline hydrolysis) the oil and measuring the resulting free phytosterols, usually by GLC (Table 7). The major phytosterols in corn germ oil are sitosterol (formerly called  $\beta$ -sitosterol) > campesterol > stigmasterol (Table 7; Figure 1). Snyder et al (50) developed

**TABLE 7. Phytosterols in Corn (germ) Oil and Corn Fiber Oil (Values Represent Free and Esterified Phytosterols, Measured After Saponification).**

Oil	mol % of Total Phytosterols							ref
	campesterol	stigmasterol	$\beta$ -sitosterol	sitostanol	$\Delta$ 5-avenasterol	$\Delta$ 7-stigmasterol	$\Delta$ 7-avenasterol	
Germ oil (RBD)	18.6–24.1	4.3–7.7	54.8–66.6	nr	4.2–8.2	1.0–4.2	0.7–2.7	37
Germ oil (RBD)	24.3	7.7	61.6	nr	3.8	0.7	0.8	48
Germ oil (RBD)	22.1	5.7	69.8	nr	2.4	nr	nr	49
(free phytosterols)								
Germ oil (RBD)	16.7	6.8	66.9	nr	8.0	nr	nr	49
(esterified phytosterols)								
Corn fiber oil (crude)	$4.9 \pm 0.4$	$1.4 \pm 0.1$	$34.3 \pm 0.1$	$43.1 \pm 0.7$	$1.8 \pm 0$	nr	nr	40
Corn aleurone oil (crude)	$4.7 \pm 0.3$	tr	$20.8 \pm 0.1$	$50.6 \pm 0.4$	$3.5 \pm 0.1$	nr	nr	40





**Figure 1.** Structures of the common sterols (phytosterols) in corn and their comparison with cholesterol, the main sterol in animals. Note that cholesterol has 27 carbons, campesterol has 28 carbons, and all of the other phytosterols shown have 29 carbons.

a supercritical CO<sub>2</sub> method to concentrate and fractionate the phytosterols in corn germ oil. Analyses of the total phytosterols in corn fiber oil revealed that the major phytosterol was sitostanol (Table 7). Sitostanol is a phytostanol (meaning that it is completely saturated—it contains no carbon–carbon double bonds, whereas

phytosterols typically contain at least one). Natural phytosterols are rare in plants, and the only reports of its presence in greater than trace amounts have been in grains and Tall Oil (a byproduct of paper pulping) (51). The phytosterols (sitosterol and campestanol) used in commercial sitosterol-ester margarines are produced by catalytic hydrogenation. Sitosterol is the phytosterol produced by the catalytic hydrogenation of the two most common plant phytosterols, sitosterol and stigmasterol. Campestanol is the phytosterol produced by the hydrogenation of campesterol (33). Our Laboratory recently reported (40) that most of the sitosterol in corn fiber oil is found as the phytostanyl ester of ferulic acid, and most of the sitosterol in corn fiber (and in corn kernels) is found in the aleurone layer (Table 7). In some grains, the aleurone layer comprises several cell layers, but in corn, it comprises a single layer of (phytosterol-rich) living cells (52). A method to enrich corn-fiber-derived aleurone cells by milling them to a small particle size, followed by floatation to separate the aleurone and pericarp was recently reported (53). The composition of corn fiber oil was also found to be influenced by the type of hybrid and the location of growth (26) and by various alternative milling technologies (54). As noted in Section 3.2, corn oil soapstocks contain almost 7% phytosterols (27) and may represent an economical feedstock for phytosterols.

Squalene is an unsaponifiable compound in corn oil that has not received much attention. Squalene was previously reported to be the major hydrocarbon in corn oil (43) and we recently reported that both corn germ oil and corn fiber oil contain about 0.2% squalene (40).

### 3.5. Tocopherols and Tocotrienols

Corn oil has long been known to be a rich source of tocopherols, with  $\gamma$ -tocopherol being the most abundant tocopherol, followed by  $\alpha$ -tocopherol and then  $\delta$ -tocopherol (Table 8). Among the tocopherols,  $\alpha$ -tocopherol has received the most attention because of its high vitamin E activity, but the other isomers also are known to have valuable antioxidant properties. Recent evidence suggests that  $\gamma$ -tocopherol may be superior to  $\alpha$ -tocopherol in preventing the oxidation of low-density lipoproteins and in delaying thrombus formation (57). Wang et al. (55) recently reported significant levels of tocotrienols (the most abundant was  $\gamma$ -tocotrienol followed by  $\alpha$ -tocotrienol) in corn kernel oil, and saponification of the kernels caused about a two-fold increase in the levels of extractable tocotrienols and  $\gamma$ -tocopherol. It is currently believed that, in addition to their valuable antioxidant properties, tocotrienols also possess cholesterol-lowering properties—probably associated with their ability to inhibit cholesterol biosynthesis (58). We recently reported high levels of  $\gamma$ -tocopherol in corn fiber oil (about 0.36 wt %), noting that heat pretreatment (150–175°C for 1 hour) of the corn fiber (prior to extraction) caused a nearly ten-fold increase in the levels of extractable  $\gamma$ -tocopherol (increasing its concentration in the oil to about 3%) (56). The HPLC method that we used to quantitatively analyze  $\gamma$ -tocopherol in this previous report (56) was based on UV-280 nm detection. Recently, we repeated our previously published measurement of  $\gamma$ -tocopherol levels in heat

**TABLE 8. Tocopherols and Tocotrienols in Corn (germ) Oil, Corn Kernel Oil, and Corn Fiber Oil.**

Oil	mg/kg oil							Ref.
	$\alpha$ -tocopherol	$\beta$ -tocopherol	$\gamma$ -tocopherol	$\delta$ -tocopherol	$\alpha$ -tocotrienol	$\gamma$ -tocotrienol	$\delta$ -tocotrienol	
Germ, crude	191	0	493	118	nr	23	nr	6
Germ, RBD	134	18	412	39	nr	nr	nr	6
Germ, RBD	23–573	0–356	268–2468	23–75	0–239	0–450	0–20	37
Kernel, crude	67–276	0–20	583–1048	12–71	46–90	60–133	nr	39
Kernel, crude (w/o saponified)	23	nr	240	nr	53	97	nr	55
Kernel, crude (with saponified)	48	nr	532	nr	124	197	nr	55
Fiber oil, crude (untreated)	nr	nr	3,600 <sup>a</sup>	nr	nr	nr	nr	56
Fiber oil (heat-pretreated)	nr	nr	34,933 <sup>a</sup>	nr	nr	nr	nr	56

<sup>a</sup> Subsequent studies in our laboratory (not yet published) have revealed that these exceptionally high  $\gamma$ -tocopherol values (obtained via UV-280 nm detection) were not accurate. When we repeated the HPLC analyses using fluorescence detection, the levels of  $\gamma$ -tocopherol in corn fiber oil (extracted before and after heat-treatment) were similar to above reported levels in corn germ oil and corn kernel oil (< 500 mg/kg oil).

**TABLE 9. Cis and Trans Fatty Acids in Refined Corn Oil and Corn Oil Margarines.**

Oil	% Fat	grams/100 g product											total trans	ref
		16:0	16:1	18:0	C 18:1	T 18:1	CC 18:2	Ct 18:2	TT 18:2	CCC 18:3	20:0	20:1		
Corn oil	100	10.9	0	1.8	24.2	0	58.0	0	0	0.7	0	0	0	38
Corn oil margarine (stick)	81.98	8.99	0	5.16	16.97	19.63	25.69	0.5	0	0.47	0.19	0.16	20.13	63
Corn oil spread, light (tub)	38.83	3.91	0.02	2.44	9.44	5.20	14.9	0.46	0	0.26	0.19	0.07	5.66	63
Corn oil spread, extra light (tub)	19.47	2.03	0.01	0.83	4.75	2.69	7.82	0.1	0	0.17	0.04	0.04	2.79	63

Abbreviations: C—cis; T—trans. For other abbreviations, see Table 5.

pretreated corn fiber using fluorescence detection (excitation at 294 nm and emission at 326 nm) and we discovered that the large UV-280 peak that we had previously identified as  $\gamma$ -tocopherol was an artifact. Our most recent results (not yet published), obtained with fluorescence detection, indicate that the levels of  $\gamma$ -tocopherol in corn fiber oil (before and after fiber heat pretreatment) are similar to the levels in corn germ oil and corn kernel oil (Table 8).

### 3.6. Carotenoids

High levels of carotenoids have been reported in corn kernels. Most of the carotenoids (74–86%) are localized in the endosperm, 2–4% in the germ, and 1% in the bran (59). The most plentiful carotenoids in corn kernels are lutein and zeaxanthin. Consuming foods that are rich in these carotenoids may decrease the risk for age-related macular degeneration (60). The levels of carotenoids in commercial corn oil are relatively low, due in part to their low concentrations in the germ and in part to their removal during the bleaching step of processing. The nutritional value of corn oil carotenoids has received little attention. Although it is generally believed that carotenoids function as antioxidants, there is evidence that, under certain conditions, carotenoids in vegetable oils and certain other food matrices may serve as pro-oxidants, especially at higher concentrations (61).

### 3.7. *Trans*-Fatty Acids

The unsaturated fatty acids in all common vegetable oils exist only in the *cis*-configuration. During the production of margarines, spreads and shortenings via catalytic hydrogenation, carbon-carbon double bonds are converted to carbon-carbon single bonds; however, the process also catalyzes the conversion of some *cis*-fatty acids to *trans*-fatty acids (62). There is a large variation in the levels of *cis*- and *trans*-fatty acids in commercial corn oil margarines and spreads (Table 9), with the levels of *trans*-fatty acids ranging from a low of 2.79 to a high of 20.13 g of total *trans*-fatty acids per 100 g of product. Concerns about possible linkages between *trans*-fatty acids and cardiovascular disease and certain types of cancer (64) have caused some groups to seek to reduce or eliminate the levels of *trans*-fatty acids in foods, either by using butter, or by using processes other than hydrogenation to raise the melting point of corn oil and thereby produce new types of margarines and spreads. With the controversy associated with *trans*-fatty acids (produced during chemical hydrogenation), some individuals may not realize that a natural group of *trans*-fatty acids, “conjugated linoleic acid” or CLA (the *trans*-double bond is produced by anaerobic rumen bacteria via “biohydrogenation”), have been discovered in dairy and beef products. Our current understanding of international research indicates that CLA may have several health-promoting properties (65). The discovery of these health-promoting properties of certain *trans*-fatty acids (CLA) may provide incentive to undertake an objective reevaluation of the risks of the *trans*-fatty acids in margarines from corn and other vegetable oils.

## 4. PROPERTIES OF CORN OIL

### 4.1. Chemical and Physical Properties

The basic properties of corn oil include its pleasing flavor, its high level of polyunsaturated (essential) fatty acids, a low level of saturated fatty acids, and a low level of linolenic acid (4). The other main physical and chemical properties of corn oil are summarized in Table 10.

### 4.2. Stability

As frying is a major use of corn oil, numerous studies have compared the stability of corn oil and other vegetable oils during frying (6, 66). One study demonstrated that when compared with canola and soybean oils, corn oil produced the lowest

**TABLE 10. Some Physical and Chemical Characteristics of Corn Oil.**

Property	Value
Iodine value	127–133 <sup>a</sup>
Saponification number	187–193 <sup>a</sup>
Free fatty acids after RBD	0.05% max <sup>b</sup>
Color lovibond	3.0 red max <sup>b</sup>
Gardner	6 max
Refractive index	
20°C	1.4753 <sup>a</sup>
26°C	1.4726 <sup>a</sup>
Specific Gravity	
25/25°C	0.91875 <sup>a</sup>
Viscosity	
40°C	30.80 cP <sup>a</sup>
60°C	18.15 cP <sup>a</sup>
Dielectric constant 26°C	3.954 <sup>a</sup>
Surface tension, 25°C	34.80 dyn/cm <sup>a</sup>
Interfacial tension, k H <sub>2</sub> O at 24°C	18.60 dyn/cm <sup>a</sup>
Thermal conductivity at 130°C	4.2017 × 10 <sup>-5</sup> J/s/cm <sup>2</sup> /°C <sup>a</sup>
Unsaponifiables	1–3%
Weight per gallon at 60°C	7.7 pounds <sup>d</sup>
Melting Point	–11 to –8°C <sup>d</sup>
Smoke Point	230 to 238°C <sup>d</sup>
Flash Point	332 to 338°C <sup>d</sup>
Fire Point	366 to 371°C <sup>d</sup>
Cloud Point	–14 to –11°C <sup>d</sup>
CAS number	8001-30-7
EINECS <sup>e</sup> number	232-278-2
Japan registry	Corn oil: 002275

<sup>a</sup>From (6).

<sup>b</sup>From (9).

<sup>c</sup>From (37).

<sup>d</sup>From (6).

<sup>e</sup>Abbreviation for "European Inventory of Existing Chemical Substances" (EU).

level of oxidation products and retained the highest level of tocopherols, during 5 days of continuous frying (44). Another oxidative stability study concluded that corn oil hybrids with higher levels of saturated fatty acids were more stable than traditional corn oils (67). A new optical method was recently developed, providing a new parameter for assessing the oxidative stability of corn oil during frying (68). A method was also developed to measure oxidative stability (PV) in corn and soybean oils using near infrared spectroscopy (NIR) (69). Sundram et al. (70) developed an oxidation-resistant proprietary blend of palm fat and corn oil.

### 4.3. Nutritional Properties

Numerous clinical studies (>30) during the last half-century have supported the hypothesis that corn oil has cholesterol-lowering properties (26). This acknowledgment of corn oil's apparent superiority over other vegetable oils in its cholesterol-lowering properties has been termed the "maize oil aberration" (71). In the 1950s and 1960s, experts believed that the high levels of polyunsaturated fatty acids in corn oil were the reason for its cholesterol-lowering properties (4). Others suggested that because corn oil contains the highest levels of unsaponifiables and phytosterols of any common vegetable oil, these components cause the cholesterol-lowering effect (72). A recent study comparing corn oil with cottonseed oil found that, although corn oil contained more unsaponifiables, cottonseed oil was more effective at lowering total serum cholesterol, which the authors attributed to the specific types of unsaponifiables in cottonseed oil (73). Lichtenstein et al. (74) reported that hydrogenation of corn oil reduces its cholesterol-lowering properties in humans. Recent clinical studies (with phytosterol and phytostanyl ester products) have indicated that a person must ingest 1.6 to 3.3 g of phytosterols per day to achieve a 5% to 10% reduction in total serum cholesterol (71). To ingest 1 g of phytosterols from corn oil, a person would need to consume about 100 g (about 1/2 cup and about 900 calories) of corn oil per day, an amount that is feasible, but probably not practical. A recent hamster feeding study (75), comparing corn oil and olive oil, presented evidence that, although both oils reduced LDL-cholesterol ("bad cholesterol"), olive oil was better at increasing HDL-cholesterol ("good cholesterol"). Other recent studies indicated that corn fiber oil is more effective than corn (germ) oil at lowering serum cholesterol in hamsters (76). Recently, Ostlund et al. (77) reported that the levels of phytosterols in commercial corn oil (about 1%) are enough to sufficiently reduce cholesterol absorption in humans. Wagner et al. (78) compared a high monounsaturated fatty acid-rich (MUFA) diet (olive/sunflower oil mixture) with a polyunsaturated fatty acid-rich (PUFA) corn oil diet and found that the latter had more influence on lipoprotein metabolism. They concluded that the hypocholesterolemic effect of the PUFA-rich diet must also be connected with the high amount of unsaponifiable matter, mainly phytosterols in corn oil (78). Obviously, more work is required to evaluate the health-promoting properties of corn (germ) oil and corn fiber oil.

Some recent clinical studies with corn oil have focused on its high levels of  $\gamma$ -tocopherol and other unique properties. A Swedish study revealed that consumption

of corn and sesame oils significantly increases the levels of serum  $\gamma$ -tocopherol (79). Recently, a U.S. patent was issued for the use of  $\gamma$ -tocopherol and  $\gamma$ -tocopherol derivatives as antioxidants and nitrogen oxide scavengers to treat and prevent high blood pressure, thromboembolic disease, cancer, natriuretic disease, the formation of neuropathological lesions, and immunomodulation (80). Schurgers et al. (81) reported that the consumption of corn oil increases the absorption and metabolism of vitamin K.

The antioxidant properties of tocopherols (such as those found in corn oil) have been suggested to be involved in treating atherosclerosis by preventing the oxidation of low-density lipoproteins (57). Another study indicated that the particular ratio of tocopherols in corn oil (a high ratio of  $\gamma$ -tocopherol/ $\alpha$ -tocopherol) may achieve better protection against DNA damage than  $\alpha$ -tocopherol alone (82). The beneficial effects of corn oil on blood pressure, platelet aggregation, and diabetes have been reported by others (22).

## 5. MAJOR FOOD USES OF CORN OIL

### 5.1. Cooking/Salad Oil

Of the 1.3 billion pounds of refined corn oil produced in the United States in 2000, approximately half was used for cooking and salad oils, and about a fourth was used for margarines and spreads (4). Corn oil has long been a popular cooking oil, because of its mild flavor, its stability (because of its low levels of linolenate), and its reputation as a healthy edible oil (because of its high levels of polyunsaturated fatty acids). Because of its higher levels of polyunsaturates than most other commodity vegetable oils (especially soy), corn oil is considered a premium oil and is sold at a premium. In recent years, with the increased popularity of monounsaturate-rich oils (olive, canola, and now NuSun sunflower oil), corn oil is still considered a premium vegetable oil, but there has been a drop in the price differential between corn oil and other commodity vegetable oils.

### 5.2. Margarines and Spreads

Corn oil margarine (common “stick” margarines contain 80% fat) and corn oil spreads (common “tub” margarine spreads contain 20% to 65% fat) are popular products. In the 1870s, Unilever began manufacturing margarine in Europe, and the U.S. Dairy Company began production of “artificial butter” in the United States (83). Sales of corn oil margarine in the United States climbed gradually and reached a level of about 1 million pounds in 1930 (4). During the period of 1950 to 1980 (when there was growing consumer interest in the health-promoting properties of the polyunsaturates in corn oil), the production of corn oil margarine in the United States climbed from 15 to 250 million pounds per year (4). During the last 20 years, there has been growing concern about possible harmful effects of the *trans*-fatty acids in margarines and spreads (which can range from 10–20% of the total fatty acids) made from corn oil and other vegetable oils, and consumer concerns about



*trans*-fatty acids have become a major issue for margarine manufacturers. Methods have been developed to produce margarines, shortenings, and spreads by interesterifying (84) or blending oils (including a recent patent that details a process to produce “*trans*-free” shortening by blending corn oil and palm fat) (70), thus eliminating the need for chemical hydrogenation and eliminating the formation of *trans*-fatty acids. Zero *trans*-fatty acid margarines and spreads currently account for a major portion of the sales in several European countries, and a growing number of U.S. manufacturers are now marketing zero *trans*-fatty acid margarines and spreads in the United States. In July 2003, the U.S. Food and Drug Administration amended its regulations on nutrition labeling to require that *trans*-fatty acids be declared in the nutrition label of conventional foods and dietary supplements on a separate line immediately under the line for the declaration of saturated fatty acids (85). This ruling will take effect on January 1, 2006.

## 6. NONFOOD USES OF CORN OIL

### 6.1. Cosmetics/Skin Care Products

Corn oil is allotted important regenerative properties because of its high level of unsaponifiables. Like all of the oils rich in essential fatty acids, it has a restructuring activity and reinforces the cutaneous barrier. It thus helps to maintain a hydrated epidermis. Corn oil is also a very good skin conditioner and an ingredient in sheathing products for dry hair, body massage oils, emollient hand creams, face care products, skin care products, after-sun oils and creams, and nourishing lip balms (86).

### 6.2. Biodiesel

In most parts of the world, the term “biodiesel” now denotes a diesel fuel that is produced by converting a vegetable oil to methyl (or ethyl) esters. In the United States soybean oil has been the primary feedstock for biodiesel, mainly because it is commonly the least expensive and most abundant vegetable oil. Although there are economic reasons why corn oil (and other U.S. vegetable oils) has not been used as feedstocks for biodiesel, there are no technical reasons why a corn oil biodiesel could not be successfully developed (personal communication. M. Haas).

### 6.3. Other Industrial Uses

Some of the other industrial uses for corn oil include insecticide formulations, paints, varnishes, rubber substitutes, rust preventatives, soaps, leather tanning, and textiles (87).

## 7. CONCLUSIONS

Corn oil’s desirable properties include its mild nutty flavor, high levels of unsaturated fatty acids, low levels of saturated fatty acids, very low levels of linolenic

acid, high levels of unsaponifiables (including phytosterols and tocopherols), and stability during frying. Although the focus of consumers has been shifting toward “high- monounsaturate”-oils (olive, canola, and NuSun sunflower oils), additional research is still needed to objectively compare the health-promoting properties of polyunsaturates versus monounsaturates. The food industry currently relies heavily on corn oil margarine and other food products that contain hydrogenated corn oils. Additional research also is needed to objectively evaluate the risks associated with *trans*-fatty acid-containing products made from partially hydrogenated corn oil, especially in light of the recent evidence that at least some types of natural *trans*-fatty acids (conjugated linoleic acids) may have multiple health-promoting properties.

## REFERENCES

1. F. D. Gunstone, *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, 2002, pp. 6, 12.
2. *Chemical Market Reporter*, June 7, 2004.
3. M. Ash and E. Dohlman. *Oil crop outlook*. (2003, April 11). Economic Research Service, USDA, Available: <http://www.ers.usda.gov>.
4. Anonymous, Corn oil, manufacturing, properties, uses and analysis of corn oil, Corn Refiners Association, St. Louis, MO, 1996, p. 24.
5. R. A. Moreau, V. Singh, S. Eckhoff, M. J. Powell, K. B. Hicks, and R. A. Norton, *Cereal Chem.*, **76**, 449–451 (1999a).
6. L. R. Strecker, M. A. Bieber, A. Maza, T. Grossberger, and W. J. Daskoczynski, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products, Fifth Edition, Volume 2, Edible Oil and Fat Products: Oils and Oilseeds*, Wiley, New York, 1996, pp. 125–158.
7. R. A. Moreau, M. J. Powell, and K. B. Hicks, *J. Agric. Food Chem.*, **44**, 2149–2154 (1996).
8. A. Maza, U. S. Patent 6,201,142, March 13, 2001.
9. Anonymous. (2003a). *2003 Corn Annual, Corn Refiners Association*. Available : <http://www.corn.org>.
10. G. R. List, J. P. Friedrich, and D. D. Christianson, *J. Am. Oil Chem. Soc.*, **61**, 1849–1851 (1984).
11. J. F. Ulrich and S. C. Anderson, U. S. Patent 6,313,328, November 6, 2001.
12. M. P. Hojilla-Evangelista, L. A. Johnson, and D. J. Myers, *Cereal Chem.*, **69**, 643–647 (1992).
13. M. P. Hojilla-Evangelista and L. A. Johnson, *J. Am. Oil Chem. Soc.*, **79**, 815–823 (2002).
14. F. Feng, D. J. Myers, M. P. Hojilla-Evangelista, K. A. Miller, L. A. Johnson, and S. K. Singh, *J. Am. Oil Chem. Soc.*, **79**, 707–709 (2002).
15. J. R. Kwiatkowski and M. Cheryan, *J. Am. Oil Chem. Soc.*, **79**, 825–830 (2002).
16. M. Cheryan, U.S. Patent 6,433,146, August 13, 2002.
17. D. D. Christianson, J. P. Friedrich, G. R. List, K. Warner, E. B. Bagley, A. C. Stringfellow, and G. E. Inglett, *J. Food Sci.*, **49**, 229–233 (1984).
18. D. D. Christianson and J. P. Friedrich, U.S. Patent 4,495,207, January 22, 1985.

19. E. Rónyai, B. Simándi, S. Tömösközi, A. Deák, L. Vigh, and Z. Weinbrenner, *J. Supercrit. Fluids*, **14**, 75–81 (1998).
20. K-D. Stolp and R. W. Stute, U.S. Patent 4,341,713, July 27, 1982.
21. M. Bocevaska, D. Karlovic, T. Jovan, and D. Pericin, *J. Am. Oil Chem. Soc.*, **70**, 1273–1277 (1993).
22. D. J. Karlovic, M. Bocevaska, J. Jakovlevic, and J. Turkulo, *Acta. Alimentaria*, **23**, 389–400 (1994).
23. D. Verser and T. Eggeman, U.S. Patent 6,509,180, January 21, 2003.
24. F. T. Orthoefer and R. D. Sinram, in S. A. Watson and P. E. Ramstad, eds., *Corn: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, MN, 1987, pp. 535–552.
25. R. A. Moreau, V. Singh, and K. B. Hicks, *J. Agric. Food Chem.*, **49**, 3793–3795 (2001).
26. V. Singh, R. A. Moreau, A. E. Haken, S. R. Eckhoff, and K. B. Hicks, *Cereal Chem.*, **77**, 692–695 (2000).
27. M. Dowd, *J. Chromatogr. A.*, **816**, 185–193 (1998).
28. R. Antoniassi, W. Esteves, and A. J. D. Meirelles, *J. Am. Oil Chem. Soc.*, **75**, 1411–1415 (1998).
29. Z. Leibovitz and C. Ruckenstein, *J. Am. Oil Chem. Soc.*, **60**, 395–399 (1983).
30. C. G. Pina, and A. J. A. Meirelles, *J. Am. Oil Chem. Soc.*, **77**, 553–559 (2000).
31. T. Verleyen, R. Verhe, L. Garcia, K. Dewettinck, A. Huyghebaert, and W. De Greyt, *J. Chromatogr. A.*, **921**, 277–285 (2001).
32. T. Verleyen, U. Sosinka, S. Ioannidou, R. Verhe, K. Dewettinck, A. Huyghebaert, and W. De Greyt, *J. Am. Oil Chem. Soc.*, **79**, 947–953 (2002).
33. K. B. Hicks and R. A. Moreau, *Food Technol.*, **55**, 63–67 (2001).
34. R. A. Moreau, K. B. Hicks, R. J. Nicolosi, and R. A. Norton, U.S. Patent 5,843,499, December 1, 1998.
35. C. M. Buchanan, N. L. Buchanan, J. S. Debenham, M. C. Shelton, M. D. Wood, M. J. Visneski, B. K. Aruunugam, J. K. Sanders, L. R. Lingerfelt Jr., and L. Blair, U.S. Patent 6,352,845, March 5, 2002.
36. C. M. Buchanan, N. L. Buchanan, J. S. Debenham, M. C. Shelton, and M. D. Wood, U.S. Patent 6,388,069, May 14, 2002.
37. D. Firestone, in *Physical and chemical characteristics of oils, fats, and waxes*, American Oil Chemists Society, Champaign, Illinois, 1999, pp. 31–32.
38. Anonymous. (2003). USDA National Nutrient Database for Standard Reference, Release 15. Nutrient Data Laboratory Home Page. Available: <http://www.nal.usda.gov/fnic/food-comp/Data/SR15/sr15.html>.
39. F. D. Goffman and T. Böhme, *J. Agric. Food. Chem.*, **49**, 4990–4994 (2001).
40. R. A. Moreau, V. Singh, A. Nunez, and K. B. Hicks, *Biochem. Soc. Trans*, **28**, 803–806 (2000).
41. P. J. White and E. J. Weber, in L. A. Johnson and P. J. White, eds., *Corn Chemistry and Technology, Second Edition*, American Association of Cereal Chemists, St. Paul, MN. In press.
42. K. J. Leto and J. F. Ulrich, U.S. Patent 6,248,939, June 19, 2001.
43. R. A. Moreau, in: F. D. Gunstone, ed., *Vegetable oils in food technology*, Sheffield Academic Press: Sheffield, 2002, pp. 278–296.

44. L. R. Strecker, A. Maza, and F. G. Winnie, in D. R. Erickson, ed., *Proceedings of the World Conference on Edible Fats and Oils Processing: Basic Principles and Modern Practices*, American Oil Chemists' Society, Champaign, Illinois, 1990, pp. 309–325.
45. W. C. Byrdwell, W. E. Neff, and G. R. List, *J. Agric. Food Chem.*, **49**, 446–457 (2001).
46. W. E. Neff, R. O. Adlof, G. R. List, and M. El-Agaimy, *J. Liq. Chromatogr.*, **17**, 3951–3968 (1994).
47. J. W. Fahey, B. A. Clevidence, and R. M. Russell, *Nutr. Rev.*, **57**, S34–S40 (1999).
48. B. Ham, B. Butler, and P. Thionville, *LC-GC*, **18**, 1174–1181 (2000).
49. R. E. Worthington and H. L. Hitchcock, *J. Am. Oil Chem. Soc.*, **61**, 1085–1100 (1984).
50. J. M. Snyder, J. W. King, S. L. Taylor, and A. L. Neese, *J. Am. Oil Chem. Soc.*, **76**, 717–721 (1999).
51. R. A. Moreau, B. D. Whitaker, and K. B. Hicks, *Prog. Lipid Res.*, **41**, 457–500 (2002).
52. V. Singh, R. A. Moreau, and P. H. Cooke, *Cereal Chem.*, **78**, 436–441 (2001).
53. V. Singh and R. A. Moreau, *Cereal Chem.*, **80**, 123–125 (2003).
54. V. Singh, R. A. Moreau, K. B. Hicks, and S. R. Eckhoff, *Cereal Chem.*, **78**, 46–49 (2001b).
55. C. Wang, J. Ning, P. G. Krishnan, and D. P. Matthees, *J. Am. Oil Chem. Soc.*, **75**, 609–613 (1998).
56. R. A. Moreau, K. B. Hicks, and M. J. Powell, *J. Agric. Food Chem.*, **47**, 2869–2871 (1999).
57. T. Saldeen, D. Y. Li, and J. L. Mehta, *J. Am. Coll. Cardiol.*, **34**, 1208–1215 (1999).
58. R. A. Parker, B. C. Pearce, R. W. Clark, D. A. Gordon, and J. J. Wright, *J. Biol. Chem.*, **268**, 11230–11238 (1993).
59. E. J. Weber, in S. A. Watson and P. E. Ramstad, eds., *Corn: Chemistry and Technology*, Am. Assoc. Cereal Chem., St. Paul, MN, 1987, pp. 311–350.
60. O. Sommerburg, J. E. E. Keunen, A. C. Bird, and F. J. G. M. Kuijk, *Brit. J. Ophthalmol.*, **82**, 907–910 (1998).
61. A. Subagio and N. Morita, *Food Res. Int.*, **34**, 183–188 (2001).
62. M. G. Enig, L. A. Pallansch, J. Sampugna, and M. Keeney, *J. Am. Oil Chem. Soc.*, **60**, 1788–1795 (1983).
63. J. Exler, L. Lemar, and J. Smith. (2001). USDA Food Nutrition and Information Center. Available: <http://www.nal.usda.gov/fnic/etext/000020.html>.
64. C. Ip, *Am. J. Clin. Nutr.*, **66**, 1523S–1529S (1997).
65. R. E. Lawson, A. R. Moss, and D. I. Givens, *Nutr. Res. Rev.*, **14**, 153–172 (2001).
66. C. Gertz, S. Klostermann, and S. P. Kochhar, *Eur. J. Lipid Sci. Technol.*, **102**, 543–551 (2000).
67. N. Shen, S. Duvick, P. White, and L. Pollak, *J. Am. Oil Chem. Soc.*, **76**, 1425–1429 (1999).
68. E. Sebben, D. C. Slaughter, and R. P. Singh, *J. Food Proc. Pres.*, **22**, 265–282 (1998).
69. G. Yildiz, R. L. Wehling, and S. L. Cuppett, *J. Am. Oil Chem. Soc.*, **79**, 1085–1091 (2002).
70. K. Sundram, D. Perlman, and K. C. Hayes, U.S. Patent 5,874,117, February 23, 1999.
71. G. W. Meijer, *Lipid Technol.*, **November**, 129–132 (1999).
72. T. J. Howell, D. E. MacDougall, and P. J. H. Jones, *J. Lipid Res.*, **39**, 892–900 (1998).
73. J. D. Radcliffe, C. C. King, D. M. Czajka-Narins, and V. Imrhan, *Plant Foods Human Nutr.*, **56**, 51–60 (2001).

74. A. H. Lichtenstein, L. M. Ausman, W. Carrasco, J. L. Jenner, J. M. Ordovas, and E. J. Schaefer, *Arterioscler. Thromb.*, **13**, 154–161 (1993).
75. A. van Tol, A. H. M. Terpstra, P. van den Berg, and A. C. Beynen, *Atherosclerosis*, **147**, 87–94 (1999).
76. T. A. Wilson, A. P. DeSimone, C. A. Romano, and R. J. Nicolosi, *J. Nutr. Biochem.*, **11**, 443–449 (2000).
77. R. E. Ostlund Jr., S. B. Racette, A. Okele, and W. F. Stenson, *Am. J. Clin. Nutr.*, **75**, 1000–1004 (2002).
78. K. H. Wagner, R. Tomasch, and I. Elmadfa, *Eur. J. Nutr.*, **40**, 161–167 (2001).
79. M. Lemcke-Norokjärvi, A. Kamal-Eldin, L-A. Appelqvist, L. H. Dimberg, M. Öhrvall, and B. Vessby, *J. Nutr.*, **131**, 1195–1201 (2001).
80. W. J. Wechter, U.S. Patent 6,242,479, June 5, 2001.
81. L. J. Schurgers, M. J. Shearer, B. A. M. Soute, I. Elmfaafa, J. Harvey, K-H. Wagner, R. Tomasch, and C. Vermeer, *J. Lipid Res.*, **43**, 878–884 (2002).
82. I. Elmadfa and E. Park, *Eur. J. Nutr.*, **38**, 286 (1999).
83. Anonymous. (2001). *National Association of Margarine Manufacturers*. Available: <http://www.margarine.org>.
84. G. R. List, T. L. Mounts, F. Orthofer, and W. E. Neff, *J. Am. Oil Chem. Soc.*, **72**, 379–382 (1995).
85. Anonymous. (2003). US Food and Drug Administration, *Federal Register* 68 (133) pp. 41433–41506. Available: [http://www.access.gpo.gov/su\\_docs/fedreg/a030711c.html](http://www.access.gpo.gov/su_docs/fedreg/a030711c.html).
86. Green cottage resource site. (2003). Available: <http://www.greencottage.com/oils/corn.html>.
87. Kentucky Corn Growers Association. (2003). Available: <http://www.kycorn.org/corn-education/cornclass/8uses.pdf>.

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## *Cottonseed Oil*

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### 1. INTRODUCTION

Cotton is both a food (cottonseed oil) and a fiber (cotton lint) crop. For each 100 kg (220.46 lbs) of cotton fiber produced, the plant also produces about 150 kg (330.69 lbs) of cottonseed. The cotton plant primarily is and has always been grown for the textile fiber (cotton) component of the plant. Consequently, the production of seed, which varies directly with cotton fiber production, is dominated by factors determining the production of cotton fiber. Cottonseed is about 15–20% of the value of the cotton crop.

A typical cottonseed crushing operation will separate the seed into oil [160 kg/t (320 lbs/t)], hulls [260 kg/t (540 lbs/t)], meal [455 kg/t (910 lbs/t)], and linters [83.5 kg/t (167 lbs/t)] (1). The hulls and meal are sources of vegetable protein feed for animals; and the linters are used as a chemical cellulose source in personal care products, in batting for upholstered furniture and mattresses, and in high-quality paper (2).

Cottonseed oil, America's original vegetable oil, dominated the United States vegetable oil market for almost 100 years. The English and European vegetable oil industry was based on a variety of oilseeds and tree fruits available in the home countries and their colonies, but cottonseed was the principal raw material for vegetable oil processors in the United States until the mid-twentieth century. In

a little more than 50 years, through research and experimentation, chemists have developed a clear, odorless, bland flavored cottonseed oil and a creamy, white shortening that set the standards for edible fats and oils worldwide. The scientific and technical advances developed to process cottonseed oil became the cornerstones of the edible fats and oils industry as it is known today. Numerous processes were developed or perfected especially for cottonseed oil, which later found application for other oils. Today, vegetable oil processors worldwide, including the United States, have a wide range of raw materials to choose from, but cottonseed pioneered the American vegetable oil industry. Table 1 identifies most of the important dates and events in the history of cottonseed oil processing and use (3).

## 2. COTTONSEED OIL INDUSTRY DEVELOPMENT

Crushing of cottonseed for oil was documented in the early Hindu medical books; a topical medicine was prepared by pounding the cottonseeds followed by boiling to extract the oil. An early Chinese process of oil extraction was the wedge press; but it is not clear if this was used to extract oil from cottonseed. For many centuries the use of cottonseed oil did not develop much beyond this crude stage and was confined to local areas. Then, during the first part of the nineteenth century, plants in Europe began to crush small quantities of Egyptian cottonseed. Fats and oils shortages, caused by rapid population increases during the Industrial Revolution and the English blockade during the Napoleonic Wars, invoked the natural law of supply and demand, which increased the cost of the available fats and oils products to levels that made it impossible for many Europeans to afford them in their diets or for illumination. The resultant demand for less expensive fats and oils products led businessmen to extract oil from a variety of oilseeds and nuts; included in these endeavors was cottonseed from Egypt and India (4, 5).

The sparsely populated United States had adequate supplies of animal fats while western Europe was experiencing shortages. Nevertheless, the extraction of oil from cottonseed became an attractive solution for another problem. Cotton cultivation had increased rapidly in the United States after Eli Whitney invented the cotton gin in 1793 creating a surplus supply of cottonseed, after that required for planting, fertilizer, and animal feed. The excess cottonseed stocks became huge, worthless, rotting piles that dotted the countryside in the southern states. A few entrepreneurs began crushing cottonseed for oil between 1820 and 1830, but none of these ventures survived more than a few years. Limited demand, as well as difficult and expensive transportation to the markets, were formidable obstacles, but probably foremost was the hull problem with the cottonseed varieties grown in America. Oil extraction from the American cottonseed was hampered by the fact that the seed of the short-staple variety grown has a tough hull covered with short cotton fibers called linters. These residual fibers and hulls absorbed valuable oil, resulting in low oil production and poor-quality, oily livestock feed. European cottonseed crushings had been with Egyptian long-staple cotton varieties, which did not have a tough hull covered with linters. In 1857, William Fee invented a huller,

**TABLE 1. Some Important Dates in the Evolution of Cottonseed Oil Processing and Utilization.**

Year	Event	Effect
1793	Eli Whitney invented the cotton gin	Cotton cultivation substantially increased
Early 1800s	Winterization process developed in France for the illumination industry	Separation of an oil into liquid and solid fractions
1857	William Fee invented the cottonseed huller	Separation equipment which solved a major oil extraction problem
1859	Petroleum industry launched	Eliminated illumination and lubrication as potential cottonseed oil applications
1880	Alexander Winters bleaching patent	Color adsorbed with Fuller's earth
1880s	Refining with caustic soda	Alkali refining introduced and accepted
1882	Olive oil adulteration	European import tariffs imposed with complete expulsion from Italian markets
1882	Cottonseed oil bottled in the U.S.	Limited acceptance due to flavor & odor
1883	O. G. Burnham patented a refrigerated chill roll	Allowed the addition of higher levels of cottonseed oil to lard
1884	Lard adulteration	U.S. label restrictions—mandatory identification of "lard compound"
1886	W. B. Albright bleaching patent	Steam used to recover oil from earth
Late 1880s	Acidity analysis	Chemists learned that FFA level in crude cottonseed oil was a good quality indicator
1891	Henry Eckstein introduced steam distillation for cottonseed oil	Removed the objectionable flavors and odors from the oil
1897	Sabatier discovered vapor phase hydrogenation using nickel catalyst	Process to convert a liquid oil to a semi-solid
1899	Wesson Oil <sup>®</sup> introduced	Cottonseed oil deodorized with a vacuum and superheated steam
1903	William Norman granted British patent for hydrogenation	Assigned to Joseph Crossfield & Son, a British concern that used the process to harden whale oil for soap manufacture
1909	Procter and Gamble acquired the U.S. rights to Norman's patent	Developed the hydrogenation process for food oil use
1911	Crisco <sup>®</sup> shortening introduced	First hydrogenated all-vegetable shortening
Early 1930s	Closed internal scraped wall heat exchanger developed by Votator <sup>®</sup>	Shortening and margarine plasticization equipment offering improved sanitation and product uniformity
1930s	Pre-Press Solvent Extraction Process Developed	Oil recovery improved from 80% to 97%
1933	Introduction of superglycerinated shortening	Initiation of the era of tailor-made fats and oils products
1941	World War II	Cottonseed oil shortages forced soybean oil utilization
1944	Soybean oil production outranks cottonseed oil for the first time	Cottonseed oil shortages and lower cost soybean oil utilization
1950	Soybean oil replaces cottonseed oil as the dominate oil for shortenings	Hydrogenation and emulsifiers allow utilization of the more economical oil
1951	Cottonseed oil loses dominance in margarine to soybean oil	Transportation and grocery store handling improvements allowed product changes
1958	Cottonseed oil use in salad oil manufacture drops below 50%	Flavor problems with more economical soybean salad oil decreased
1960s	Expander Solvent Extraction Technology Introduced	Improved oil recovery and quality



which effectively separated the tough, linty hulls from the meats to solve the major extraction problem with American cottonseed. (6, 7). Fee also invented an improved hydraulic press, and although many advances were made in the mechanical process of cottonseed crushing, the basic technology available remained unchanged well into the twentieth century.

Cottonseed oil usage for illumination purposes in lamps and candles to supplement whale oil and lard was curtailed by the petroleum industry in 1859. Apart from its use as a smokeless oil for miner's lamps during the late nineteenth century, cottonseed oil would have no future as an illuminant (8). However, the processing techniques developed in connection with lighting ultimately became important to the food industry. Candles require a wax or fat with a high melting point to remain solid until melted from the flame heat; conversely, a lamp oil must be a clear liquid to maintain a flame. During the early nineteenth century, a fractionation process was developed in France to make vegetable oils more like whale oil and tallow. This technique, called winterization, was achieved by holding the oil in outside tanks during the winter. The low winter temperatures caused the higher melting fractions to crystallize and settle to the bottom of the tank, leaving a top layer of clear oil. The clear oil could be obtained by either decanting from the top or filtering the mixture to separate the liquid and solid fractions (9). The hard fractions found application as a tallow replacement in candle making, and the liquid fraction could be used as a lamp oil.

Even with all of the early setbacks, cottonseed crushing and refining became a profitable venture in the United States after 1870. Large quantities of cottonseed oil were exported for use in soap manufacture and some found its way into cooking, salad oils, and the oleomargarine product developed by the French chemist, Hippolyte Mege-Mouries. Adulteration of olive oil with cottonseed oil resulted in import tariffs in the olive growing countries and complete expulsion from Italy in 1883. These restrictions curtailed cottonseed oil exports to create an oversupply of cottonseed oil, which again decreased the value (6).

High lard prices offered an opportunity to increase the domestic consumption of cottonseed oil. Lard was the choice fat for cooking and baking in many parts of the western world. One of the chief reasons was the particular consistency, which was optimum for incorporation into breads, cakes, pastries, and other baked products. It had become common practice to mix beef fat or tallow with lard, so adulteration with cottonseed oil was a natural step for the use of the inexpensive oil. Initially, meat packers secretly added cottonseed oil to lard. This practice was uncovered in 1884, when Armour and Company, seeking to corner the lard market, found that it had purchased more lard than the existing hog population could have produced. Public disclosure of this practice led to a Congressional investigation, which made identification of these mixtures with the descriptive name "lard compound" or "compound" mandatory (6, 10).

Major improvements in oil processing had to take place before substantial quantities of cottonseed oil could replace lard as the preferred fat for baking and frying. Crude cottonseed oil was unsuitable for use in most food applications because of its dark color, high free fatty acid content, and objectionable flavor and odor.

Cottonseed oils crushed in Europe and Asia prior to the nineteenth century did not have the objectionable qualities experienced after technology improvements to improve yield were implemented. Oilseed milling became an established trade in Europe, without the benefit of any substantial technology advancements since the days of the Roman Empire. These inefficient practices produced an oil with a mild flavor and odor due to a very low level of nontriglycerides. Oil quality changed with the improvements in milling machinery and technology during the seventeenth and eighteenth centuries. Extraction technology progressed from edgestone to wedge press to hydraulic press during this period. Hydraulic pressing was the predominant means used to separate oil from cottonseed for most of the nineteenth century. Economics promoted research activity to recover the oil left in the press cake; mechanical pressing left nearly 20% of the available oil in the press cake. This effort led to the prepress solvent extraction process in the 1930s that was able to recover better than 97% of the available oil in cottonseed. Further productivity and quality demands brought expander-solvent extraction technology into cottonseed crushing in the late 1960s (11). The more efficient high-pressure expression and solvent extraction systems provided higher oil yields, which contained substantial quantities of nontriglyceride materials like gums, color bodies, and other impurities with strong flavors and odors. As a result of the presence of these impurities, these oils require more processing to provide a palatable product (12, 13).

Acid-refining techniques were introduced in the United States during the last half of the eighteenth century but never assumed importance. Alkali-refining procedures developed in Europe around 1840 using calcium and potassium hydroxides, also never found acceptance in the United States. Later, during the 1880s, alkali refining using caustic soda was introduced and found acceptance. Cottonseed oils were alkali refined in open kettles, and the foots were separated by gravitation until continuous systems utilizing centrifuges were introduced (14–16). Refining with caustic soda reduced both free fatty acid content and a large portion of the red-yellow color pigment, gossypol, in cottonseed oils. Caustic-refined cottonseed oils were found acceptable for packing sardines and marginally acceptable for mixing with lard when it was scarce and expensive. Neutralized cottonseed oil after caustic refining still had an acrid flavor and somewhat unpleasant odor, which limited consumer acceptance (17).

Additional color removal from cottonseed oil was necessary to make it more acceptable for edible oil applications. People expected lard to be white; therefore, cottonseed oil used to dilute lard or as an ingredient to replace lard had to be light in color. At first, refiners used a bleaching technique previously used to remove the orange color from palm oil. This bleaching process was accomplished by exposing the oil to sunlight in large shallow tanks for up to 18 months on factory roofs. This method had serious drawbacks: the lengthy process tied up capital for months, and the sunlight deteriorated the keeping quality of the oil. Cottonseed oil processors then began to experiment with bleaching agents, such as carbon and different kinds of clays or bleaching earths, to adsorb the color pigments. The use of bleaching earth for vegetable oil decolorization was an American achievement. In 1880, Alexander Winters obtained a U.S. Patent for purifying animal and vegetable fats

by treatment with pulverized fuller's earth. Six years later, William B. Allbright secured a patent for fuller's earth applied with steam to facilitate removal of oil from the used earth.

Although caustic refining removed free fatty acids and other impurities and bleaching with fuller's earth solved the color problem, the unpleasant flavor and odor limited the acceptance of cottonseed oil as a salad or cooking oil, as an ingredient for margarines, and the levels that could be used in lard compounds or substitutes. Attempts to chemically remove the offensive flavor or mask them with spices or flavors were unsuccessful. The first successful attempt at actually removing odor and flavor consisted of blowing a current of live steam through oil at elevated temperatures and atmospheric pressure. Reportedly, Henry Eckstein, a Fairbank Company chemist, observed this technique at an English firm and understood its importance for the future of cottonseed oil. He introduced this technique around 1891, and it was quickly adopted by most American processors with other refinements identified by Boyce, Cuff, and others. Later, David Wesson perfected and introduced a deodorization process that exposed oil to superheated steam in a vacuum. This process provided a bland-flavored oil with improved stability or keeping quality. Deodorization enabled processors to increase the amount of cottonseed oil in lard compounds to 80% or more (7, 8, 17, 18).

Cottonseed oil was bottled in the United States as early as 1882, some of it with labels printed in French, Spanish, and Italian to make immigrants think that they were buying olive oil from the country of their origin. Acceptance of liquid cottonseed oil was limited by the acrid flavor and unpleasant odor (9). In 1899, David Wesson convinced the Southern Cotton Oil Company to process, package, and distribute Wesson Oil. This cottonseed salad oil was caustic refined, adsorption bleached, and deodorized with the Wesson technique after winterization. The winterization process, a nineteenth-century French development to make whale oil substitutes, removed the high melting fractions from cottonseed oil to maintain clarity at refrigerator temperatures. This requirement became a necessity with the introduction of mechanical refrigeration and the increased popularity of mayonnaise, salad dressings, and other emulsified sauces. Oils that solidify at refrigerator temperatures cause emulsions to break, resulting in an unsightly product separation.

Introduction of the catalytic hydrogenation process gave the cottonseed oil processors independence from the meat-packing industry and initiated a new era. Compounds were dependent on oleo stearine as the stiffening agent, which were supplied by the meat packers who recognized their monopolistic situation and maintained a high cost for this material. Hydrogenation provided a means for converting liquid cottonseed oil to semisolid with a consistency similar to the meat-fat stearine products. Sabatier discovered vapor-phase hydrogenation, using nickel as a catalyst, in Paris in 1897. Six years later, William Norman, a German working in England, obtained a British patent for hydrogenation, and in 1906 commercial hydrogenation of whale oil began. Procter & Gamble acquired the U.S. rights to the Norman patent in 1909 and produced the first all-vegetable shortening in 1911. The product was named kispo, which was later changed to Crisco, short for crystallized cottonseed oil. Crisco is still the leading household shortening in

the year 2004 and even though it has undergone several composition changes, it still utilizes some cottonseed oil in the formulation. The success of this hydrogenated cottonseed oil inclined other manufactures to litigation actions, which led to a U.S. Supreme Court decision invalidating Procter & Gambles exclusive use of the Norman patent and cleared the way for all processors to employ the hydrogenation process (8, 19).

Meat packers continued to offer lard compounds, later shortened to “compounds”, employing the hydrogenation process only for the production of hardened oils to serve as occasional substitutes for oleostearine. Cottonseed oil producers had the foresight to abandon the lard substitute product concept and description to offer their hydrogenated products as a new food ingredient that has become known as shortening. Thereafter, shortening development followed two divergent courses; all-hydrogenated or a blended type formulation somewhat similar to the compound concept.

The hydrogenation process gave cottonseed oil shortenings a definite advantage over the compound shortenings offered by the meat packers. This key process permitted them to change the composition of the inherently liquid oil products to provide a more consistent product. The properties of the hydrogenated cottonseed oil shortenings could be controlled, whereas, the consistency of lard varied according to the time of the year, the variety and age of the hogs, and the type of feed. The superior neutrality, oxidative stability, and uniformity of the hydrogenated cottonseed oil shortenings found favor with commercial bakers and homemakers alike at the expense of the meat-fat products. This was confirmed by the consumers willingness to consistently pay higher prices for the hydrogenated cottonseed oil shortenings.

Solidification of shortening and margarine into smooth plastic products also posed formidable challenges for the edible oil industry. Pure lard contains relatively little high-melting fractions; therefore, slow cooling was a satisfactory procedure for production of smooth, plastic lard products. Agitation of shortening products in jacketed, water chilled tanks, which had been the usual practice for lard, resulted in grainy products with the lard compounds containing cottonseed oil as well as the hydrogenated shortenings. An internally refrigerated chill roll developed for crystallizing lard compounds was patented by O. G. Burnham in 1883. The chill roll consisted of a revolving hollow metal cylinder chilled by circulating cold or brine water. A thin layer of lard compound or shortening applied to the revolving drum solidified and was scraped off into a mixing unit, called a picker pan, where crystallization continued and air was incorporated. After picking, the product was forced through a small orifice at high pressure to complete the homogenization of the air and oil. This crystallization process allowed incorporation of higher levels of cottonseed oil in lard compound products (20).

Initially, margarine was chilled by pouring or spraying molten emulsions into a vat of running cold water. The emulsion entered at one end of the vat and solidified before it reached the other end. The solidified material floated to the surface in the form of a flaky mass, which was skimmed off. After the excess water was drained from the solidified emulsion, it was worked to achieve the desired consistency. The

chill roll used to plasticize lard compounds and shortenings was adopted and used in nearly the same manner (21).

In the early 1930s, development of improved heat-transfer equipment for freezing ice cream led to the perfection of a closed, continuous, internal scraped wall chilling unit, which replaced chill rolls for plasticizing shortening and margarine products. Votator<sup>®</sup> equipment chilled and plasticized shortenings and margarines in seconds as it flowed continuously through a closed, mechanically controlled system. Adoption of this system offered a number of advantages: (1) improved sanitation afforded by a closed system; (2) improved product uniformity provided by more accurate control of nitrogen injection, temperature, pressure, agitation, and throughput; (3) cost reductions resulting from labor savings, reduced space requirements, and lower refrigeration demand; and (4) a reduction in product losses (22). Versions of these closed internal chilling systems are still employed to plasticize shortenings and margarines.

In 1933, the introduction of superglycerinated shortening brought about significant changes for the baker and the shortening industry. These shortenings contained monoglycerides and diglycerides, which promoted emulsification of fat in water. Emulsified shortenings allowed bakers to produce more moist, higher volume, longer shelf life cakes with a fine grain and texture, lighter icings and fillings with higher moisture levels, and yeast-raised bread and rolls with an extended shelf life. This development added a new dimension to the fats and oils industry, which ushered in the era of tailor-made shortenings. Shortening applications were expanded beyond baking usage to snacks, dairy analogs, confections, foodservice, and other areas. At the beginning of World War II, the United States domestic use of cottonseed oil was 65% in shortenings, 8% in margarines, and 18% for miscellaneous food products, which included salad dressing, mayonnaise, salad oil, and packing of fish and cured meats (23).

## 2.1. Post World War II Cottonseed Oil Markets

Cottonseed oil was the edible oil of choice in the United States for many years, both as a liquid for salad and cooking oils and hydrogenated to produce shortenings and margarines. During the beginning years of the U.S. soybean oil industry, its flavor and reversion problems were cottonseed oil's allies to support cottonseed oil's dominant position and premium pricing. This dominant position was maintained well into the 1950s until the boll weevil and New Deal crop restrictions opened the door to U.S. markets for competing vegetable oil by limiting the availability of cottonseed oil (6). Cottonseed oil shortages and an increased demand for edible oil encouraged research by government and industrial laboratories to develop processes to make soybean oil more palatable. Cottonseed oil lost its dominant position but retained its premium pricing over soybean oil and the food fat it originally challenged for market share—lard.

Cottonseed oil shortages during World War II allowed soybean oil production to grow despite serious flavor, stability, and reversion problems. Reminiscent of cottonseed oil's early days when it was used to adulterate lard, fats and oils processors incorporated as much soybean oil as possible into cottonseed-oil-based formula-

tions to take advantage of the 4–9 cent per pound lower cost. Initially, the amount that could be blended into margarine and shortening products was restricted to less than 30% to ensure that an objectionable flavor and odor would not develop.

Soon after World War II, soybean surpassed cottonseed as the principal source of vegetable oil in the United States. In the early 1950s, researchers identified the cause of soybean oil's offensive flavor as the 7–8% linolenic fatty acid content with a classic experiment. Nine percent linolenic fatty acid was intersterified into cottonseed oil, which typically contains less than 1% of this unsaturated fatty acid. Blind flavor panels profiled this modified cottonseed oil as soybean oil. This finding gave processors a means to improve the acceptance of soybean oil: hydrogenation to reduce the linolenic fatty acid content (24). Margarine and especially shortening products, were the most likely candidates for hydrogenated soybean oil utilization because these formulations already employed hydrogenated cottonseed oil base-stocks. However, the beta crystal habit of soybean and most other vegetable oils could not duplicate the smooth, creamy consistency contributed by the beta-prime crystal habit of cottonseed oil. Therefore, it was necessary to formulate soybean oil-based shortenings requiring a smooth, plastic consistency with some hydrogenated cottonseed oil or another beta-prime crystal promoter. However, this was not the case with margarine; the use of multiple high-*trans* basestocks, uniform low-temperature shipping, storage, and display practices in the United States allowed table-grade margarines and spreads to be formulated with 100% soybean oil.

Soybean salad oil with an iodine value similar to winterized cottonseed salad oil and the linolenic fatty acid reduced to 3–4% was introduced to the U.S. market around 1958. This product was lightly hydrogenated and subsequently winterized to remove the hard fractions developed during hydrogenation. Even with additional hydrogenation costs, this salad oil cost less than winterized cottonseed salad oils. Specially processed soybean salad oil was eventually accepted as a replacement for cottonseed salad oil, but most processors proceeded cautiously, offering blends with cottonseed during the introductory periods, beginning with 20–30% soybean salad oil and gradually increasing the proportion, eventually reaching 100% several years later.

With the success of soybean oil and a growing consumer appreciation for nutritional foods, other vegetable oils began to more actively compete for a share of the vegetable oil market. The importance of the essential fatty acids, linoleic and linolenic, were recognized and diet modification to restrict intake of cholesterol, saturated fatty acids, *trans* fatty acids, and total fat calories have been recommended to lower serum cholesterol levels in order to reduce the risk of heart attacks. The fats and oils usage data on Table 2 (25–28) reflects these trends: (1) a move away from animal fats to vegetable oils; (2) replacement of previously established fats and oils with different source oils; (3) introduction of new vegetable oils; (4) a rise and fall of some individual source oils; (5) source oil changes reflecting the results of medical studies; (6) introduction of new oil seed hybrids; and more. Ironically, the implications in this table could be used as the basis for a social commentary on life in the United States in the late twentieth, early twenty-first century. For cottonseed oil, these data show a rapid decline in U.S. domestic usage volume after 1950 to a low point in the 1980s when it started to regain domestic market share.

**TABLE 2. U.S. Edible Fats and Oils Disappearance and Per Capita Consumption.**

Year	1000 Metric Tons					
	1950	1960	1970	1980	1990	2000
Canola					261.7	791.1
Coconut			357.4	468.1	406.9	439.1
Corn	101.2	140.6	201.9	305.3	521.2	776.1
Cottonseed	655.4	555.7	404.2	237.2	386.0	305.7
Olive	35.8	23.1	30.4	26.3	95.7	206.4
Palm		0.5	82.6	135.6	116.1	170.1
Palm Kernel	11.8	24.0	42.6	NR	164.2	110.2
Peanut	46.7	28.1	87.5	50.8	89.4	110.7
Safflower			45.4		26.3	46.3
Soybean	655.9	1365.8	2836.3	4134.1	5517.6	7352.8
Sunflower				29.0	90.7	161.9
Lard	929.9	856.8	746.2	464.0	374.2	436.4
Tallow	70.8	148.8	235.0	451.3	433.2	679.5
Butter	601.9	504.9	487.6	461.3	496.7	463.6
Total	3167.9	3726.3	5557	6763.1	8979.9	12049.8
Per capita consumption, kg						
Vegetable oils	10.9	12.1	17.7	20.4	23.9	28.6
Animal fats	9.9	8.4	6.4	5.5	4.3	5.2
Total	20.8	20.5	24.0	25.9	28.2	33.8

NR = not reported

This renewed popularity was most likely at the expense of tallow and palm oil, which lost consumer appeal due to unfavorable publicity highlighting nutritional concerns with saturated fatty acids or cholesterol; both of these edible fats and oils crystallize in the beta-prime habit like cottonseed oil.

Two primary contributing factors to the supply of cottonseed for processing, and thus cottonseed oil, in the United States are (1) the supply of seed is determined by the demand for cotton lint, which has been heavily influenced by the cotton provisions of the U.S. Farm Program, and (2) the raw material usage of cottonseed has changed dramatically at the end of the twentieth century. Cotton acreage planted in the United States is largely determined by the support programs of the USDA and is based on world and domestic stocks and the expected lint requirements. Thus, the supply of cottonseed cannot respond to market signals the way soybean production has since the 1940s. The second influence on cottonseed stocks for crushing has been the competition for whole seed by the dairy-feeding industry. Shifts in dairy production in the past two decades have put a premium on cottonseed's unique combination of protein, energy and fiber, and this has affected the availability of whole cottonseed for dairy feeding. The tonnage of cottonseed crushed in the United States dropped by a third between 1980 and 2002 while the total supply of seed rose during the same period. These divergent trends resulted in approximately 74% of the cottonseed available in 1980 in the U.S. being crushed for products as opposed to crush levels near 36% twenty-two years later. These shifts are indicated in Table 3 (29, 30).

**TABLE 3. Cottonseed Supply and Disappearance Data for Selected Years (1,000 Metric Tons).**

Year Beginning Aug. 1	Supply			Disappearance					Ending Stocks
	Beginning Stocks	Production	Total	Crush	Amount of Supply Crushed (%)	Exports	Feed, Seed and Residual	Total	
1899–1900	—	3,767	—	2,249	53%	—	—	—	—
1904–1905	—	5,413	—	3,035	56%	—	—	—	—
1914–1915	—	6,491	6,501	5,244	81%	3	1,255	6501 <sup>a</sup>	—
1929–1930	38	5,812	5,870	4,551	78%	—	1,279	5830 <sup>a</sup>	—
1937–1938	38	7,116	7,154	5,739	81%	—	1,112	6848 <sup>a</sup>	—
1940–1941	36	4,795	4,832	3,990	83%	—	723	4713 <sup>a</sup>	119
1950–1951	261	3,724	3,986	3,380	85%	5	543	3926 <sup>a</sup>	60
1960–1961	95	5,340	5,435	4,855	89%	5	405	5264 <sup>a</sup>	171
1970–1971	73	3,690	3,763	3,382	90%	35	148	3,565	198
1980–1981	960	4,056	5,016	3,698	74%	121	836	4,655	361
1990–1991	332	5,415	5,749	3,056	53%	48	2,054	5,158	591
2000–2001	249	5,839	6,427	2,498	39%	213	3,329	6,039	396

<sup>a</sup>Calculated.



The export market for U.S. cottonseed oil also affects supplies (31–33). Available cottonseed oil is consumed domestically or exported, most likely to the most profitable market. Supply and demand practices have channeled the available supplies to the highest bidders which long maintained a premium price structure, while disposing of the available cottonseed. Cottonseed oil exports were a strong market during the initial years of the U.S. cottonseed crushing industry and again in the 1980's. Foreign tariffs in 1882 and U.S. imposed tariffs in 1921 caused export declines (6). In the 1990's, price competition from South American countries and the above-mentioned domestic seed supply problems again ate away at export volumes.

The technologies, processes, and methods developed to produce shortening, salad oil, margarine, and other edible oil products with cottonseed oil have been adapted for use with soybean, corn, canola, and other oilseeds as well as the animal fats; lard and tallow. However, fats and oils interchangeability is limited by the physical and chemical characteristics of the individual source oil. Each source oil has distinctive flavor characteristics, fatty acid compositions, and triglyceride structures. Cottonseed oil's properties have helped to maintain it as an important source oil for food products worldwide. U.S. cottonseed oil has enjoyed a strong export market, along with the high demand for its performance characteristic in specific food products. Cottonseed oil's functional characteristics; such as a pleasing flavor described as nutty, good flavor stability resulting from an absence of tri-unsaturates, which oxidize rapidly, and a beta-prime crystal habit probably due to a high-palmitic fatty acid content, have helped to maintain it as a desirable vegetable oil. These characteristics and the fact that it was America's original vegetable oil has made it the standard to which other oils are compared.

## 2.2. World Production of Vegetable Oils

Cottonseed oil dominated the U.S. and world edible oil markets until just before World War II, when it was displaced by soybean oil. Cottonseed oil had occupied this dominant position because of ample supplies, generally a lower price than competing oils, and manufacturers preferred cottonseed oil for most edible products. The major reason for the loss of this dominant position was an increased demand for edible oils, which cottonseed oil could not meet. An adequate supply of raw material was not available due to a declining demand for cotton. The cotton fiber surplus resulted in cotton farm acreage being converted to growing soybeans. Initially, oil demand influenced soybean cultivation to fill the edible oil shortage. Later, recognition of a world shortage of food protein changed this to make soybean oil production secondary to the demand for meal.

About 80 countries in the world grow cotton (34). Planting time for cotton varies by locality, varying from February to June in the Northern Hemisphere; harvest time is in the late summer or early/late fall. In the Western Hemisphere, cotton is cultivated between about 37° N and 32° S latitude and in the Eastern Hemisphere, between about 47° N and 30° S. Cultivation of cotton differs markedly from one country to another, depending on the degree of mechanization (35). The People's

**TABLE 4. World Vegetable Oil Production.**

2001/2002 World Vegetable Oil Production (Metric Tons)					
Rank Order	With U.S. Usage		Without U.S. Usage		% U.S. Usage
1	Soybean	28660.1	Palm	24614.4	0.86
2	Palm	24828.1	Soybean	20968.0	26.84
3	Canola	12174.5	Canola	11497.3	5.56
4	Sunflower	7554.2	Sunflower	7384.1	2.35
5	Peanut	4291.0	Peanut	4177.6	2.64
6	Cottonseed	3812.0	Cottonseed	3464.1	9.13
7	Coconut	3253.2	Coconut	2754.2	15.34
8	Palm Kernel	3103.5	Palm Kernel	2942.5	5.19
9	Olive	2524.7	Olive	2318.3	8.17
10	Corn <sup>a</sup>	1115.4	Corn	512.6	45.96
	Total	91316.8	Total	80633.2	11.70

<sup>a</sup>U.S. production only.

Republic of China, the United States, India, Pakistan, and Uzbekistan are the largest producers of cotton and cottonseed (34) and presumably the largest user of cottonseed oil. Turkey, Brazil, and Egypt also grow cotton and produce cottonseed oil, mostly for domestic use (36). Brazil, Argentina, and the United States are the major exporters of cottonseed oil.

The world vegetable oil markets and cottonseed oil have experienced many changes over time. One of the more interesting changes for cottonseed oil is a decrease in position of vegetable oil predominance from second place to sixth while the quantities produced have steadily increased. Soybean oil has maintained a dominant position over the past 40 years but has competition from oils grown primarily for their oil content; sunflower oil from Europe, ground nut (peanut) oil from Africa, rapeseed oil from Canada and Europe, and palm oil from tropical countries. Another interesting point, illustrated in Table 4, is that palm oil easily becomes the world leader when the U.S. consumption is taken away from the world market statistics.

### 3. COTTONSEED OIL PROPERTIES

Cotton is a warm-weather shrub or tree of the *Malvaceae* family, the tribe *Gossypieae*, and the genus *Gossypium* that grows naturally as a perennial but for commercial purposes is grown as an annual (34). Botanically, cotton bolls are fruits (37). The principal domesticated species of cotton of commercial importance are *hirsutum*, *barbadense*, *arboreum*, and *herbaceum* (38). Many different varieties of these species have been developed through conventional breeding to produce cotton plants with improved agronomic properties and with improved cotton fiber and cottonseed properties (39).

The overwhelming proportion of cottonseed oil in the United States is obtained from the seeds of *Gossypium hirsutum*, which is the shorter staple upland cotton (about 92% of world cotton production and about 97% of U.S. production). *Gossypium barbadense*, the extra long staple, or Egyptian, cotton, comprises only about 3% of the U.S. crop and about 8% worldwide. *Gossypium aboreum* and *herbaceum* are of very minor importance but are grown in China, India, and Pakistan. Crude cottonseed oil has a strong, characteristic flavor and a dark, reddish brown color from the presence of highly colored material extracted from the seed. It is a member of a particularly useful group of vegetable oils whose fatty acids consist substantially of 16 and 18 carbon fatty acids containing no more than two double bonds. Cottonseed oil is stable in the beta-prime crystal form, which is desirable in most solidified products because it promotes a smooth, workable consistency usually referred to as plasticity. Deodorized cottonseed oil's reverted flavor is usually described as nutty or nut-like, which is acceptable at higher degrees of oxidation than other vegetable oils. Its characteristics make it a highly desirable food oil for use in salad and cooking oils, shortenings, margarines, and specialty fats and oils products.

A number of factors are responsible for minor variations in the composition of cottonseed oil before it is extracted from the seed. These include climate, growing region, variety of cotton grown, the agronomic practices employed, and the handling/storage of the cottonseed before crushing. Due to the interaction of all these factors in any one sample of oil, it is difficult to make clear generalizations about quality variations. Factors influencing cottonseed oil quality before it is extracted from the seed are discussed below.

**Climate.** Cotton is very productive at high temperatures when adequate water is available. Free fatty acid levels may rise during hot, humid conditions, but heavy irrigation above normal levels appears to have no effect on oil hydrolysis to produce free fatty acids. Abundant rainfall, particularly in May and June, favor high seed-oil content. Low rainfall tends to increase the proportion of protein, but prolonged drought results in smaller seeds. Increased maturity, rather than warm weather, seems to be responsible for higher oil content in late-harvested seeds. Protein is accumulated in the seeds gradually, but oil is produced just before the boll opens. Stansbury and co-workers (40) found that high temperatures during seed development influenced saturated fatty acid development. They determined that the average iodine value decrease (reduction in unsaturated fatty acids) per °F temperature was 0.76 during boll development and 1.172 during seed development.

**Geographical Regions.** Differences in climate and soil conditions cause geographical regions to have a major influence on cottonseed composition, which can be greater than that caused by the variety of seed grown (41). Tharp (42) reviewed the variations in oil and protein levels from four regions of the United States and found that qualitative differences can occur. For example, oil from the Mississippi Delta region is usually more unsaturated than oil from Texas (43). The iodine value of the oils tends to increase in seeds grown farther north (42). Meara and Steiner (44) examined several American, Indian, African, and West Indian cottonseed oils and concluded that the geographic location had a major effect on the degree of

unsaturation. The iodine values of 25 cotton varieties ranged from about 97 to 112. Certain varieties appear to be better suited for certain geographical regions. During the 1978–1982 Regional Cotton Variety Tests, the variety Acala SJ-5 had higher oil contents than Stoneville 213, but this difference was larger in the western regions, particularly in the San Joaquin Valley (41).

**Fertilizers.** Nitrogen, phosphorus, and potassium in various percentages are the components of most crop fertilizers. Other elements are vital to plant growth, but their effects on oil content and composition have not been studied as closely. High nitrogen levels favor increased protein content in seeds along with decreased lint and higher number of seeds per boll (42). In a comparison of two levels of nitrogen application, lower levels produced seeds with higher oil content and viability (42, 45, 46). Phosphorus has no clear effect on oil and protein levels and potassium seems to cause a slight increase in oil content. Potassium can increase the oil resulting from a given land area by both increasing the seed cotton produced and the oil percentage, the latter perhaps at the expense of seed protein (47). Interactions among all three nutrients and any growth regulators can occur and a balance is important (46).

**Seed Handling and Storage.** Cottonseed oil from improperly stored cottonseeds will develop a dark color that requires additional processing. Boatner (48) noted that the following conditions favor the development of colored or “reverted” oil: harvest of immature (bollie) seeds, extremes of moisture or temperature, or other damage to the cotton plant during cultivation or harvest. Oxidation of gossypol and other pigments has been proposed as the chemical cause of the color (49). To avoid this color change, processors avoid intermingling seed known to be damaged or immature with good-quality seeds. Seeds with higher moisture or higher than normal free fatty acid (FFA) content are usually processed first. Only seeds with good quality are stored to be processed throughout the crop year before the next crop season begins. The moisture, temperature, and FFA level of seeds in storage are monitored periodically and serve as the basis for further processing and handling decisions.

**Varieties.** Glandless cotton varieties tend to contain more oil in the seed than do glanded varieties (50), and the glandless oil is slightly more unsaturated (51). Although both types of cottonseed contain approximately equal levels of cyclopropanoid fatty acids, the higher gossypol content of glanded seeds causes higher refined and bleached oil colors compared to glandless. Despite these problems, glanded seed types are by far the most common. Glanded varieties of cottonseed normally contain numerous tiny glands (30–70 micrometers) which hold the natural pigment, gossypol, and other gossypol-like compounds. Gossypol, a polyphenolic pigment (sesquiterpene), is a known antinutrient to livestock and thought to be a source of color problems to cottonseed oil, has been the focus of both scientific and practical research (23). However, gossypol is not detectable in RBD cottonseed oil using the most sensitive analytical techniques currently known. Research efforts have been devoted to removing pigment glands from cottonseed by processing or breeding. Screw press, expeller, liquid cyclone (LCP), and air classification processes have been developed to reduce the free gossypol content or to remove the

gossypol pigment glands from cottonseed meal (52). All these processes are able to produce cottonseed meal with a low enough free-gossypol content ( $< 450$  ppm) for food applications. However, except for a small amount of screw pressed meal, neither LCP nor air classified cottonseed meals have been made commercially available. Glandless cottonseed, a new variety seed without the pigment glands, was developed with traditional breeding research and introduced in the 1960s (53). Lusas and Jividen (54) have provided a thorough review of the discovery and history of glandless cottonseed.

Like other agricultural crops, cotton has been the subject of traditional breeding and genetic modification programs to develop new varieties with improved characteristics. Cotton is one of the leading crops to be genetically engineered (55) and since its introduction in 1996, transgenic cotton has been one of the most rapidly adopted technologies ever (56). In 2002/2003, transgenic cotton varieties comprised about 20% of the harvested cotton acres and about 27% of the cotton produced in the world (57). Presently, it is being grown in the United States, China (Mainland), Australia, South Africa, Argentina, Mexico, India, and Indonesia. In the United States, in 2002, about 78% of the planted cotton acreage was transgenic cotton. Genetic engineering is being used to produce transgenic cottons with insect resistance (e.g., Bollgard<sup>®</sup>; “Bt cottons” incorporating genes from *Bacillus thuringiensis* for boll worm/bud worm resistance), herbicide tolerance [e.g., bromoxynil (Buctril<sup>®</sup>; “BXN cotton”), and glyphosate (Roundup<sup>®</sup>; “Roundup Ready<sup>®</sup>; cottons” (59)) tolerant cottons] (57, 58). A second wave of transgenic cottons is imminent, with plants containing two Monsanto Bt genes in a “stack” debuted in 2003, followed by glufosinate (Ignite<sup>®</sup>) herbicide-resistant cottons and plants with better tolerance to glyphosate about 2006. Other countries, e.g., Egypt and Pakistan, are evaluating the performance of transgenic cottons (60) and some are preparing to plant them on a commercial scale (61–63).

Research is underway to produce transgenic cottons with other improved agronomic traits as well as improved seed and fiber quality properties (56). Transgenic technology can provide a means for modifying the lipid profile of cottonseed oil to improve it nutritionally (e.g., high oleic) and provide the functional properties for various food and industrial applications (e.g., high stearic) (64). Elimination of gossypol from cottonseed would both enhance the feed value of the meal and could reduce the processing cost of cottonseed oil (65). Genetic engineering may allow the reduction or elimination of gossypol only in the seed without affecting its levels in other mature parts of the plant where it has a beneficial function (66). Efforts to eliminate or reduce gossypol in cottonseed are in progress through sense and antisense transgenic approaches (67, 68).

Cottonseed oil, with the minimum amount of processing for a food product, is refined, bleached, and deodorized (RBD) similarly to all vegetable oils that are used for human consumption (69). Due to the processing, RBD cottonseed oil should not contain any detectable aflatoxin, pesticides, or gossypol using current analytical techniques. In addition, RBD cottonseed oil contains no detectable protein or DNA, so oil from the transgenic plant should be identical to that from nontransgenic plants (55). The current focus of concern about transgenic plants is on

food products (70), which have already impacted the use of cottonseed oil, meal, and cake in the European Union. However, in 2002, the use of cottonseed oil derived from plants containing the Bollgard<sup>®</sup> gene or Roundup Ready<sup>®</sup> gene were approved by the European Commission for human consumption (71).

### 3.1. Cottonseed Oil Glyceride Composition

Chemically, all fats and oils are esters of glycerol and fatty acids; nevertheless, the physical properties of natural fats and oils vary widely. This is because; (1) the proportion of the fatty acids vary over wide ranges and (2) the triglyceride structures vary for each individual oil and fat. Fats and oils are commonly referred to as triglycerides because the glycerin molecule has three separate points where a fatty acid can be attached. All triglycerides have identical glycerol components that leave the fatty acids to contribute the different properties. Three aspects can differentiate the fatty acid components: (1) chain length, (2) the number and position of the double bonds, and (3) the position of the fatty acids with regard to the glycerol. Variations in these characteristics cause a large portion of the chemical and physical differences experienced with edible fats and oils. Fats and oils, for all practical purposes, contain fatty acids with carbon chain lengths between 4 and 24 carbon atoms with zero to three double bonds. The fatty acids occurring in edible fats and oils are classified according to their degree of saturation:

- Saturated fatty acids, which contain only single carbon-to-carbon bonds, are chemically the least reactive and have a higher melting point than corresponding fatty acids of the same chain length with one or more double bonds. Most of the natural saturated fatty acids have an unbranched structure with an even number of carbon atoms. Fatty acids with carbon chain lengths from 2 to 30 have been reported, but the most important saturated fatty acids are butyric (C4:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0), behenic (C22:0), and lignoceric (C24:0). The melting point of saturated fatty acids increases with chain length. Saturated fatty acids with 10 and longer carbon chains are solids at room temperature.
- Unsaturated fatty acids, which contain one or more carbon-to-carbon double bonds, are the most chemically reactive and those with the most double bonds are the most reactive. A fatty acid containing only one double bond is called monounsaturated; the most notable is oleic (C18:1). When a fatty acid contains more than one double bond, it is identified as polyunsaturated. The notable polyunsaturated fatty acids are the essential fatty acids linoleic (C18:2) and linolenic (C18:3). These fatty acids are essential in the sense that the human body needs them and yet cannot either synthesize at all or in sufficient quantities. In nature, the double bonds are *cis*-form, which has both hydrogen atoms on the same side of the double bond. *Trans*-form fatty acids, with the hydrogen atoms on opposite sides of the double bond, are thermodynamically more stable. *Trans*-fatty acids are *cis*-form fatty acids that have been isomerized by oxidation or hydrogenation (72).

TABLE 5. Summary of Cottonseed Oil Fatty Acid Compositions.

Fatty Acid, %		References						
		a	b	c			e	f
				CA	TX	d		
Myristic	C14:0	0.8	0.9	0.7	0.9	0.8	0.8	0.7
Palmitic	C16:0	27.3	24.7	22.7	25.2	23	22.7	21.6
Palmitoleic	C16:1	0.8	0.7	0.6	0.8	0.6	0.8	0.6
Stearic	C18:0	2.0	2.3	2.3	2.7	2.3	2.3	2.6
Oleic	C18:1	18.3	17.6	17.3	17.5	15.6	17	18.6
Linoleic	C18:2	50.5	53.3	55.8	52.6	55.6	51.5	54.4
Linolenic	C18:3		0.3			0.3	0.2	0.7
Arachidic	C20:0		0.1					

a. (73).

b. Durkee Industrial Foods, Cleveland, Ohio.

c. (74).

d. (75).

e. (76).

f. Capital City Products Co., Columbus, Ohio.

**Cottonseed Oil Fatty Acid Composition.** The specific fatty acid profile of the triglycerides in cottonseed is dependent on the variety of cotton grown, growing conditions such as temperature and rainfall, and the analytical method used to determine the profile. Table 5 summarizes the fatty acid composition observations of several research and commercial groups. Cottonseed oil is typical of the oleic-linoleic group of vegetable oils, because those two fatty acids comprise almost 75% of the total fatty acids. Although oleic acid makes up 22% and linoleic makes up 52%, less than 1% linolenic acid is present. Palmitic fatty acid makes up about 24% of the fatty acids. Minor amounts of other saturated fatty acids are also found.

Bailey (23) noted that the composition of American cottonseed oils will rarely fall outside of these limits: 23–28% total saturated fatty acids, 22–28% oleic acid, and 44–53% linoleic acid. The Food and Agriculture Organization of the World Health Organization (FAO/WHO) has determined a range of fatty acid contents for commercial fats and oils. The acceptable range of fatty acids prescribed by Codex in 1997 for cottonseed oil is shown in Table 6 (77).

Cherry et al. (78) examined the variation in cottonseed oil content and composition as part of a study of genetic and location effects on Texas cottonseed. Both factors were found to have a significant effect on oil quantity. The oil content of moisture and lint-free seeds ranged from 23.2% to 25.7% depending on location. The variation in the six key fatty acids of cottonseed oil was significant within both cultivars and location, and five of them had significant interactions between cultivar and location. Linoleic acid varied from 49.07% to 57.64% with a mean of 54.54%. Palmitic acid varied from 21.63% for an Acala variety grown in Lubbock to 26.18% for a Lockett variety grown in Corpus Christi, whereas the mean was 23.68%. The authors also indicated that agronomic practices as well as weather conditions at the specific location may play a part in the observed variation. Such variations in oil quantity are not well understood and have not

**TABLE 6. Codex Fatty Acid Ranges for Cottonseed Oil.**

Fatty Acids, %		Typical	Range
Lauric	C12:0		0–0.2
Myristic	C14:0	0.7	0.6–1.0
Palmitic	C16:0	21.6	21.4–26.4
Palmitoleic	C16:1	0.6	0–1.2
Stearic	C18:0	2.6	2.1–3.3
Oleic	C18:1	18.6	14.7–21.7
Linoleic	C18:2	54.4	46.7–58.3
Linolenic	C18:3	0.7	0–0.4
Arachidic	C20:0	0.3	0.2–0.5
Gadoleic	C20:1		0–0.1
Eicosadienoic	C20:2		0–0.1
Behenic	C22:0	0.2	0–0.6
Erucic	C22:1		0–0.3
Docosadienoic	C22:2		0–0.1
Lignoceric	C24:0		0–0.1

been carefully studied in cotton, and more work needs to be done. Cherry et al. (79) have provided a good review of the existing information.

**Cottonseed Oil Triglyceride Composition.** The triglyceride structure of an edible fat or oil is affected by which carbon atom of the glycerol has the fatty acid linked, whether the three fatty acids are the same or different, and the position of each. Triglycerides with three identical fatty acids are called simple or monoacid triglycerides. Triglycerides containing more than one type of fatty acid are called complex or mixed triglycerides. A mixed triglyceride containing three different fatty acids has three isomeric forms, depending on which fatty acid is in the middle, 2, or beta position of the glycerol portion of the molecule and which fatty acids are in the alpha or outside positions. Therefore, both the chemical and physical properties of fats and oils are largely determined by the fatty acids that they contain and their position within the triglyceride structure.

As linoleic, oleic, and palmitic fatty acid account for over 90% of the fatty acids in cottonseed oil, most of the triglycerides contain some combination of these fatty acids. Table 7 lists the possible combinations of composition and position of saturated, oleic, and linoleic acids in cottonseed oil triglycerides. These ten types of triglycerides account for 92% of the total triglycerides found (73). The predominant type is SLL (saturated and linoleic fatty acid in the 1, 2, and 3 positions, respectively), which accounts for over 22 mol% of the triglyceride molecules.

The predominant pair of these ten types includes palmitic acid as the saturated acid in acyl positions 1 and 3, whereas position 2 is occupied by oleic or linoleic acid. This is illustrated in position distribution data from Jurriens and Kroesen (73) that indicates the middle acyl position was occupied by linoleic acid 64.3% of the time.

The amounts and the types of fatty acids and the interpositional and intrapositional distribution result in various triglyceride forms that contribute to the various



**TABLE 7. Triglyceride Composition of Cottonseed Oil.**

Fatty Acid Pattern	Number of Double Bonds	Mol %
SOS	1	4.5
SOO	2	4.8
SLS	2	12.4
SOL	3	9.4
SLO	3	8.4
OOL	4	4.1
SLL	4	22.5
OLL	5	6.4
LOL	5	6.5
LLL	6	13.0
Other		8.0
Total		100.0

S = saturated; O = oleic; L = linoleic.

functional properties of cottonseed oil. Bland et al. (80) used more recent separation techniques to identify the triacylglycerides and positional isomers of a sample of cottonseed oil. Their results are presented in Table 8.

**Cyclopropenoid Fatty Acids.** Cotton, and other plants in the Malvaceae family, contain a pair of unique cyclopropene fatty acids (CPFA). These two fatty acids, sterculic and malvalic acid, are generally referred to collectively as cyclopropenoid fatty acids. Sterculic acid is the most active of the two fatty acids whose general action is to inhibit the desaturation of stearic to oleic fatty acid in the animal body with a resultant alteration in membrane permeability or an increase in the melting point of fats.

**TABLE 8. Cottonseed Oil Triglyceride Structure.**

Triacylglyceride	GLC, %	HPLC, % <sup>a</sup>
PPL	25.7	27.5
LLL	16.1	19.0
POL	14.0	14.0
OLL	12.9	12.5
PPL	8.7	7.1
OOL	4.4	3.1
POO	3.3	3.1
PPO	2.5	2.2
OOO	2.4	1.6
SLL	2.4	1.4
SPL	2.1	1.5
SOL	1.5	1.3

P = palmitic; O = oleic; S = stearic; L = linoleic; GLC = gas liquid chromatography; HPLC = high-performance liquid chromatography.

<sup>a</sup>Corrected using GLC analysis of fatty acid methyl esters.

**TABLE 9. Cottonseed Oil Cyclopropenoid Fatty Acid Levels.**

Cottonseed Oil Source	Cyclopropenoid Fatty Acid, %			Comment	Reference
	Malvalic	Sterculic	Total		
Crude Oil	0.52			Expeller Extraction	(84)
Crude Oil	0.56–0.90			Hexane Extraction	(84)
Crude Oil	0.58–0.98			Petroleum Ether Extraction	(85)
Crude Oil	0.7–1.5	0.3–0.5			(86)
Crude Oil	0.22–1.44	0.08–0.56	0.3–2.0		(81)
Crude Oil	0.64			Glanless Variety	(84)
Refined Oil	0.62				(84)
RBD Oil	0.015–0.324	0.005–0.126	0.02–0.45		(81)
RBD Oil			0.04		(87)
Winterized Salad Oil	0.04–0.42			Brand Name Salad Oils	(84)
Winterized Salad Oil	0.1–0.23			Various Commercial Brands	(88)

Sterculic and malvalic acids are 18 and 17 carbons long, respectively, and include one double bond at the site of the propene ring, either at the 9, 10 position or 8, 9 position. The cyclopropene ring is the physiologically active entity of the two fatty acids (81, 82). The physiological activity of sterculic acid is reported to be greater than that of malvalic acid (82). The ratio of malvalic acid to sterculic acid in cottonseed oil is usually about 3 to 1. The cyclopropenyl structure is highly strained, which apparently accounts for its reactivity (83). Such activity makes it highly susceptible to the inactivation that regularly occurs during processing. In cottonseed oil, the CPFAs are reduced in processing with the result that cottonseed oil in commercial channels contains a negligible level. Examples of levels of CPFA found in cottonseed oil products are given in Table 9 (81, 84, 85, 87, 88, 89). Deodorization and hydrogenation are the processing steps responsible for the greatest inactivation of CPFA.

### 3.2. Cottonseed Oil Nonglyceride Components

The primary constituents in crude vegetable oils are the triglycerides, but they also contain varying amounts of nonglyceride materials. Cottonseed oil is unusual for the amount and variety of nonoil substances in the crude oil. Its content of nonglyceride substances, exclusive of free fatty acids, commonly amounts to 2% or more in the crude state. These minor components, identified as the unsaponifiable fraction, consist of phospholipids, tocopherols, sterols, resins, carbohydrates, pesticides, gossypol, and other pigments. Some, but not all, of the nonglyceride materials are undesirable. Therefore, the objective in all edible oil processing is to remove the objectionable impurities with the least possible damage to the desirable constituents.

**Gossypol.** Cottonseed oil is unique among the commercially important fats and oils because of the presence of a relatively complex system of pigments. Most of the pigments are of the gossypol type, a biologically active terpenoid substance present in discreet glands in the seed, leaf, bract, stem, taproot, bark, and root of the cotton plant. The adaptive function of the compound is believed to be insect resistance (90). During seed processing, the glands are ruptured, allowing the gossypol and other similar substances to mix with the protein and oil. Fortunately for the oil processor, most of the gossypol is bound to the protein. However, because gossypol and its chemically related compounds are strong pigments, it is a major objective during caustic refining and bleaching processes to remove as much of the pigments as possible. Gossypol compounds give crude cottonseed oil a red color so dark that it usually appears to be black. The characteristic yellowish amber color of refined, bleached, and deodorized cottonseed oil is primarily caused by the remaining gossypol after processing.

Recognition of gossypol's role as a fertility control agent heightened interest in this polyphenolic compound. In the 1960s, small batches of cold-pressed cottonseed oil consumed without further processing caused infertility in Chinese men. The cooking step, in industrial extraction processes, binds some of the gossypol to the protein to keep the level below its physiologically active level. Caustic refining segregates gossypol into the soapstock to levels high enough to impact its use in animal feeds. After refining, the active earths employed in bleaching are effective in gossypol removal. Deodorization, by purging the oil with live steam under vacuum, removes many impurities and odor-causing compounds. Few foods are as chemically clean as an oil or fat that has been refined, bleached, and deodorized. Analyses have indicated that alkali refining and bleaching reduced the gossypol content of cottonseed oil to less than 1 ppm from 0.05% to 0.42% in solvent extracted oil and 0.25% to 0.47% for screw-pressed oil (91, 92).

As a practical matter, the presence of gossypol in commercially available cottonseed oil conflicts with the refiner's need to sell a light, clear product, so there is a significant incentive to do a good job of removing the gossypol from the oil. A variety of cotton bred through traditional techniques for its glandless characteristic became available in the early 1960s. It is generally devoid of gossypol-containing glands. Glandless cottonseed produces a light colored oil with less-restrictive processing conditions (54). However, this variety has never become popular, because the fiber-producing characteristics of cotton varieties are more important to growers than the seed genetics, and the market for vegetable proteins has been readily met by soybean and other products.

**Phospholipids.** These components are better known to oil processors as phosphatides and are frequently referred to, together with small quantities of carbohydrates and resins, as "gums" that have adverse effects on product quality and refined oil yield. Phosphatides are emulsifiers, and so hinder the separation of oil and water phases in the caustic-refining process. The phosphatides are broadly separated into hydratable and nonhydratable types. As the name implies, hydratable phosphatides can be removed by treatment with water, and the nonhydratable compounds, which are salts or coordination compounds of calcium and magnesium primarily

with phosphatidic acid, can only be rendered insoluble in the oil by the use of chemical reagents, the most commonly used being phosphoric acid.

Phosphatide contents are normally calculated from the determination of total phosphorus and the use of a factor relating the molecular weight of phosphorus to the mean molecular weight of the phosphatides in the oil. Typically, the amount of phospholipids in cottonseed crude oil varies from about 0.7% to 0.9%. The phosphatides can also be beneficial: They act as synergists for the tocopherols to inhibit the autoxidation of vegetable oils (93). This synergistic effect is partly responsible for the oxidative stability of crude cottonseed oil.

**Tocopherols.** Various tocopherol isomers that act as naturally occurring antioxidants are found in cottonseed. Nature's fat soluble antioxidants can exist in at least seven forms with alpha-, beta-, delta-, and gamma-tocopherol predominating in vegetable oils. Alpha-tocopherol contributes Vitamin E activity and some oxidation resistance, but the gamma and delta forms are the most effective antioxidants. Typically, crude cottonseed oil contains about 1000-ppm tocopherols, but up to a third can be lost during processing. The tocopherol content decreases during each stage of processing, with the highest reductions occurring during chemical refining and deodorization. Caustic refining can remove as much as 10% to 20% of the tocopherols, and 30% to 60% of the remaining natural antioxidants can be lost during deodorization. Typical tocopherol contents for selected vegetable oils after processing are compared in Table 10 (94, 95).

**Sterols.** Sterols are crystalline, neutral, unsaponifiable, high-melting alcohols with multiple-ring structures. Sterols, minor components of all natural fats and oils, are the major constituents of the unsaponifiable matter remaining in processed vegetable oils; the remainder consists essentially of saturated and unsaturated hydrocarbons. The sterols are colorless, heat stable, and relatively inert, so they do not contribute any important property to a fat or oil. Chemical refining removes a portion of the sterols, but more effective separation requires fractional crystallization, molecular distillation, or high-temperature steam distillation. Vegetable oil soapstocks from caustic refining and deodorizer residue are rich sources for sterol reclamation. An evaluation, comparing caustic-refined cottonseed oil with

**TABLE 10. Typical Tocopherol Contents of Selected Vegetable Oils.**

Vegetable Oil	Total Tocopherols, ppm	Tocopherols, %			
		Alpha	Beta	Gamma	Delta
Cottonseed Oil	830-900	41	trace	58	1
Canola Oil	690-695	35		63	2
Corn Oil	870-2500	16	2	79	3
Olive Oil	30-300	93		7	
Palm Oil	360-560	30	trace		
Peanut Oil	330-480	51	1	44	4
Soybean Oil	900-1400	10	3	63	24
Sunflower Oil	630-700	96	2	2	

TABLE 11. Sterol Content of Several Vegetable Oils (mg/kg oil).

Sterol	Cottonseed Oil <sup>a</sup>	Soybean Oil		Sunflower Oil <sup>a</sup>
		Crude	Refined	
Cholesterol	0.5	0.5	nd <sup>b</sup>	0.5
Brassicasterol	0.5	0.5		0.5
Campesterol	276.0	563.0	470.0	242.0
Stigmasterol	17.3	564.0	470.0	236.0
beta-Sitosterol	3348.0	1317.0	1230.0	1961.0
delta-5-Avenasterol	85.1	46.1	10.0	163.0
delta-7-Stigmasterol	0.7	92.0	10.0	298.0
delta-7-Avenasterol	17.9	63.2	5.0	99.4
24-Methylene-cycloartenol	0.5	53.0		204.0
Total	3746.5	2699.3	2195.0	3204.4

<sup>a</sup>Crude or refined oil not specified.

<sup>b</sup>nd = nondetectable.

deodorized cottonseed salad oil, showed a reduction of total sterols from 0.574 to 0.397 mg/100 g of oil (96). Recovered sterols from oil-processing byproducts are the starting materials for the synthesis of sex hormones and the preparation of synthetic Vitamin D. The sterols of vegetable oils are mixtures called phyosterols. The sterols in most vegetable oils include beta-sitosterol, campesterol, stigmasterol, and others in lesser amounts. The most extensively investigated and consumer-recognized sterol is most likely cholesterol. Trace amounts of cholesterol have been found in some vegetable oils, but animal fats like tallow are routinely found to contain 1077 mg/kg (97). The sterol content of several vegetable oils are presented in Table 11 (75, 98).

**Pesticides.** Pesticides have been used for increased agriculture production throughout the world. Studies have shown that the majority of the pesticides applied eventually reach the soil surface, where they gradually spread, translocate to other environments, or degrade eventually. Translocation to oil-bearing plant seeds has also been demonstrated by studies. Processing studies have shown that neither solvent extraction nor bleaching affected the pesticide levels in the vegetable oils. However, it was found that pesticides were removed by volatilization during hydrogenation and/or deodorization (99–101). U.S. government agencies have recognized that the insecticides are distilled from edible oils during the deodorization process.

**Trace Metals.** Vegetable oils contain varying levels of trace metals, depending on exposure during the growing season as well as during extraction and processing. Metals can be encountered throughout processing; these reduce the efficiency of the process and are harmful to product quality and human health. Trace quantities of copper, iron, manganese, and nickel substantially reduce the oxidative stability of oils, whereas calcium, sodium, and magnesium reduce the efficiency of refining, bleaching, and hydrogenation systems. The effects of the metals can be diminished

by the use of chelating agents at various process points to sequester the trace metals (102). The most widely used chelating agents are citric and phosphoric acids.

### 3.3. Cottonseed Oil Physical Characteristics

The physical properties for all fats and oils, including cottonseed oil, are determined by their individual chemical compositions. Physical properties are of practical importance because most applications depend on the melting behavior, solubility, flavor, density, appearance, and the other physical properties to provide functionality for finished products. Analytical and physical evaluation methods are used to measure these attributes for identification, trading, and control purposes.

**Melting Point.** The usual definition for melting point is the temperature at which a material changes from a solid to a liquid. Determination of a fat or oil's melting point is difficult because natural fats and oils do not have true melting points; the different components melt at different temperatures. Fats and oils are complex mixtures of triglycerides that pass through a gradual softening before becoming completely liquid. The melting point procedure is further complicated by the fact that fat crystals can exist in several polymorphic modifications, depending on the specific triglycerides involved and the temperature-time pretreatment or tempering of the sample. For a melting point value, one point within the melting point range must be selected. Several methods to determine the melting point have been standardized by AOCS and other organizations, each providing different values: capillary melting point, softening point, slipping point, Wiley melting point, Mettler dropping point, and others. The temperature range at which cottonseed oil changes from a solid to a liquid is 50°F to 60°F or 10°C to 16°C.

**Solid Fat Index.** This analysis has become the most important criterion for the melting behavior and crystalline structure of fats and oils products. It determines the proportion of solid and liquid materials at a given temperature. The solid fat index (SFI) analysis is an empirical measure of the solid fat content. It is calculated from the specific volume at various temperatures using a dilatometric scale graduated in units of milliliters times 1000. Values for the solid contents are usually determined at 50°F, 70°F, 80°F, 92°F, and 104°F or 10°C, 21.1°C, 26.7°C, 33.3°C, and 40°C. Unlike the tropical oils, cottonseed and the other oleic- and linoleic-classification oils do not contain any significant quantity of triglycerides made up of two or three saturated fatty acids; therefore, the solid fat index at the lowest temperature usually measured would have minimal values. Natural cottonseed oil can have a solid fat index content at 50°F or 10°C but not at the higher temperature measurements.

**Cold Test.** The ability of an oil to withstand refrigerator storage is determined by the cold test analysis; crystallization resistance is measured as the time in hours before the oil appears cloudy at 32°F or 0°C. Standardized AOCS Method Cc 11-53 requires that dry filtered oil be placed in a sealed 4-ounce bottle and submerged into an ice bath (103). A go/no-go examination after 5.5 hours for clarity is stipulated by the Official AOCS Method; however, most laboratories practice the alternative procedure, which continues the clarity examinations until a cloud

appears. The cold test procedure was developed to evaluate cottonseed oil for the production of mayonnaise and salad dressings. An oil that solidifies at refrigerator temperatures will cause an emulsion break with a resultant separation of the oil and water phases. Currently, the cold test is also used to assure that bottled oils for retail sales will not develop an unattractive appearance on the grocery shelf.

**Cloud Point.** An empirical cloud point analysis is performed by stirring a sample of fat while it is being cooled until the oil has clouded enough to block a light beam of known intensity. Both cloud point and congeal point values are more closely related to consistency than melting points. A definite relationship exists between the cloud point results and the solid fat index values at 92°F or 33.3°C. Cottonseed oil that has not been winterized or hydrogenated will have a cloud point of 30°F to 38°F or  $-1.1^{\circ}\text{C}$  to  $3.3^{\circ}\text{C}$ . Winterized cottonseed salad oil, with the hard fraction removed, will have a cloud point of approximately 22°F to 26°F or  $-5.6^{\circ}\text{C}$  to  $-3.3^{\circ}\text{C}$ .

**Titer.** The titer test, AOCS Method Cc 12-59 (103), measures the solidification point of the fatty acids. First, a fat sample must be saponified and dried before determining the titer value. Then, a titer tube is filled to the 57-mm mark with dried fatty acids and suspended in an air bath, which is surrounded by a water bath at 15°C to 20°C below the expected titer result. The sample is stirred until the temperature begins to rise or remains constant for 30 seconds, after which the stirring is stopped and the endpoint is determined as the maximum temperature that the fat starts crystallizing or solidifying. Titer analyses are used predominantly in the soap and fatty acid industries. For edible oils, titer values are commonly specified for an oil that has been hydrogenated to almost complete saturation. Cottonseed oil hydrogenated to a 5 iodine value or less should have a titer value of  $60^{\circ}\text{C} \pm$ .

**Pour Point.** A vegetable oil's pour point is the temperature at which the oil just remains pourable. Actually, this analysis is another melting point determination. For crude or natural cottonseed oil, the pour point is between 25°F and 32°F. The pour point temperature increases as the oil is saturated; for hydrogenated cottonseed, the pour point will be higher than for the unhardened oil and can be as high as 140°F depending on the degree of saturation.

**Refractive Index.** The refractive index of fats and oils is an important characteristic because of the ease and speed with which it can be determined precisely, the small amount of sample required, and its relationship to structure. It is useful for source oil identification, for observing progress of reactions rapidly, and for establishing purity. The general relationship between refractive index and the composition of an oil product with minor exceptions are as follows (104):

- Refractive indices increase as the carbon chain length increases.
- Refractive indices increase as the number of double bonds increases.
- Refractive indices are higher for glycerides than those of fatty acids.
- Refractive indices of mixed glycerides are close to corresponding simple glycerides.
- Refractive indices are higher for monoglycerides than for corresponding triglycerides.

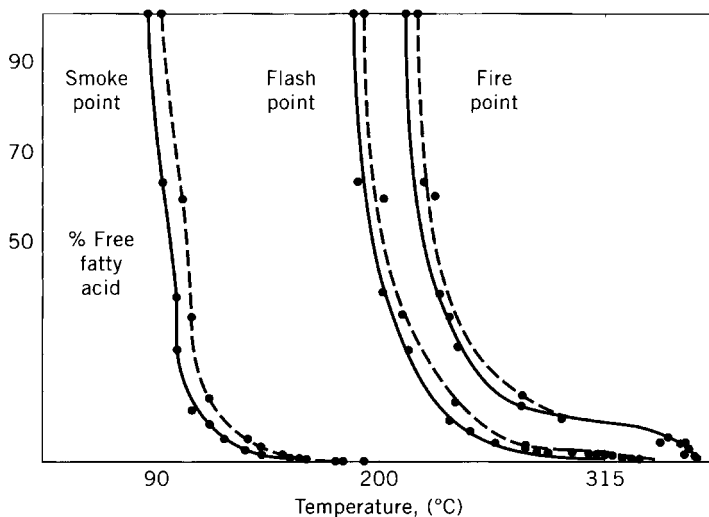
Refractometers equipped with temperature controls are used for fats and oils. The measurements are usually made at 25°C for soft oils, and the higher melting point products require temperature adjustments to 40°C or 60°C, depending on the melting point of the product. The refractive index values decrease as the temperature is increased but still increases with the length of the carbon chains and the number of double bonds present in the sample. By reference to a predetermined curve relating the refractive index at temperature measured to iodine value, a rapid estimation of the iodine value may be made. One source of error in this method is that *trans*-acids formed during hydrogenation affect refractive index values but not iodine value.

**Viscosity.** The physical property of a fluid or semifluid that enables it to develop and maintain a certain amount of shear stress, dependent on the velocity of flow, and then to offer continued resistance to flow is defined as viscosity. The viscosity of an oil is temperature dependent; flow increases as the temperature increases. For edible fats and oils, viscosity decreases as saturation decreases and with shorter carbon-chain lengths that have lower molecular weights. Most vegetable oils in the linoleic category have similar viscosities; however, cottonseed oil should be slightly less viscous by measurement because of a higher saturation level than other oils in this category. The lauric oils have an easily detected lower viscosity than other vegetable oils, caused by their high level of short-chain fatty acids. This difference in viscosity with the use of coconut and palm-kernel oils may be demonstrated by coverage from coating using these oils versus the longer chain vegetable oils.

**Specific Gravity.** The density of a substance compared with water is called specific gravity. Density or specific gravity is the ratio of the weight of a volume of an oil to the weight of an equal volume of water at the same temperature. Cottonseed oil's density measurement results are affected by both temperature and fatty acid composition. The oil's density decreases about 0.000638 units for each °C or 0.000355 for each °F increase when heated in the 150–500°F range. At lower temperatures, the change is greater, about 0.00069 units per °C for cottonseed oil between 0 and 40°C. The specific gravity results of vegetable oils have an inverse relationship with molecular weight and a direct relationship with the degree of unsaturation. Lund (105) developed an equation based on saponification and iodine values to predict the specific gravity of liquid vegetable oils at 15°C: Specific Gravity = 0.8475 + 0.0003 (Saponification Value) + 0.00014 (Iodine Value). Therefore, a liter of cottonseed oil with a specific gravity of 0.917 will weigh 917 g or 917 kg/m<sup>3</sup>. A U.S. gallon of cottonseed oil with this specific gravity would weigh 7.66 lbs.

**Smoke Point.** As oils or fats are heated, a thin bluish smoke appears. The smoke point is the lowest temperature, under controlled conditions, that the smoke becomes visible. Cottonseed oil's smoke, fire, and flash points, like other fats and oils, are almost entirely dependent on the free fatty acid content. Fats and oils' smoke point results decrease when the triglycerides are split during hydrolysis to form free fatty acids and glycerol. The glycerol portion decomposes to form acrolein, which is the major portion of the smoke evolved from heated fats and oils. Like other long-chain fatty acid oils, cottonseed oil with 0.01% free fatty acid will have a smoke point of approximately 450°F. Additions of monoglycerides





**Figure 1.** Relationship between free fatty acid content and smoke, flash, and fire points of cottonseed and peanut oils. —, Refined cottonseed oil; - - -, peanut oil.

and diglycerides lower the smoke point because of its free glycerol content. AOCS Method Cc 9a-48 measures the temperature at which smoke is first detected in a laboratory apparatus protected from drafts and equipped with special lighting (103). The temperature at which smoking will be observed in actual use will be somewhat higher than the test result. Figure 1 shows the relationship among free fatty acid content, smoke, flash, and fire points for processed cottonseed and peanut oils.

**Flash and Fire Point.** Flash point is the temperature at which the volatile products are evolved at such a rate that they are capable of being ignited but not supporting combustion. At the fire point, the accumulated breakdown products are capable of supporting a flame on their own. A crude cottonseed oil with a fatty acid content of 1.8% was found to have a flash point of 560°F or 293.3°C. Solvent-extracted oils can have a low flash point because of a solvent residue. A flash point analysis would identify this crude oil deficiency to prevent an accidental fire or explosion in an atmosphere that was not explosion proof. Crude vegetable oil shipments received with a flash point below 250°F are rejectable by most trading rules. Figure 1 shows the relationship between free fatty acid content, smoke, flash, and fire points of processed cottonseed and peanut oils.

**Color.** Crude cottonseed oil has a dark reddish-brown color because of the presence of highly colored material extracted from the seed. After processing, it typically has a rich golden yellow color that is lighter than peanut and corn oils but darker than soybean, sunflower, canola, and safflower oils. Some of the color found in cottonseed oil comes from carotene, but most of the color is caused by a minimal residual level of gossypol and gossypol derivatives. Although the carotene pigments

are rendered colorless by heat bleaching and some of the pigments may be removed by adsorption bleaching, gossypol can only be removed by alkali refining. The color removal level is dependent on the cottonseed handling and storage conditions prior to extraction. Cottonseed oil darkens when exposed to high temperatures, which sets the color and makes it impossible to remove, even with caustic refining. Vegetable oil trading rules recognize the color removal problems with cottonseed oil, with premium grades specifying bleach color as well as refining loss. The trading rules have established price adjustments for higher colors and refining losses. The best crude cottonseed oil grade is "prime crude cottonseed oil," which requires that the oil be capable of refining to not less than 7.6 red on the Lovibond scale. Maximum colors for "basis prime crude," "off crude," and "reddish off crude" oils are 12, 20, and 30 red, respectively. No maximum red color is specified for "low grade cottonseed oil."

The Wesson method, which is the principal color method for the U.S. edible oil industry, has been used for many years primarily because of its simplicity. AOCS Method Cc 13b-45 (103) determines the color of a melted fat or oil product by comparison with red and yellow Lovibond glasses of known characteristics. This method, originally developed in England for measuring the color of beer, is only intended to assess the degree of redness. Yellow is necessary for assessment of redness by allowing the colors to closely match with that of oil sample; the amount of yellow was considered unimportant for this method, and a fixed yellow ratio of 10 yellow to 1 red was adopted for oils with red colors below 3.5 and higher yellow settings were specified for the darker oils.

**Flavor.** One of the most important palatability parameters for edible fats and oils users is flavor. Generally, the flavor of an edible oil product should be completely bland, so that it can enhance the food product's flavor rather than contribute its own. Cottonseed oil is well known for its initial bland flavor and the nutty flavor it develops with oxidation. It has been used as the standard for comparison with other oils for both flavor and odor. The nutty flavor developed with oxidation is more pleasant than the oxidized flavor of some of the other oils in the oleic linoleic classifications; for example, soybean oil reverts to a painty, green, watermelon type flavor with oxidation. Another major cause of off-flavors in food oils is hydrolysis. The free fatty acids liberated with hydrolysis have a distinct flavor and odor that are more disagreeable when the fatty acid chain length is shorter than 14 carbons. Cottonseed oil that contains mostly C-16 and C-18 fatty acids does not become unpalatable until the free fatty acids exceed 1.0%.

**Consistency.** Fats and oils are polymorphic, which means that with cooling, a series of increasingly organized crystal changes occur until a final crystal form is achieved. Each fat and oil has an inherent crystallization tendency, either beta or beta-prime. The tiny, uniform tightly knit, needle-like, beta-prime crystals produce smooth-textured shortening, margarine, and specialty solidified oil products with good plasticity, heat resistance, and good creaming properties. The large, high-melting, self-occluding, coarse, stable beta crystals produce grainy, sandy, brittle solidified oil products that can experience separation of the liquid oil portion. Crystal habit can be controlled by source oil selection as shown on Table 12. Cottonseed

**TABLE 12. Crystal Habit of Hydrogenated Oils and Fats.**

Beta Type	Beta-Prime Type
Canola	Butter Fat
Cocoa Butter	Cottonseed
Coconut	Menhaden
Corn	Palm
Lard	Rapeseed (High Erucic)
Olive	Rice Bran
Palm Kernel	Tallow
Peanut	
Safflower	
Sesame	
Soybean	
Sunflower	

oil is on the shorter list of hydrogenated oils that crystallize in the beta-prime crystal form. Almost all of the other domestic oils in the United States crystallize in the beta form.

### 3.4. Cottonseed Oil Chemical Characteristics

Cottonseed oil, like all oils and fats, is made up of glyceridic materials with some nonglyceridic material in lesser quantities. It is the chemical composition that defines the chemical and physical properties of all fats and oils, which in turn will determine the suitability of the oil in various processes and applications.

**Free Fatty Acid.** Oil chemists learned that the free fatty acid (FFA) content was a good indicator of crude cottonseed oil quality in the 1880s. Hydrolysis causes the triglyceride molecule to split at the ester linkage to form FFA, diglycerides and monoglycerides, and eventually free glycerine. This reaction is normally induced by the presence of moisture and accelerated by heat, but it can also be caused by certain enzymes. The liberated free fatty acids have a distinct flavor and odor, which are more disagreeable when the fatty acid chain length is shorter than 14 carbons. Cottonseed oil, which contains mostly C-16 and C-18 fatty acids, does not become unpalatable until the FFA level exceeds 1.0%.

Crude oil from the best quality cottonseed will have an FFA content of 0.5% to 0.6%. During a good season, the FFA content will be less than 1.0%; however, during unfavorable climatic conditions, the FFA content may average 5% or higher. Dry weather during cotton picking favors low FFA development. The oil in moist seeds, either in the field or in storage, undergoes rapid hydrolysis. Extremely poor oil may contain 15% to 25% FFA. As the refining process neutralizes or removes free fatty acids to a level of 0.05%, this impurity has a direct relationship with refining loss, i.e., the amount of usable oil enclosed in the soapstock. For chemical refining, the quantity of sodium hydroxide for neutralization is based on the FFA level of the crude cottonseed oil. Refining losses as low as 2.5% to 3.0% are encountered

with cottonseed oils containing 0.5% to 0.6% FFA, but oils with high free fatty acid contents may have refining losses as high as 40% to 50% (23).

**Peroxide Value.** Oxidation of oils is a major cause of their deterioration. Hydroperoxides are the primary products formed by the reaction between oxygen and the unsaturated fatty acids. Hydroperoxides have no flavor or odor but break down rapidly to form aldehydes, which have a strong, disagreeable flavor and odor. The peroxide concentration, usually expressed as peroxide value (PV), is a measure of oxidation or rancidity in its early stages. PV measures the concentration of substances, in terms of milliequivalents of peroxide per 1000 grams of sample, that oxidize potassium iodide to iodine. AOCS Method Cd 8-53 (103) is the official method for peroxide value determinations.

Peroxide value is one of the most widely used chemical tests for the determination of fats and oils quality. It has shown good correlation with organoleptic flavor scores. However, a peroxide value determination does not provide a full and unqualified evaluation of oil quality because of the transitory nature of peroxides and their breakdown to nonperoxide materials. Although a linear relationship has been observed between peroxide values and organoleptic flavor scores during the initial stages of lipid oxidation, this method alone is not a good flavor quality indicator because peroxide increases to a maximum and then decreases as time increases. Therefore, a high peroxide value indicates oxidation to produce a poor flavor, but a low peroxide value is not always an indication of a good flavor.

**Anisidine Value.** Anisidine value is a measure of secondary oxidation or the past history of an oil. It is useful in determining the quality of crude oils and the efficiency of processing procedures, but it is not suitable for the detection of oil oxidation or the evaluation of an oil that has been hydrogenated. AOCS Method Cd 18-90 has been standardized for anisidine value analysis (103). The analysis is based on the color reaction of anisidine and unsaturated aldehydes. An anisidine value of less than ten has been recommended for oils upon receipt and after processing (94).

**Inherent Oxidative Stability.** The unsaturated fatty acids in all fats and oils are subject to oxidation, a chemical reaction that occurs with exposure to air. The eventual result is the development of an objectionable flavor and odor. The double bonds contained in the unsaturated fatty acids are the sites of this chemical activity. An oil's oxidation rate is roughly proportional to the degree of unsaturation; for example, linolenic fatty acid (C18:3), with three double bonds, is more susceptible to oxidation than linoleic (C18:2), with only two double bonds, but it is ten times as susceptible as oleic (C18:1), with only one double bond. The relative reaction rates with oxygen for the three most prevalent unsaturated fatty acids in edible oils are:

Fatty Acid		Reaction Rate With Oxygen
Oleic	C18:1	1
Linoleic	C18:2	10
Linolenic	C18:3	25

Oxidation deterioration results in the formation of hydroperoxides, which decompose into carbonyls, dimerization products, and polymerized gums. It is accelerated by temperature, oxygen pressure, prior oxidation, metal ions, lipoxidases, hematin compounds, antioxidant reductions, absence of metal deactivators, time, and ultraviolet or visible light. Extensive oxidation will eventually destroy the beneficial components contained in many fats and oils, such as the carotenoids or vitamin A, the essential fatty acids (linoleic and linolenic), and the tocopherols or Vitamin E. Fats and oil oxidative reactions are directly related to the fatty acid composition, or more specifically, to the unsaturation type and amount. Oxidative stability estimates can be made from the iodine value measurement, calculated iodine value from the fatty acid composition, or an oxidative stability formula. The oxidative stability estimates, determined for the major natural vegetable oils, are presented in Table 13 (106). Inherent oxidative stability rating is the combined reaction rates of an oil's unsaturated fatty acids. It is determined by multiplying the decimal fraction of each unsaturated fatty acid by its relative oxidation rate and then summing the results (107). As indicated in Table 13, deodorized cottonseed oil, with approximately 26% saturates, resists oxidation and subsequent reversion better than other less saturated oils containing higher quantities of linolenic fatty acids (C18:3).

**AOM Stability.** The active oxygen method (AOM) is the most commonly used analytical method for measuring oxidative stability of fats and oils products. AOM employs heat and aeration to accelerate oxidation of the oil by continuously bubbling air through a heated sample. Periodic peroxide value analyses are performed to determine the time required for the oil to oxidize under the AOM conditions. This method requires close attention to detail to produce reproducible results, and even then, the variation between laboratories is  $\pm 25$  for a 100-hour AOM sample.

**TABLE 13. Vegetable Oils Oxidative Stability**

Rating <sup>a</sup>	Inherent Oxidative Stability <sup>b</sup>	Vegetable Oil Source	Calculated Iodine Value	Total Double Bonds
<i>Best</i>	0.3	Coconut	9.6	11
	0.4	Palm Kernel	17.2	20
	1.5	Palm	52.2	61
	1.6	Olive	82.4	96
	3.7	Peanut	97.0	113
	5.3	Canola	116.5	135
	5.8	Cottonseed	112.4	130
	6.5	Corn	128.4	148
	7.2	Sunflower	136.2	157
	7.5	Soybean	132.5	153
<i>Worst</i>	8.0	Safflower	146.1	169

<sup>a</sup>A perfect inherent oxidative stability value is zero.

<sup>b</sup>Calculated Inherent Oxidative Stability = Sum of the decimal fraction of each unsaturated fatty acid times its relative oxidation rate.

**Oil Stability Index.** Two conductivity instruments, Rancimat and The Oxidative Stability Instrument, have been developed as alternatives to AOM Stability analysis. These instruments measure the increase in deionized water conductivity resulting from trapped volatile oxidation products produced when the oil product is heated under a stream of air. The conductivity increase is related to the oxidative stability of the products. These instruments provide a more reproducible measurement of oxidation stability with less technician time and attention.

**Iodine Value.** Iodine value (IV) is a simple and rapidly determined chemical constant that measures the unsaturation of an oil, but it does not define the specific fatty acids. The iodine value procedure determines the grams of iodine absorbed by 100 g of oil. A higher iodine value indicates a greater number of double bonds. The iodine value results for cottonseed oil vary somewhat from year to year, sections of the country, and by growing season. A cooler growing season provides oil with a higher than average linoleic fatty acid (C18:2) content with a lower oleic fatty acid (C-18:1) content; warmer growing seasons reverse this trend. These variations increase or decrease the number of double bonds, which affects the iodine value. Typically, cottonseed oil iodine values range from 103 in Texas to 112 in other regions of the United States.

**Halphen Reaction.** The halphen test is a very sensitive and reliable method for detecting the presence of cottonseed oil in another oil. A reaction with sulfur in carbon disulfide mixed with equal amounts of amyl alcohol gives a cherry red color when cyclopropanoid fatty acids unique to the *Malvaceae* family, which includes cottonseed and okra, are present. This test is capable of detecting 0.25% or less cottonseed oil in an oil blend. The oil is no longer responsive to the halphen test after hydrogenation, which decreases the iodine value 2–5 units.

**Unsaponifiable Matter.** Unsaponifiable matter are those substances dissolved in an oil that cannot be saponified by alkalis but are soluble in nonpolar solvents. These materials are made up of sterols, hydrocarbons, tocopherols, pigments, and higher materials that are insoluble in water. The level of unsaponifiable matter in good-quality cottonseed oil usually ranges from 0.5% to 0.7%. It may decrease slightly in deodorized oils due to slight reductions of sterols with alkali refining and high-temperature deodorization.

**Saponification Value.** Saponification value is useful in predicting the type of glycerides in an oil by measuring the alkali-reactive groups. It is a measure of the average molecular weight of the glycerides in the oil. Glycerides containing short-chain fatty acids have higher saponification values than those with longer chain fatty acids. Cottonseed oil saponification values range from 189 to 198 with an average of 195. Independent of any other analytical measurement, the saponification value results overlap too much to identify individual fats or oils; most oleic and linoleic-classification oils have saponification values in the 180 to 200 range. In edible oil processing, saponification value analyses have been replaced almost entirely by fatty acid composition analysis via gas-liquid chromatography.

**Fatty Acid Composition.** The classical method for determining the fatty acid composition of an oil used a combination of its iodine value, relative density, refractive index, and saponification value. This method has been replaced with

**TABLE 14. Fatty Acid Compositions of Oleic/Linoleic Classification Vegetable Oils.**

Fatty Acid		Olive	Canola	Peanut	Corn	Cottonseed	Soybean	Sunflower
Myristic	C14:0		0.1	0.1	0.1	0.8	0.1	0.1
Palmitic	C16:0	9.0	4.0	11.1	10.9	23.2	10.6	6.3
Palmitoleic	C16:1	0.6	0.3	0.2	0.2	0.7	0.1	0.1
Stearic	C18:0	2.7	1.8	2.4	2.0	2.1	4.0	5.1
Oleic	C18:1	80.3	58.8	46.7	25.4	16.9	24.0	17.8
Linoleic	C18:2	6.3	21.4	32.0	59.6	55.8	52.9	69.1
Linolenic	C18:3	0.7	10.3		1.2	0.2	7.7	0.4
Arachidic	C20:0	0.4	0.6	1.3	0.4	0.3	0.3	0.4
Gadoleic	C20:1		1.5	1.6				0.1
Behenic	C22:0		0.3	2.9	0.1		0.3	0.6
Erucic	C22:1		0.7					
Lignoceric	C24:0		0.2	1.5				

fatty acid composition analysis determined by gas-liquid chromatographic (GLC) patterns. The GLC procedure identifies the percentage of each individual fatty acid with one analytical procedure, which applies equally well to refined and unrefined oils. The fatty acid composition analysis by GLC provides a rapid and accurate means of determining the fatty acid distribution of fat and oil products. This information is beneficial for all aspects of product development, process control, and marketing because the physical, chemical, and nutritional characteristics of fats and oils products are determined by the kinds and proportions of the component fatty acids and their position on the glycerol moiety. The physical characteristics of an oil depend on the degree of unsaturation, the carbon length, the isomeric fatty acid forms, and the molecular configuration. Each fatty acid has an individual melting point that increases with chain length and decreases as the fatty acids become more unsaturated. The unsaturated fatty acids are chemically more active than the saturates because of the double bonds, and this reactivity increases as the number of double bonds increase. The double bonds are subject to oxidation, polymerization, hydrogenation, and isomerization. Usually, fats and oils products are liquid at room temperature when the unsaturates are high and solid when the level of unsaturates are low. However, this generalization can be complicated by the *trans*-isomers formed during hydrogenation (108). Table 14 compares the typical fatty acid composition of the oleic-linoleic classification oils.

### 3.5. Cottonseed Oil Analytical Characteristics

All of the edible fats and oils vary considerably in their chemical structure, which determines the physical characteristics that provide functionality. The physical, chemical, and performance analyses are the tools available to the fats and oils processor for the evaluation of the products produced, development of new products, purchase of raw materials, and identification of specific customer requirements.

**TABLE 15. Typical RBD Cottonseed Oil Analytical Characteristics.**

Characteristic	Typical	Range
Specific Gravity at 25/25°C		0.916 to 0.918
Refractive Index at 25°C		1.468 to 1.472
Iodine Value	108.0	98.0 to 118.0
Saponification Number		189 to 198
Unsaponifiable Number		< 1.5
Titer, °C	34.9	30.0 to 37.0
Melting Point, °C	13.0	10 to 16
Solidification Point, °C		+12.0 to -13.0
Cloud Point, °C	3.0	-1.0 to 3.0
Cold Test, hours	none	
AOM stability, hours	16.0	16 to 19
Tocopherol Content, ppm		
alpha-tocopherol	355	340 to 369
gamma-tocopherol	502	481 to 522
delta-tocopherol	8	8 to 9
Fatty Acid Composition, %		
Myristic C14:0	0.7	0.6 to 1.0
Palmitic C16:0	21.6	21.4 to 26.4
Palmitoleic C16:1	0.6	0 to 1.2
Stearic C18:0	2.6	2.1 to 3.3
Oleic C18:1	18.6	14.7 to 21.7
Linoleic C18:2	54.4	46.7 to 58.2
Linolenic C18:3	0.7	0 to 1.0
Arachidic C20:0	0.3	0.2 to 0.5
Gadoleic C20:1		0 to 0.1
Behenic C22:0	0.2	0 to 0.6
Erucic C22:1		0 to 0.3
Lignoceric C24:0		0 to 0.1
Triglyceride Composition, %		
Trisaturated (GS <sub>3</sub> )	0.1	0 to 0.1
Disaturated (GS <sub>2</sub> U)	13.2	14.0
Monosaturated (GSU <sub>2</sub> )	58.4	50.0 to 58.0
Triunsaturated (GU <sub>3</sub> )	28.3	28.0 to 36.0
Hydrogenated Crystal Habit	beta prime	

G = glycerides; S = saturated; U = unsaturated.

Table 15 lists the typical cottonseed oil analytical characteristics, including fatty acid and triglyceride compositions and ranges (43, 80), which allow for the different varieties, growing conditions, and analytical error.

#### 4. COTTONSEED HANDLING, OIL EXTRACTION AND PROCESSING

Nature has provided plants with systems to synthesize, utilize, and store food lipids. Improper handling and storage of oilseed prior to extraction can have deteriorous effects on the oil quality. Therefore, control of cottonseed transportation, storage,



segregation of lots, and moisture are the first processes for processing a desirable edible oil. Oil extraction has a long development history, while most of the oil processing methods were largely introduced during the twentieth century. Until the recent past, crude oil extraction and oil processing were two separate industries. However, during the last quarter century, sheer economics and product synergy have caused both horizontal (merger of similar operations) and vertical integration (combination of different but related operations) of these activities to occur. Now, companies continue to increase their crushing capacity and many extract and refine the oil as a continuous operation. Most of these operations have integrated miscella refining with sodium hydroxide to produce a prime-bleachable-summer-yellow (PBSY) cottonseed oil with consistently lighter color. PBSY, the caustic-refined cottonseed oil, is a trading definition of the National Cottonseed Products Association with an AOCS official bleach color of no greater than 2.5, no more than 0.25% free fatty acid, and no more than 0.10% moisture and volatile matter. The oil processor is guaranteed quality via this trading rule with its 2.5 laboratory bleach color.

#### 4.1. Cottonseed Handling and Storage

Once a cotton boll opens, the cottonseeds within are susceptible to deterioration. The living seeds respire, producing carbon dioxide and heat. Other biological processes occur in the seeds as well. Triglycerides are split by enzymes via hydrolysis to release free fatty acids, which the embryo plant uses for energy. The production of free fatty acids is undesirable for the oil processor because seeds high in FFA contain low-quality oil, which usually results in a higher refining loss. The rate of hydrolysis is dependent on temperature and moisture, but the hydrolytic enzymes may be inactivated by heat. Oxidation of the fatty acids in the oil can result from heating (109). Altschul (110) comprehensively reviewed the biological processes that take place in the cottonseed before and after harvest, and more recent reviewers (111) have considered cottonseed development.

Another factor in cottonseed storage is the control of mold development on the seeds. Cottonseed is particularly susceptible to the fungus *Aspergillus flavus*, which produces aflatoxin. Aflatoxin formation in cottonseed is generally, but not exclusively, initiated in the field, rather than during storage. The rigors of normal processing, along with the fact that the toxin is associated with the protein segment of the seed, preclude aflatoxin from the oil, but the residual meal from affected seed is still contaminated. The use of contaminated meal for animal feed is strictly controlled by both state and federal regulation. For health and economic reasons, it is important to minimize conditions that favor the growth of *A. flavus* and to monitor for its presence. An excellent review of the subject of aflatoxin control in cottonseed has been provided by Park et al. (112).

Ideally, cottonseed should be stored at a moisture content of less than 10% (49). Dehulled seeds should contain no more than 9% moisture and 1% FFA (109). Prior to storage, cottonseeds must be sampled for moisture analysis. The American Oil Chemists' Society (AOCS) method Aa 3-38 specifically describes a procedure for

determining moisture in cottonseeds, although other methods may be used. Seeds with 10–11% moisture may be stored immediately, but seeds with higher moisture require additional drying. Drying may take place at either ambient temperatures or up to 104.5°C (220°F), to 12% or less. There is no benefit to drying the seeds below 9% moisture. Although dryers may not be needed every season, they are a necessity when wet seeds are delivered to the mill (113). As the seeds are hygroscopic, the relative humidity of the air in storage must be monitored to maintain the desired seed moisture level. Treatment of high-moisture seeds with propionic acid to retard deterioration has been investigated, although it is not widely practiced (114).

The most common type of cottonseed storage facility is the seed house, however, some silos are used; and west of the Mississippi, seed is also stored outside on solid slabs and covered with canvas. An air-cooling system is vital to the successful storage of cottonseed. The temperature of the seeds is dependent on the ambient temperature and degree of ventilation in the storage area (115). As the seeds are respiring, heat can build up, particularly if the seeds have a high moisture or FFA level (49). Overheated seeds must be cooled to below 60°F (15.6°C) to prevent further deterioration (116). Storage for over one year is possible provided that the seeds are held under adequate conditions.

## 4.2. Cottonseed Oil Extraction

Cottonseed was one of the earliest oils to be extracted from the seed. The extraction of cottonseed oil slowly progressed from edgestone to wedge press to hydraulic press (23). Hydraulic pressing was the predominant means used to separate the oil from cottonseed for most of the nineteenth century. As improved cotton spinning, weaving, and ginning operations during the eighteenth century made more cottonseed available for crushing, the labor-intensive hydraulic-press operation quickly yielded to the continuous screw press in the early 1900s. As edible oil commanded a respectable market price and because either the hydraulic press or the screw press still left nearly 20% of the available oil of the seed in the press-cake, much research activity was initiated to find an acceptable solvent to extract the remaining oil from the cake. This effort led to the prepress solvent extraction process in the 1930s. This combination of mechanical press and solvent extraction of the press-cake was able to recover better than 97% of the available oil in the cottonseed. Further demand in productivity and oil quality brought expander- and miscella-refining of cottonseed oil into the crushing business in the 1970s. Toward the late 1980s, more than 80% of the cottonseed crushed in the United States was accomplished by the expander solvent extraction operation (3). The flow sequence for cottonseed oil extraction most practiced in the United States is illustrated in Figure 2 (69).

**Cottonseed Preparation.** Most oilseeds require some degree of cleaning and preparation before the oil is separated from the solid portion of the seed. After the cotton fiber is removed from the seed by the ginning operation, the seed still has short linters with a white appearance. This is called white or fuzzy cottonseed in the

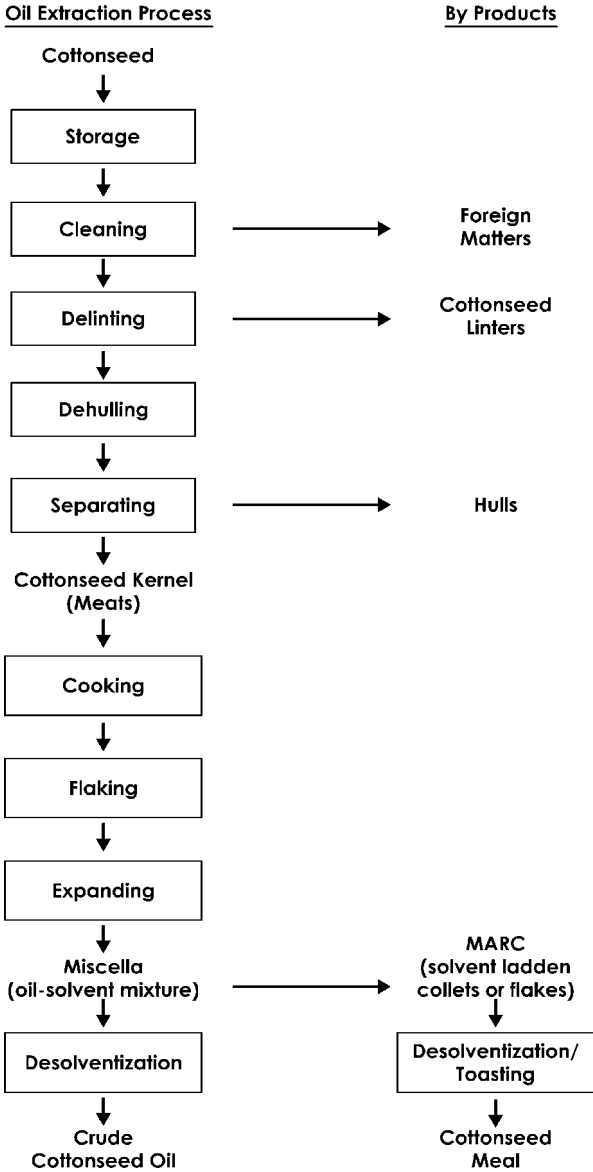


Figure 2. Cottonseed oil extraction flow sequence.

trade. On a dry basis, white cottonseed is composed of 12.7% linter, 31.8% hull, and 55.5% kernel (23). As illustrated in Figure 2, cottonseeds are usually stored uncleaned when received at the oil mill. When the cottonseeds are removed from storage for extraction, dirt and other trash must be removed. Several seed-cleaning systems are used, which are all based on some type of screening. Trash that is

lighter or smaller than the seeds will be aspirated in pneumatic systems or sifted out mechanically. The larger pieces of trash are screened out and magnets are used to remove ferrous metal. Foreign materials that have the same size and density as the seed can still be carried on through the process stream (117).

**Delinting.** This step is unique to cottonseed among all the oilseeds. The short cellulose linter fibers must be removed from the seeds because leaving them on the seed would lower the yield of oil due to absorption of the oil by the cellulose fibers. Linters, are bulky and tend to hold the neutral oil or occupy valuable extractor space during the extraction. Chemicals, such as sulfuric acid, have been used to remove the linters, especially when seeds are prepared for replanting. However, all commercial mills remove the linters mechanically as these short fibers have many nonfood applications, serving as the starting material for pure cellulose, plastics, and rayons, and are used in high-quality paper, batting or padding in bedding and furniture, and automotive uses.

**Hulling.** Once the lint is removed, the hulls are separated from the seeds. Hulls that are allowed to remain with the kernels absorb oil during extraction and lower the quality of the meal produced by lowering the protein level. The hulls cannot be completely eliminated without a loss of kernel, so an acceptable level of hull retention must be determined, depending on the desired protein level of the final meal.

Two types of hullers are used in the industry. The bar huller consists of a bar- or knife-studded cylinder that rotates within another cylinder having similar knives protruding from its interior. The hulls are cut as the seeds pass around the inner cylinder. The seed decorticator has two hardened steel rolls, both of which have longitudinal grooves cut in the surface. The seeds are fed between the rolls and then cut by the grooves and the difference in speed between the two rolls. The hulls and uncut seeds are removed from the kernels by screening and the hulls are aspirated so that the seeds may be returned to the huller.

**Flaking.** After hulling, the meats, or kernel, are reduced in size or flaked to facilitate oil removal. This rolling process minimizes the distance through which the free oil must pass, but it does not necessarily rupture the walls of the oil cells. Proper moisture content of the seeds is essential for flaking, and if the moisture level is too low, the seeds must be conditioned to raise the moisture to about 11% (116). Cottonseeds may be flaked by passing between two rolls mounted side-by-side; however, they are more often flaked in a series of five stacked-crushing rolls because a thinner flake may be achieved with the vertical rolls. The ultimate thickness of the flake is determined by the method of extraction used. For mechanical pressing, a thickness of 0.127–0.254 mm (0.005–0.010 inch) is common, and for solvent extraction, flakes of not less than 0.230–0.254 mm (0.008–0.010 inch) are common (49). Thinner flakes tend to disintegrate during the solvent process.

**Cooking.** Prior to extraction, the flakes are heated or cooked. Ward (118) summarized the purpose of cooking the flakes as follows: (1) cell walls are broken down allowing the oil to escape; (2) oil viscosity is reduced; (3) moisture content is controlled; (4) protein is coagulated; (5) enzymes are inactivated and micro-organisms are killed; (6) gossypol is bound to protein, to some extent, by the action of heat in combination with moisture and physical treatment and thus some portion of it

is detoxified; and (7) certain phosphatides are fixed in the cake, which helps to maintain subsequent refining losses.

Cottonseed flakes are usually cooked in stack cookers that are 4–8 kettles high. The sides and floors of each kettle are steam-jacketed to heat the flakes. The flakes are fed into the top kettle, heated for a specific time, and then swept into the kettle below. The temperature of lower kettles are usually maintained at higher temperatures than the top kettle. If the flakes are relatively dry, moisture may be added to the top kettle to reach a level of 11–12%. As the flakes progress toward the bottom kettle, water is evaporated and removed by vents in each of the lower kettles until the final moisture level is reached. The desirable level is 5–6% moisture for seeds to be hydraulically pressed and about 3% for seeds to be expeller or screw pressed (49). Cooking seeds at low temperatures and moisture content may result in less protein binding of gossypol. Higher gossypol levels will then develop in the oil and affect the color of the crude oil (49).

The flakes are heated to over 190°F (87.8°C) in the upper kettle. Flakes with high phosphatide content may benefit from being cooked at slightly lower temperatures to avoid elevating refining losses. The temperature of the flakes is raised to 230–270°F (110–132.2°C) in the lower kettles. The seeds are cooked for up to 120 minutes and, depending on the size of the cooker, 81–136 metric tons (90–150 short tons) of meats may be cooked in a 24-hour period.

Overcooking lowers the nutritional quality of the meal and darkens both the oil and the meal. Poor-quality seeds with high levels of free fatty acids cannot be cooked for as long a period as high-quality seeds because of darkening. Darker oil requires additional refining to achieve the desired bleach color.

**Oil Extraction.** Four types of processing systems are used to extract oil from oil-bearing materials: (1) hydraulic press, (2) expeller or screw press, (3) prepress solvent extraction, and (4) direct solvent extraction. These systems employ the two techniques in common practice for the extraction of cottonseed oil. These are mechanical by means of a press or the solvent process with the use of hexane. Mechanical pressing is normally applied to oilseeds that are relatively high in extractable oil. Hull-free cottonseed kernels contain as much as 34% oil and are suitable for the mechanical extraction process (3). The prepress solvent system employs a combination of the two techniques, where seeds are lightly screw-pressed to reduce the oil by one-half to two-thirds of its original level before solvent extraction completes the job. After 1980, more than 80% of the cottonseed crushed in the United States was accomplished by the expander solvent extraction procedure. In most cases, solvent extraction and refining processes are coupled together for quality and efficiencies as reviewed in Miscella Refining of Section 4.3. On a worldwide basis, due to available transportation infrastructure, hardware, solvent, and skilled labor, cottonseed is still being processed with all four extraction systems.

**Hydraulic Pressing.** Batch pressing was the earliest commercial method of oil extraction. Hydraulic equipment replaced the mechanical operations and the method became known as hydraulic pressing (49). In open presses, oilseed meals were wrapped in cloths and placed between plates, which were then gradually

**TABLE 16. Comparative Yield of Cottonseed Oil from Different Extraction Processes.**

Method	Kg of Oil per MetricTon of Cottonseed
Hydraulic Press	154
Continuous Screw Press	163.5
Solvent Extraction Including Prepress Solvent Extraction	180.5–188

compressed to squeeze the oil from the seeds. Box-type presses were most often used for cottonseed, and this method was fairly labor intensive. Today, worldwide, very little cottonseed is hydraulically pressed and none in the United States. The relative efficiency of this type of extraction to more modern methods is shown in Table 16 (49). Wrenn (5) presented the historical context of hydraulic pressing of cottonseed oil and included pictures of the equipment in operation.

**Screw Pressing.** The screw-press process has been used since the early 1900s in place of the hydraulic press to separate oil from cottonseed meats. With this system, pressure is gradually applied to the flakes as a screw conveys them from the feed end to the discharge end of the expeller barrel. A plug of the compressed meal develops at the discharge end and a drainage barrel surrounds the press to collect the oil expressed during the passage of the flakes. About 3–4% oil remains in the cake that results from screw pressing. Anderson (Cleveland, Ohio) expellers have both vertical and horizontal presses to maximize pressure, and French (Piqua, Ohio) screw-presses generally consist of a horizontal, water-cooled cage. Both types of presses exert 680–1089 atm (5–8 tons per square inch) pressure on the flakes (49). A disadvantage of the screw press method compared with the now outdated hydraulic pressing was the tendency of screw-pressed cottonseed oil to have higher color due to the lower moisture content of the cooked meats prior to pressing (49).

**Direct Solvent.** This process is based on the use of a nonpolar solvent, specifically hexane, to dissolve the oil without removing proteins and other compounds. The flakes are mixed with hexane in a batch or continuous operation. The resulting oil-solvent micelle and the residual meal are heated to evaporate the solvent, which is collected and reused. Solvent extraction yields about 11.5% more oil than does the screw-press method (49), and 1% or less oil remains in the meal. However, direct solvent extraction is problematic for the cottonseed industry because the high oil content of cottonseed flakes causes them to break into fires during extraction after the oil is removed; occasional overheating of the oil-solvent miscella will cause irreversible color changes in the oil; the meal requires additional heating to bind gossypol, if it is destined for use as poultry or swine feed; the solvent poses fire problems and is expensive; hexane poses environmental pressures as a volatile organic compound (VOC); and the main component of hexane, n-hexane, is also classified as a Hazardous Air Pollutant (HAP) by the U.S. Environmental Protection Agency and is strictly regulated in the United States (55, 119).

**Prepress Solvent Extraction.** Solvent extraction is relatively costly and is not well suited for the high-oil content of cottonseed. Mechanical pressing leaves about 5% oil in the press-cake, and it is desirable to recover as much oil as possible. A logical processing step was to combine the two extraction techniques. With prepress solvent extraction, cottonseeds are pressed to remove most of the oil and then the oil remaining in the press-cake is extracted with solvent. This solvent extraction operates on a reduced volume of feed stock (i.e., press-cake, as opposed to full-fat flakes) and, therefore, requires a modest size extractor with modest amounts of desolventizer and solvent.

**Expander-Solvent Extraction.** The most recent development in cottonseed extraction is the use of expanders. The expander is a low-shear extruder that heats, homogenizes, and shapes oilseeds into porous collets or pellets with a high-bulk density. Steam is injected into the oilseed flakes or cake in the expander while under pressure, and then this mixture is extruded through plates to the atmosphere. The collets expand when released to the atmosphere, hence the name expander. Some expanders have a drainage cage to reduce the oil content of high-oil seeds to less than 30%, thus enabling the production of intact collets for direct solvent extraction, instead of the prepress extraction process (120). Watkins (121) reported that after hexane extraction, the collets had 25–45% less solvent holdup and 15 times less oil than did traditionally prepared flakes. Solvent capacity is enhanced because the extruded cottonseed requires solvent-feed ratios of 1:1, compared with 1.8:1 for direct extraction of cottonseed meats (122). After extraction, the meal contains 0.10–0.20% free gossypol, which is about half the amount found in flakes. As of 2000, essentially all commercial cottonseed extraction in the United States employs the expander solvent extraction process.

### 4.3. Cottonseed Oil Processing

Processing flow sequences for six different product groups are illustrated in Figure 3. One finished product that is still used in some parts of the world was omitted from Figure 3: extracted and filtered cottonseed oil. Cottonseed oils that did not have any processing after extraction were purchased at local bazaars in Central Asia in 1998. These oils would be unacceptable in the west, where the consumers have been conditioned to prefer edible oils that are light in color, bland flavored, have a high smoke point, maintain a clear appearance both on the grocery shelf and under refrigeration, contain additives to prolong flavor and frying stability, are modified to provide a specific performance characteristic, and attractively packaged for convenient handling. The processes responsible for these and other oil product qualities are presented in this section.

**Refining.** As used here, the term *refining* refers to any purification treatment designed to remove FFA, phosphatides, gossypol, and other gross impurities in cottonseed oil; it excludes any other process, such as bleaching or deodorization. The refining process probably has more impact on a vegetable oil's quality and economic performance than any of the other processes during the conversion to a finished product. Inadequately refined oils will affect the operation of all succeeding pro-

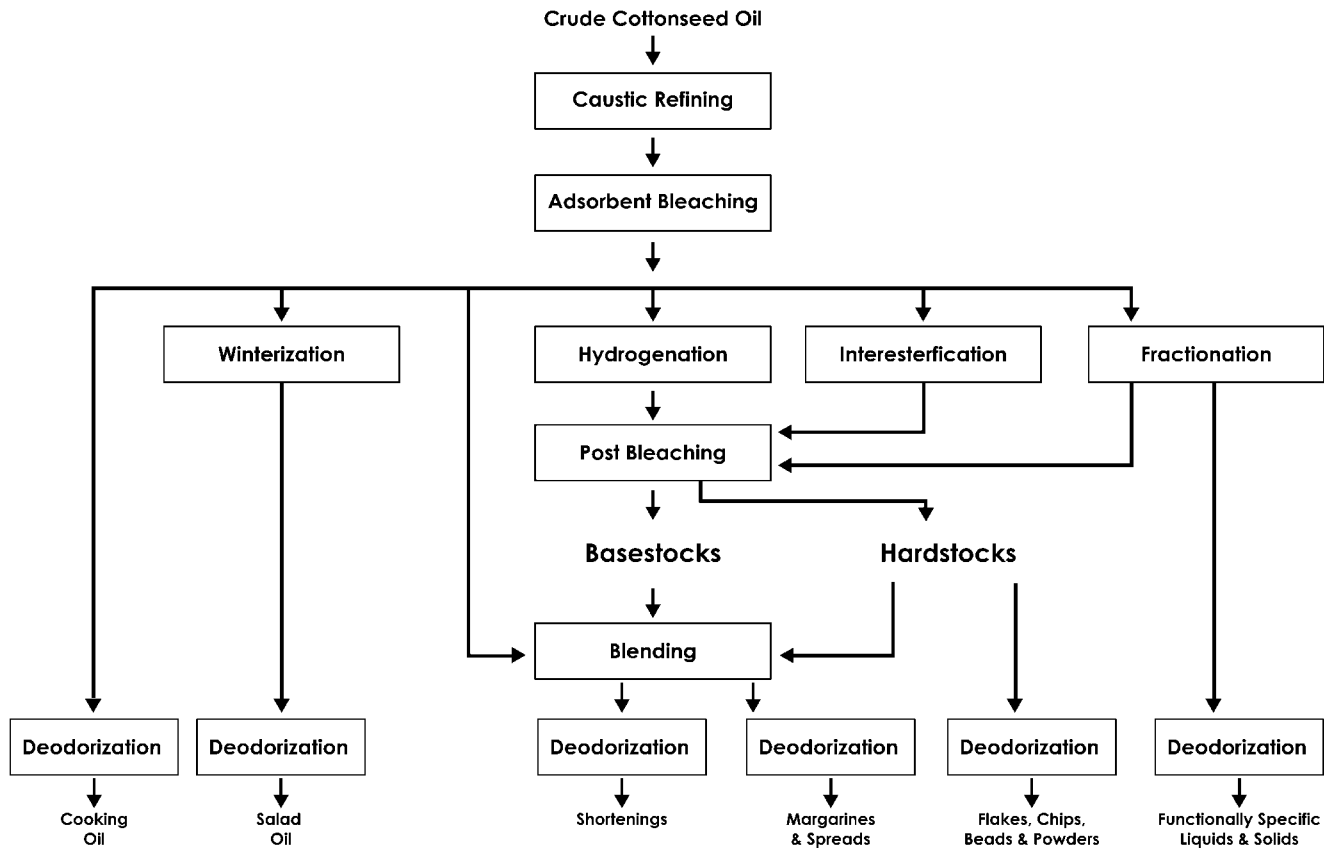


Figure 3. Cottonseed oil processing—flow sequence.



cesses and the quality of the finished product. Additional processing and handling required because of poorly refined oils will increase the costs of a suspect quality finished product beyond that of one produced with a properly refined oil that has a good quality.

Two different refining systems are currently used to refine vegetable oils; chemical and physical refining. Some shortcomings experienced with physical refining have maintained alkali refining as the preferred vegetable oil purification technique in the United States. Some oils, like cottonseed, contain nonglyceride materials that cannot be removed adequately by the processes employed with physical refining. Gossypol and related pigments in cottonseed oil readily combine with caustic soda and, thus, are removed most effectively by alkali-refining. Several different versions of the alkali-refining process are practiced in the United States and other countries: long-mix, short-mix, miscella, and the Zenith process. This discussion will be limited to the conventional, or long-mix, caustic-refining process, which evolved to provide extended reaction times with caustic soda for the effective removal of gossypol and phosphatides from cottonseed oil and the miscella process.

**Conventional or Long-Mix Caustic-Soda Refining.** The conventional caustic-soda refining process is the most widely used and best known refining system. The addition of an alkali solution to a crude oil brings about a number of chemical and physical reactions. The alkali combines with the free fatty acid present to form soaps. The phosphatides and gums absorb alkali and are coagulated through hydration or degradation. Much of the coloring material is degraded, absorbed by the gums, or made water soluble by the alkali, and the insoluble matter is entrained with the other coagulable material. With heat and time, the excess caustic can also bring about the saponification of the neutral oil. Therefore, selection of the NaOH strength, mixing time, mixing energy, temperature, and the quantity of excess caustic all have an important part in making the alkali-refining process operate effectively and efficiently.

The current alkali-refining techniques are a result of the gradual application of science to the basic art of batch refining originally performed in open-top, cone-shaped kettles. Efficient separation of soapstock from the neutralized oil is the significant factor in alkali refining, and the technique of using centrifugal separators materially improved the yield from 1.5% to 2.5%. The caustic soda continuous system that evolved has the flexibility to efficiently refine all crude edible oils presently used in the world. The system may be outlined as follows (106).

- **Crude Oil Conditioning**—Crude oils with high levels of phosphatides and trace metals are usually treated with food-grade phosphoric acid for 4 to 8 hours before refining. The purpose of the acid pretreatment is to: (1) help precipitate phosphatidic materials; (2) precipitate natural calcium and magnesium as insoluble phosphate salts; (3) inactivate trace metals, such as iron, copper, and others that may be present in the oil; (4) reduce the neutral oil losses; and (5) improve the color and flavor stability of the finished deodorized oil.

- **Caustic Treatment**—The crude oil is continuously mixed with a proportioned stream of dilute caustic-soda solution and heated to break the emulsion formed. Selection of the caustic treatment is determined by the type of crude oil, FFA content, past refining experience with similar oils, and the refining equipment available. In general, the minimum amount of the weakest strength necessary to achieve the desired endpoint should be used to minimize saponification of the neutral oil and prevent emulsions during separation. Usually, the best results are obtained with relatively weak caustic solutions for low-FFA oils and with stronger concentrations for high-FFA oils. Phosphatide reduction during refining is determined largely by the amount of water present in the caustic solution. Higher excess caustic treatments remove more phosphatides, but the increase in removal is caused more by the increased water than the sodium hydroxide (NaOH). The strength of the caustic solution is measured in terms of specific gravity expressed in degrees Baumé (Bé). Therefore, the caustic treat selected for the crude oil will vary with the FFA content and the level of caustic excess over the theoretical quantity determined for each oil. The theoretical quantity of caustic is based on the ratio of molecular weights of NaOH to oleic fatty acid. This factor is determined as follows:

$$\text{Factor} = \frac{\text{NaOH Molecular Weight}}{\text{Oleic Fatty Acid Molecular Weight}} = \frac{40}{282} = 0.142.$$

Thus, the formula for caustic treatment is:

$$\% \text{ Treat} = \frac{\% \text{FFA} \times 0.142 + \% \text{Excess} + \text{Acid Addition}}{\% \text{NaOH in caustic}} \times 100.$$

The refining conditions for cottonseed oil are chosen more for the improvement of color because of the presence of gossypol. This pigment is sensitive to heat and oxidation, forming colored compounds that are difficult to remove from the oil other than by reaction with caustic. Therefore, a larger excess of a more concentrated NaOH solution treat is used for cottonseed oil than most other vegetable oils; good quality cottonseed oils generally require 0.2% excess, while darker colored oils may require up to 0.4% excess treat (29).

- **Caustic Oil Mixing**—After the caustic reagent has been proportioned into the crude oil, it must be adequately blended to ensure sufficient contact with the FFA, phosphatides, and color pigments. The gums are hydrolyzed by the water in the caustic solution and become oil-insoluble. The caustic solution and oil are mixed at 30–35° or 86–95°F in a dwell mixer with a 5–15-minute residence time. High-oil temperatures during the caustic addition must be avoided because they can increase the neutral oil saponification and reduce the refined oil yield. After the caustic mixing phase is complete, the mixture is delivered to the centrifuges at a temperature suitable for optimum separation,

normally the oils are heated to 74°C or 165°F to provide the thermal shock necessary to break the emulsion.

- **Soap-Oil Separation**—From the caustic oil mixer, the resultant soap-in-oil suspension is fed to the centrifuges for separation into light- and heavy-density phases. There are various types of centrifuges used in vegetable oil refining, however, most of them contain a bowl or hollow cylinder that turns on its axis. The flow of material enters the rotating bowl and is forced outward to a disc stack. The heavier density soapstock is forced to the outside of the bowl and flows over the top disc and out the discharge port. The lighter neutral oil phase moves to the center of the bowl for discharge from the neck of the top disc. Refining yield efficiency is dependent on this primary separation step.
- **Water Washing**—Sodium soaps remaining from the primary centrifugation phase are readily washable and easily removed from the oils with either a single or double wash. The refined oil from the primary centrifuge is mixed with softened water heated 5°C to 8°C or 10°F to 15°F above the oil temperature at a rate of 10% to 20% of the oil flow. Softened water must be used to avoid the formation of insoluble soaps. The water-oil mixture passes through a high-speed, in-line mixer for maximum soap transfer from the oil to the water phase. The soapy water-oil mixture is centrifuged to separate the two phases.
- **Vacuum Drying**—Refined oil from water washing is usually dried if it is to be stored before bleaching to remove the traces of water remaining after water washing to avoid hydrolysis. Typically, washed oil at approximately 85°C or 185°F is sprayed through nozzles into a vacuum chamber controlled at 70 cm of mercury. This weak vacuum reduces the moisture content to below 0.1%, most often in the range of 0.05%. Oils that are vacuum bleached immediately after water washing can bypass the drying step.
- **Re-Refining**—In handling dark oils, it frequently happens that no matter how efficient the original caustic refining of a crude cottonseed oil may be, the bleached color is less than desired. Under these circumstances, it becomes necessary to re-refine the oil for additional color removal. Usually, 1% or 2% of a 16° Bé caustic treat is sufficient to provide a bleach color of 2.0 to 2.5 Lovibond red color (123).

**Miscella Refining.** Since 1960, miscella refining has become the most common method for the treatment of cottonseed oil in the United States. Facilities with an existing oilseed solvent extraction system found miscella refining to be advantageous by using the same solvent recovery unit for both purposes. Miscella is the solution or mixture that contains the extracted oil. Both continuous and batch miscella-refining processes are suitable for most fats and oils. Miscella refining is especially beneficial for cottonseed oil to provide an oil with a lighter red color and a high-neutral-oil yield. This type of refining should be done at a solvent extraction plant as soon as possible, preferably within 6 hours after the oil is extracted

from the oilseed or animal tissue. The advantages for miscella refining, as compared with conventional continuous caustic-soda refining, are (1) higher oil yield, (2) lighter color oil without bleaching, (3) elimination of the water wash step, and (4) extraction of the color pigments before solvent stripping has set the color (124).

For this purification process, the crude miscella source may be from (1) the pre-evaporator of a direct-solvent extraction plant, (2) a blend of prepressed crude oil and solvent-extracted miscella from the press-cake, or (3) a reconstituted blend of crude oil with solvent. In the process, a mixture of approximately 40% to 58% oil in solvent is heated or cooled to 104°F (40°C) and filtered to remove meal, scale, and other insoluble impurities. Two solvents that have been used commercially for miscella refining are hexane and acetone.

Hydrolysis of phosphatides and pigments in the crude oil miscella requires an acid pretreatment, which usually varies between 100 ppm and 500 ppm by weight of the oil, depending on the quality of the crude oil. An acid such as phosphoric or glacial acetic has been found effective in improving oil quality and reducing refining losses. Phosphoric acid is used more commonly because of its less corrosive properties and its availability. The acid is mixed with the miscella in a static mixer to provide an intimately dispersed acid phase that immediately reacts with the crude miscella.

The pretreated crude miscella is then alkali refined using dilute caustic soda with a 16–24°Bé and a 0.2–0.5% NaOH excess over the theoretical amount required to neutralize the free fatty acids. The reaction of the caustic soda with the free fatty acids proceeds rapidly at 130–135°F (54–57°C), using homogenizers with a shear-mixing intensity capable of homogenizing milk, hydrolyzing the phosphatides and pigments with the caustic soda to produce a two-phase mixture. The miscella temperature is adjusted to 135°F (57°C) to obtain the best separation of the heavy phase or soapstock from the oil or the light phase with the centrifuge. The neutral oil is then filtered through a diatomaceous earth precoated pressure-leaf filter. At this point, the refined and filtered miscella can be stripped of the solvent to produce a neutral yellow oil, or it can be further processed as miscella to dewax, fractionate, or hydrogenate the oil (125, 126).

**PreBleaching.** Bleaching is popularly and correctly regarded as the partial or complete removal of color; however, it is also a purification process to prepare the oil for further processing. Bleaching is relied on to clean up the traces of soap, phosphatides, and pro-oxidant metals remaining after caustic neutralization and water washing that hinder filtration, poison hydrogenation catalysts, darken the oil, and adversely affect the flavor of the finished oil. Another function, considered primary by many processors, is the removal of peroxides and secondary oxidation products. These impurities compete for space on the adsorbent surface of the filter media. The key process parameters for bleaching include (1) procedure, (2) bleaching media, (3) temperature, (4) time, (5) moisture, and (6) filtration (106).

- Procedure—The three most common types of contact bleaching methods are batch atmospheric, batch vacuum, and continuous vacuum. Although vacuum

bleaching is preferred, atmospheric bleaching can produce quality bleached oils. Vacuum bleaching, either batch or continuous, is more effective than atmospheric bleaching because it can use less clay, operates at lower bleaching temperatures, effects quicker moisture evacuation for less FFA development, and does not expose the oil to oxidation at high temperatures. Batch bleaching is preferred to continuous operations when a variety of source oils are processed in the same system. However, continuous systems are more efficient and effective for systems dedicated to single source oils.

- **Bleaching Agents**—Chemical agents have been proposed and some used, but practically all edible oil decoloration and purification is accomplished with adsorptive earths or carbons. The basic kinds of adsorbents used in edible oil bleaching are neutral clays, activated earths, and activated carbon. Particle size is a major physical parameter affecting bleaching earth performance because adsorption theory considers adsorption as a surface phenomena. In general, the finest particle size earths have the best performance; however, too small particles create severe filtration problems and oil retention is increased. The natural bleaching earths, usually referred to as “fullers earth,” are bentonite clays that exhibit adsorptive properties in the natural state with only physical processing. Activated bleaching earths have been treated with organic or inorganic acids to enlarge the surface and pore volume to make it selectively attractive to the detrimental components in refined oils. Also, the activated bleaching earths normally contain 10% to 18% moisture, which supports the montmorillonite layers in the clays. Activated carbon is effective in adsorbing certain impurities not affected by earths, but it is used sparingly due to problems with filtration, relatively high cost, and a high oil retention.
- **Bleaching Media Dosage**—The amount of bleaching material used depends on the type of adsorbent used and the impurities to be removed. Bleaching earth requirements vary in wide range from 0.15% to 3.0%. On the basis of adsorbent activity, the acid-activated earths are generally 1.5 to 2 times more effective than the natural earths. Carbon is rarely used alone but sometimes employed in admixture with a bleaching earth in a ratio of 10–20 parts bleaching earth to 1 part carbon (49).
- **Temperature**—Bleaching earth activity increases as the temperature is increased by reducing the viscosity of the oil, but decoloration declines after the optimum temperature is reached. Temperature also affects other properties of the oil, which dictate that it should be kept as low as possible to minimize product abuse, but high enough for adequate adsorbance of the impurities and pigments. The optimum bleaching temperature for nearly all edible fats and oils ranges between 70°C and 110°C or 160°F to 230°F. Fewer problems are encountered when the bleaching temperature is maintained below 110°C or 230°F with vacuum bleaching. Secondary oxidation products begin to develop at temperatures above 110°C or 230°F. Low temperatures favor the retention of the adsorbed pigment on the bleaching earth surface. Although higher temperatures favor the movement of pigment molecules into the pores where chemisorption occurs, it will also promote structural changes of the unsatu-

rated fatty acid groups. Under extremely high temperatures, isomerization of the unsaturated fatty acid groups and excessive FFA could develop.

- Time—In theory, adsorption should be practically instantaneous; however, in practice, this is not the case. The rate of decoloration is very rapid during the first few minutes after the adsorbent comes in contact with the oil and then decreases to a point where equilibrium is reached and no more color is adsorbed. Usually 15 to 20 minutes contact time is adequate at a bleaching temperature above the boiling point of water. Contact time is made up of two time periods: (1) the time in the bleaching vessel and (2) the time in the filter during recirculation or final filtering.
- Filtration—After an adsorbent has selectively captured the impurities, it must be removed from the oil before it becomes a catalyst for color development or other undesirable reactions. Filtration, the separation method most often used for spent bleaching media removal, is the process of passing a fluid through a permeable filter material to separate particles from the fluid. Examples of the filtration materials used are filter paper, filter cloth, filter screen, and membranes. Filter aid, such as diatomite, perlite, or cellulose, are usually used in conjunction with the permeable filters for surface protection. Traditionally, either plate and frame or pressure-leaf filters have been used for spent bleaching media removal. Currently, self-cleaning, closed filters that operate on an automated cycle are available.

**Winterization.** When cottonseed oil is designed for use as salad oil, it must be winterized, that is, a considerable portion of the more saturated glycerides must be removed so that the material will remain clear when exposed to reduced temperatures, such as those likely to be encountered with refrigeration. If the saturated glycerides in cottonseed oil are not removed, it will solidify at temperatures encountered in a refrigerator;  $45\pm^{\circ}\text{F}$  or  $7.2\pm^{\circ}\text{C}$ . The composition of the products resulting from winterization of cottonseed oil are presented in Table 17 (127). The solid-fat fraction that settles out is referred to as the stearine.

Winterization is a narrow form of fractionation. Both fractionation and winterization processing operations for edible oils basically consist of the separation of

**TABLE 17. Characteristics of Products from Cottonseed Oil Winterization.**

Cottonseed Oil Product	Unwinterized Cooking Oil	Winterized Salad Oil	Winterized Stearine
Fatty acid composition, %			
Myristic C14:0	0.8	0.7	0.6
Palmitic C16:0	24.2	22.6	32.4
Palmitoleic C16:1	0.6	0.4	0.3
Stearic C18:0	1.6	2.8	2.4
Oleic C18:1	21.0	19.8	17.2
Linoleic C18:2	51.8	53.7	47.1
Iodine value	107.8	111.4	98.0
Cold test, hours	0	5½ plus	0

oils into two or more fractions with different melting points. In the winterization process, the oils are cooled in a simple way, kept at the low temperatures for some time to crystallize solid-fat fractions that would normally cloud when the oil is held at refrigerator temperatures, and generally separated by filtration. With fractionation processes, cooling of the oil and the separation of the fractions are performed with more sophisticated techniques and controlled conditions to provide substances with unique properties.

The descriptive term of winterization evolved from the observation that refined and bleached cottonseed oil stored in outside tanks during the winter months physically separated into clear and hard fractions. Topping or decanting the clear oil from the top of the tanks provided an oil that remained liquid without clouding for long periods at cool temperatures. A need for a liquid oil with these characteristics was created by the introduction of the refrigerator for home use and the requirements of the mayonnaise and salad dressing industry. The indoors process developed to simulate the natural winter process consisted of a chilled room held at 42°F, or 5.6°C, with deep, narrow, rectangular tanks to provide the maximum surface exposure to cooling. Warm, dry, refined, and bleached cottonseed oil pumped into the chill room tanks began to cool and crystallize out stearine immediately but slowly. Convection heat transfer simulated the outside storage conditions. Agitation was avoided because it fractured the crystal, causing formation of small, soft crystals that were difficult to filter. Cooling with the 42°F, or 5.6°C, room temperature, which simulated mild winter conditions closely, required 3 days to produce the desired large crystals for filtering. After the oil temperature equated with the room temperature, it was held for several hours to allow the stearine or hard fraction to precipitate more fully. The stearine was separated from the liquid oil by filtering with plate and frame presses. Normally, the oil was gravity fed to the filters to avoid breaking up the crystals. Winterization is still performed with the classic technique described above. However, most processors have made equipment and process modifications to improve efficiency; such as jacketed, enclosed tanks equipped with programmable cooling and agitation, better filtration, improved pumping methods, and so forth.

**Fractionation.** Cottonseed oil has melting points spanning a range from -13.3°C, or 8°F, to 35°C, or 95°F, due to its triglyceride composition. Cottonseed oils triglyceride composition, as determined by high-performance liquid chromatography (80), liquidity zones (106), triglyceride melting points, and functionality (128) of each are compared in Table 18 (3). This range of melting points limits the applications for cottonseed oil, as well as all other edible fats and oils. Application potential can be increased with fractionation, a thermomechanical process by which an oil is separated into two or more portions. Thermomechanical separation processes include distillation and crystallization. Distillation is commercially unsuited for the separation of triglyceride mixtures because of their low vapor pressure and relatively low stability at high temperatures. However, separation can be effected by crystallization.

All edible fats and oils are polymorphic in their crystalline behavior. The three forms of importance are alpha, beta-prime, and beta, which are of increasing

**TABLE 18. Cottonseed Oil Triglyceride Functionality.**

Liquidity	Triglyceride Composition	HPLC Analysis, %	Melting Point		Functionality
			°F	°C	
Heated	PPO	2.2	95.0	35.0	Structure and Moisture Barrier
	SPL	1.5	86.0	30.0	
	PPL	7.1	81.0	27.2	
Room Temperature	POO	3.1	60.0	15.6	Room Temperature Clarity
	SOL	1.3	43.0	6.1	
	OOO	1.6	42.0	5.6	
	SLL	1.4	34.0	-1.1	
	OOL	3.1	30.0	-1.1	
Cool	POL	14.0	27.0	-2.8	Refrigerated Clarity
	PLL	27.5	22.0	-5.6	
	OLL	12.5	20.0	-6.7	
	LLL	19.0	8.0	-13.3	

P = Palmitic; S = Stearic; O = Oleic; L = Linoleic.

stability in that order. The rate of crystallization of the alpha form is greater than that of the beta-prime, which is greater than that of the beta polymorph. If supercooling is carried out too rapidly, crystallization of the alpha form occurs resulting in a mass of very small crystals. To obtain good separation of cottonseed oil fractions, crystallization is required in the beta-prime form. This is because the beta-prime crystals agglomerate into large aggregates that are firm and of uniform spherical size that separate well with filtration.

Fractional crystallization is a thermomechanical separation process wherein component triglycerides of oils are separated, usually as a mixture, by partial crystallization in a liquid phase. In the fractionation processes, three successive stages are recognized:

1. Cooling of the liquid oil to supersaturation, resulting in the formation of nuclei for crystallization;
2. Growth of the crystals by gradual cooling to a shape and size that permits efficient separation; and
3. Separation, isolation, and purification of the resultant crystalline and liquid phases.

In the fractionation process, the minor components of the original oil become concentrated in the separated fractions. This concentration has a considerable effect on the oxidative stability of the individual fractions. Relative to the starting oil, the liquid or soft fraction is enriched in tocopherols and depleted of trace metals. The reverse occurs with the hard or stearine fraction, which becomes appreciably more susceptible to oxidation despite its lower content of unsaturates. The stearine fraction is also the recipient of other impurities remaining in the oil after refining and bleaching, such as phosphatides and soap.



There are three processes in commercial use for the fractionation of edible fats and oils:

- Dry Fractionation - The principal of this fractionation process is based on the cooling of oil under controlled conditions without the addition of chemicals or solvents. The liquid and solid phases are separated by filtration.
- Detergent Fractionation - The oil is crystallized on its own similar to the dry fraction technique, but separation is affected by employing an aqueous detergent solution and centrifugation.
- Solvent Fractionation - The separation of component triglycerides that differ in solubility is accomplished by fractional crystallization of a solution of oil in an organic solvent, followed by separation of the solids from the liquid by filtration, and finally, removal of the solvent from the separated fractions by steam stripping. This process is the most versatile of the fractionation techniques presented.

Some processors have employed solvent fractionation systems to produce salad oil, which has demonstrated three major advantages over the traditional winterization process: (1) a considerably lower viscosity, which allows a faster crystal growth for more rapid stearine separation; (2) the cottonseed salad oil produced has a better resistance to clouding at cool temperatures for longer cold tests; and (3) less liquid oil is trapped in the stearine component for higher salad oil yields. An operational continuous solvent process was described by Cavanagh (129) for winterization of cottonseed oil. A 50% solution of oil in hexane is cooled rapidly with a heat exchanger to 20°F to 26°F, or -6.6°C to -3.3°C. After cooling, it passes through a continuous winterization column that cools with a series of agitated trays over a 40–60 minute period to temperatures as low as -40°F or -40°C. The stearine or solid stream is separated from the liquid miscella stream with a continuous solids discharge centrifuge. The solvent is removed from the salad oil portion with an evaporator system before deodorization. The comparison in Table 19 of

**TABLE 19. Cottonseed Salad Oil Stearine Analysis.**

Winterization Process	Conventional	Solvent
Iodine value	95.5	71.6
Solids fat index, % at:		
10° or 50°F	21.6	52.3
21.1°C or 70°F	1.3	33.7
26.7°C or 80°F		1.2
33.3°C or 92°F		0.1
Fatty acid composition, %		
Myristic C14:0	0.7	0.6
Palmitic C16:0	34.6	52.1
Palmitoleic C16:1	0.6	0.8
Stearic C18:0	2.1	1.9
Oleic C18:1	15.8	9.1
Linoleic C18:2	46.2	35.5

conventional versus solvent process produced stearine confirms that the solvent system produces a harder, firmer, more compact stearine crystal with less entrapped oil than the conventional process.

Fractionation technology, in particular solvent fractionation, has been utilized to produce some very highly specialized edible oil products. High-stability liquid oils, with AOM stability results of 350 hours minimum without the benefit of added antioxidants, and cocoa butter equivalents are two examples of products that can be produced with fractionation technology. Fractionation technologies may also be used to produce basestocks for utilization as components in finished products for various applications.

**Hydrogenation.** The hydrogenation process is an important tool for the edible fats and oils processors. With hydrogenation, cottonseed oil can be converted from a liquid oil into a plastic or solid fat more suitable for numerous applications. There are two reasons to hydrogenate fats and oils: (1) to convert naturally occurring oils into physical forms with melting and handling characteristics more suited to the desired product functionality; and (2) to improve oxidative stability, which provides prolonged organoleptic acceptability. A wide range of edible oils products can be produced with the hydrogenation process, as shown previously (see Figure 3), depending on the conditions used, the starting oils, and the degree of saturation or isomerization.

During the hydrogenation process, hydrogen is chemically attached at the double bond sites on the carbon chain of the unsaturated fatty acids. This reaction eliminates a double bond and converts an unsaturated fatty acid to a more saturated fatty acid. Isomerization during the hydrogenation process can also create *trans*-isomers. Both of these chemical changes increase the melting point of the reacted oil.

Typically, an unhardened cottonseed oil is composed of 2.5% stearic fatty acid and 22–23% palmitic fatty acid, which are the principal saturated fatty acids. The unsaturated fatty acids are composed of approximate 18% oleic, 54% linoleic, and less than 1% each of linolenic and palmitoleic fatty acids. If the hardening reaction was completely selective, before any hydrogen was absorbed by oleic fatty acid the linoleic fatty acid would have to be completely converted to oleic. On the disappearance of the linoleic fatty acid, the oleic would next absorb hydrogen to be converted to the fully saturated stearic fatty acid. This degree of selectivity is never attained in practice but how closely it is approximated depends on the catalyst type and dosage, temperature, and pressure.

Hydrogenation can take place only when the three reactants have been brought together: unsaturated oil, catalyst, and hydrogen gas. The hydrogen gas must be dissolved in the liquid before it can diffuse through the liquid to the solid catalyst surface. Each absorbed unsaturated fatty acid can then react with a hydrogen atom to complete the saturation to the double bond, shift it to a new position, or twist it to a higher melting *trans*-form. When the unsaturated oil to be hydrogenated contains mono-, di-, and tri-unsaturates, there is competition for the catalyst surface. The di- and tri-unsaturates are preferentially absorbed from the oil to the catalyst surface and partially isomerized or hydrogenated to a mono-unsaturate until their

concentration is very low, permitting the mono-unsaturate to be absorbed and reacted. The variables that can affect the hydrogenation reaction are temperature, agitation, hydrogen pressure, type and quantity of catalysts, feedstock quality, and the fatty acid composition of the source oil. The effects of these variables are (106) described below.

- Temperature - Hydrogenation is operated at temperatures ranging from 150–230°C or 300–450°F. Hydrogenation, like most chemical reactions, proceeds at a faster rate at higher temperatures. The hydrogenation rate is increased as the temperature is elevated resulting from an increase in hydrogen gas solubility in the liquid oil. Higher reaction temperatures increase selectivity and *trans*-isomer formation and hydrogenation rate to provide steep solid-fat index curves. Hydrogenation is an exothermic reaction; it creates heat as long as the reaction is active. The reaction temperature is increased by 1.6–1.7°C, or 2.9–3.1°F, for each iodine value drop.
- Agitation - The function of agitation is to supply dissolved hydrogen gas to the catalyst surface. Agitation decreases selectivity and isomerization by keeping the catalyst supplied with sufficient hydrogen to increase the reaction rate.
- Hydrogen Pressure - Most edible oil hydrogenations are performed at hydrogen pressures ranging from 0.8–4.0 atmospheres. At low pressure, the hydrogen gas dissolved in the oil does not cover the catalyst surface, while at high pressure, hydrogen is readily available for saturation of the double bonds. Higher pressure increases the saturation rate, which results in a decrease in *trans*-formation and selectivity to produce flatter SFI curves.
- Catalyst Level - Hydrogenation reaction, selectivity and *trans*-isomer formation increase as the catalyst concentration is increased, up to a point where it levels off. The rate increase is caused by increased activity at the catalyst surface. The maximum is reached when hydrogen will not dissolve quickly enough to supply the catalyst levels.
- Catalyst Type - Nickel metal catalyst, sometimes promoted with copper, aluminum oxide, or sulfur, are commonly used in commercial hydrogenation. These catalysts are prepared by a variety of techniques, some proprietary to the catalyst supplier, to provide the surface activity necessary for the desired selectivity. Precious metals have been found to be effective hydrogenation catalysts, which are more active at lower temperatures and produce less *trans*-isomers. However, their use has been deterred by the initial cost and recovery problems associated with the minute quantities required.
- Source Oil - Hydrogenation selectivity also depends on the type of unsaturated fatty acids available and the number of unsaturated fatty acids per triglyceride. Those oils with high-linolenic and high-linoleic fatty acid levels hydrogenate more rapidly and to higher melting points than oils with high-oleic fatty acid levels. The relative hydrogenation reactivity for the 18-carbon fatty acids was described by Hastert (130),

Fatty Acid		Relative Reactivity
Linolenic	C18:3	40
Linoleic	C18:2	20
Oleic	C18:1	1

Cottonseed oil with a high-linoleic fatty acid content and a saturated fat level higher than any of the other linoleic-classification oils requires less hydrogenation to attain the same degree of hardness.

**Hydrogenation Systems.** Batch hydrogenation is most commonly used for edible oil processing because of the simplicity and flexibility for use with different source oils. Essentially all that is required is a reaction vessel, usually referred to as a converter, that can withstand 7- to 10-bar pressure, with an agitator, heating and cooling coils, a hydrogen gas inlet, piping and pumps to move the oil in and out, and a sample port for process control of the reaction. The converter must also be provided with the means to control the three reaction variables of pressure, temperature, and rate of reaction.

Continuous hydrogenation systems have been available for quite some time, but their commercial usage has been limited for several reasons. The maximum value for any continuous operation is realized when it is used to produce large quantities of the same product. As most edible oil processors produce a variety of products, several different basestocks are routinely required that can be produced more efficiently, with better uniformity using batch hydrogenation systems.

**Basestock Systems.** Most prepared foods are formulated with ingredients designed for their application or, in many cases, specifically for the particular product or processing technique employed by the food processor. These customer-tailored products have expanded the product base for edible oil processors from a few basic products to literally hundreds. Each of these products could be formulated to require a slightly different hydrogenated oil. This practice, with the ever increasing number of finished edible oil products, could be an inventory nightmare with a large number of product heels remaining from overproduction waiting for the next production run. Basestock systems with a limited number of hydrogenated stock products for blending to meet the finished product requirements are used by most processors for better control and efficiency.

Basestock requirements will vary with each processor, depending on the customer requirements, which dictate the finished products produced. A basestock system can include several source oils, or be limited to a single source oil if that oil's composition can provide all of the properties necessary for the finished product's functionality. Cottonseed oil is the closest to meeting these qualifications for most product applications: It has a good oxidative stability because of the higher level of natural saturated fatty acids; it has a high-palmitic fatty acid content, which helps to make it stable in the beta-prime crystal form; and it is a rich source of linoleic acid, an essential fatty acid that the human body cannot synthesize, when not hydrogenated to a great extent. Table 20 (131) presents a basestock system that uses only cottonseed oil. Use of this basestock system should enable a processor to

TABLE 20. Cottonseed Oil Basestock System.

Basestock Type	RB	~ ~ Flat ~ ~	~ ~ ~ Steep ~ ~ ~	Saturated			
Iodine Value	110	80	75	70	65	58	>5
Mettler dropping point, °C	~	32.0±	34.0±	36.0±	38.0±	43.5±	Too Hard
Solids fat index, % at:							
50°F or 10°C	~	20.5±	37.0±	47.0±	63.0±	Too Hard	
70°F or 21.1°C	~	8.0±	10.5±	21.5±	30.5±	51.0±	Too Hard
80°F or 26.6°C	~	5.0±	7.0±	14.0±	18.0±	40.0±	Too Hard
92°F or 33.3°C	~	1.5±	2.5±	3.5±	10.5±	25.0±	Too Hard
104°F or 40°C	~	~	~	~	~	10.5±	Too Hard
Titer, °C	~	~	~	~	~	~	60.0±
Fatty Acid Composition, %							
Myristic C14:0	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Palmitic C16:0	22.5	22.6	22.7	22.7	22.6	22.9	23.2
Palmitoleic C16:1	0.7	0.6	0.6	0.5	0.5	0.5	~
Stearic C18:0	2.5	5.7	6.8	3.9	7.0	7.4	71.3
Oleic C18:1	18.2	48.2	51.0	61.4	62.6	65.0	4.3
Linoleic C18:2	54.1	21.5	17.7	10.0	6.2	2.4	~
Linolenic C18:3	0.8	0.2	~	0.3	~	~	~
Arachidic C20:0	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Behenic C22:0	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Hydrogenation conditions:	None	Nonselective	~ ~ ~ Selective ~ ~ ~	Nonselect			
Gassing temp., °F	~	~ ~ 300 ~ ~	~ ~ ~ ~ 300 ~ ~ ~ ~	300			
Hydrogenation temp., °F	~	~ ~ 350 ~ ~	~ ~ ~ ~ 440 ~ ~ ~ ~	450			
Pressure, bar	~	~ 2.0 to 3.0 ~	~ ~ ~ 0.7 to 1.0 ~ ~ ~	5.0			
Catalyst, % Nickel	~	~ ~ 0.02 ~ ~	~ ~ 0.04 to 0.08 ~ ~	0.4 to 0.8			
Agitation	~	~ ~ Fixed ~ ~	~ ~ ~ Fixed ~ ~ ~	Fixed			

± Stands for a range of values.

produce most formulated edible oil products by blending two or more of the basestocks, except for some specialty products that can only be made with special hydrogenation conditions or a lauric oil. The basestocks represent saturation levels, starting with natural cottonseed oil followed by six hydrogenated basestocks ranging from an iodine value of 80 to almost complete saturation. The basestock types are as follows:

- RB—RB is an abbreviation for *refined and bleached*. Oils are refined and bleached before hydrogenation to remove impurities and catalyst poisons.
- Flat—Many food products require a shortening, margarine, or tailored formulated oil product that has an extended plastic range with a good oxidative stability. These products must be relatively soft, with a plastic or workable consistency at room temperature while still possessing some body at temperatures of 37.8°C or 100°F, and with a melting point only slightly above body temperature. So-called nonselective hydrogenation conditions are used to produce a flat SFI curve basestock for these products. A flat SFI curve are those SFI values that provide east to west (x-axis) directional slopes when plotted on a graph.

- Steep—Poor plasticity but good mouthfeel and oxidative stability are indicated by a north-to-south (y-axis) directional SFI slope, usually referred to as steep. A narrower span of temperatures with higher SFI values provides sharper melting tendencies for an improved palatability and a good oxidative stability. The preferred bland flavor is retained longer because of a lower unsaturated fatty acid content. However, steep SFI curves produced with hydrogenation usually indicate a higher *trans*-fatty acid content.
- Saturated—Technically, an oil should have to have a zero iodine value to be designated as fully saturated. In theory, it is possible to attain a zero IV, but it is impractical in production. These basestocks are also referred to as fully hydrogenated fats, low iodine value hardfats, stearines, or hardfats. Beta prime hardfats are added to softer basestocks to extend the plastic or workable range, to improve the product's tolerance to high temperatures, and to establish the finished product's crystal habit. Hydrogenation conditions to produce hardfats is the least critical of all hydrogenation operations because neither selectivity nor isomerization are factors, and unsaturation is very minor because the oil is almost totally saturated.

**Post Bleaching.** A separate bleaching operation immediately after the hydrogenation process has three purposes: (1) to ensure that all traces of the prooxidant hydrogenation catalyst that may have escaped the filtration system after hydrogenation have been captured, (2) to remove undesirable colors that may have been accentuated during hydrogenation, and (3) to remove peroxide and secondary oxidation products. Postbleach systems can be exact duplicates of the prebleach process. One difference is that a chelating acid, either citric or phosphoric, should be used to ensure that any residual nickel content is reduced to the lowest possible level. Batch systems are preferred for postbleaching over continuous for the same reasons as for hydrogenation systems—production of a wide variety of hydrogenated basestocks (132). Postbleaching systems are also used to remove undesirable impurities formed during other modification processes, such as interesterification, fractionation, and blending.

**Interesterification.** The least known and practiced processing technique available to the fats and oils processor for modification of the physical properties of an oil is interesterification, often referred to as rearrangement. The ability to modify the melting point and functional crystallization characteristics without changing the fatty acid composition makes interesterification a process with a number of unique possibilities. The benefits of the random interesterification processes are summarized in Table 21 (133).

**TABLE 21. Random Interesterification Process Benefits.**

Modified Characteristics	Unaffected Characteristics
Melting point	Iodine value
Solids fat index	Fatty acid composition
Crystal habit	<i>Trans</i> -isomer content
Oxidative stability	Nutritional aspects

**TABLE 22. Interesterification Process Comparison.**

Process	Random	Directed
Type reaction	Nonselective	Selective
Reaction time	13 to 30 minutes	6 to 24 hours
Catalyst usage, %	0.05 to 0.1	0.1 to 0.3
Temperature, °F	90 to 140	20 to 60
Filtration required	none	yes

One of the most important types of interesterification with respect to applications is chemically catalyzed random-ester interchange. Natural fats and oils have specific fatty acid distributions and positional arrangements that determine their physical and functional characteristics such as melting point and oxidative stability. During the interesterification process, the triglyceride ester bonds are broken, the resulting fatty acids mix together, and eventually they reattach. If the fatty acids reattach to the same glycerol molecules, the reaction is called *intraesterification*. When the fatty acids attach to different glycerol molecules, it is called *interesterification*. A random equilibrium arrangement of the fatty acids is reached if the reaction is allowed to continue long enough. The primary benefits are modification of the physical properties, such as, melting point and solid fat index values, and modification of the crystal form tendencies. Similar types of physical changes are possible with hydrogenation or fractionation, but both of these processes change the fatty acid distribution of the final product.

Two basic types of chemical interesterification are practiced: random and directed. Both involve the use of transition metals, such as sodium, or more commonly derivatives, such as sodium methoxide, as a catalyst. The differences between these two interesterification reactions are summarized in Table 22.

Random chemical rearrangement of fats and oils can be accomplished with either a batch or continuous process. Both perform the three important rearrangement steps: (1) pretreatment of the oil, (2) reaction with the catalyst, and (3) deactivation of the catalyst. A typical batch rearrangement reaction vessel is equipped with an agitator, coils for heating and cooling, nitrogen sparging, and vacuum capabilities. The process steps are as follows:

1. Heat the oil to 250°F to 300°F or 120°C to 150°C in the reaction vessel under a vacuum to dry the oil. Drying is critical because moisture deactivates the catalyst.
2. After drying, cool the oil to the reaction temperature, suck in the catalyst with the vacuum, and agitate for 30 to 60 minutes or until the distinctive brown color is formed indicating randomization. Analyze the reaction mixture with a refractometer to determine if the reaction is complete or requires additional catalyst and/or time to reach the predetermined analytical endpoint.

3. When reaction completion is confirmed, the catalyst is neutralized either with the addition of phosphoric acid or water washing.
4. The neutralized interesterified oil must be postbleached to remove the brown color formed during the reaction before blending and/or deodorization.

In directed rearrangement processes, one or more of the trisaturated glycerides are selectively removed from the ongoing reaction when the mixture is cooled below its melting point. This selective crystallization upsets the equilibrium, forcing the reaction to produce more trisaturated glycerides to reestablish equilibrium. Continuous processes are normally used for directed interesterification because the batch process is difficult to control and a number of reaction vessels would be required. Scraped-wall heat exchangers cool the mixture to initiate crystallization and later remove the heat liberated when the trisaturates crystallize. The trisaturates formed can be separated by filtration, or the reaction can be controlled to produce the trisaturate level desired for a compound-type shortening formulation. In either case, the reaction is usually terminated by adding water to inactivate the catalyst. After neutralization of the catalyst, the liquid soap phase is normally removed with centrifugation and vacuum drying followed by bleaching to remove the undesirable impurities formed.

Table 8 lists the triglyceride composition for cottonseed oil as determined by HPLC. The triglyceride distribution for cottonseed oil is considered even or nonrandom because the saturated fatty acids are positioned predominately in the *sn*-1 or -3 positions with the unsaturated fatty acids in the *sn*-2 position. Random rearrangement shuffles the fatty acid distribution to effect a melting point increase from 50.9°F or 10.5°C to 93.2°F or 34°C (134). With directed interesterification, a solid fat may be produced using natural cottonseed oil as the feedstock. The trisaturated triglycerides are crystallized and precipitate as they are formed. The unsaturated portion that remains may be used as a salad oil, as most of the saturated fatty acids have been removed (29). Interesterification can be used to produce basestocks similar to those produced with hydrogenation or fractionation for blending to formulate shortenings, margarines, high-stability liquid oil products, and other specialty products for the food processor.

**Blending.** Various basestocks are blended to produce the specified composition, consistency, and oxidative stability for edible fats and oils products, such as shortenings, frying fats, margarine oils, specialty products, and even some salad oils. The basestocks may be composed of natural source oils and/or modified oils produced with hydrogenation, fractionation, or interesterification processes. Blends are made to meet both the composition and analytical consistency controls identified by the product developers and quality assurance. The consistency controls can include specific limits for SFI, IV, melting point, fatty acid composition, or other analysis specific to the physical characteristics of the particular product. The blending process requires storage tanks to inventory the basestocks and scale tanks and/or meters to proportion the basestocks accurately for each different product. The blend tanks should be equipped with agitators and heating coils to assure a uniform blend (106).



**Deodorization.** Edible fats and oils retain undesirable flavors and odors after refining, and they develop other organoleptic undesirables during bleaching, hydrogenation, fractionation, and interesterification processing. Deodorization is basically a vacuum-steam distillation process operated at elevated temperatures to remove FFA and other volatile odoriferous components that cause the undesirable flavors and odors. Additional deodorization benefits include heat bleaching to destroy carotenoid pigments, pesticide removal, and cyclopropanoid fatty acid reduction to a negligible level, all of which ensure oil purity. Deodorization is the last major processing step where flavor, odor, and many of the other qualities of an edible fat and oil product can be controlled. From this point forward, all efforts are directed toward retaining the quality of the deodorized product.

Experience has shown that edible fats and oils flavor and odor removal correlates well with the reduction of FFA. The odor and flavor of an oil with a 0.1% FFA will be eliminated when the FFA is reduced to 0.01% to 0.03%, assuming a zero peroxide value. Therefore, all commercial deodorization consists of steam stripping the oil for FFA removal. Typical conditions practiced in the United States for the three deodorizer system types are shown in Table 23. The four interrelated operating variables that influence deodorizer design are vacuum, temperature, stripping rate, and retention time at deodorization temperatures.

**TABLE 23. Typical Deodorization Conditions.**

Deodorization Conditions	Range
Vacuum, absolute pressure, mbar	2 to 4
Deodorization temperature, °F	410 to 500
Deodorization temperature, °C	210 to 260
Retention time at deodorization temperature:	
Batch deodorizer, hours	3 to 8
Continuous deodorizer, minutes	15 to 120
Semicontinuous deodorizer, minutes	15 to 120
Stripping steam, weight percent of oil	
Batch deodorizer	5 to 15
Continuous deodorizer	1 to 5
Semicontinuous deodorizer	1 to 5
Drop temperature	
Liquid oils, °F	100 to 120
Liquid oils, °C	37.8 to 48.9
Higher melting products:	
°F above melting point	10 to 15
°C above melting point	5.5 to 8.5
Product free fatty acid, %	
Feedstock	0.05 to 6.0
Deodorized Product	0.02 to 0.03
Product peroxide value, meq/kg	
Feedstock	2.0 max
Deodorized product	zero
Deodorized product flavor	bland

- Vacuum—Deodorization must be carried out at an extremely low absolute pressure to promote boiling of fatty acids like palmitic, stearic, and oleic to permit distillation of the odoriferous substances.
- Temperature—Increases in temperature combine with decreases in pressure to accelerate deodorization. However, an excessive increase in temperature promotes polymerization, *trans*-isomer development, excessive removal of tocopherols and sterols, color reversion, and the formation of odoriferous low-boiling products.
- Stripping Steam—Injection of steam into the oil modifies the vapor pressure of the materials to be distilled to effect deodorization. Adequate stripping steam, consistent with the temperature and pressure, is required; however, too much live steam may cause hydrolysis, recreating FFA.
- Retention Time—Retention time is the period during which the oil is at deodorization temperature and subjected to stripping steam. Factors such as temperature, vacuum, depth of the oil layer, and product type can vary the retention time required to properly deodorize an oil.

Deodorization is a multistep process that includes deaeration, heating, deodorization/deacidification, cooling, metal chelating, and polish filtration. Deodorization equipment in current use can be classified into three principal types: batch, continuous, and semicontinuous. Batch deodorization, the original method used for edible oils, consists of a vacuum vessel containing internal heating and cooling coils, stripping steam injection apparatus, steam-jet ejector vacuum equipment, high-temperature heating media source, and polish filtration. These units are now less popular because of (1) high stripping steam requirements, (2) high vacuum ejector motive steam consumption (3) long deodorization cycle time, and (4) the finished product quality is not consistent. Batch systems are still used in circumstances where low construction costs, small capacities, and specialized or gourmet end products are involved.

Continuous deodorization systems are used when the feedstock is constant and the same material is processed for several days. These systems generally have a modest investment cost with the greatest energy efficiency and, therefore, the lowest operating cost. The continuous flow allows uniform temperatures during heating and cooling, permitting smaller ancillary equipment. The general approaches for continuous deodorizer design are to carry out heating, cooling, and heat recovery in exchangers external to the deodorizer, or to do the heating, cooling, and heat recovery within the deodorizer unit. The internal approach is less efficient for heat recovery but affords a more efficient, more reliable method for product changes than the external heat exchange. Continuous deodorization benefits are lost with as few as two or three stock changes in a 24-hour period because of loss of production time and likelihood of commingling products.

Semicontinuous systems operate on the basis of handling finite batches of oil in a timed sequence of deaeration, heating, holding-steam stripping, and cooling so that all of the oil is completely subjected to each condition before proceeding to the next

step. Most semicontinuous deodorizers consist principally of a tall cylindrical shell of carbon steel construction with five or more type-304 stainless steel trays stacked inside of the outer shell. Each tray is fitted with a steam sparge and is capable of holding a measured batch of oil. By means of a measuring tank, oil is charged to the top tray, where it is deaerated while being heated with steam to about 320°F to 330°F or 160°C to 166°C. At the end of the heating period, the charge is automatically dropped to the second tray, and the top tray is refilled with oil. In the second tray, the oil is heated to the operating temperature and, again after a timed period, is automatically dropped to the tray below. When the oil reaches the bottom tray, it is cooled to 100°F to 130°F or 38°C to 54°C and discharged to a drop tank from which it is pumped through a polishing filter to storage.

**Deodorization Process Control.** Deodorization is the last processing step, in which flavor, odor, and many of the stability qualities of an edible fat and oil product can be controlled. To produce quality deodorized products, attention must be focused on all of the factors involved with the process. The deodorization physical process removes the volatile, odoriferous materials present in the oils. The other factors that influence the quality of the deodorized oil products are as follows (106):

- **Undeodorized oil preparation**—The first process control requirement is to assure that the processing of the oil prior to deodorization has been preformed properly. Preparation of the oil before deodorization has a significant effect on the product after deodorization. For example, deodorization will remove the hydroperoxides from abused oils, but the secondary oxidation products formed will accelerate the rate of oxidation during storage to compromise the flavor and odor. With proper process control, the abused oil would have been bleached prior to deodorization to remove the aldehydes and ketones that make up the secondary oxidation products. Two other deleterious impurities that deodorization will not remove are soap and phosphatides, which must be eliminated in the up-stream processes.
- **Air elimination**—Oils must be protected from air before, throughout, and after the deodorization process. At deodorization temperature, the oil reacts instantly with oxygen to become oxidized, which, in turn, causes flavor reversion. Following are recommended procedures to eliminate potential air sources before and during deodorization: (1) Deaeration of the feedstock is essential to remove oxygen from previous exposure; (2) air leaks at fittings below the oil level and in external pumps, heaters, and coolers should be checked and stopped; and (3) the stripping steam must be generated from deaerated water to be oxygen-free. After deodorization, the oils must be protected from air to preserve the deodorized oil quality. The usual procedure is to replace air with nitrogen. Oxygen contact can be reduced considerably by keeping the entire handling system after deodorization protected with an inert gas like nitrogen. Gas-free finished oil can be delivered from the deodorizer to a storage tank under a complete nitrogen blanket, or the oil can be sparged with nitrogen at the deodorizer exit. Thereafter, effective protection against

oxidation requires the product to be protected by nitrogen gas in the storage tanks and bulk transports, as well as in packaging.

- **Metal chelating**—Trace metals may be absorbed from the soil by the plants during the growing season, and later in the oil from contact during crushing, storage, and the other processes and transfers. Many of the trace metals promote autoxidation, which results in off-flavors and odors accompanied by color development. Studies have identified copper as the most harmful metal, with iron, manganese, chromium, and nickel following. The effects of prooxidants can be diminished by using chelating agents before and after deodorization. The most commonly used chelating agents are citric acid, phosphoric acid, and lecithin. Deodorized oils are usually treated with citric acid during the cooling cycle at 50 to 100 ppm.
- **Oil polishing**—The final stage of deodorization is filtration of the oil to remove any fine particles of soaps, metallic salts, rust, filter aid, polymerized oil, or any other solid impurities.
- **Antioxidant addition**—Vegetable oils contain tocopherols that are natural antioxidants. Tocopherols are removed in the refining, bleaching, and deodorization processes, but enough survive to provide the optimum stability available from the natural antioxidants. Several phenolic compounds have been identified that can also provide oxidative stability and longer shelf life for fats and oils by delaying the onset of oxidative rancidity. Phenolic substances or antioxidants function as free radical acceptor, thereby terminating oxidation at the initial step. Several antioxidant compounds are available, but tertiary-butylhydroquinone (TBHQ) is the most effective antioxidant for vegetable oils.

## **5. REGULATORY CONSIDERATIONS: COTTONSEED OIL EXTRACTION AND PROCESSING**

Many workplace, environmental, food safety, and other regulations apply to oilseed and oil processors (55). Some of the regulations required in the United States are discussed. Many other countries have similar requirements, but if they do not, it would be prudent for oilseed solvent extraction operations and vegetable oil processors to consider meeting these regulations and for these industries to have environmental, health and safety, and quality management programs (135, 136).

### **5.1. Workplace Regulations (OSHA)**

Workplace regulations are promulgated and enforced in the U.S. by the Occupational Safety and Health Administration (OSHA), which is part of the U.S. Department of Labor, under the Occupational Safety and Health Act (OSH Act; PL 91-596 as amended by PL 101 552; 29 U.S. Code 651 et. seq.). OSHA general industry health and safety standards (29 CFR 1910) apply to oilseed extraction and oil

refining. In addition, even if there is not a specific standard, OSHA can site a facility under the “general duty clause” [Sec. 5(a)(1) of the OSH Act], because the OSH Act requires the employer to maintain a safe and healthful workplace.

Some health and safety standards that affect cottonseed oil extraction and processing are as follows:

1. *OSHA Health Standards:*

- a. Air Contaminants Rule, 29 CFR 1910.1000 (the permissible exposure limit [PEL] for n-hexane is 500 ppm [1800 mg/m<sup>3</sup>], 8-hr time-weighted-average; there are PELs for sodium hydroxide, sulfuric acid, vegetable oil mist, nuisance dust, etc.)
- b. Hazard Communication Standard, 29 CFR 1910.1200
- c. Cotton Dust Standard, 29 CFR 1910.1043 (cottonseed oil mills have medical surveillance, recordkeeping, and reporting requirements)
- d. Bloodborne Pathogens, 29 CFR 1910.1030
- e. Occupational Exposure to Hazardous Chemicals in Laboratories, 29 CFR 1910.1450

2. *OSHA Safety Standards:*

- a. Process Safety Management, 29 CFR 1910.119 (for n-hexane)
- b. Emergency Action Plan, 29 CFR 1910.38(a)(1)
- c. Fire Prevention Plan, 29 CFR 1910.38(b)(1)
- d. Fire Brigades, 29 CFR 1910.156
- e. Permit-required Confined Space, 29 CFR 1910.146
- f. Lockout-Tagout, 29 CFR 1910.147
- g. Occupational Noise Exposure, 29 CFR 1910.95 and Hearing Conservation Program, 29 CFR 1910.95(c)
- h. Personal Protection Equipment:
  - General Requirements, 29 CFR 1910.132
  - Eye and Face Protection, 29 CFR 1910.133
  - Respiratory Protection, 29 CFR 1910.134
  - Head Protection, 29 CFR 1910.135
  - Foot Protection, 29 CFR 1910.136

## 5.2. Environmental Regulations (EPA)

The U.S. Environmental Protection Agency (EPA) administers all regulations affecting the environment and chemicals in commerce. EPA regulations are intended to protect human health and welfare and the environment. The individual states and state environmental regulatory control boards implement and enforce most of the regulations.

The legislation that serves as the basis for the regulations can be divided into:

1. Statutes that are media-specific [Clean Air Act (CAA) and Clean Water Act (CWA)]
2. Statutes that manage solid and hazardous waste [Resources Conservation and Recovery Act (RCRA) and Comprehensive Environmental Response, Compensation and Liability Act (CERCLA; “Superfund”)]
3. Statutes that directly limit the production rather than the release of chemical substances [Toxic Substances Control Act (TSCA) and Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)]

Some of the more important environmental regulations that affect oilseed extraction and processing are as follows:

1. *Clean Air Act (CAA; 42 U.S. Code 7401 et seq.)*. States and state air control boards are required to implement regulations and develop state implementation plans (SIP) (137). Hazardous air pollutants (HAP), such as n-hexane, are regulated with *National Emissions Standards for Hazardous Air Pollutants (NESHAP)* and criteria pollutants [e.g., ozone (O<sub>3</sub>), particulate matter (PM), nitrogen oxides (NO<sub>x</sub>), sulfur oxides (SO<sub>x</sub>), carbon monoxide (CO), and lead (Pb)] are regulated with *National Ambient Air Quality Standards (NAAQS)*.

n-Hexane is a regulated HAP but isohexane and acetone are not. Regulated criteria pollutants, such as O<sub>3</sub>, PM, CO, and NO<sub>x</sub>, also are emitted during the extraction and refining of cottonseed oil.

- a. *Hazardous Air Pollutants (HAP) or Air Toxics (40 CFR 61)*: If a facility is a major emitter of n-hexane, the EPA requires sources to meet national emissions standards (119). The air toxic control measures for source categories are technology-based emission standards (not health based) established for major sources (10 tons/yr of one HAP or 25 tons/yr of total HAP) that require the maximum degree of reduction emissions, taking costs, other health and environmental impacts, and energy requirements into account. Compliance with a NESHAP involves the installation of Maximum Achievable Control Technology (MACT), which essentially is maximum achievable emission reduction. The NESHAP for Solvent Extraction for Vegetable Oil Production (4/12/01, 65 FR 34252; 40 CFR 63 subpart GGGG) requires all existing and new solvent-extraction processes that are major sources to meet HAP emission standards, as a 12-month rolling average based on a 64% n-hexane content. Solvent-extraction facilities covered are those that produce crude vegetable oil and meal products by removing crude oil from listed oilseeds (corn germ, cottonseed, flax, peanuts, rapeseed, safflower, soybeans, and sunflower) through direct contact with solvent. HAP emission standards (solvent loss factor) for cottonseed oil production are: for cottonseed, large

(process > 120,000 tpy) 0.5 gal/t and for cottonseed, small (< 120,000 t/yr) 0.7 gpt. Facilities have until 4/12/04 to get into compliance. As the emission loss factor values are 12-month rolling averages, the first compliance report would be due no sooner than 48 months after the standard was promulgated (i.e., 4/12/05).

- b. *NAAQS*: The NAAQS are set at levels sufficient to protect public health, including the health of sensitive populations (primary air quality standards) and public welfare (secondary air quality standards) from any known or anticipated adverse effect of the pollutant with an adequate (appropriate) margin of safety.

Volatile organic compounds (VOC) are essentially considered the same as the criteria pollutant ozone (119). n-Hexane and hexane isomers are VOCs. Most U.S. cottonseed oil extracting facilities would be major sources of VOCs and would be covered by the requirements for ozone emissions and attainment, unless they used a solvent that was not classified as a VOC (e.g., acetone).

Most vegetable oil production facilities are major sources of particulate matters (PM). Depending on the oilseed processed, PM emissions can be 0.1–0.3 lbs of total suspended particulate (TSP), which is about 50% PM<sub>10</sub> (PM smaller than 10 microns) and less than 2% PM<sub>2.5</sub> (PM smaller than 2.5 microns), per ton of seed processed. PM controls would also have to be part of a facility's federal and state permits. Cottonseed oil production facilities probably also have to include NO<sub>x</sub>, SO<sub>x</sub>, and CO emissions in their federal and state permits.

Any new or significantly modified facility would have to comply with the new source review (NSR) requirements. NSR is a preconstruction permitting program. If new construction or making a major modification will increase emissions by an amount large enough to trigger NSR requirements, the source must obtain a permit before it can begin construction.

- c. *Odor*: There are no specific federal regulations for odor. States can, however, regulate odor if they choose to.
- d. *Federal Permits (40 CFR 70)*: All major sources of regulated solvents are required to have federally enforceable operating permits (FOP) (137) (also referred to as Title V permits).
- e. *State Permits*: Most states require state permits for facilities that emit listed air pollutants (119). In some states, federal permits and state permits are combined, while in other states, facilities are required to have both a state or county (air district) permit and a federal permit. As part of annual emission inventory reporting requirements, many states already require reporting of HAP and VOC because of their state implementation plan (SIP).
2. *Clean Water Act (CWA; 33 U.S. Code 1251 et seq.)*. Under the CWA, the U.S. EPA establishes water quality criteria used to develop water quality standards, technology-based effluent limitation guidelines, and pretreatment standards

and has established a national permit program [National Pollution Discharge Elimination System (NPDES) permits; 40 CFR 122] to regulate the discharge of pollutants. The states have responsibility to develop water quality management programs.

Oilseed processing and oil refining are covered by the following (138):

- Basic discharge effluent limitations that require NPDES permits (40 CFR 122)
  - Stormwater regulations that require a stormwater permit (40 CFR 122 and 123)
  - Oil spill prevention and response plans (40 CFR 112)
3. *Resource Conservation and Recovery Act (RCRA; 42 U.S. Code 6901 et seq.)*. RCRA Subtitle D covers nonhazardous wastes. Subtitle C (40 CFR 261) is a federal “cradle-to-grave” system to manage hazardous waste (including provisions for cleaning up releases and setting statutory and regulatory requirements). Materials or items are hazardous wastes if and when they are discarded or intended to be discarded. Hazardous wastes are either listed wastes (40 CFR 261.30-.33) or characteristic wastes (40 CFR 261.21-.24). The U.S. EPA defines four characteristics for hazardous waste: ignitability (40 CFR 260.21); corrosivity (40 CFR 260.22); reactivity (40 CFR 260.23); and toxicity (40 CFR 260.24).
- a. *Spent bleaching clay* is not a RCRA hazardous waste (40 CFR 302). It is usually disposed of by taking it to a regular landfill. There sometimes can be a spontaneous combustion (oxidation of unsaturated fatty acids in the retained oil causing self heating leading to combustion) problem when it is taken to the landfill. The potential for spontaneous combustion in bleaching earth depends on the type and amount of oil retained and rises with increasing unsaturation of the fatty acids in the retained oil. U.S. DOT classifies materials liable to spontaneous combustion as Class 4.2 hazardous materials [49 CFR 173.124 (b) and Appendix E 3]. Spent bleaching clay can be finely ground and put in small quantities into the animal meal in operations that do oil extraction. With the increased concern about dioxin in food and feed product by FDA and EPA, this is discouraged.
- b. *Spent nickel catalyst* is not considered a RCRA hazardous waste (40 CFR table 302.4). No reporting of release of this substance is required if the diameter of the solid metal released is equal to or exceeds 100  $\mu\text{m}$ . The RQ for particles greater than 100  $\mu\text{m}$  is 100 lbs. Most, if not all, spent nickel catalyst is recycled.
4. *Emergency Planning and Community Right-to-Know Act (EPCRA; 42 U.S. Code 11001 et seq.)* EPCRA requires states to establish emergency planning districts with local committees to devise plans for preventing and responding to chemical spills and releases.



*Section 313 (40 CFR 372), Toxic Release Inventory (TRI):* Businesses are required to file annual reports with federal and state authorities of releases to air, water, and land above a certain threshold for chemicals on the TRI/Section 313 list (40 CFR 372.65) by July 1 each year for the previous year's releases (139). TRI requirements are triggered if a facility is involved in manufacturing with 10 or more full-time employees, manufactures, processes, or otherwise uses with one or more listed substances in a quantity above the statutory reporting threshold of 25,000 lbs./yr (manufactured or processed) or 10,000 lbs./yr (otherwise used). Beginning with the 1991 reporting year, such facilities also must report pollution prevention and recycling data for such chemicals pursuant to Section 6607 of the Pollution Prevention Act (42 U.S. Code 13106). n-Hexane was added to the TRI list in 1994 with reporting for 1995 emissions (135). Isohexane is not on the TRI list.

5. *Toxic Substances Control Act (TSCA; 15 U.S. Code 2600 et seq.)* If a chemical's manufacture, processing, distribution, use, or disposal would create unreasonable risks, the U.S. EPA, under the TSCA (40 CFR section 700, et seq.), can regulate it, ban it, or require additional testing.

*Inventory Update Rule (IUR) (40 CFR 710).* The IUR was established in 1986 to require manufacturers and importers of chemicals listed on the master TSCA Inventory to report current data every four years on the production volume of chemicals imported or produced. Food and feed products produced from natural agricultural product, such as oilseeds, are not required to be reported but all oil and meal products obtained by solvent extraction that is sold for other than food or feed use (e.g., oils as chemical raw materials and meal as fertilizer) are. Cottonseed oil, soap stocks, acidulated soap stocks, deodorized distillates, hydrogenated cottonseed oil are some of the substances reported by extraction and refining operations under IUR. EPA amended this rule in 2003 (1/9/03; 68 FR 848). Cottonseed oil is on the list of partially exempt substances, which are not subject to the new reporting requirements for processing and use data but continue to have to report the current IUR information as well as manufacturing exposure-related information.

### 5.3. Food Safety (FDA)

The U.S. Food and Drug Administration (FDA) regulates all aspects of food, including food ingredients and labeling in the United States. An extraction solvent or other processing substances that can be in food are subject to premarket approval by FDA unless its use is generally recognized as safe (GRAS) by qualified experts. Oilseed extraction solvents and food processing substances, to be legally used in the United States, must have been subject to an approval by the U.S. FDA or the U.S. Department of Agriculture (USDA) during 1938–1958 for this use (“prior sanction”); be GRAS for this use by FDA [“GRAS affirmation” (21 CFR 170.35); substances don't have to be specifically listed and there are several ways to determine GRAS]; or be used in accordance with food additive regulations promulgated by the U.S. FDA [21 CFR 170.3(h)(I)]. Food additives generally fall into two broad

categories: 1) those added directly to food (21 CFR 172), and 2) those that are added indirectly to food through contact with packaging materials, processing equipment, or other food-contact materials (21 CFR 174-178).

Many prior sanctions and early GRAS determinations are not codified in the U.S. FDA regulations. Extracting solvents used in food manufacturing, such as n-hexane or isohexane, have been labeled as food additives or incidental additives (i.e., “additives that are present in a food at insignificant levels and do not have any technical or functional effect in that food”). Incidental additives can be “processing aids,” (i.e., “substances that are added to a food during processing but removed from the food before it is packaged”). Most food-processing substances, including solvents, can be regarded as “incidental additives” and, thus, are exempt from label declaration in the finished food product.

As vegetable oil and other human food grade oils undergo refining, bleaching, deodorization, (steam distillation) and sometimes other purification processes as part of the manufacturing process prior to being used as a food product, they should not contain any (< 100 ppb) of the extraction solvent, if proper manufacturing practices are followed.

“Commercial hexane,” containing about 50–85% n-hexane (the rest is the hexane isomers that make up “commercial isohexane”), has been mostly used since the 1940s as an oilseed-extraction solvent on the determination that it is GRAS and it may also be subject to a prior sanction. Like many other food-processing substances, there is no U.S. FDA regulation specifically listing n-hexane as GRAS or prior sanctioned. However, under FDA regulations, hexane has been cleared as a solvent (residue not more than 5 ppm) for use in many products (140). Isohexane/hexane isomers also are not specifically listed as GRAS or prior sanctioned.

In Europe, the maximum residue limit (MRL) in vegetable oils has been established as 5 ppm n-hexane (141). There is no MRL for isohexane/hexane isomers.

In summary, extraction solvents can be considered incidental additives/processing aids that are exempt from label declaration. GRAS status may be determined by a company or an industry (“GRAS self-determination” (142) or “GRAS notification” (April 17, 1997; 62 FR 18938)), an independent scientific organization (e.g., “FEMA GRAS” (143), or the U.S. FDA (GRAS affirmation (21 CFR 170.35)). The Federal Food, Drug and Cosmetic Act (FFDCA; 21 U.S. Code 321 et seq.) does not provide for the U.S. FDA to approve all ingredients used in food, and the U.S. FDA explicitly recognizes that its published GRAS list is not meant to be a complete listing of all substances that are, in fact, GRAS food substances. Although there is no requirement to inform the U.S. FDA of a GRAS self-determination or to request FDA review or approval on the matter, the U.S. FDA has established a voluntary GRAS affirmation program under which such advice will be provided by the agency. Solvents that do not have prior sanction, a GRAS determination of somekind, or a tolerance set probably should be evaluated for compliance under food safety requirements if a facility is considering changing its extracting solvent.

## 6. COTTONSEED OIL PRODUCTS FINISHING TREATMENT

Not too many years ago, fats and oils finished products were only available packaged in containers no larger than 55-gallon drums. Then, large industrial users of cooking and salad oils that could pump oils at ambient temperatures installed bulk handling facilities. These installations were followed by fats and oils handling, which required equipment in addition to storage facilities; i.e., specifically equipment for chilling and plasticization of shortening and margarine-type products. Serious consideration must be given to the means selected to deliver the product to the consumer or ingredient user, be it shipped by rail or truck, or packaged before shipment in smaller quantities. Quality, cost, and handling by both the fats and oils supplier and the consumer are dramatically affected by the means selected. An oil product that has been carefully processed to attain optimum palatability, nutrition, and performance can be maintained or lost by the selection of packaging, crystallization, cooling, filling, or bulk shipment choices.

### 6.1. Cooking and Salad Oil Filling and Packaging

The salad and cooking oil package contains and protects the oil on its journey from the processor through usage by the consumer. Salad and cooking oils are usually packaged shortly after deodorization in containers for home, restaurant, or food processor use. It is not customary to store large quantities of deodorized oils; common practice is to package these oils as soon as possible. The preservation measures necessary are nitrogen protection, temperature control, light avoidance, and the addition of any additives required by product or customer specification. A cost-effective package is one that delivers the product to the satisfaction of the user at the least cost.

Early in the twentieth century, glass bottles replaced tin-plated cans in Europe and the United States for cottonseed and other salad oils packaged for the household consumer. Glass packaging was well established as the efficient package for retail consumer oils until the mid-1980s. Then, plastic replaced glass due to consumer preference for lightweight, unbreakable, and easily handled containers with added benefits of improved economics effected by lower package costs, reduced transportation costs, and equalivent product protection. Consumer liquid oils in the United States are currently packaged in 8-, 16-, 24-, 32-, 38-, 48-, and 64-ounce clear, rectangular shaped, stretch-blown polyethylene terephthalates (PET) containers, as well as 1-gallon opaque plastic containers. The same oils and some additional products with additives, such as antifoamers and antioxidants, are packaged in 35-pound or 5-gallon plastic jugs and 425-pound closed head drums for food service and food processor customers. High-speed automatic filling, capping, labeling, and casing machines are used for packaging the smaller containers. Slower speed twin spout automatic fillers are normally used for the steel drums.

**TABLE 24. Crystallization Process Conditions' Influence on Shortening Consistency.**

Plastic	Process	Brittle
13 ± 1%	Gas incorporation	None
Cold	Chilling	Warm
More	Mechanical working	Less
High	Pressure	Low
85 ± 5°F	Tempering	Cold

## 6.2. Shortening Plasticization, Packaging, and Tempering

Plasticized shortening products can be defined as fats with a consistency than can be readily spread, mixed, or worked. Considerably more is involved in the plasticization of shortening and margarine than merely lowering the temperature to cause solidification. Slow cooling of these products produces a grainy, pasty, nonuniform mushy product that lacks the appearance, texture, and functional characteristics associated with plasticized products. Development of these characteristics is a function of controlled crystallization or plasticization. The final consistency of a shortening is the culmination of all the factors influencing crystallization and plasticization: chilling, working, tempering, pressure, and gas incorporation. Table 24 summarizes the effects of the crystallization processes upon shortening consistency (144).

The plasticization process involves the rapid chilling and homogenization of the shortening mixture. Most shortenings are quick-chilled in closed thin-film scraped-wall heat exchangers with extrusion valves to deliver a smooth homogenous product to the package at 17–27 atm pressure. Nitrogen is injected at 13 ± 1% into most shortenings to increase the product's workability and provide a white, creamy appearance. After packaging, many processors temper shortenings at temperatures slightly above the packaging temperature to allow the crystal structure of the hard fraction to reach equilibrium and form a stable matrix. After tempering, shortenings are usually stored and shipped at controlled temperatures of 70–80°F or 21.1–26.7°C to avoid crystal change and loss of the plastic properties (145). Figure 4 depicts a typical shortening plasticization process flowsheet in the United States, beginning when the deodorized shortening blend has been transferred to the chilling unit supply tank and all of the specified additives have been incorporated (106).

**Packaging of Shortening.** Plasticized shortenings for commercial use are usually packaged in plastic film-lined 50-pound corrugated cartons, commonly referred to as a cube. The 50- and 110-pound tinplated cans and the 380–400-pound open head drums, both steel and composite, that were favorite packages for many years have almost completely disappeared. For home consumption, vegetable shortening is packed in 1- and 3-pound composite or plastic cans, and occasionally in larger 8-pound tinplated lithographed cans with a recloseable pressure-applied lid.

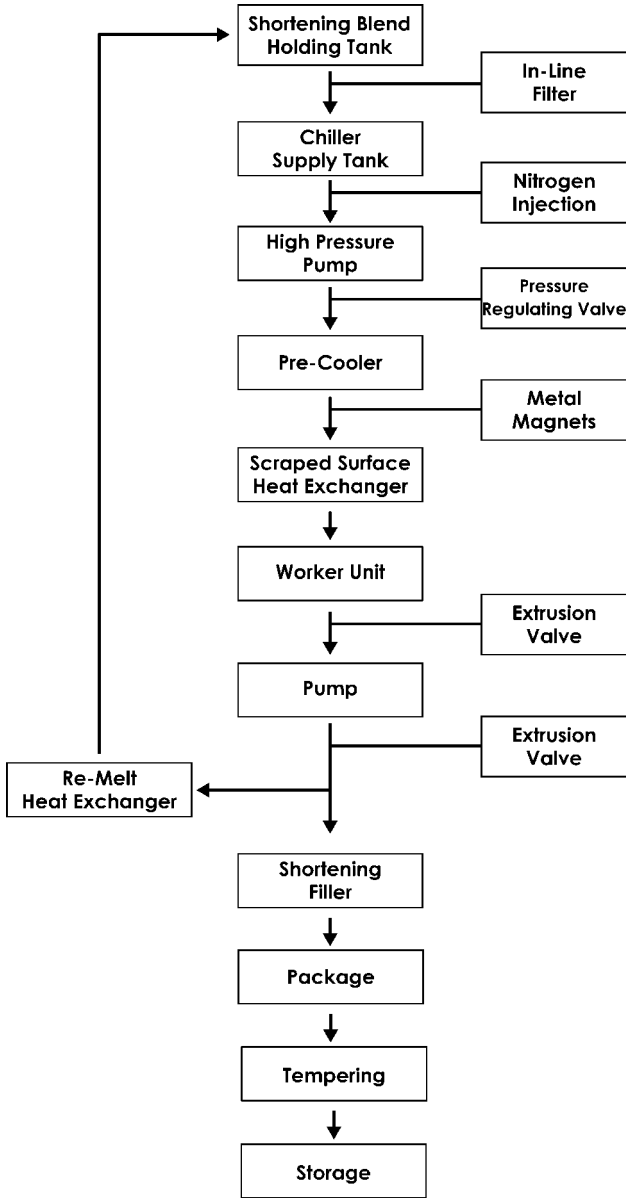


Figure 4. Typical process flow for shortening plasticization.

The 50-pound cube containers are generally filled directly from the texturing valve on scales and automatically filled to the package declared weight. Household shortenings are usually packed in the smaller containers using volumetric type filling machines. The seamed lid applied on tinned household shortening cans has been replaced with a foil seal and a plastic overcap on the composite and plastic

containers. After filling, all of the shortening products are tempered to stabilize the crystal structure in the desired form.

### 6.3. Margarine Mixing, Chilling, and Packaging

Margarine is a flavored food product containing 80% fat, made by blending selected basestocks with other ingredients, fortified with vitamin A, to produce a table, cooking or baking fat product that serves the purpose of dairy butter but is different in composition and can be varied for different applications (146). Margarine was developed and continues to be a butter substitute but now, spreads have been developed as margarine substitutes. The major difference between spreads and margarine is that spreads are not legally required to contain a minimum of 80% fat.

Processing for margarines and spreads begins with the preparation of an emulsion of the ingredients. Emulsions are prepared by adding the oil-soluble ingredients to a heated margarine oil formulation in an agitated emulsion tank. Concurrently, a pasteurized aqueous phase is prepared by mixing all of the water-soluble ingredients together in another vat. The water phase is then added to the oil phase to make the emulsion. The emulsion is rapidly chilled with scraped-wall heat exchangers similar to those used for shortening products. The plasticized products are then formed into convenient sizes and shapes wrapped with paper, which sometimes are called prints, or filled into the various containers for consumer, restaurant, or food processor use. Most margarine and spread products are stored at refrigerator temperatures immediately after packaging, except for some specialized baking products (106).

### 6.4. Flaking and Spray Chilling

Fat flakes describe the higher melting fat and oil products solidified in a thin flake form for ease of handling, quick remelting, or for a specific function in a food product. Flakes are solidified on a chill roll, which has been described as an endless moving chilling surface held at a temperature below the crystallization point of the applied fat or oil product to form a congealed film on the outer surface. Chill rolls and processed oil formulations have been adapted to produce several different flaked products that can provide distinctive performance characteristics in specialty formulated foods. The flaked products, produced almost exclusively for restaurant and food processor consumers, are hardfats or stearines, shortening chips, icing stabilizers, confectioners fats, hard emulsifiers, and other customer specific products. The flake products are packaged in kraft bags, corrugated cartons with vinyl liners, or other suitable containers for storage and shipment (145).

Spray chilled or powdered fats are specialized products developed for ease of incorporation, handling, melting efficiency, uniform delivery with addition systems, encapsulation, and other special-purpose uses. The spray chilling process consists of atomizing a molten fat in a crystallization zone or tower, maintained under temperature conditions where a very fine mist of the melted fat is contacted with cooled air or gas to cause crystallization without marked supercooling (106).

## 6.5. Bulk Fats and Oils Shipments

Food processors that use fats and oils in large quantities often have the facilities to handle this ingredient liquid in bulk. All of the products packaged for shipment and use can be provided to the customers in tank cars or tank trucks, except margarine and spread mixes that contain milk and salt. The customers for these bulk products must have fats and oils bulk handling systems to receive, store, and handle the liquid products. Chilling equipment is also necessary for the shortening or margarine type products to develop the crystal structure necessary for functionality.

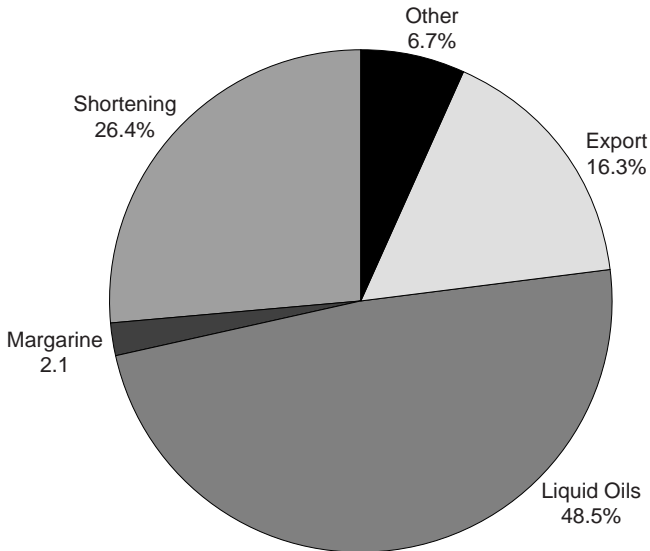
## 7. COTTONSEED OIL UTILIZATION

Edible fats and oils usage can be separated into four product categories: (1) liquid oils, (2) shortenings, (3) margarines, and (4) specialty products. Until World War II, cottonseed oil had been the major oil source for all four food product categories in the United States. The U.S. vegetable oil industry was developed with cottonseed oil as the original source oil, and it dominated this market for almost 100 years. Many of the prepared food products available today were developed with a shortening, margarine, or an oil product containing cottonseed oil. Soybean oil became the dominate vegetable oil in the world as a result of availability and economics, not overall performance. Unlike other source oils, cottonseed oil is a more universal source oil to provide the desired functionality for most products; in fact, cottonseed oil or another beta-prime crystal former is necessary in most product formulations with soybean, sunflower, canola, and corn oils to effect a smooth, plastic consistency.

Cottonseed oil is still used in all four of the fats and oils product categories, as shown in Figure 5 (28). However, competition from other vegetable oils grown domestically and imported has decreased the popularity of cottonseed oil from a dominant position to that of a specialty oil, as indicated previously in Table 2. In the United States, soybean oil is the undisputed volume leader for all of the fats and oil products with 61.0% of the consumption in 2000. Collectively, the animal fats still have the second highest consumption, but individually as lard, tallow, and butterfat, their usage is less than three individual vegetable oils; soybean, canola, and corn oils. A reduced usage of animal fats along with an increased usage of liquid vegetable oils indicates an effort on the part of the U.S. consumers to eat a healthier diet. Consumers have reduced their usage of animal fats to avoid cholesterol and are decreasing their shortening usage in favor of liquid oils to reduce saturated fats. Margarine usage has also suffered due to consumers efforts to reduce visible fat consumption. Consumer table spread popularity has shifted to low-fat spreads while this product category is experiencing a volume decline.

### 7.1. Oil and Fat Products Requirements

Fats and oils are very versatile raw materials. Processors have developed methods to make them even more useful to the food industry and analytical chemists have



**Figure 5.** U.S. Cottonseed oil utilization.

devised methods to qualify the products produced. Satisfactory performance of oil products is dependent on several important elements that determine suitability. Formulators must identify the attributes that are important for each individual finished product and then effectively use process modification capabilities to satisfy the prepared foods requirements. Successful production of these products relies on the manipulation of the basestock blends to produce suitable physical properties and to prevent undesirable changes during and after processing. The important vegetable oil performance characteristics that must be considered and modified to fit the finished product's requirements are as follows:

- **Flavor.** Generally, the flavor of a cottonseed or a substitute oil product should be completely bland to enhance the food product's flavor rather than contribute a flavor.
- **Flavor Stability.** The oil ingredient must have the identified degree of resistance to oxidative and lipolytic flavor degradation to maintain a bland flavor and odor throughout the shelf life and use life of the prepared food product. Cottonseed oil reverts to a nutty flavor that is not as objectionable as the reversion flavors of the other oils with high levels of linoleic and oleic fatty acids.
- **Physical Characteristics.** The proper blend of basestocks and hardstocks must be identified that provide the physical, functional, and organoleptic properties required by the finished product. These characteristics are usually identified analytically with solids fat index, melting point, and iodine value determinations.
- **Crystallization.** Cottonseed oil crystallizes in the beta-prime crystal form, which is preferred for plasticity, heat resistance, and creaming properties in



shortening, margarine, and most of the specialized product formulations. This characteristic provides a more universal functionality for cottonseed oil; it can be the sole source oil in a product or provide the impetus to force the crystal habit to the desired beta-prime form in blends with other oils that crystallize in the beta form.

- **Nutritional Concerns.** All fats and oils are recognized as important nutrients for both humans and animals because they provide a concentrated source of energy, contain essential fatty acids, and serve as carriers for fat-soluble vitamins. However, significant research has been done on the relationship between fats and the incidence of coronary heart disease. Diet modifications, including reductions in fat consumption, saturated fats, cholesterol, and *trans*-isomers, have been recommended. Cottonseed oil is a good source of the essential fatty acids because it contains 53% to 55% linoleic fatty acid. Like other vegetable oils, it also has the advantage of being essentially cholesterol free while providing the highest saturated fatty acid content (26%) of the other vegetable oils in its classification. Unhardened cottonseed oil with a typical iodine value of 111 can be used in some formulations to provide saturation and oxidative stability without the *trans*-isomers developed in other oils during hydrogenation to reach this point.

Current chemical and physical processing techniques provide the processor with the capability of modifying one or more of an oil's properties. It is possible to change the functionality of an oil product to provide the ability to formulate tailor-made products to suit a particular product or process. Further, the processing techniques provide the processor with a wider range of alternative raw material sources to improve commercial viability. The main objectives for applying the available modification or processing techniques are as follows:

1. Produce an oil product to meet certain performance characteristics not possible with natural oils.
2. Production of basestocks with lower processing costs to duplicate the functionality of a more costly alternative.
3. Oxidation stability improvements through the elimination of the reaction sites.
4. Palatability improvement.
5. More nutritionally acceptable products; i.e., reduce saturates and *trans*-acids while increasing polyunsaturates.

## 8. LIQUID OILS

Clarity at or below ambient room temperature is the primary characteristic of a liquid oil. Natural vegetable oils that are liquid at room temperatures in temperate climates,  $75 \pm 5^\circ\text{F}$  or  $23.9 \pm 2.8^\circ\text{C}$ , contain high levels of unsaturated fatty acids

with low melting points. Fatty acids with one or more double bonds and 18 carbon atoms are the most important unsaturated fatty acids for liquid oils. Oleic (C-18:1), a monounsaturated fatty acid, is the most widely distributed and most stable 18 carbon chain-length unsaturated fatty acid. Linoleic (C-18:2) and linolenic (C-18:3) are the most widely distributed di- and triunsaturated fatty acids. Both of these polyunsaturated fatty acids are termed essential because they cannot be synthesized by the human body and must be supplied in the diet. Complete exclusion of the essential fatty acids results in scaly skin, loss of weight, kidney lesions, and death.

The physical state of a fats and oils product may be natural or the result of processing. Changes to either a solid or a liquid can be effected by processes that change the melting or solidification points. These physical properties can be modified by several different processes available to the fats and oils industry: (1) hydrogenation, which saturates the unsaturated fatty acids to increase both the oxidative stability and melting point; (2) interesterification, which rearranges the fatty acids among the triglycerides to change the melting, oxidative stability, and crystallization tendencies of the oil; (3) fractionation, which physically separates hard and soft fractions to provide different functional properties; and (4) genetic engineering procedures, which modify the oil's fatty acid composition during the development of the seed.

Three major oil types have been developed that maintain different degrees of clarity or oxidative stability at and below room temperature in temperate climates. These liquid oils are identified by their functionality traits; cooking, salad, and high stability. The definition for each of these classifications is as follows:

**Cooking Oil.** An edible oil that is liquid and clear at room temperature, 75°F or 23.9°C, that may be used for cooking. Cooking oils are typically used for pan frying, deep-fat frying, sauces, gravies, marinates, and other nonrefrigerated food preparations where a clear liquid oil has application. Cooking oils usually congeal or solidify at refrigerator temperatures. Refined, bleached, and deodorized cottonseed oil is considered a cooking oil because it contains saturated fatty acids that cause the oil to cloud and solidify slightly below room temperature.

**Salad Oil.** An edible oil that is suitable for the production of a mayonnaise or salad dressing emulsion, which will remain liquid at refrigerated temperatures; 40°F or 4.4°C. This requirement has been refined to require a minimum cold test of 5 and one-half hours; this measurement requires that a sample of the oil remain clear and brilliant while submerged in an ice bath. Cottonseed oil must have a portion of the saturated triglycerides removed to meet the requirements of a salad oil; winterization is the process normally used to produce a cottonseed salad oil.

**High Stability Oils.** An edible oil that is clear at room temperature and possesses an exceptional oxidative and flavor stability. The usual measure for oxidative stability is the Active Oxygen Method (AOM), AOCS Method Cd 12-57. High-stability oils will withstand the AOM abuse for periods in excess of 75 hours, and some longer than 300 hours, as opposed to a 15-hour AOM result for cottonseed cooking oils. High-stability oils can be produced by hydrogenation followed by fractionation or by genetic engineering. Most high-stability oils are considered specialty products.

TABLE 25. U.S. Source Oil Consumption as Salad and Cooking Oils.

Oil Source	Year (Metric Ton)				
	1960	1970	1980	1990	2000
Canola				W	233.6
Corn	112.0	112.0	158.8	288.5	227.7
Cottonseed	341.1	239.0	208.7	208.7	137.9
Olive	23.1	28.1	26.3	96.6	208.2
Peanut	12.7	63.5	67.1	63.0	W
Safflower		5.4			
Soybean	402.3	1120.8	1833.4	2114.7	3338.9
Sunflower			NR	NR	NR
Unidentified	0.5	2.3	49.4	15.0	23.1
Total	891.8	1571.3	2343.7	2786.4	4169.5
kg per person	4.2	7.8	9.7	11.0	15.3

W = withheld; NR = not recorded.

### 8.1. Cooking and Salad Oil Sources

A steady growth in the consumption of cooking and salad oils is evident from the USDA Economic Research Service *Oil Crops Situation and Outlook Reports* for domestic consumption of salad and cooking oils in the United States. The consumption data by source oil is summarized in Table 25 (25, 33). Deodorized cooking and salad oils are principally prepared from soybean, cottonseed, corn, canola, sunflower, and peanut oils. Olive oil is technically a cooking oil and is considered a gourmet product by many due to its distinctive flavor and odor, which would be destroyed by deodorization, considered mandatory for the other liquid oils.

Cottonseed oil contains only minor amounts of linolenic fatty acid and a fair amount of saturates, approximately 26% to 27%, to make it a relatively stable cooking oil. Cottonseed salad oil production requires winterization to remove the stearine fraction caused by the high level of saturated fatty acids. The unsaturates are 53.3% linoleic fatty acid and 18.3% oleic fatty acid. The natural nutty or buttery flavor is an additional benefit that can mask less desirable flavors in food products. Cottonseed oil was the principal cooking and salad oil used in the United States until the late 1950s. As a source oil, it had lost its dominance for usage in shortenings and margarines in the 1940–1950 era, but remained the preferred liquid oil due to the flavor problems associated with soybean oil. Demand for cottonseed salad and cooking oil decreased both in use percentage and amount after technology was developed to overcome the soybean oil flavor problems. The actual pounds of cottonseed oil used domestically for salad and cooking oils have decreased 60% since 1960, and the market portion has decreased from 38.25% in 1960 to 3.3% in 2000. One of the factors that led to the decreased consumption in the United States was the lucrative export market for cottonseed oil. Export demand provided an outlet for a high percentage of the available cottonseed oil at good income levels. These exports helped to maintain the premium pricing for cottonseed oil marketed domestically. Currently, a major usage for liquid cottonseed oils is as a cooking oil

for snack foods. The unique cottonseed oil flavor imparts a pleasant, stable flavor to potato chips and other salty snacks unattainable with other frying oils that lack the nutty fried flavor note.

## 8.2. Liquid Oil Markets

Liquid oils enter into three major areas of food preparation: (1) retail, (2) foodservice, and (3) food processor. For some uses, there is a similarity in product requirements, but there are also major differences in performance requirements in the three areas. Package sizes are obviously different, with the smallest designed for home use and the largest being the food processor shipments of 150–180 thousand pound tank cars. The retail market consists of bottled oils sold in grocery stores or other retail outlets for home use. The foodservice industry is composed of restaurants, hotels, institutions, and other mass feeding operations. In many cases, foodservice oils are specialized products designed for the intended use; i.e., frying, salads, sauces, etc. The food processor market is made up of prepared food manufactures of products sold through retail outlets or used by the foodservice industry. The food processor oils are normally specifically designed for the individual operation to provide the desired performance for the product as well as for the process.

**Retail Consumer Oils.** None of the retail consumer oils currently available have been chemically modified to change their physical characteristics. Processing for the majority of the household bottled oils consists of refining, bleaching, and deodorization with additional dewaxing required for canola, corn, safflower, and sunflower oils and winterization for cottonseed oil to meet the cold test requirements for a salad oil. Soybean oil is a natural winter oil that meets the requirements of a salad oil and requires only refining, bleaching, and deodorization processing. Peanut oil gels, when chilled to the extent that it cannot be separated, into an oil and hard fraction with winterization to produce a salad oil. Therefore, both peanut and olive consumer bottled oils will become a semisolid at refrigerator temperatures.

The broad dietary shift from animal fats and then saturated fats has favored liquid oil products. The U.S. consumer has become increasingly aware of the fats and oils role in coronary heart disease. As a result, consumers have replaced solid shortenings with liquid oils. The liquid oils favored by consumers tend to reflect the findings of the most recent publicized study. Initially, when it was thought that only polyunsaturated oils were useful in lowering serum cholesterol corn oil began its rise in popularity. Reports that monounsaturates were equal to polyunsaturates in lowering serum cholesterol appeared to help peanut and olive oil sales. Later, the “Mediterranean diet” helped to improve the sales of olive oil. Canola oil introductions capitalized on the low saturate level; i.e., 94% saturates free. In 1991, an oil composed of soybean, sunflower, and canola oils blended to one-third the saturate level of soybean oil was introduced (147). Blended retail oils are still marketed by the major branded suppliers but with different compositions. One blended oil is composed of soybean and canola oils with 7% saturates and another is a blend of corn and canola oils with 15% saturates. The advent of *trans*-fatty-acid labeling requirements is shifting consumer attention to that parameter.

Throughout all these changes, cottonseed oil has not fared well. It has dropped from the leading consumer bottled oil to almost become a gourmet type oil in most retail grocery stores sharing space with exotic oils like safflower, avocado, walnut, and grape seed oils. The major reasons for this decreased presence are probably the high level of saturates, the winterization requirement for a clear and brilliant salad oil, and the higher cost that cottonseed oil commands.

**Foodservice Liquid Oils.** The foodservice industry consists of restaurants, hotels, and institutions where food is prepared for in-house service or delivery to the consumer. This industry had an inflation adjusted growth rate of 2.6% per year in contrast to a retail food sales growth rate of 0.7%. The fast-food segment of this industry continues to represent the largest and fastest rising share of sales among separate eating places (148). Edible fats and oils are major foodservice ingredients, which includes all of the liquid oil types: cooking, salad, and high-stability oils. Foodservice kitchens may use liquid oils for a number of different applications, such as deep-fat frying, pan frying, grilling, pan release, seasoning, preparation of salad dressings, sauces, gravies, some types of baking, and other food applications.

Vegetable oils became the leading U.S. foodservice frying media in 1990 when the major fast-food restaurants switched from tallow to vegetable oils for frying french fries. The vegetable oil products used for deep-fat frying range from hydrogenated plasticized frying shortenings, to cooking or RBD oils. RBD liquid oils, in most cases, have the lowest initial cost but also have the poorest frying stability of all the available products. In general, cooking oils will perform satisfactorily when the fried food volume is extremely high and an oily product appearance is desired. However, nature did not create all of the vegetable oils alike; some source oils have a better frying stability than others and, in some cases, a particular oil type is chosen for the distinctive flavor that the slightly reverted oil contributes.

The major criteria for comparing the frying stability of different oils is the level and type of unsaturates and the fried flavor characteristics. Oxygen combines with the unsaturated fatty acids, causing oxidation and a flavor reversion back to the original characteristics. The rate that an oil oxidizes is dependent on the amount and type of the unsaturated fatty acids. Frying temperatures accelerate the oxidation reaction, but in comparison testing, all of the cooking oils would be treated the same. In general, the more unsaturated the fatty acid, the faster its rate of oxidation. Natural vegetable oils that are liquid at room temperature generally contain high levels of polyunsaturated fatty acids with low melting points. These polyunsaturated fatty acids, predominately linoleic (C18:2) and linolenic (C18:3), tend to be very liquid but are also highly susceptible to oxidation and, therefore exhibit a poor frying life in foodservice applications. Oxidation precedes polymerization in the frying oil deterioration cycle; polymerization increases an oil's viscosity, which eventually results in persistent foaming after the oil thickens to the point that it will not allow the moisture from the food to escape. Table 26 compares the levels of unsaturates, the inherent oxidative stability, and the oxidized or reverted flavors for the most common cooking oils used in the United States. Cottonseed oil has a median stability rating but has the advantage of a somewhat pleasant, nutty oxidized flavor. Soybean cooking oil has the poorest stability rating and develops an

**TABLE 26. Frying Performance Considerations for Cooking Oils.**

Oil Source	Fatty Acid Composition, %				Inherent Oxidative Stability	Fried Flavor
	Saturates	Oleic	Linoleic	Linolenic		
Peanut oil	19.4	46.7	32.0		3.7	Peanut
Canola oil	7.3	60.9	21.0	8.8	4.9	Earthy
Cottonseed oil	25.7	18.6	54.4	0.7	5.8	Nutty
Corn oil	13.6	25.4	59.6	1.2	6.5	Musty
Sunflower oil	12.8	18.7	67.5	0.8	7.1	Nutty
Soybean oil	15.3	23.3	53.7	7.6	7.5	Fishy

objectionable oxidized flavor and odor; even with these characteristics, it can be utilized in high-use situations that do not require a long shelf life.

Another clear liquid frying oil type available to the foodservice operator is the high-stability frying oils. The term high-stability oils describes triglyceride mixtures that are both liquid and stable in the temperature range of 60–85°F, or 15.6–29.4°C. In nature, this combination rarely occurs. Natural oils that are liquid under these conditions are generally much less stable with respect to frying stability than solid fats. The oxidative/frying stability of vegetable oils, as referred to above, depends on a combination of factors, including the following: (1) degree of unsaturation or number of double bonds; (2) nature of unsaturation, or the position of the double bonds; (3) pro-oxidant or trace metals content; (4) antioxidant content; and (5) exposure to oxygen. Two entirely different techniques have been developed to enhance stability while maintaining liquidity, these being processing or plant breeding. Using a combination of hydrogenation and fractionation techniques, it is possible to produce oils that are both stable and liquid from otherwise unstable polyunsaturated raw materials. Plant breeding technology has developed genetically superior varieties of oilseeds that yield oils more-suitable for high temperature frying than the conventional vegetable oils. The high-stability oils, both those produced with processing or plant breeding, have excellent frying stabilities, which approach the stability of a hydrogenated plasticized shortening. However, high-stability oils produced with either technology have not become as popular as the solid or liquid frying shortenings, probably due to higher product costs.

Most of the foodservice liquid cooking and high-stability oils designed for frying are stabilized with dimethylpolysiloxane, a combination antifoaming and antioxidant agent added to extend the frying stability. It can extend frying life by three to ten times, with the degree of increase dependent on the original frying life without the antifoamer; the more stable products initially show greater increases in frying life than the less stable products.

Another primary use for a salad and even cooking oil in most foodservice operations is for making salad dressings. Cooking oils are generally not satisfactory for salad dressing use in industrial preparations, but can be used in foodservice operations where the salad dressing is prepared fresh and is not expected to withstand prolonged storage periods at refrigerated temperatures. In making salad dressings,

ease of blending with other ingredients is quite often an advantage. Most salad dressings depend on the oil to coat the salad ingredients and to hold the flavors of herbs, spices, and vinegar.

Other uses for salad oils include pan frying, griddling, sauces, gravies, dips, and some types of baking. When liquid oils are used in baking, the general use is to make chiffon or sponge type cakes and other all-purpose baking applications where a lubricant is beneficial.

**Food Processor Liquid Oils.** Food processors often purchase their fats and oils ingredients in bulk quantities to obtain a cost advantage generated by elimination of packaging, lower shipping rates, and reduced labor costs. Liquid oils offer a definite advantage for bulk handling as a result of their absence of solid fractions at ambient temperatures and below. A major concern with fats and oils bulk handling is that the product may deteriorate before use. This is particularly true of shortenings that must be held in a molten state. Melted products are generally more susceptible to deterioration than packaged products or liquid oils, which are normally fluid and pumpable at ordinary ambient temperatures. Autoxidation increases markedly with heating. It has been determined that the rate of oxidation doubles for each 25°F or 15°C increase in temperature within the range of 70°F to 140°F or 20°C to 60°C. Exclusion of oxygen during storage represents an effective method for limiting quality deterioration for all types of fats and oils. The usual procedures replace oxygen with nitrogen during transit and storage before use.

All three of the liquid oil types (salad, cooking, and high-stability) are utilized by food processors as ingredients for specific product applications. The choice of an oil is the result of a synthesis of many parameters, which explain the variety of chosen solutions. Liquid oils offer a choice of cooking, salad, or high-stability oil types and functional variations occur within each regarding flavor, degree of unsaturation, fatty acid distribution, essential fatty acids, oxidative stability, frying stability, cost, availability, etc. (132).

Food Processors utilize cooking oils predominately for frying snack foods, nuts, fish, poultry, meats, potatoes, and other food products for dry, refrigerated, and frozen distribution to retail and foodservice consumers. Food processors must evaluate the contributions of each cooking fat or oil to their product and identify the most suitable ingredient for use. One role of fat in cooking or frying is essentially to provide an efficient heat-transfer medium, transmitting heat rapidly and uniformly to the surface of the food being cooked. Additionally, the oil contributes flavor and palatability to the food fried. Every fat and oil product will experience reversion to characteristic flavor, which may or may not complement the food product. Many potato chip manufacturers use cottonseed cooking oil for frying their product because of its stability compared with other oils and the characteristic nutty flavor developed with oxidation.

Cooking and frying oils are used at high temperatures, often in the presence of hydrolyzing conditions, namely water and steam. Hydrolysis causes free fatty acid development, which results in more acidic flavors. Some products that are eaten shortly after preparation do not need as stable a frying oil as other foods that are packaged and require an atmospheric shelf life of several weeks. Products that

remain frozen until the consumer thaws them do not require a high degree of oxidative stability. Surface appearance of the fried food is also affected by the frying media; liquid oils provide a shiny, wet, soft appearance, whereas solid fats impart a dry, somewhat dull appearance. Polymerization, either thermal or oxidative, causes a thicker viscosity, which increases the oil absorption giving a greasier product.

Successful use of liquid oils in baked products has been a technological advancement for the baking industry. This technology involved the use of emulsifiers to provide the functionality lacking with the liquid oils. Baked products prepared with liquid oils alone had low volume, fair softness, and poor grain; plastic fats provided dough strength, gas retention, aeration, volume, symmetry, fine grain, and even texture. It was found that the use of proper emulsifier systems with liquid oils provided these functions with some added benefits. The liquid oils allowed the bakers to merchandise their products with “all-vegetable” and “polyunsaturated” labeling. Cooking oils are appropriate for these products because refrigeration is not an issue and the shelf life of baked products is well within the oxidative stability of polyunsaturated oils.

Pourable and spoonable salad dressings, prepared for retail sale or foodservice outlets, are a major use for food processor salad oils. Pourable salad dressings may be prepared as an emulsified form or a two-phase system of oil and water such as Italian style. As most dressings are stored in the refrigerator after being opened, it is important to use a salad oil to resist clouding at these temperature conditions. Aside from the unattractiveness of a cloudy product when refrigerated, it is necessary that no solid crystals be present that would give a waxy, tallowy tasting sensation in the mouth. Homogenized, pourable salad dressings require the use of noncrystallizing salad oils to prevent separation during refrigeration.

Mayonnaise and spoonable salad dressings are another major food processor use for salad oils. Oil constitutes 80% of most mayonnaise formulations and is responsible for the body and viscosity of the product. Spoonable salad dressings have only 35% to 50% oil whose function is to modify the mouthfeel of the starch paste that imparts the body. In both cases, a smooth, creamy, nonoily mouthfeel is desired that will not occur if crystallization occurs. These emulsions are very unstable and the presence of fat crystals will break the emulsion, rapidly causing oil pockets to form (128).

**Food Processor High Stability Oils.** The primary characteristics of a high-stability oil are liquidity at ambient temperatures and resistance to oxidation. Most oils that are liquid at room temperature contain high polyunsaturated fatty acid levels, which are the most susceptible to oxidation. Two technologies have been developed to enhance the stability of liquid oils while retaining the functional and nutritional properties. The first technology involves processing with hydrogenation to change the polyunsaturates to monounsaturated fatty acids followed by fractionation to separate the stearine, or hard fractions, formed during hydrogenation from the olein, or soft fraction. The olein fraction, high in oleic fatty acids, becomes the high-stability oil. The alternate technology for producing high-stability oil is the use of biotechnology and plant breeding techniques to produce



oilseeds with low levels of polyunsaturates and saturates but high levels of monounsaturates.

High-stability oils produced by either technology can be used by food processors wherever liquidity and oxidative stability influence the finished product's quality or handling conditions. The identified functionalities of the monounsaturated oils in specific applications are as follows (106):

- Frying oils—The high proportion of oleic fatty acid increases frying stability by limiting the opportunities for oxidation and polymerization. High-stability oils have exhibited frying stability results close to the selectively hydrogenated heavy-duty frying shortenings.
- Spray oils—When applied to the surface of food products, the high-stability oils protect the product from moisture and oxygen invasion, prevent clumping, and impart a glossy appearance. Specific applications include raisins and other fruits, breakfast cereals, nut meats, croutons, bread crumbs, spices, seasonings, crackers, and others.
- Pan release agents—Aerosol and brushed lubricants for cooking skillets, baking pans, confectionery products, and other food products where liquidity and oxidative stability are beneficial.
- Product carrier—Colors, spices, flavors, vitamins, and other additives may be dispersed in high-stability oils to preserve the flavor, color, or activity during extended shelf life periods with fluidity.
- Product compatibility—High-stability oils are compatible with all other fats and oils products because crystal type is not a concern. Liquid oils do not have a crystal structure.

## 9. SHORTENINGS

Shortening is an American invention developed with cottonseed oil to replace lard, the solid fat of choice. Cottonseed oil was plentiful and inexpensive in the latter decades of the nineteenth century as a direct result of the growth of the cotton industry in the United States. As the shortening product category developed, it expanded from a limited application to include baked and other product types. In 1948, H. C. Black defined shortening as a semisolid plastic material made wholly from fats and oils for use in cooking, baking, and frying (17). Today, shortening has become virtually synonymous with fat to include many other edible fats or oils products designed for all prepared foods.

Initially, shortenings were produced to resemble the consistency and plasticity of lard. Now, shortenings are designed to satisfy individual specific requirements for all of the food industry as well as offering products with broad general appeal. With this broader application, shortening consistency varies from wide workable ranges to brittle products with sharp melting characteristics, from very firm consistencies to liquid or pumpable products, or from creamy, smooth textures to grainy structures depending on the requirements of the application.

In most cases, products identified as shortening are 100% fat; however, there are exceptions, such as the roll-in shortenings, which may contain moisture. In some cases, a fat or oil system may be identified as a shortening to distinguish it from a margarine product. Generally, in the United States, if a fat product contains at least 80% fat and has the required Vitamin A content, it is a margarine. Products that resemble margarine but contain less than 80% and not more than 40% fat are required to be labeled as spreads. Products that do not meet these criteria have been identified as shortening since it does not have identity standards. Currently, a description for shortening would be: processed edible fats and oils that affect flavor, oxidative stability, shelf life quality, eating characteristics, nutrition, and the eye appeal of prepared foods by providing emulsification, lubricity, structure, aeration, moisture barrier, flavor medium, or heat transfer (20, 106, 149).

### 9.1. Shortening Attributes

Shortenings are functional ingredients that contribute heavily to the success of the food product prepared. Shortenings can be tailor made for a specific food product and process or designed as a general purpose product that must perform in varying conditions, product types, processes, and formulations. Adequate performance of a shortening for a food application is dependent on a number of factors. These requirements differ for each customer or application dependent on the product formulation, equipment, processing, preferences, and other considerations. Therefore, performance is dependent on the interrelated elements that determine acceptability.

**Flavor.** Generally, the flavor of a shortening should be completely bland, so that it can enhance the food products flavor rather than contribute its own flavor. The bleaching and deodorization processes remove primary and secondary oxidation products from shortenings. In some cases, shortening is the carrier for a flavor desired in the finished product. Flavor additives to shortening products are usually butter-like flavors; for example, butter flavors are incorporated into most pan and grill shortenings. Also, the flavor of the source oil may be desired for a specific food preparation; for example, the nutty fried-in flavor contributed by cottonseed oil.

**Flavor Stability.** The bland, typical, or formulated flavor must remain stable throughout the shelf life and use life of the prepared food product. Reverted or oxidized and hydrolyzed flavors and odors of most fats and oils are objectionable. Shortenings must possess the identified degree of resistance to both oxidative and lipolytic flavor degradation. Flavor stability is built into shortenings by selection of saturated or monounsaturated components or processing to decrease the unsaturated fatty acid content. The processes that influence flavor stability are hydrogenation, fractionation, or interesterification. Flavor stability can be preserved after it has been established with the use of antioxidants.

**Physical Characteristics.** Shortenings are pictured as solid materials but, in reality, are predominantly fluids. A plastic shortening consists of approximately one-quarter crystalline-solid triglycerides suspended in liquid triglycerides. The ratio of these two phases determines the consistency of a shortening as it relates to firmness, softness, and spreadability. Fats and oils processors change the consistency of a

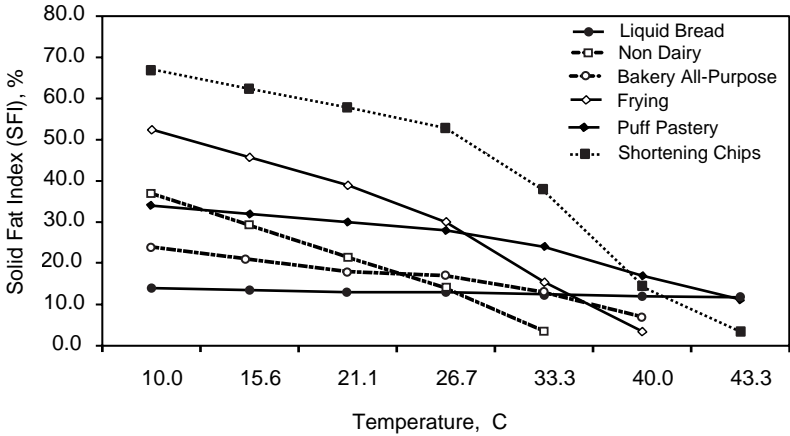


Figure 6. Shortening solid fat index profiles.

shortening by manipulating the solid-to-liquid: ratio over a range of temperatures. Three processes capable of altering the consistency of a shortening component are hydrogenation, fractionation, and interesterification. Fractionation processes make physical separations of the hard and soft fractions. Interesterification rearranges the position of the fatty acids in the triglycerides to affect melting characteristics. Hydrogenation, the most popular process for modifying solid-to-liquid ratios, saturates the unsaturated fatty acids to affect melting characteristics. The solid-to-liquid proportions of the shortening components can be somewhat selectively altered with these procedures to provide the physical characteristics most suited to the desired functionality. Figure 6 compares the solids fat index curves for six shortenings, designed for different applications, to illustrate changes in physical characteristics that can be affected for shortenings. Solid fat index (SFI) analysis is an empirical determination of when a fat passes from a solid to a liquid at the measurement temperatures. A plot of the results produces a curve that illustrates the changes in solids or liquid content to indicate the following:

- Consistency—The factor most directly influencing the consistency of a shortening is the proportion of the product in the solid phase. The consistency of a shortening at use, preparation, and storage temperature conditions materially affects the performance of the prepared food product. SFI curves characterize the firmness of the shortening over a range of temperatures and liquidity at mouth temperature for development, selection, and control purposes.
- Plastic Range—Shortenings are normally plastic and workable for SFI values between 15 and 25. At an SFI value above 25, shortenings start to become brittle and below 15, they become too fluid. The range of temperatures where the SFI values remain within the 15% to 25% solids is usually referred to as the plastic range.

**TABLE 27. Characteristics of the Polymorphic Forms of Monoacid Triacylglycerols.**

Characteristics	Alpha-Form	Beta-Prime Form	Beta Form
Chain packing	hexagonal	orthorhombic	triclinic
Short spacing, (Å)	4.2	3.8 and 4.2	4.6
Characteristic infrared spectrum	single band at 720/cm	double at 727 and 719/cm	single band at 717/cm
Density	least dense	intermediate	most dense
Melting point	lowest	medium	highest

- Flat Solids Curves—SFI values that provide east to west (x-axis) directional slopes generally have a better plasticity because the product is maintained at the ideal consistency over a greater temperature range. This same attribute contributes a slower “get away” in the mouth due to melting points above body temperature caused by higher SFI contents over a greater temperature span.
- Steep Solids Curves—Poor plasticity but good mouthfeel and oxidative stability are usually indicated by a north to south (y-axis) directional SFI slope. A narrower span of temperatures for the higher solids values provides sharper melting tendencies for better palatability and good oxidative stability. The preferred bland flavor is retained longer due to a lower unsaturated fatty acid content. However, steep SFI curves from hydrogenated products usually indicate a higher *trans*-fatty acid content.

**Crystalline Behavior.** Fats and oils are polymorphic, which means that, with cooling, a series of increasingly organized crystal changes occur until a final crystal form is achieved with no change in chemical structure. Typically, triglycerides exhibit three major crystalline forms: alpha, beta-prime, and beta. Characteristics of each are shown in Table 27 (150). The alpha crystal has a short life and readily transforms into the beta-prime crystal form. Thereafter, each source oil has an inherent crystallization tendency of either beta or beta-prime. The tiny, uniform, tightly knit, needle-like beta-prime crystals produce smooth textured shortenings with good plasticity, heat resistance, and good creaming properties. The large, high-melting, self-occluding, coarse, stable beta crystals produce visibly grainy, sandy, brittle shortenings that can experience separation of the liquid-oil portion. Both of these crystal habits provide physical conditions desirable for particular functionalities; for example, beta-prime for cake and icing shortenings requiring good creaming or air entrapment capabilities and beta for pie crust shortenings where a grainy consistency helps provide a flaky texture. The affinity of a fat and oil product to form beta-prime crystals is dependent on several factors: (1) the amount of palmitic fatty acid in the product, (2) the distribution and position of both palmitic and stearic fatty acids on the triglyceride molecule, (3) the degree of hydrogenation (beta-prime crystallization increases with the degree of hydrogenation and *trans*-isomers development), and (4) the degree of randomization of the triglyceride (151). Crystal development is complemented by the plasticization conditions employed: (1) chilling, which initiates the crystallization process, and (2) tempering, where the desirable crystal nuclei are developed and stabilized.

**Appearance.** Appearance is one of the collective results of all the processes used to convert crude edible fats and oils into shortening: (1) refining, bleaching, and deodorization are all involved in the removal of the color pigments; (2) hydrogenation, fractionation, or interesterification affect the solid fat index (SFI), which helps determine product consistency; (3) formulation determines crystal structure and SFI content for texture and consistency control; (4) chilling and tempering initiate, develop, and stabilize the crystal nuclei; and (5) storage and transportation under proper conditions prevent product damage.

Nearly all plasticized shortenings contain 12% to 14% nitrogen by volume added during the chilling process. The gas is finely dispersed to enhance creaming properties, control texture, and improve the appearance of the shortening. Shortenings containing creaming gas are creamy white in contrast to a yellow or green cast characteristic of petroleum jelly when no nitrogen is present.

**Emulsification.** Shortening emulsifying properties are accomplished by adjustment of the fat structure or the addition of surface-active agents like mono- and diglycerides. Food emulsifiers supplement and improve the functionality of a properly developed shortening to act as a lubricant, emulsify fat in batters, build structure, aerate, improve eating qualities, extend shelf life, modify crystal development, improve antisticking properties, act as a dispersant, alter moisture retention, and more. Obviously, no single surfactant can perform all of these different functions. Emulsifier selection requires the same attention to functionality used to identify the other components for shortenings.

**Antioxidants.** The oxidation rate for a shortening depends primarily on the number of double bonds and their arrangement in the triglyceride. However, the oxidation process can be slowed down with the preservation of the natural antioxidants or the addition of synthetic antioxidants. Antioxidants are chemical compounds that delay the onset of oxidation. They function by inhibiting or interrupting the free radical mechanism of autoxidation; they function as a free radical acceptor, thereby terminating oxidation at the initial step. Tocopherols are the natural antioxidants contained in most vegetable oils. Phenolic compounds that provide antioxidant activities are BHA (Butylated Hydroxyanisole), BHT (Butylated Hydroxytoluene), and TBHQ (Tertial Butyl Hydroxyquinone). Much of the success of the antioxidants depends on their being in chemical contact with the product they are protecting; therefore, various combinations of different antioxidants and chelating agents are generally combined for use. These combinations provide a synergistic effect to increase the antioxidants' effectiveness and allow the incorporation of a chelating agent to sequester prooxidant trace metals.

**Trace Metals.** Vegetable oils pick up metals from the soil. Many of the metals picked up from where the plants are grown and later from contact during crushing, processing, and storage promote oxidation, which results in off flavors, odors, and off-color development. Studies have identified copper as the most harmful with nickel, manganese, chromium, and iron following. Metal scavengers, added at low levels during processing prior to filtration, facilitate removal of the harmful metals. The most widely used chelating agent is citric acid, which is usually added at a rate of 50–100 parts per million (ppm).

**Foaming.** Polymerization occurs during frying to produce three dimensional polymers, which result in an increased frying shortening viscosity. Foam develops on the surface when the frying shortening will no longer release moisture but keeps it trapped as steam vapor. Polymerization can be inhibited with the use of dimethylpolysiloxane, which has been labeled as an antifoam agent. Dimethylpolysiloxane may be safely used in processed foods at levels not exceeding 10 ppm. The effective usage range to inhibit foaming for frying shortening has been identified as 0.5–2.0 ppm, which must be strictly controlled because concentrations above 10 ppm will promote foaming. Additional control is also necessary to segregate this additives use for frying shortenings only; unintentional additions to bakery shortenings can cause cake and icing failures, glazes may not adhere to donuts, and snack foods may lack crispness.

**Nutrition.** Fats and oils are recognized as important nutrients for both humans and animals because they provide a concentrated source of energy, contain essential fatty acids, and serve as carriers for fat-soluble vitamins. Research studies have also indicated a relationship between saturated fats, cholesterol, and *trans*-isomers and the incidence of coronary heart disease. In many cases, shortening functionality can be maintained with formulations limiting cholesterol, the identified saturated fatty acids, and *trans*-isomers. Shortening formulation can also aid in reductions of fat consumption by development of more effective products to reduce the levels required to produce the desired functionality and finished product quality.

## 9.2. Shortening Formulation

Most shortenings are identified and formulated according to usage. Figure 6 illustrates the diverse SFI or solids-liquids relationships among different fats and oils products. The SFI slopes indicate the differences in plastic range necessary to perform the desired function in the finished food products. Shortenings with the flattest SFI slopes have the widest plastic range for workability at cool temperatures as well as elevated temperatures. The all purpose and puff pastry shortenings in the illustration have the widest plastic range of the plasticized or solidified products. The frying and nondairy shortenings illustrated have relatively steep SFI slopes, which will provide a firm, brittle consistency at room temperature but are fluid at only slightly elevated temperatures. The very flat SFI slope represents a fluid opaque liquid or pumpable shortening that has become popular due to the convenience offered, handling cost savings, and lower saturated fatty acid levels. Liquid shortening systems can be produced with cottonseed oil but the most successful products have been prepared with beta crystal forming hardfats, which produce a stable fluid product with lesser amounts of processing. The most recent addition to the shortening classification is shortening chips. These specialty products are modifications of fat flakes, which were previously limited to hardfats or almost fully saturated fats and oils. Shortening chips are formulated with steep SFI contents to provide solid-to-liquid ratios high enough to flake but low enough for good eating characteristics after melting into the food product (106).

**Wide Plastic Range Shortenings.** The basic all-purpose formulation has been the building block for shortenings where creaming properties, a wide working range, and heat tolerance are important. The functionality of an all-purpose product at any temperature is largely a function of the solids content at that temperature. The all-purpose products are formulated to be not too firm at 50–60°F, or 10–15.6°C, and not too soft at 90–100°F, or 32.2–37.8°C. Initially, a liquid oil was blended with a hardfat to make a compound shortening that had a very flat SFI curve. This blend provided an excellent plastic range similar to but slightly firmer than the liquid shortening SFI curve illustrated on Figure 6. However, the low oxidative stability of these shortenings precluded their use for most products requiring a long shelf life. Currently, most of these products are formulated with a nonselectively hydrogenated basestock and a low iodine value (IV) cottonseed oil hardstock. Beta-prime ( $\beta'$ ) hardfats are added to beta ( $\beta$ ) crystal tending basestocks both to extend the plastic range, which improves the tolerance to high temperatures, and for crystal type and stability. The beta-prime ( $\beta'$ ) crystal forming cottonseed oil hardstock functions as a plasticizer for improved creaming properties, texture, and consistency.

Hydrogenation of a fat and oil basestock increases the oxidative stability. As a rule, the lower the base IV the better the oxidative stability. However, as base hardness is increased, the level of hardstock required to reach a desired consistency must decrease. Hardstock reduction reduces the plastic range and heat tolerance. Therefore, oxidative stability improvements are achieved at the expense of plasticity and a wide plastic range can be at the expense of oxidative stability. The extent that one attribute can be compromised to improve another must be determined by the requirements of the intended food product. Oxidative stability is directly related to the level and type of unsaturated fatty acids present; therefore, oxidative stability results do not average. For example, a 50:50 blend of a 40-hour AOM basestock and a 100-hour AOM basestock will not have an AOM stability of 70 hours, but rather will be closer to the component with the lower AOM stability (152).

Plastic range is important for bakery use fats and oils products intended for roll-in as well as creaming applications because of the consistency changes with temperature. Fats and oils products become brittle above the plastic range and soft below the range; both conditions adversely affect creaming and workability alike. Shortenings are normally plastic and workable at SFI values between 15 and 25. Therefore, products with flatter SFI slopes fall within the plasticity window for a much greater temperature range than those with steep SFI slopes. The all-purpose shortening in Figure 6 has a 40.7°F, or 22.6°C, plastic range from less than 50°F to almost 92°F, or less than 10°C to almost 33.3°C; the frying shortening charted would have an equivalent workability if it was used within the 5.8°F, or 3.2°C, range from 84.2–90°F, or 29–32.2°C. The frying shortening's use as a roll-in would require very strict controlled temperature use probably not available in bakeries and at a temperature detrimental to the laminated baked product. The 40.7°F, or 22.6°C, working range for the all-purpose product is decidedly more practical.

The use of a partially hydrogenated base plus hardfat to produce a wide plastic range with good creaming properties has been expanded into a whole family of

**TABLE 28. Wide Plastic Range Applications.**

Nonemulsified	Emulsified
All-purpose	Household
Danish roll-in	Cake & icing
Puff pastry	Icing & filling
Cookie	Cake mixes
Donut frying	Specialty cakes
Yeast raised	Yeast raised

specialized shortenings. The development of these products has involved the selection of the most suitable hydrogenated basestock and hardfat to produce the desired plastic range and AOM stability. These developments have taken two directions: (1) the addition of an emulsifier or an emulsifier system to an all-purpose fat and oil base or (2) formulating nonemulsified products for a specific functionality. Table 28 outlines some of the current applications for these two categories (106).

**Narrow Plastic Range Shortenings.** Plasticity is of minor importance and can be a detriment for products requiring sharp melting characteristics or a high oxidative stability. Fat and oil products designed for specific frying situations, nondairy systems, cookie fillers, and confectionery fats require an eating character and flavor stability not possible with blends of nonselectively hydrogenated oils with hardfats. These products require as low an iodine value as possible for oxidative stability with a steep SFI slope to provide a melting point lower than body temperature for good eating characteristics. There are two alternative routes to obtain a steep SFI curve: (1) nature has provided the lauric oils with a steep SFI and a sharp melting point or (2) most liquid oils can be selectively hydrogenated to provide the desired solids melting relationships. Selective hydrogenation is a progressive diminution of the most unsaturated fatty acid groups. When the overall hydrogenation effect is that the fatty acids with three double bonds are nearly all reduced to two double bonds, before those with two are nearly all reduced to one, before the monounsaturates are saturated, then good or high selectivity exists (153).

The frying shortening illustrated in Figure 6 meets the restaurant industry requirements for a stable heat-transfer media that becomes a part of the food to supply texture, mouthfeel, and an enhancement to food flavor. Solid frying shortenings are usually composed of a single hydrogenated base or possibly two selectively hydrogenated bases chosen for a slightly different slope than what is available with a single base. In the case of two bases, both have high solids at the lower temperature readings but fall off rapidly to a low melting point, which will result in the desired eating characteristics or mouthfeel with an excellent oxidative and frying stability. For example, the frying shortening on Figure 6 is a blend of the 58 and 70 iodine value cottonseed oil basestocks detailed previously on Table 20. It could also be produced by selectively hydrogenating cottonseed oil to a 65 iodine value.

Nondairy shortenings are used to replace butter fat in applications such as imitation cheese, mellorine, an ice cream substitute, coffee whiteners, vegetable-oil-based whipped toppings, dip bases, milk analogs, etc. The solid fat index values



and melting characteristics of these shortenings usually closely approximate those of butter. The solids contents are relatively high at the cooler temperatures to provide structure for the food products but drop off rapidly to melt completely about body temperature for good eating characteristics (106).

**Liquid Shortenings.** Prior to the introduction of emulsifiers, antioxidants, and antifoamers, shortenings relied on fatty acid composition, crystal habit, plasticization, and tempering to provide functionality. The aeration function of plastic shortenings was correlated with the polymorphic form of the triglyceride while shelf life and frying stability were attained by the level of saturation to resist oxidation. Plastic shortenings that exist in the small beta-prime crystal form aerate batters much more thoroughly than those in the large beta form, and saturation of the unsaturated fatty acids eliminates a reaction site for oxygen to extend flavor and frying stability. These rules are still applicable but emulsifiers, antioxidants, and antifoamers have significantly reduced the dependence on the plastic consistency for functionality. This reduced dependence has allowed the development of liquid shortenings that combine the functional characteristics of plastic shortenings with the bulk handling characteristics of a liquid oil.

A liquid shortening is a stable dispersion of solids with the proper polymorphic form in a continuous oil phase that is both flowable and pumpable over a temperature range of 60–90°F, or 15.6–32.2°C. The solids are derived from either hardfats or emulsifiers and sometimes both. For some applications, the primary function of the liquid shortening is as a delivery system for emulsifiers that alter the characteristics of the food product. Like the plastic shortenings, liquid shortenings are designed for the specific end-use application. Fluid shortenings require a wide range of functional and physical attributes. For optimum fluidity the base oil should be liquid at room temperature and have no suspended solids. Oxidative stability is dependent on the base oil, which may require the use of a high-stability oil. Utilization of a hydrogenated basestock reduces fluidity and requires more exacting processing to prevent product separation. Antioxidant and antifoamer additions improve oxidative stability and frying life, but the improvement does not approach the stability of a plastic shortening with these same additives.

Saturated fatty acid composition of the hardfat or fat-based emulsifiers added to the suspension are important to the functionality and physical form of the fluid shortening system. Palmitic fatty acids are more soluble in oil, but tend to supercool more and take longer to crystallize. Stearic fatty acids do not supercool as much but are not very soluble. For the greatest emulsifier loading in the system, emulsifiers high in palmitic fatty acid content are the best. However, for the most stable viscosity over time, stearic fatty acids are preferred as they readily crystallize out of the liquid oil and form a stable crystalline matrix. Therefore, in contrast to plastic shortenings, it is desirable to formulate nonemulsified liquid shortenings with beta-stable blends whose large crystals tend to form a stable dispersion. Normally, a low-iodine-value, beta-forming hardfat is used to seed crystallization for liquid shortenings. The hardstock levels can vary from as low as 1.0% to higher levels, as required to produce the required viscosity, usually no higher than 10%. The ease in which beta-forming hardfats convert to the stable beta crystal form make them ideal for

liquid shortening crystallization. The beta crystals do not intertwine to form a matrix that can enmesh the liquid phase and form a thick product as found with beta-prime hardfats. The liquid opaque shortening SFI slope plotted on Figure 6 is typical of a liquid bread shortening formulated with 10% soybean hardfat crystallized in 90% unhardened liquid oil. In contrast, emulsified liquid shortenings should be formulated with surfactants made from cottonseed oil, which has a high palmitic fatty acid content, to take advantage of the greater emulsifier loading capability (154).

**Shortening Flakes and Chips.** Another shortening form is represented by products crystallized with a chill roll. Edible oil products crystallized on a chill roll solidify into a thin flake form that affords ease of handling of these higher melting products. For quite some time this crystallization process was limited to hardfats commonly referred to as stearines. Now, chill-roll use has been expanded to include several different products for various applications. These shortening products use both the steep and flat SFI slopes produced with selective and nonselective hydrogenation techniques depending on application.

- **Stearines, Titters, or Low-Iodine-Value Flakes**—Melting before use is the standard procedure for these high-melting, almost completely saturated oil products. Cottonseed hardfat with a 5 or less IV has a melting point of approximately 140°F or 60°C. Some of the applications for these flakes are: melting point adjustment, lubricant, encapsulation base, bread stabilizer, chewing gum ingredient, and so forth.
- **Hard Emulsifiers**—Distribution in frozen desserts, whipped toppings, pasta, peanut butter, yeast-raised products, shortenings, and other applications require that these high-melting surfactants be added as a liquid. The thin flake form allows the melting process to be accomplished at a quicker rate with less damage to the product.
- **Shortening Chips**—These thicker and larger chips are incorporated into baked products to provide a flaky product similar to danish pastry without the labor intensive roll-in process. Normally, the thickness of these chips is controlled at a mean of 0.05 inches while the standard flake thickness is no more than half this thickness. Shortening chips are formulated to resist incorporation into doughs and batters during mixing and still have a palatable mouthfeel. Steep SFI basestocks with high solids contents at 50–80°F, or 10–26.7°C, and declining rapidly thereafter provide this functionality.
- **Stabilizers**—These slightly high-melting, flat SFI profile flakes were designed to stabilize roll icings, syrups, donut glazes, and butter creme icings. An icing stabilizer's performance requires a flat SFI slope to maintain a soft consistency with solids contents high enough to facilitate a rapid set. The rapid set allows the icing to resist fingerprinting when handled while the flat SFI slope allows the icing to retain an elasticity to prevent product brittleness and flaking. A range of melting point products, usually from 110°F, or 43.3°C, to 125°F, or 51.7°C, are made for this application, which has been extended to confections, cake mixes, and other food products.

- **Confectionery Fats**—Confectionery fats require a very steep SFI curve, which makes them brittle with a short melting range that ensures a quick meltdown and a pleasant mouthfeel. These fats, formulated to resemble the characteristics of cocoa butter, are usually referred to as “hard butter.” Quality hard butters have a relatively high solid fat index at room temperature of above 50%, as lower levels can provide a greasy or tacky feel. The SFI then falls off quickly for a complete melt for most products at 95–102° F. (35–39.2° C). Processes used to prepare hard butters include hydrogenation, interesterification, solvent and dry fractionation, and blending. The hard butters can use both lauric and nonlauric source oils.

### 9.3. Cottonseed Oil Utilization

Shortenings are a unique food ingredient in that a high degree of interchangeability among the raw materials is possible for many products and uses. However, in order for a particular oil to substitute for another in a given product, it may be necessary for it to undergo additional processing steps, which may increase its cost to become an adequate replacement. After this additional processing, if the replacement fat or oil can be substituted in terms of physical and analytical properties in the end product, then price becomes a major consideration for the employment of the raw material replacement. Experience has shown that small cost differences in competing source oils can markedly change the proportion of the oils used in a shortening.

Shifts in the use of various fats and oils in the composition of an individual shortening were more common in the past than at present. Source oil labeling requirements have made alternate formulations for the same product difficult. However, shortening customers may still substitute alternative source oil produced shortenings that have comparable performance characteristics. Table 29 tracks the changes in the source oil use for U.S. shortenings from 1940 through 2000 (13, 26–28).

**TABLE 29. U.S. Shortening Source Oils Usage (Metric Ton).**

Year	1940	1950	1960	1970	1980	1990	2000
Coconut oil	8.2	9.1	4.5	20.4	46.7	15.4 <sup>a</sup>	
Corn oil	0.5	0.5	1.8	5.4	W	122.5	12.2
Cottonseed oil	373.3	249.0	165.6	125.2	85.7	114.3	75.3
Palm oil	NR	NR	NR	40.8	85.3	94.8 <sup>a</sup>	84.8 <sup>a</sup>
Peanut oil	10.4	5.4	0.9	7.3	NR	NR	NR
Soybean oil	96.2	381.5	530.3	989.7	1202.5	1816.2	2591.9
Lard	7.7	80.3	217.7	195.0	171.5	119.7	108.4
Tallow	26.3	14.1	121.6	236.8	305.3	288.9	128.8
Unidentified	20.0	12.2	1.4	3.2	8.2	6.4	
Total	542.5	783.4	1043.7	1623.9	1905.1	2578.2	3001.5
Per capita, kg		5.0	5.7	7.8	8.3	10.1	10.5

<sup>a</sup>Estimated.

W = withheld.

NR = none recorded.

Shortenings were developed as lard substitutes to provide an outlet for the then plentiful and inexpensive cottonseed oil. The first shortenings, prepared by blending liquid cottonseed oil with stearines, were no better than the product imitated. Introduction of the hydrogenation process enabled cottonseed oil processors to develop shortenings that outperformed the natural lard products. Shortenings, initially marketed as economic lard replacements, became the desired product that commanded premium pricing. Specialty shortening development was enhanced with the introduction of mono- and diglyceride emulsifiers in 1933. Throughout these developments, cottonseed oil retained its position as the dominant source oil for shortenings until soybean oil rose from a minor, little known, problem-related oil before 1940 due to a shortage of cottonseed oil created by World War II. A plentiful supply of lower cost soybean oil and the advanced technologies developed during the war years made it impossible for cottonseed oil to regain its dominant source oil position for shortenings. However, it could not be totally replaced, cottonseed oil has some definite advantages over soybean and most other vegetable oils, one being its beta-prime ( $\beta'$ ) crystal habit. A smooth consistency, fine texture, wide plastic range, good creaming properties, and a tolerance to high temperatures have become the standard for shortening. Most of these desirable characteristics are contributed by the tiny, needle-like beta-prime ( $\beta'$ ) crystals that pack together into dense, fine grained, ridged structures to form a three-dimensional network capable of immobilizing a large amount of liquid oil. Soybean oil, as well as most of the other available vegetable oils, has a beta ( $\beta$ ) crystal habit, which are large, coarse, high-melting, self-occluding crystals that clump, allowing separation of the liquid oils, and responsible for a visible grainy appearance. A beta-prime ( $\beta'$ ) crystal form can be induced with the addition of hydrogenated cottonseed oil, or another oil with a beta-prime ( $\beta'$ ) crystal habit (see Table 12 Crystal Habit of Hydrogenated Oils and Fats), usually at levels of 10% or higher.

## 10. MARGARINE AND SPREADS

Margarine is a flavored food product containing 80% fat, made by blending selected fats and oils with other ingredients, fortified with Vitamin A, to produce a table, cooking, or baking fat product that serves the purpose of dairy butter but is different in composition and can be varied for different applications (145). Margarine was developed to fill both an economic and a nutritional need when it was first made as a butter substitute. The ability to be physically altered to perform in many varied applications was a major factor in the growth of margarine. There are over ten different types of margarine produced today, including regular, whipped, soft tub, liquid, diet, spreads, no fat, restaurant, bakers, and specialty types, which are packaged in as many different packages. These margarines are made from a variety of fats and oils, including cottonseed, soybean, palm, corn, canola, safflower, sunflower, lard, and tallow. Margarine products cater to the requirements of all the different consumers; retail, foodservice, and food processor.

TABLE 30. U.S. Margarine and Spread Source Oil Usage.

Year	Metric Ton (MT)					
	1950	1960	1970	1980	1990	2000
Corn oil	0.5	30.8	83.9	101.2	94.3	25.4
Cottonseed oil	232.7	76.2	30.8	11.3	D	D
Safflower oil		5.9	10.0			
Soybean oil	173.3	621.4	639.6	749.8	793.3	758.9
Animal fats	7.3	34.5	44.9	47.2	15.9	5.9
Unidentified	11.3		4.5	15.4	49.9	5.9
Total	425.0	768.8	813.8	924.9	953.5	796.1
Per Capita, lbs	2.8	4.3	5.0	5.2	4.9	3.8

D = data withheld by census.

### 10.1. Cottonseed Oil Utilization

Cottonseed oil lost its dominant source oil position for margarine oils in 1951. Soybean oil became the leading margarine source oil due to the technologies developed for use of this lesser cost raw material. Cottonseed oil use in margarines steadily decreased thereafter to also be surpassed by corn oil in 1963. Margarine manufacturers found that cottonseed oil was no longer required in the formulation to maintain a beta-prime crystal habit, due to the use of multiple high-*trans* basestocks and the improvements in shipping, storage, and grocery store display refrigeration capabilities. This eliminated the 10% or more hydrogenated cottonseed oil in most consumer margarine oil formulations. Most of the industrial margarine oil formulations still maintain a hydrogenated cottonseed oil hardstock in the formulation to provide the plasticity and consistency desired. The changes in source oil use in margarine and spread base oils are outlined in Table 30.

### 10.2. Margarine Oil Formulation

The physical and functional aspects of a margarine product are primarily dependent on the characteristics of the major ingredient—the margarine oil or marbase. Margarine consistency, flavor, and emulsion stability depend on crystallized fat. In the United States, hydrogenation is the preferred process used to change the solid-liquid relationship of margarine basestocks. A direct relationship exists between the fat solids content and the structure, consistency, and plasticity of the finished margarine. SFI values at 50°F, 70°F, and 92°F, or 10.0°C, 21.1°C, and 33.3°C, are used by most margarine manufacturers in the United States for margarine consistency control. The SFI values are indicative of the crystallization tendencies and the finished product quality as shown in Table 31 (106).

**Consumer Margarine Oil Formulations.** Consumer margarines are formulated by blending two or more basestocks with different degrees of hardness. This permits the margarines to be spreadable directly out of the refrigerator and to maintain a solid consistency at room temperature. Hydrogenated cottonseed oil was a

**TABLE 31. Consumer Margarine Solid Fat Index (SFI) Affect on Product Characteristics.**

Solid Fat Index (SFI)		Characteristic Affected	Solid Fat Index (SFI) Influence
Temperature	% Solids		
10°C or 50°F	10 to 28	Spreadability	Optional range for spreadability at refrigerator temperatures.
10°C or 50°F	10 to 28	Printability	Low SFI = Colder Chilling Temp. Required High SFI = Channeling Possible with Cooling
21.1°C or 70°F	5 to 18	Consistency	Body and Resistance to Oil Separation Too High = Brittle, Firm, Poor Spreadability Too Low = Soft, Soupy, Oil Separation
33.3°C or 92°F	3.5 max	Mouth Feel	Quality Consumer Tablegrade Margarine Melts Quickly With a Cooling Sensation Too High = Lingering Pasty, Greasy, Waxy, Sensation Due to Palate Coating

component of most vegetable oil margarines to induce a beta-prime crystal habit to prevent graininess for quite some time. However, the development of more spreadable margarines, the use of multiple basestocks, and uniformly low cold storage temperatures has reduced the transition of margarines formulated with soybean and corn oils to the beta crystal form that causes sandiness.

The hydrogenated vegetable oil basestocks best suited for table-grade products have steep SFI slopes to provide the desired eating, melting, and nonoiling physical characteristics along with machinability. The cottonseed oil basestocks previously presented in Table 20 may be used to produce margarine oils as well as shortenings. Margarines have been able to conform to the increased health consciousness of the U.S. consumer by responding with increased polyunsaturated fatty acids and liquid-oils levels. Although polyunsaturate level is less emphasized now, apparently it is still perceived as healthy by the consumer. Therefore, it is still advantageous to have a high polyunsaturate-to-saturates ratio contributed by a high liquid-oil level. Table 32 identifies the typical SFI values for six table-grade marbases; three are

**TABLE 32. Typical SFI Values for Margarine Products.**

	10°C	21.1°C	26.7°C	33°C	40°C
Solids Fat Index:	50°F	70°F	80°F	92°F	104°F
Consumer prints:					
Soft stick	22.0	13.5		2.0	
All hydrogenated	28.5	17.5		3.0	
High liquid	30.0	17.0		1.5	
Consumer Tub:					
All hydrogenated	12.0	7.5		3.0	
High liquid oil	11.0	5.5		0.7	
Consumer liquid	7.0	6.0	6.0	5.5	5.0
Bakers margarine	24.0	18.0	17.0	13.0	7.0
Roll-in margarine	29.0	24.0	22.0	16.0	10.0
Puff pastry	34.0	30.0	28.0	24.0	17.0

for stick products, two are soft tub types and a liquid or squeezable margarine. The soft stick marbase probably represents the softest stick type margarine that can be packaged successfully. The present day packaging equipment will deposit, wrap, and carton product that is softer, but the margarine could not withstand the normal abuse after packaging in storage and distribution. This product was introduced after the soft tub products in an effort to take advantage of the soft concept in the old familiar stick package (106).

The all-hydrogenated stick table-grade product represents the type that was the main consumer margarine product for quite some time. It is still the most preferred table fat product for baking and some cooking due to better oxidative stability. This product also prints well due to the high 50°F or 10°C SFI, especially in equipment that doesn't deposit the margarine into preformed quarters parchment. The high liquid-oil stick marbase represents the majority of the stick margarine production. The high liquid oil in the formula provides a marketing claim for low saturates but reduces the finished margarine's oxidative stability and therefore shelf life. The surface of the margarine develops a darker color from oxidation that is quite noticeable when the surface layer is scraped away during use.

Soft tub marbase compositions are somewhat like a compound shortening formulation: a blend of a soft and a hard basestock. The hard basestock can't be as saturated as the low-iodine-value hardfats used for shortenings and should have a steep SFI slope for good eating characteristics. Two different soft tub compositions are shown in Table 32. The all-hydrogenated product provides the oxidative stability and the firmest margarine consistency. Slight consistency differences can be the difference between a soft tub margarine with a picture perfect surface appearance or one with excessive "lid slosh." The all-hydrogenated margarine oil base has a better chance of retaining a smooth surface because it should set quicker than the product formulated with liquid oil.

Liquid margarine has been marketed for quite some time but it has never achieved significant consumer acceptance. Food processors have accepted and used this product for specialty applications more so than consumers. Liquid margarine has been prepared using both beta and beta-prime type hardfats. Beta-prime ( $\beta'$ ) hardfats, like cottonseed oil, have been found more suitable for the product packaged without a tempering for crystallization process stage. Product prepared with beta crystal forming hardfats requires tempering of the supercooled mixture at an elevated temperature for a period of time under agitation to develop and stabilize the beta crystal. This liquid margarine process resembles the liquid shortening process closely. The beta crystal formulation and procedure produces a more fluid, less viscous product with better suspension stability than the beta-prime product but costs more to produce (151). The beta-prime hardfat direct process provides a better mouthfeel and flavor but requires constant refrigeration to avoid separation.

**Industrial Margarines.** Foodservice and food processor margarines and spreads are considered industrial products. These products are formulated or packaged for more specific applications than the consumer products. The most popular

foodservice margarine is the consumer stick margarine formulation packaged in one-pound solids for use in food preparation. Individual serving or portion control soft spread products are popular foodservice dining room products. The major difference that affects the desired functionality is the margarine oil composition.

The consumer table-grade margarine formulation is also packaged in 50 pound and larger containers for food processor applications. It is plasticized like a shortening instead of the margarine print procedure for roll-in and other applications. Baker's margarine is designed to have a wide plastic range with good creaming properties like the standard all-purpose shortening. In fact, the marbase for bakers margarine can be the same composition as an all-purpose shortening using an 80–85 IV hydrogenated cottonseed or soybean oil basestock with a fully hydrogenated beta-prime crystal forming cottonseed oil hardfat. Special roll-in margarines for danish and other pastries are prepared with fat blends similar to anhydrous or shortening products. Margarine formulations provide a buttery flavor and color to the finished product as well as moisture to produce steam during baking to help expand the dough layers and improve flakiness. Several other specialty products also rely on the marbase formulation for functionality and the margarine process to provide flavor, color, and moisture for functionality (106).

### 10.3. Spread Formulations

All products resembling margarine that contain less than 80% but more than 40% fat are required to be labeled as spreads. However, these products must conform with the margarine standard in all respects except the fat content and that safe and suitable ingredients not provided for in the standard may be added to improve functional characteristics so that the spreads are not inferior to margarine. Soft tub and stick spreads containing 40% to 75% fat are usually formulated from the same fat and oil blends as those used for regular consumer margarines. The other ingredients used are also basically the same with the following exceptions (155, 156):

- Milk protein acts as an oil-in-water emulsifier. Consequently, the use of milk, casein, or caseinates can result in a phase reversion. Therefore, most spread formulations are milk and milk-protein free.
- Emulsifier levels are increased slightly to improve the physical characteristics of the emulsion and its stability; typically 0.4% to 0.6% alpha monoglyceride levels with mono- and diglyceride use. Soft mono- and diglycerides are essential in protein-free spreads. Mono- and diglyceride and polyglycerol emulsifier systems have been found effective in spreads containing significant quantities of protein.
- Lecithin use in low fat spreads may decrease the emulsion stability and increase the tendency to oil off; however, it also functions to slow the emulsion breakdown in the mouth. Therefore, the use of lecithin and the level of use must be evaluated for each formulation.



- Gelatin, pectin, carrageenans, agar, xanthan gum, starch alginates, or methylcellulose derivatives are gelling or thickening agents used in some spreads to improve the body.
- The higher emulsifier levels used for spread can produce tighter emulsions and the gelling or thickening agents can affect the rate and order that flavors are perceived. The flavor content and types must be defined to produce oral responses similar to the high-fat products.
- Preservatives are more important in spreads than in regular margarines as a result of the higher moisture content.
- The light reflection of a spread is different from that of regular margarine as a result of the increased number of water droplets present. Therefore, it is necessary to add about twice the amount of color used in normal margarine to obtain the same color intensity.

## 11. OTHER COTTONSEED OIL USES

Major changes are continually occurring in the markets for fats and oils as discussed previously. Food applications have been a major use for cottonseed oil but it has also been used in soap, lubricants, sulfonated oil, pharmaceuticals, protective coastings, rubber, as a carrier for nickel catalysts, and, to a lesser degree, in the manufacture of leather, textiles, printing ink, polishes, synthetic plastics, and resins (23). More recently, cottonseed oil was used in the synthesis of sucrose polyesters as a zero-calorie fat substitute that has a trade name of Oleans, or a common name of Olestra. This product, developed by Proctor & Gamble, was approved by the U.S. Food and Drug Administration in 1996 (157–160). Cottonseed oil was chosen for this application because it imparted a pleasing nutty flavor to the fat substitute.

## REFERENCES

1. Anonymous, *INFORM*, **11**(8), 820–839 (2000).
2. S. R. Gregory, E. Hernandez, and B. R. Savoy, in C. W. Smith J. T. and Cothren, eds., *Cottonseed Processing. Cotton Origin, History, Technology, and Production*, John Wiley & Sons, New York, 1999, pp. 793–823.
3. R. D. O'Brien and P. J. Wan, in R. F. Wilson, ed., *Proceedings of the World Conference on Oilseed Processing and Utilization*, AOCS Press, Champaign, Illinois, 2001, pp. 90–140.
4. National Cottonseed Products Association, Inc., *Cottonseed And Its Products*, 9th ed., 1989, pp. 4–7.
5. L. B. Wrenn, *Cinderella of the New South A History of the Cottonseed Industry, 1855–1955*, The University of Tennessee Press, Knoxville, Tennessee, 1995, pp. 9, 15, 44, 73–74, 76, 80, and 81.

6. L. A. Jones and C. C. King, eds., in *Cottonseed Oil*, National Cottonseed Products Association, Inc. and the Cotton Foundation, Memphis, Tennessee, 1990, pp. 1–2, 5–7, 9–10, and 14.7. L. B. Wrenn, *INFORM*, **4**(1), 6–19 (1993).
7. L. B. Wrenn, *INFORM*, **4**(1), 6–19 (1993).
8. L. B. Wrenn, *INFORM*, **6**(1), 98 (1995).
9. L. B. Wrenn, *INFORM*, **9**(1), 100–106 (1998).
10. L. H. Meyer, in *Food Chemistry*, The AVI Publishing Co., Westport, Connecticut, 1975, p. 55.
11. R. D. O'Brien, in F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, CRC Press, Boca Raton, Florida, 2002, pp. 217–223.
12. W. H. Goss, in Proceedings of A Six-Day Short Course in Vegetable Oils, The American Oil Chemists' Society and The University of Illinois, Champaign, Illinois, 1948, pp. 53–55.
13. K. F. Mattil, in D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, 3rd ed., Wiley Interscience New York, 1964, pp. 249–263, and 296.
14. E. M. James, U. S. Patent 2,050,844, 1936.
15. B. Clayton, U.S. Patents 2,100,276 and 2,100,277, 1937.
16. B. H. Thurman, U.S. Patent 2,150,733, 1939.
17. H. C. Black, A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience Publisher, Inc., New York, 1948, pp. 732–762.
18. C. E. Morris, *J. Amer. Oil Chem. Soc.*, **26**(10), 607 (1949).
19. R. C. Hastert, *INFORM*, **9**(12), 1140 (1998).
20. R. D. O'Brien, in P. J. Wan et al., eds., *Introduction to Fats and Oils Technology*, 2nd ed., AOCS Press, Champaign, Illinois, 2000, pp. 421–426.
21. L. C. Brown, *J. Amer. Oil Chem. Soc.*, **26**(10), 634 (1949).
22. J. E. Slaughter, Jr., in *Proceedings of A Six-Day Short Course in Vegetable Oils*, The American Oil Chemists' Society and The University of Illinois, Champaign, Illinois, 1948, pp. 119–131.
23. A. E. Bailey, in A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience Publisher, Inc., New York, 1948, pp. 364–408.
24. H. J. Dutton, *J. Amer. Oil Chem. Soc.*, **58**(3), 234–236 (1981).
25. USDA, *Fats and Oils Situation*, April 1971, pp. 24–25, 27.
26. USDA, *Fats and Oils Situation*, July 1977, pp. 14–15.
27. USDA, *Oil Crops Situation and Outlook Yearbook*, August 1985, p. 8. USDA, *Fats and Oils Situation Report*, January 1981, Table IX.
28. USDA, *Oil Crops Situation and Outlook Yearbook*, October 2002, pp. 40, 44, 53, 62–66, and 68.
29. L. A. Jones and C. C. King, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., Wiley-Interscience, New York, 1996, pp. 188–189.
30. National Cottonseed Products Association, *The Cottonseed Industry Statistical Handbook*, Memphis, Tennessee, 2002.
31. C. E. Lund, in C. E., in A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience Publisher, Inc., New York, 1948, pp. 58, 62, and 64.
32. USDA, *Oil Crops Situation and Outlook Yearbook*, October, 1997.

33. USDA, *Oil Crops Situation and Outlook Yearbook*, October, 1998, p. 72.
34. P. J. Wakelyn, in *Kirk-Othmer Encyclopedia of Chemical Technology*, 5th ed., John Wiley & Sons, Inc. New York, 2002.
35. R. J. Kohel and C. F. Lewis, eds., *Cotton*, American Society of Agronomy, Crop Society of America, Soil Society of America, Madison, Wisconsin, 1984.
36. F. D. Gunstone, in F. D. Gunstone, ed., *Vegetable Oils in Food Technology*, CRC Press, Boca Raton, Florida, 2002, p. 11.
37. K. Esau, *Anatomy of Seed Plants*, John Wiley & Sons, Inc., New York, 1977.
38. A. E. Percival, J. E. Wendel, and J. M. Stewart, in C. W. Smith and J. T. Cothren, eds., *Cotton Origin, History, Technology, and Production*, John Wiley & Sons, New York, 1999, pp. 33–63.
39. D. S. Calhoun and D. T. Bowman, in C. W. Smith and J. T. Cothren, eds., *Cotton Origin, History, Technology, and Production*, John Wiley & Sons, New York, 1999, pp. 361–414.
40. M. F. Stansbury, A. F. Cucullu, and G. T. Den Hartog, *J. Agric. Food Chem.*, **2**, 693 (1954).
41. H. H. Ramsey, in Proceedings 33rd Oilseed Processing Clinic, New Orleans, Louisiana, January 16–17, 1984.
42. W. H. Tharp, in A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience Publishers, Inc., New York, 1948, pp. 117, and 142–149.
43. N. O. V. Sonntag, in D. Swern, ed., *Bailey's Industrial Oils and Fat Products*, Vol. 1, 4th ed., Wiley Interscience Publishing Co., New York, 1979, pp. 289, 352–362.
44. M. L. Meara and E. H. Steiner, *Studies on the Fatty Acid Composition and the Crystallizing Behavior of Some of the Major Components, to Obtain Fundamental Information That Will Contribute to the Development of Improved Edible Products and Hence to Expanded Utilization of Cottonseed Oil*, British Food Manufacturing Industries Research Association, Leatherhead, Surrey, U.K., 1966.
45. H. R. Leffler, in Proceedings 33rd Oilseed Processing Clinic, New Orleans, Louisiana, January 16–17, 1984.
46. Z. M. Sawan, A. A. El-Farra, and S. A. El-Latif, *J. Agron. Crop Sci.*, **161**, 50 (1988). M. K. Schwitzer, *Margarine and Other Food Fats*, Interscience Publisher, New York, 1956, p. 64.
47. J. F. O'Kelly, W. W. Hull, and M. Geiger, Technical Bulletin 20, Mis. Agr. Exp. Sta., State College, Mississippi, 1933.
48. C. H. Boatner, in A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience Publisher, Inc., New York, 1948, p. 213.
49. F. A. Norris, in D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, Wiley Interscience New York, 982, pp. 175, 253, 295, and 479.
50. L. C. Dasilva-Ramos and R. J. Kohel, *J. Amer. Oil Chem. Soc.*, **64**, 1337 (1987).
51. C. M. Lawhon, Cater, and K. F. Mattil, *J. Amer. Oil Chem. Soc.*, **54**, 75 (1977).
52. P. J. Wan, *Oil Mill Gaz.*, **103**(11), 38–45 (1998).
53. Agricultural Research Service and National Cottonseed Products Association, in Proceedings of a Conference, Southern Regional Research Center, ARS, USDA, New Orleans, Louisiana, 1978.
54. E. W. Lusas and G. M. Jividen, *J. Amer. Oil Chem. Soc.*, **64**, 839 (1987).

55. P. J. Wakelyn and P. J. Wan, in C. Tzia, ed., *Extraction Optimization*, Marcel Dekker, New York, 2003, pp. 391–427.
56. T. A. Wilkins and R. Mishra, in Proc. 11th International Plant and Animal Genome Conference, Scherago Int., New York, 2003, p. 61.
57. International Cotton Advisory Committee, *ICAC Recorder*, **XX**(4), 8–12 (2002).
58. International Cotton Advisory Committee, *Report of the Second Expert Panel on Biotechnology of Cotton*, 2004.
59. D. L. Nida, K. H. Kolacz, R. E. Buehler, W. R. Deaton, W. R. Schuler, T. A. Armstrong, M. L. Taylor, C. C. Ebert, G. J. Rogan, S. R. Padgett, and R. L. Fuchs, *J. Agric. Food Chem.*, **44**, 1960–1966 (1996); D. L. Nida, S. Patzer, P. Harvey, R. Stipanovic, R. Wood, and R. L. Fuchs, *J. Agric. Food Chem.*, **44**, 1967–1974 (1996a).
60. O. A. Momtaz, A. A. Diab, and M. A. Madkour, in *Proceedings. Beltwide Cotton Conference*, National Cotton Council, Memphis, Tennessee, 2000, pp. 513–517.
61. R. G. Cantrell, in *Proceedings World Cotton Research Conference 3*, Capetown, South Africa, International Cotton Advisory Committee. Washington, D.C., 2003.
62. Y. L. Cousins, B. R. Lyon, and D. J. Llewellyn, *Aust J. Plant Physiol.*, **18**, 481–494 (1991).
63. X. Y. Wu and X. H. Xiang, *J. Hunan Agric. Univ.*, **24**, 494–498 (1998).
64. Q. Liu, S. Singh, and A. Green, *J. Amer. Coll. Nutr.*, **21**(3), 205S–211S (2002).
65. K. S. Rathore, in *Proceedings Beltwide Cotton Conference*, National Cotton Council, Memphis, Tennessee, 1997.
66. J. H. Benedict and D. W. Altman, in J. J. Jenkins and S. Saha, eds., *Emerging Technologies in Cotton Breeding*, Science Publishers, Enfield, New Hampshire, 2000, pp. 137–201.
67. C. R. Benedict, J-L. Lu, G. S. Martin, J. Lu, and R. D. Stipanovic, in C. R. Benedict and G. M. Jividen, eds., *Genetic Control of Cotton Fiber and Seed Quality*, Cotton Incorporated, Cary, North Carolina, 2000, pp. 244–249.
68. G. S. Martin, C. R. Benedict, J. Liu, and L. Puelchaber, in C. R. Benedict and G. M. Jividen, eds., *Genetic Control of Cotton Fiber and Seed Quality*, Cotton Incorporated, Cary, North Carolina, 2000, pp. 262–270.
69. P. J. Wakelyn and P. J. Wan, *Oil Mill Gaz.*, **106**(8), 14–22 (2001).
70. K. Blaine, S. Kamalden, and D. Powell, *J. Food Sci.*, **67**(9), 3200–3208 (2002).
71. European Commission, *GM-Derived Cottonseed Oils Notified for Marketing in the EU*, Midday Express Notification, December 19, 2002.
72. P. J. Wan, in R. D. O'Brien, W. E. Farr, and P. J. Wan, ed., *Introduction to Fats and Oils Technology*, 2nd ed., American Oil Chemists' Society, Champaign, Illinois, 2000, pp. 20–48.
73. G. Jurriens and A. C. J. Kroesen, *J. Amer. Oil Chem. Soc.*, **42**, 9 (1965).
74. J. P. Cherry, *J. Amer. Oil Chem. Soc.*, **60**, 360 (1980).
75. P. J. Van Niekerk and A. E. C. Burger, *J. Amer. Oil Chem. Soc.*, **62**, 531 (1985).
76. J. B. Reeves and J. C. Weirauch, *Composition of Foods, Agriculture Handbook No. 8-4*, United States Department of Agriculture, Washington, D.C., 1979.
77. D. Firestone, *Physical and Chemical Characteristics of Oils, Fats, and Waxes*, AOCS Press, Champaign, Illinois, 1999, pp. 32–33.

78. J. P. Cherry, J. G. Simmons, and R. J. Kohel, in Proceedings of Belwide Cotton Production Research Conference, 1978.
79. J. P. Cherry, R. J. Kohel, L. A. Jones, and W. H. Powell, in J. R. Mauney and J. M. Stewart, eds., *Cotton Physiology*, The Cotton Foundation, Memphis, Tennessee, 1986, p. 557.
80. J. M. Bland, E. J. Conkerton, and G. Abraham, G., *J. Amer. Oil Chem. Soc.*, **68**, 840 (1991).
81. R. A. Phelps, F. S. Shenstone, A. R. Kemmerer, and R. J. Evans, *Poult. Sci.*, **44**, 358 (1965).
82. E. Allen, A. R. Johnson, A. C. Fogerty, J. A. Pearson, and F. S. Shenstone, *Lipids*, **2**, 419 (1967).
83. F. L. Carter and V. L. Frampton, *Chem. Rev.*, **64**, 497 (1964).
84. J. S. Harris, F. C. Magne, and E. L. J. *Amer. Oil Chem. Soc.*, **41**, 309 (1964). R. C. Hastert, *The Partial Hydrogenation of Edible Oils*, AOCS Short Course, Phoenix, Arizona, 1988.
85. A. V. Bailey, J. A. Harris, and E. L. Skau, E. L., in *J. Amer. Oil Chem. Soc.*, **43**, 107 (1966).
86. F. S. Shenstone and J. R. Vickery, *Nature*, **190**, 168 (1961).
87. P. H. Eaves, H. P. Depuy, L. L. Holenthol, E. T. Rayner, and L. E. Brown, *J. Am. Oil Chem. Soc.*, **45**, 293 (1968).
88. V. L. Frampton, *Poult. Sci.*, **45**, 527 (1966).
89. R. J. Evans, D. H. Bauer, and C. J. Flegal, *Poult. Sci.*, **54**, 824 (1975).
90. L. Marchlewski, *J. fuer Praktische Chemis*, **60**, 81 (1899).
91. W. A. Pons, Jr., C. L. Hoffpauir, and O'Connor, *J. Amer. Oil Chem. Soc.*, **28**, 8 (1951).
92. Y. Yabe, S. Tan, T. Ninomiya, and T. Okada, *J. Food Hygien. Soc. Jpn.*, **25**, 264 (1984).
93. B. J. F. Hudson and M. Ghavami, *Lebensm. -Wiss. -u Technol.*, **17**, 191 (1984).
94. N. A. M. Eskin, B. E. McDonald, R. Przybylski, L. J. Malcolmson, S. Scarth, T. Mag, K. Ward, and D. Adolph, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., Wiley-Interscience, New York, 1996, pp. 52–53 and 67.
95. T. Harper, in R. F. Wilson, ed., *Proceedings of the World Conference on Oilseed Processing and Utilization*, AOCS Press, Champaign, Illinois, 2001, p. 25.
96. L. N. Norcia and B. E. Rosenthal, *JAOCS*, **43**, 168–170 (1996).
97. J. A. Pennington, *Bowes and Church's Food Values of Portions Commonly Used*, J. B. Lippincott Co., New York, 1994.
98. E. H. Pryde, in D. R. Erickson et al., eds., *Handbook of Soy Oil Processing and Utilization*, Soybean Assoc. and Am. Oil Chem. Soc., St Louis, Missouri and Champaign, Illinois, 1980, p 13.
99. K. J. Smith, P. B. Polen, D. M. DeVries, and F. B. Coon, *J. Amer. Oil Chem. Soc.*, **45**(9), 866–869 (1968).
100. T. L. Mounts, C. D. Evans, H. J. Dutton, and J. C. Cowan, *J. Amer. Oil Chem. Soc.*, **46**(9), 482–484 (1969).
101. M. M. Chaudry, A. I. Nelson, and E. G. Perkins, *J. Amer. Oil Chem. Soc.*, **53**(11), 695–697 (1976).
102. F. J. Flider and F. T. Orthoefer, *J. Amer. Oil Chem. Soc.*, **58**, 270–272 (1981).

103. *Official Methods and Recommended Practices of the AOCS*, 5th ed., 2nd printing, AOCS, Champaign, Illinois, 1999.
104. D. Swern, in D. Swern, ed., *Bailey's Industrial Oils and Fat Products*, 3rd ed., Interscience Publishers, New York, 1964, pp. 128–131.
105. C. E. Lund, in A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience Publishers, New York, 1948, pp. 58, 62, and 64.
106. R. D. O'Brien, *Fats and Oils Formulating and Processing for Applications*, Technomic Publishing Co., Lancaster, Pennsylvania, 1998, pp. 1–4, 47–54, 53–121, 129–131, 168–172, 182–183, 188–190, 199–200, 251–253, 258–260, 264–266, 266–290, 327–341, 437–458, 483, and 525.
107. D. R. Erickson and G. R. List, in T. H. Applewhite, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 3, 4th ed., John Wiley & Sons, Inc., New York, 1985, pp. 275–277.
108. R. D. O'Brien, *Fats and Oils: Formulating and Processing for Applications*, Technomic Publishing, Lancaster, Pennsylvania, 1998.
109. L. A. Johnson, Report to Cotton Inc., Raleigh, North Carolina, 1981.
110. A. M. Altschul, in A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience Publisher, Inc., New York, 1948, p. 157.
111. J. R. Mauney and J. M. Stewart, *Cotton Physiology*, The Cotton Foundation, Memphis, Tennessee, 1986.
112. D. L. Park, L. S. Lee, R. L. Price, and A. E. Pohland, *J. Assoc. Off. Anal. Chem.*, **71**, 685 (1985).
113. F. A. Norris, L. J. Hilfman, and C. E. Land, *J. Amer. Oil Chem. Soc.*, **35**, 684 (1958).
114. K. C. Rhee, E. W. Lusas, L. R. Watkins, and D. H. Kinard, in Proceedings 37th Oilseed Processing Clinic, New Orleans, Louisiana, March 14–15, 1988.
115. R. A. Rusca and F. L. Gerdes, U.S. Department of Agriculture Circular 651, 1942.
116. O. H. Alderks, in A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience Publisher, Inc., New York, 1948, p. 615.
117. J. P. Galloway, *J. Amer. Oil Chem. Soc.*, **53**, 271 (1976).
118. J. A. Ward, *J. Amer. Oil Chem. Soc.*, **53**, 261 (1976).
119. P. J. Wan and P. J. Wakelyn, *INFORM*, **9**(12), 1155–1160 (1998).
120. K. F. Carlson and J. D. Scott, *Inform* **2**, 1034–1060, 1991.
121. L. R. Watkins, Proceedings 37th Oilseed Processing Clinic, New Orleans, Louisiana, March 14–15, 1988.
122. D. P. French, in Proceedings 37th Oilseed Processing Clinic, New Orleans, Louisiana, March 14–15, 1988.
123. E. M. James, in A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience Publisher, Inc., New York, 1948, pp. 705–706.
124. L. S. Crauer, *J. Amer. Oil Chem. Soc.*, **41**, 656–661 (1964).
125. G. C. Cavanagh, *J. Amer. Oil Chem. Soc.*, **53**, 361–363 (1976).
126. W. B. Hendrix, *J. Amer. Oil Chem. Soc.*, **61**, 1369–1372, 1984.
127. T. J. Weiss, in *Food Oils and Their Uses*, AVI Publishing Co., Inc., Westport, Connecticut, 1983, p. 39.
128. T. R. Bessler and F. T. Ortheofer, *J. Amer. Oil Chem. Soc.*, **60**(10), 1765–1766 (1983).

129. G. C. Cavanagh, *Food Processing*, **22**(4), 38–45 (1961).
130. R. C. Hastert, *AOCS short course*, Phoenix, Arizona, 1988.
131. R. D. O'Brien, in *Fats and Oils Formulating and Processing for Applications*, CRC Press, Boca Raton, Florida, 2003.
132. R. D. O'Brien, in P. J. Wan et al., eds., *Introduction to Fats and Oils Technology*, 2nd ed., AOCS Press, Champaign, Illinois, 2000, pp. 463–485.
133. D. Lampert, in P. J. Wan, ed., *Introduction to Fats and Oils Technology*, 2nd ed., AOCS Press, Champaign, Illinois, 2000, pp. 208–234.
134. F. A. Norris, *J. Amer. Oil Chem. Soc.*, **25**(8), 274–275 (1947).
135. P. J. Wakelyn and P. K. Adair, in S. S. Koseoglu, K. C. Rhee, and R. F. Wilson, eds., *Emerging Technologies, Current Practices, Quality Control, Technology Transfer, and Environmental Issues*, Vol. 1, Proc. of the World Conference on Oilseed and Edible Oil Processing, AOCS Press, Champaign, Illinois, 1998, pp. 305–312.
136. P. J. Wakelyn, P. K. Adair, and S. R. Gregory, *Oil Mill Gaz.*, **103**(6), 23–30 (1997).
137. P. J. Wakelyn, *Cotton Gin Oil Mills Press*, **92**(17), 12–15, 18 (1991).
138. P. J. Wakelyn and L. A. Forster, Jr., *Oil Mill Gaz.*, **99**(12), 21–30 (1994).
139. U. S. EPA, The Toxic Release Inventory (TRI) and Factors to Consider when Using TRI Data. [http://www.epa.gov/tri/2002\\_tri\\_brochure.pdf](http://www.epa.gov/tri/2002_tri_brochure.pdf).
140. P. J. Wakelyn, in P. J. Wan, D. O'Brien, and W. Farr, eds., *Introduction to Fats and Oils Technology*, 2nd ed., AOCS Press, Champaign, Illinois, 2000, pp. 302–325.
141. European Union (EU), *J. Eur. Commun. L*, **157**, 0028–0033 (1988).
142. A. H. Allen, *Food Product Design* (1996).
143. B. L. Oser and R. A. Ford, *Food Technol.*, **27**(1), 64 (1973).
144. R. D. O'Brien, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, 5th ed., Wiley, New York, 1996, pp. 190–191.
145. R. D. O'Brien, Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 3, 5th ed., Wiley-Interscience, New York, 1996, pp. 190–191.
146. S. F. Riepma, *The Story of Margarine*, Public Affairs Press, Washington, D.C., 1970, p. 5, 109, 148, and 153.
147. Anonymous, *INFORM*, **2**(4), 373 (1991).
148. C. C. Price, *Food Rev.*, **19**(3), 11 (1996).
149. R. J. Bell, R. J., Shortening and Margarine Products, in P. J. Wan, ed., *Introduction to Fats and Oils Technology*, American Oil Chemists' Society, Champaign, Illinois, 1991, pp. 183–210.
150. W. W. Nawar, in O. R. Fennema, ed., *Food Chemistry*, 2nd ed., Marcel Dekker, New York, 1985, p. 139.
151. L. H. Weideman, *J. Amer. Oil Chem. Soc.*, **55**(11), 823 (1978).
152. E. G. Latondress, in D. R. Erickson et al., eds., *Handbook of Soy Oil Processing and Utilization*, Am. Soybean Assoc. and Am. Oil Chem. Soc., 1980, pp. 146–154.
153. H. B. Patterson, *Oleagineux*, **28**(12), 585 (1973).
154. A. G. Herzing, *INFORM*, **7**(2), 165–167 (1996).
155. M. M. Chrysam, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 3, 5th ed., Wiley-Interscience, New York, 1996, pp. 67–68 and 92–96.

156. A. Moustafa, in D. R. Erickson, ed., *Practical Handbook of Soybean Processing and Utilization*, AOCS Press and United Soybean Board, Champaign, Illinois and St. Louis, Missouri, 1995, pp. 352–353.
157. D. M. Prince and M. A. Welshenbach, *J. Amer. Diet. Assoc.*, **98**(5), 565–569 (1998).
158. J. B. Klis, *Food-Technol.*, **50**(2), 124 (1996).
159. J. Giese, *Food Technol.*, **50**(3), 130–131 (1996).
160. D. Buss, *New-Nutrition-Business*, **6**(10), 7–10 (2001).



# 6

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## *Flax Oil and High Linolenic Oils*

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### **1. INTRODUCTION**

Many species in the Europhorbiaceae and Labiatae families produce seeds with a high content of oil and contribution of linolenic acid of up to 76% (1). Flaxseed has been used for years in the production of paints, varnishes, inks, and linoleum. In food applications, flaxseed is more often used than oil because of its better stability and because of the presence of fiber, lignans, and  $\alpha$ -linolenic acid (ALA), which have health benefits. Cold pressed flaxseed oil is not considered suitable for deep-frying, although Chinese use it in stir-frying (2). In this chapter, oilseeds of flax, perilla, camelina, and chia are discussed as sources of oils with elevated content of ALA. These oilseeds are produced in industrial quantities and can be considered as potential sources of new oils with specific nutritional and functional properties.

## 2. FLAX

### 2.1 Origin

Flax, widely adapted to warm and cool climates, has been cultivated for centuries in various parts of the world for its stem fiber, linen cloth, and seed. Linseed is an alternative name used for flax. Crops grown for seed are termed linseed in India and in the United Kingdom and flaxseed in Canada and the United States, and flax oil or flax seed is used in many European countries.

Flaxseed/linseed is the annual cultivar of *Linum usitatissimum* L. Flax is a member of the *Linaceae* family that includes ten genera and more than 150 species (3). Approximately 200 species of *Linum* are known (3).

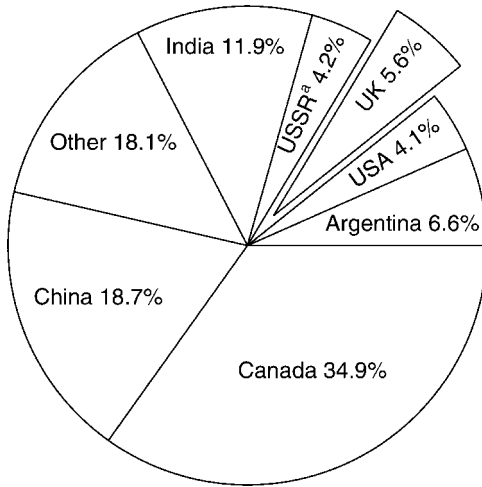
The crops grown for both seed and fiber are generally called dual-purpose flax. Initially, the same variety was used for both oil and fiber production. Today, oil and fiber varieties are different and specifically designed to serve the actual end use. Fiber varieties usually have longer stem, 80–120 cm tall, with fewer branches, fewer seed capsules, and smaller seeds. Although oil type has shorter and heavily branched stems, 60–80 cm tall, with a higher number of seed capsules and larger seeds.

All registered flax varieties in Canada have a dark brown seed coat. There are available yellow seed-coated varieties grown in other countries such as the Omega variety in the United States. Transition to different color is mainly esthetic, lighter colored flaxseed flour is produced from these seeds, and appearance of the product is less affected when it is applied as an ingredient.

### 2.2 Production

More than 60 years ago, the average world production of flaxseed was about 3.4 million metric tons (MMT), which was more than sunflower, 2.5 MMT, and slightly lower than rapeseed, 3.8 MMT. In the same period, soybean was produced at a level of 12.6 MMT (4). In those years, flaxseed was the third-most produced oilseed in the world by volume. Since then, world production of flaxseed has remained between 2 and 3 MMT, and the production of other oilseeds has increased considerably (4). In 2000–2001, world production of flaxseed was 2.34 MMT, with Canada being the largest producer and exporter of this oilseed (See graph in Canola chapter).

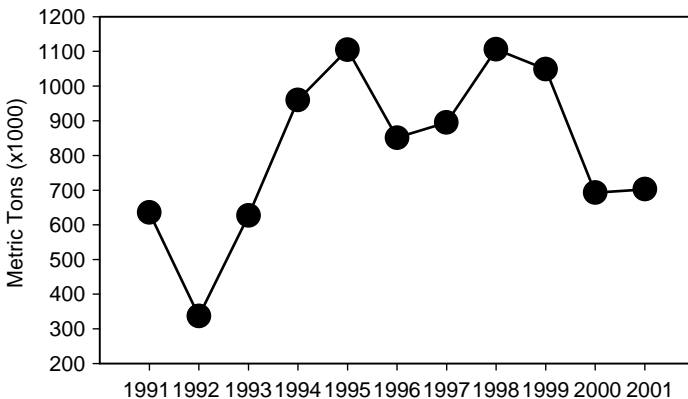
The total average yearly world production of flaxseed for the past ten years was 2.52 MMT (5). The principal growing areas for flaxseed are Canada, China, India, Argentina, the United States, the United Kingdom, former USSR, and some European countries (5). The average contribution of mentioned countries in the world production of flaxseed is presented in Figure 1. Among mentioned producers, Canada, China, and India contributed 34.9%, 18.7%, and 11.9%, respectively, to the world production. The eight main flaxseed producers listed contributed up to 82% of the total yearly flaxseed production.



**Figure 1.** Major world producers of flaxseed (Ten-year average from 1990 to 2000). Production averaged 2.52 million metric tons/year. <sup>a</sup>Former U.S.S.R. Source: Canadian Grains Council, Statistical Handbook 2001 (5).

Canada is one of the major flaxseed producers and exporters, where a minimal amount of seeds is crushed to produce flax oil. Flax oil is mainly considered as a health food product but not a commodity oil. Figure 2 shows yearly production of flaxseeds in Canada for the past ten years. On average, Canada is producing above 800,000 MT (metric tons) of flaxseed per year (5). Part of this production is low linolenic acid varieties, which contribute from 10% to 15% to the total production.

Recently, the food industry in North America and Europe has shown an increased interest in utilization of flaxseed in food product formulations. This is



**Figure 2.** Flaxseed production in Canada. Data include low linolenic flaxseed. Source: Canada Grains Council Statistical Handbook 2001 (5).

mainly because of the presence of ALA, dietetic fiber, and plant lignans, which according to scientific evidence provide important health benefits.

### 2.3. Physicochemical Properties of Flax Oil

Some physicochemical properties of conventional flaxseed oil and low linolenic varieties are presented in Table 1. The higher specific gravity of 0.935 observed for flaxseed oil than other vegetable oils can be directly attributed to the high contribution of linolenic acid. It is in line with the specific density of fatty acids that increases from 0.895 to 0.9038 and to 0.914 for oleic, linoleic, and linolenic acids, respectively (7).

The amount of polyunsaturated fatty acids (PUFA) affects both melting and flashpoints of vegetable oils. Melting temperature of oil is directly related to the melting point of fatty acids, which decreases with unsaturation (7). The flash point of flaxseed oil is relatively low compared with other vegetable oils; this can be attributed to a high contribution of PUFA.

Unsaponifiable matter content, saponification value, and iodine value are characteristic for a high contribution of PUFA in the flax oil. The content of unsaponifiable matter in flax oil is similar to other vegetable oils.

### 2.4. Chemical Composition of Flax Oil

Main components of vegetable oils, including flax oil, are triglycerols and usually contribute more than 90% of all components (Table 1). Minor components in flax oils were found to be at the similar level as in canola and soybean oils (10). The presence of chlorophyll in flax oil usually indicates immaturity of flaxseed.

**TABLE 1. Properties of Flaxseed Oils (6–9).**

Parameter	Flaxseed Oil	Linola™	
		Crude	RBD
Relative density (20°C/water at 4°C)	0.925–0.935	0.921	0.920
Refractive Index ( $n_D$ 20°C)	1.475–1.475	1.4657	1.4665
Melting Point (°C)	–20 to –24		
Flash Point, min. (°C, open cup)	120–135		
Viscosity (cp)		46.8	46.4
Iodine Value	182–203	142	144
Unsaponifiable Matter (%)	0.1–1.7	1.2	0.6
Saponification Value (mgKOH/g)	187–195	185	185
Phosphorus (ppm)	1.0–30	300	1.0
Chlorophyll (ppm)	0.0–1.5	0.4	0.1
Free Fatty Acids (% of oleic)	0.1–2.0	0.3	<0.02
Triglyceride (%)	94–98	93–98	96–98

RBD—Refined, bleached, and deodorized.

**TABLE 2. Composition of Flaxseed and Major Oils (6, 10, Przybylski Unpublished Data).**

Component	Flax	Linola™	Canola	Soybean	Sunflower
<b>Fatty Acids (%)</b>					
C16:0	5.3	6.1	3.8	11.2	6.0
C18:0	3.3	3.8	1.7	4.1	4.0
C18:1	17.9	15.5	58.2	24.3	16.5
C18:2	14.7	71.3	20.1	54.6	72.4
C18:3	58.7	2.0	9.6	8.3	0.5
SFA	9.0	10.0	6.2	15.6	11.2
MUFA	18.1	17.1	64.2	23.4	16.7
PUFA	72.9	72.9	29.6	61.0	72.1
<b>Tocopherols (ppm)</b>					
Alpha	20	15	272	116	613
Gamma	200	200	423	737	19
Delta	7	5	—	275	—
Plastochromanol-8	120	110	75	—	—
Total	347	330	770	1128	632
<b>Phytosterols (%)</b>					
Brassicasterol	1	1	14	—	—
Campesterol	27	23	28	18	7
Stigmasterol	8	4	1	15	7
$\beta$ -Sitosterol	50	54	52	54	58
$\Delta^5$ -Avenasterol	10	18	5	2	4
<b>Total sterols (g/kg)</b>	2.3	2.2	6.9	2.6	3.1

**Abbreviations:** Fatty Acids: SFA—saturated; MUFA—monounsaturated; PUFA—polyunsaturated; Plastochromanol-8—derivative of gamma tocotrienol with longer side chain.

Fatty acid composition of regular flax oil is different from other commercial oils because of the very high contribution of ALA, usually above 50% (Table 2). Because of the high content of this unique fatty acid, flaxseed and flax oil are often used as food supplements, where enrichment with omega-3 fatty acids is needed. This fatty acid is susceptible to oxidation; it oxidizes 20–40 times faster than oleic acid and 2–4 times faster than linoleic acid (8). This property makes the oil a good material for paint and plastic production where fast oxidation is required. Flax oil contains low amounts of saturated fatty acids (SFA) compared with low linolenic flax oil (Linola), soybean, and sunflower oils; however, it is higher than canola oil (Table 2). Canola oil contains the lowest amount of SFA among all commercial oils.

The contribution of linolenic acid in flaxseed oil showed a wide range and was affected by the growing conditions. Flax varieties grown in Western Canada, average from 495 samples analyzed, contained 5% palmitic acid (16:0), 3% stearic acid (18:0), 17% oleic acid (18:1), 15% linoleic acid (18:2), and 59% linolenic acid (18:3) (11). Although similar varieties were grown in North Dakota, the 11 cultivars assessed showed the following fatty acid composition: 5–6% of 16:0, 3–6% of 18:0, 19–29% of 18:1, 14–18% of 18:2, and 45–52% of 18:3 (12).

Cool temperatures during the 10–25 days after flowering are the main cause for higher amounts of linolenic acid in flax oils (14). For the same reason, flaxseed grown in the Canadian prairies, northern latitude, produce oils with higher levels of polyunsaturated fatty acids and lower contributions of oleic acid and saturated fatty acids. This phenomenon was also observed for other oilseeds such as sunflower, canola, and soybean (7, 13, 14). Similarly, a wide variation in fatty acid composition in Australian flaxseed samples was observed: 13–25% of 18:1 and 46–64% of 18:3 (6).

Analysis performed on varieties of flaxseeds collected from different flax growing regions of the world and later grown in Morden, Manitoba, Canada, showed even wider distributions of oleic acid 14–60%, linoleic acid 3–21%, and ALA 31–72% (13). All of these data indicate that within flax, there is a wide distribution of fatty acids, and this variability can be used for developing specialty oils based on traditional breeding and to avoid GMO oils.

Flaxseed oils contain much lower amounts of tocopherols, half of the amount present in sunflower and canola oils and one-third of that present in soybean oil (Table 2). A lower content of these antioxidants makes these oils even more susceptible to oxidation. Gamma-tocopherol was found as the main tocopherol in flax oils, with a contribution of about 80% to the total amount. This makes flax oil comparable with soybean oil. Among unique antioxidants detected in flax oils was plastochromanol-8. This compound is a derivative of gamma tocotrienol with twice as long unsaturated side chain. Plastochromanol-8 was found to be a more efficient antioxidant than any tocopherols isomer (15). A low content of tocopherols in flaxseed did not make them more susceptible to oxidation; experiments showed that milled flaxseed could be stored for 28 months at ambient temperatures without measurable changes in oxidation products. This can be attributed to the presence of antioxidants other than tocopherols in the seeds (16).

Sterols or phytosterols are present in flax oils at a level lower than those in many vegetable oils, 2.3 mg/g in flaxseed oil versus 4.1 to 6.9 mg/g in other oils (Table 2). The composition of sterols was similar to other oils, where  $\beta$ -sitosterol was the main component followed by campesterol and  $\Delta^5$ -avenasterol. Brassicasterol was found in trace amounts in flax oil. This phytosterol is characteristic to plants from the *Brassica* family and often is used as a marker for oil adulteration (Table 2).

As discussed above, triacylglycerols are the main components of vegetable oils and the composition of flax acylglycerols is presented in Table 3.

As expected from fatty acid composition, the main triacylglycerols contain linolenic acid in their molecules and 84% of all triacylglycerols have this acid in their structure (Table 3). Among them, 21% of total acylglycerols contained three ALA in molecule, second by contribution were acylglycerols with two ALA, and linoleic acid had the second-most abundant fatty acid present in the flax oil (17).

Flaxseed is the richest source of plant lignans containing 75–800 times more than that in other oilseeds, cereals, legumes, fruits, and vegetables (18). These plant origin components act in mammals as hormone-like phytoestrogens. Lignans are compounds with a dibenzylbutane skeleton, which have been found in many higher plants (18–20). Plant lignans, namely, secoisolariciresinol diglycoside (SDG) and

**TABLE 3. Composition of Triacylglycerols in Flaxseed Oil (17).**

Triacylglycerols <sup>1</sup>	Contribution (%)
PLnLn	7.6
PLLn	6.7
PLL	1.5
POL	1.6
LnLnLn	20.9
LLnLn	13.8
LLLn	3.7
OLnLn	8.4
LLL	0.9
OLLn	5.3
OLL	0.9
SLLn	1.1
OOL	3.4
OOLn	7.3
POLn	4.0
SLnLn	3.2
POL	1.6
PLL	1.5
OOO	3.3

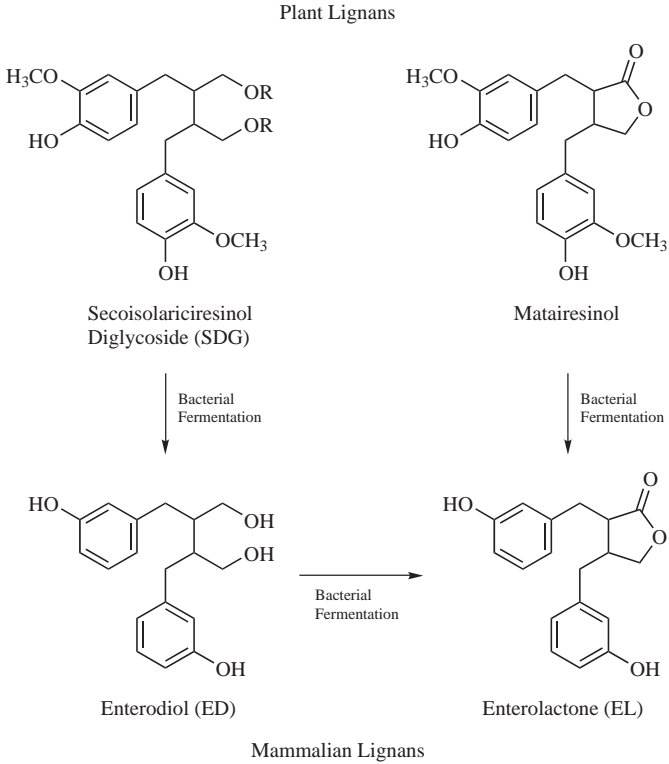
<sup>1</sup>Abbreviations of fatty acid: P—palmitic; Ln—linolenic; L—linoleic; O—oleic; S—stearic.

matairesinol (MAT), are the main compounds among flaxseed lignans. Both are structurally different from animal and human lignans, enterodiol (ED) and enterolactone (EL). Mammalian lignans are formed by intestinal microorganisms from plant precursors (Figure 3). The concentration of mammalian lignan precursors is measured by adding a particular food ingredient to the model of the intestinal microorganism and assessing the amounts of released ED and EL (18). Similarly, excretion of animal lignans in urine may be measured (18, 19). Figure 4 shows urinary excretion of ED and EL when different plant components were included in the diet. Flax oil is the second dominant source of mammalian lignans excreted after flaxseed, in far higher amounts than other oils, oilseeds, and cereals.

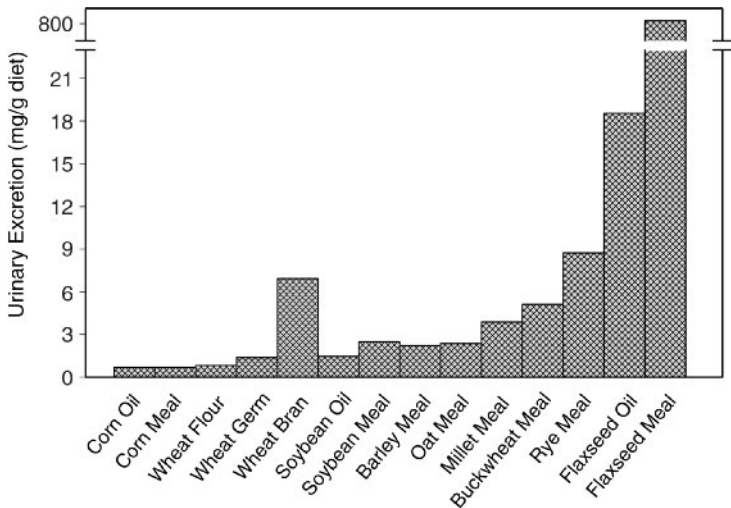
Lignans from flaxseed have been shown to reduce mammary tumor size by more than 50% and tumor number by 37% in carcinogen-treated rats (19, 20). Furthermore, it has been suggested that lignans have antimiotic, antiestrogenic, antiviral, antibacterial, antifungal, and antioxidant properties (20–33).

The presence of plant lignans in flax oil makes it nutritionally more valuable than any other oil. When high levels of ALA and linoleic acid are considered in the whole equation, flaxseed oil serves as the best oil in terms of its nutritional and health value.

The Food and Drug Administration (FDA) regulations allow inclusion of flaxseed in food products, but the amount allowed is limited to 12% (34).



**Figure 3.** Mammalian lignan formation in digestive tract and their plant precursors (19).



**Figure 4.** Total excretion of human lignans in the urine of rats after diet was supplemented with various foods (18).



## 2.5. Low Linolenic Flaxseed Oil

Low linolenic acid varieties with yellow-seed coat flax trademarked Linola were developed by the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia and distributed elsewhere under this name by United Grain Growers, Canada (4). The Linola seed color has been changed to yellow to make it distinguishable from the traditional flaxseed dark brown color. The generic common name Solin has been assigned by Flax Council of Canada for all low linolenic flax varieties produced in Canada. Developmental work on Solin (Linola is a brand name within Solin family) is continuing mainly to reduce saturated fatty acid and to increase linoleic acid content above the 70% level and to increase the content of antioxidants as well as to enhance nutritional properties of the meal.

The new oilseed crop is grown wherever flax and linseed varieties are currently cultivated (35, 36). The climate in northern Europe is highly suitable for production of Linola, where sunflower and corn/maize cannot be produced. Linola seed can be processed by existing crushing plants using similar processing parameters. Linola meal is used for ruminant feed in the same way as linseed meal.

The fatty acid composition of the new crop has been modified, and the level of linolenic acid has been reduced from over 50% to 2% (6). This greatly improves oxidative stability of the oil, which by fatty acid composition is very close to sunflower and soybean oils (Table 2). Linola has been found to be more resistant to oxidation than regular flax oil, and its stability is comparable with soybean, canola, and sunflower oils (Przybylski, unpublished data).

Refining of crude Linola oil by conventional steps, namely, degumming, alkali refining, bleaching, and deodorization, produces colorless and odorless oil, which has good oxidative stability (9). In addition, properties of crude and refined, bleached, and deodorized (RBD) Linola oil are comparable with other commodity oils (Table 1).

The FDA granted Generally Recognized as Safe (GRAS) status for Solin/Linola oil in 1998 (38). This oil can be used as an ingredient in food product formulations such as salad oil, cooking, and frying oil, and in fat phase to formulate margarine, spreads, and shortenings (19, 37).

Because of several beneficial nutritional properties, mainly related to the high level of linoleic acid and lignans, there is a growing interest to use Linola seeds and oil in bakery and confectionery applications. The golden-yellow-colored Linola seeds can serve as an attractive and appealing topping for baking goods. It seems evident that Linola/Solin seed and oil can have promising future applications in food products (35).

## 2.6. Processing of Flaxseed and Oil

Flaxseed is covered with fibrous hull accounting for 25 to 45% of the seed weight and contains 2–7% by weight of water-soluble carbohydrates. These components called mucilage can interfere during processing (38). Flaxseed contains approximately 25% protein, 10% moisture, and 35–45% of oil (6, 38, 11). In immature

seeds, cyanogenic glucosides such as linamarin, linustatin, and neolinustatin can be present at the level of 200–650 mg/100 g of seeds (9). Enzyme linase is always present in flaxseed, and it decomposes glucosides to many products, including hydrocyanic acid, one of the most toxic substances. Newly developed varieties of flax have lower amounts of glucosides in the seed. During processing, small amounts of glucoside can be transferred into oil, whereas these compounds are water-soluble.

Flaxseed contains a high amount of oil, but expressing oil from it is difficult and often double pressing is required to efficiently remove oil from the seeds. Processing steps for flax oil production are presented in Figure 5. Before crushing, cleaned seeds are tempered to achieve a moisture level of 9.5% to 10%, this will minimize the formation of fine particles when seeds are cracked or flaked and will maximize removal of oil from them. Moisturized seeds are passed through sets of corrugated and smooth rolls to be cracked and flaked, respectively. From the next processing step, production of flax oil is differentiated from that for Solin/Linola oil (7). The flax oil for human consumption is cold-pressed, and further purification of oil is not applied. According to industry standards, cold pressing is achieved when the temperature of oil coming from the extruder does not exceed 35°C and pressing is performed under protection from oxygen, usually under a blanket of nitrogen. Good practice requires utilization of expellers, which have the ability to cool parts of the press, which are in contact with seeds and oil to control the temperature during processing (38).

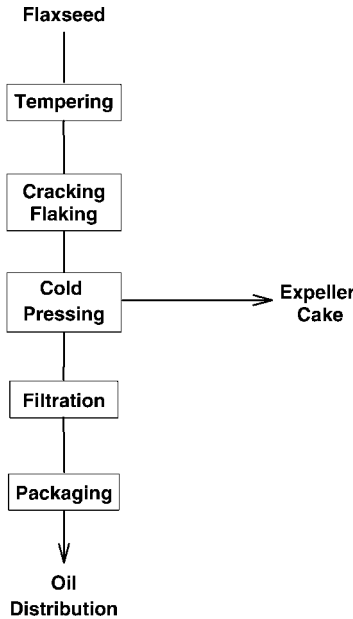
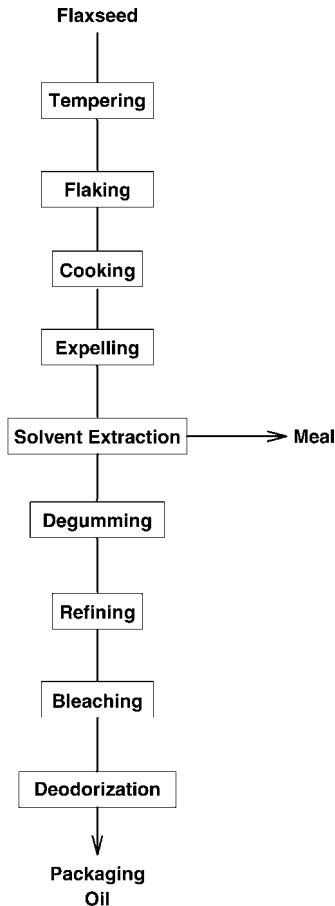


Figure 5. Processing of flaxseed to produce cold-pressed flax oil.

Oil from expeller is filtered, packaged under nitrogen or other neutral gas into bottles protecting from light exposure, and ready for distribution. Flax oil is very susceptible to oxidative deterioration, and treatment to eliminate oxygen needs to be applied. On the North American continent, flax oil is considered as a health food oil.

When flax oil is processed for industrial use, standard processing steps are applied as described in Figure 6. Flaxseeds are tamped and then flaked, passing through a set of smooth rolls. Flaked seeds are sent to a cooker where they are heated to a temperature of 80–100°C to inactivate enzymes and help release the oil during pressing. At this stage, formation of toxic substances is prevented. The cooked seeds are transferred to the expeller, and expelled oil through filtration is placed in a storage tank, where it is combined with oil from solvent extraction. Cake/meal after pressing is fed to the solvent extractor, where hexane is used as



**Figure 6.** Processing of flaxseed to produce refined, bleached, and deodorized flax oil.

a solvent. From the extractor, cake is moved to the desolvatizer where the solvent is removed at 100°C. Meal is then cooled and used as an animal feed ingredient. Combined oils are purified by the standard refining process, typical to all vegetable oils (7). Degumming is applied to remove phospholipids, refining to lower the content of free fatty acids, bleaching to eliminate chlorophylls and other pigments as well as to decompose hydroperoxides, and deodorizing to make the oil odorless through elimination of oxidation products (Figure 6). Processing of low linolenic flaxseed oil is similar to that described for flax oil and other commodity oils.

### 3. PERILLA OIL

#### 3.1. Origin and Application

Perilla, *Perilla frutescens*, L. Britton, is a member of the mint family, Lamiaceae (Labiatae). This plant is a common annual weed in the eastern United States (1). In Asia, perilla is considered a commercial crop where seeds are used to produce oil and plant parts are used as garnish, flavoring, and sources of nutritional components in combination with cereals or vegetables. In the United States, perilla food products are available in the Korean ethnic markets, and red-leafed plants are used in landscaping. After the Second World War, the United States imported perilla oil, which was used as a drying oil (1). Perilla plant and seed is used in Asia as seeds for birds and human consumption; seed oil is used as a fuel, a drying oil, or a cooking oil; leaves are used as a pot-herb, for medicine, food coloring, flavoring dishes, and source of functional nutrients; foliage is distilled to produce an essential oil for flavoring.

Wilson et al. (39) isolated the toxin, perilla ketone, which causes pulmonary edema (fluid in the lung cavity) in many animal species, although not in pigs and dogs (40). In Japan, 20–50% of long-term workers in the perilla industry developed dermatitis on their hands because of contact with perillaldehyde (41). Small amounts of these components have been detected in perilla oil where it works as an efficient antioxidant.

Perilla was never grown commercially as an oilseed in the United States; however, several agronomists have investigated the crop (42, 43). Rabak and Lowman (43) determined that perilla is well adapted to the climate of the southeastern United States; it would be unprofitable to cultivate it, unless seed shattering can be controlled. Seed yields ranged from 220 to 1400 kg/ha in Illinois (44), 1020 to 1440 kg/ha in Korea (45), and 1110 to 1670 kg/ha in Japan agricultural production (41). Perilla was also experimentally grown as a crop in many parts of the British Empire (46, 47). Production of perilla seeds and oil has been continued in Korea for a long time (48, 45). Annual production of perilla seed is approximately 40,000 MT, and perilla oil is the third largest among edible oils used in the Korean market (49). Perilla plant and seed is widely used in Asian countries as food ingredients, including Japan, China, and India.

### 3.2. Perilla Seed and Oil

The seed of perilla contains 31–51% of oil, which is similar in composition to flaxseed oil, with a higher contribution of PUFA of over 70% (Table 4). The oil is highly unsaturated, with an iodine value of 192–208-g iodine /100-g oil (Table 4). Perilla oil contains over 60% linolenic acid with equal amounts of both linoleic and oleic acids (Table 4). Specific gravity of this oil is higher than flax oil because of a higher contribution of PUFA. Other physical parameters of this oil reflect the composition of its fatty acids.

**TABLE 4. Composition and Properties of Perilla, Camelina, and Chia Oils.**

Parameter	Perilla <sup>a</sup>	Camelina <sup>b</sup>	Chia <sup>c</sup>
<b>Fatty Acids (%)</b>			
C16:0	7	6	6
C18:0	2	2	3
C18:1	14	13	7
C18:2	17	16	20
C18:3	61	39	63
Saturated	8	12	9
Monounsaturated	14	34	8
Polyunsaturated	78	54	83
<b>Tocopherols (ppm)</b>			
α-Tocopherol	31	46	
γ-Tocopherol	461	420	
δ-Tocopherol	7	10	
Total	499	500	
<b>Lipid Classes (%)</b>			
Sterol Esters	2		
Glycerides	91		97
Glycolipids	4		2
Phospholipids	2		0.9
<b>Sterols (%)</b>			
Cholesterol		5	
Brassicasterol		4	
Campesterol		25	
Stigmasterol		3	
β-Sitosterol		52	
Δ <sup>5</sup> -Avenasterol		11	
<b>Total Sterols (mg/kg)</b>		3604	
<b>Physicochemical Properties</b>			
Refractive Index (n <sub>D</sub> 20°C)	1.4761	1.4698	1.4753
Specific Gravity (at 15.5°C/15.5°C)	0.937	0.925	0.936
Iodine Value	192–208	127–155	190–199
Saponification Value (mgKOH/g)	188–197	180–190	180–192
Unsaponifiable Matter (%)	1.3–1.5	1.2–1.5	1.1–1.3
Oil Content (%)	35–50	35–42	32–40
Protein Content (%)	17–28	25–30	20–30

Camelina contains 15% of eicosenoic acid (C20:1) and 3–5% of erucic acid (C22:1).  
 Source: <sup>a</sup>(49); <sup>b</sup>(50, 51); <sup>c</sup>(50–54).

The amount of tocopherols in perilla oil is higher compared with flax oil, and a similar contribution of gamma-tocopherol, above 90%, was observed (Table 4). Shin and Kim (49) analyzed perilla oil for lipid composition and established that it contained more than 90% triacylglycerols, 4% glycolipids, and 2% of each phospholipids and sterol esters.

Perilla oil has been used as a drying oil in paints, varnishes, linoleum, printing ink, lacquers, and for protective waterproof coatings on cloth. It has also been used for cooking and as fuel (56). The meal produced after oil extraction is often used as an animal feed ingredient.

### 3.3. Perilla Oil Processing

Perilla oil in Korea is processed like other cold-pressed oils, where pressing and filtration are the main processing steps. To improve the flavor of perilla oil used in food applications, roasting of seeds is practiced. This will provide oil with a distinctive roasted, nutty flavor and improved stability. Roasting of perilla seeds is often applied in Korea and China (57). Kim et al. (57) analyzed different parameters of roasting and established that temperature above 170°C provided the best flavor and stability for the oil. Nonenzymatic browning components are mainly responsible for flavor and antioxidant activity (52). When perilla oil is produced for the industrial applications, additional processing such as refining, bleaching, and deodorization is carried out (58).

## 4. CAMELINA

Standard oilseed crops are not often suitable to marginal lands where factors such as low moisture, low fertility, and saline soils play an important role in the possible crop to be grown. In recent years, there has been increasing interest in developing agronomic systems with low requirements for fertilizer, pesticides, and energy, which provide better soil erosion control than conventional systems. Camelina can grow in these extreme conditions and provide oilseed with enhanced nutritional value (59, 60).

### 4.1. Origin

*Camelina sativa* (L.) Crantz., plant from the Brassicaceae family, known as false flax, linseed dodder, and Gold-of-Pleasure, originated in the Mediterranean area and Central Asia (61). Seeds are small (0.7 mm × 1.5 mm), pale yellow-brown, oblong, rough, and with a ridged surface. Camelina is listed as being adapted to the flax-growing region on the Prairies, in Europe, and other countries (59, 62). It is primarily a minor weed in flax, which does not have seed dormancy (63). Camelina is short-seasoned, 85–100 days, so it could be incorporated into double cropping systems during cool periods in warmer environments (55).

Cultivation of camelina probably began in Neolithic times, and by the Iron Age in Europe, when the number of crop plants approximately doubled, this crop was

commonly used as an oil-supplying plant. Cultivation, as evidenced from carbonized seed, has been shown to occur in regions surrounding the North Sea during the Bronze Age (64). Camelina monoculture occurred in the Rhine River Valley as early as 600 B.C. Camelina probably spread in mixtures with flax and as monocultures, similar to small grains, which also often spread as crop mixtures. It was cultivated in antiquity from Rome to Southeastern Europe and the Southwestern Asian (64).

Camelina production declined during medieval times because of unknown factors, but it continued to coevolve as a weed with flax, and this is the possible introduction of it to the Americas. Like rapeseed oil, camelina oil has been used as an industrial oil after the industrial revolution (64). The seeds have been fed to caged birds, and the straw has been used for fiber. There has been scattered production of camelina in Europe in modern times, mostly in Germany, Poland, and the USSR. In the 1980s, breeding and germplasma screening were applied to modify fatty acid composition and the content of glucosinolates in camelina seeds (65–69).

Camelina has been evaluated in Canada, North Dakota, and Minnesota for its agronomical performance (63, 70, 50). Recent interest in the species is mainly because of the demand for alternative low-input oilseed crops with the potential for food and nonfood utilization of the seed oil (60, 71). Unique agronomic features such as compatibility with reduced tillage and cover crop and competitiveness with weeds or winter surface seeding showed suitability of camelina for sustainable agriculture systems. Furthermore, the species has a potential as a low-cost crop for green manuring (60).

Long-term yield of camelina cultivars in North America has been averaging from 1100 to 1200 kg/ha with a maximum of about 2000 kg/ha. It should be noted that the yield of many commodity oilseeds, especially *B. napus*, has been improved through plant breeding, whereas camelina has not been modified yet (63).

## 4.2. Seed Composition

The oil content of camelina seed ranges from 29% to 45% in North American crops, and in Germany, it is between 37% and 44%. The seed protein content varies from 23% to 30% (60, 50, 71, 72). Camelina protein content and composition is similar to flax, although higher sulfur content has been observed for camelina oil (63). Camelina meal is comparable with soybean meal, containing 45–47% crude protein and 10–11% fiber (73). Like other cruciferous plants, camelina meal contains glucosinolates at levels of 15–20  $\mu\text{mol/g}$  (74). This is a low content of glucosinolates compared with other brassicaceous species, hence making the utilization of meals easier (73, 75).

## 4.3. Fatty Acid Composition and Use of the Oil

Camelina oil has a unique fatty acid pattern and is characterized by a linolenic acid (C18:3) content ranging from 30% to 40%, eicosenic acid (C20:1) content

of around 15%, and less than 4% erucic acid (21). The fatty acids in camelina oil are primarily unsaturated, with only about 12% being saturated (Table 4). About 54% of the fatty acids are polyunsaturated, primarily linoleic (18:2) and linolenic (18:3), and 34% are monounsaturated, primarily oleic (18:1) and eicosenoic (20:1).

The fatty acid composition of camelina oil can be influenced by both environment and variety, although the effects detected were small. Nine varieties were tested, and the maximum differences between oleic, linoleic and linolenic acid levels were 3%, 2.4%, and 2.2%, respectively (76). Also, a 2% less linolenic acid was observed in camelina grown during a dry warm year compared with the normal year. Although these differences are statistically significant, they are relatively small in absolute terms and have no significant effect on the properties of the extracted oil (68, 50, 76).

With its high contribution of polyunsaturated fatty acids, mainly linoleic and linolenic, and relatively low saturated fatty acid content, camelina oil could be considered a high-quality edible oil. Camelina oil is less unsaturated than flax oil but more than sunflower or canola oils (Tables 2 and 4). This oil seems to be unique among vegetable oils in having a high content of 11-eicosenoic acid. Most of the camelina lines assessed contain 2–4% erucic acid (Table 4), which is higher than the maximum limits for canola-quality rapeseed oil. However, screened germplasm of camelina showed that lines with zero erucic acid content are available and, through plant breeding, zero erucic varieties can be obtained.

Plant sterols identified in this oil consist mainly of  $\beta$ -sitosterol and campesterol (Table 4). About 4% brassicasterol was detected in the oil, which is typical for Brassica family plants (51). The total content of sterols in oil is comparable with other commercial oils (Tables 2 and 4). The presence of cholesterol in camelina oil makes it unique among vegetable oils, where only a trace has been detected in some tropical oils (51).

Composition and content of tocopherols in camelina oil was similar to perilla oil, where more than 80% of all tocopherols were gamma isomer (Table 4). Alpha and delta tocopherols were detected as minor antioxidants (77). The total content of tocopherols was comparable with perilla oil, and higher than that in flax oil (Tables 4 and 2). The total content of tocopherols in camelina oil is higher than canola, flax, soybean, and sunflower.

#### 4.4. Processing of Camelina Seed, Oil Stability, and Utilization

Cold-pressed camelina oil had an attractive yellow color, a mustard-like taste, and a characteristic pleasant odor. This type of flavor is acceptable in India and other Asian countries, but in Europe and North America, it is difficult to find acceptability among consumers, mainly because of a different expectation from vegetable oils. However, commercial camelina oil needs to be refined and deodorized to produce an odorless and colorless product as expected by consumers (76). Crude camelina oil, refined following typical steps as described for flax oil (Figure 6), afforded a product similar to typical commercial oils (76).



To establish storage stability of camelina oil, an accelerated Schaal Oven storage test was carried out at 65°C with crude and refined canola and linseed and camelina oils (76). Conjugated dienes, peroxide, and *p*-anisidine values were determined. The results indicated that the storage stability of camelina oil was similar to flax oil, but it was less stable than canola oil. Crude camelina oil showed a higher oxidative stability than the refined product (76). During storage, refined camelina oil had a 30% higher peroxide level when compared with crude camelina oil (76). Comparison with fish oil, which is rich in omega-3 fatty acids, proved that camelina oil is much more resistant to oxidative deterioration than fish oil (76). At room temperature, crude camelina oil was far more stable than could be expected from its high linolenic acid content. This unusual oxidative stability can be attributed to the presence of natural antioxidants. However, the content of tocopherols discussed above was in the middle range compared with other commercial oils but slightly higher than that of flax oil (Tables 2 and 4). Oxidative stability is not only related to the content and composition of tocopherols, but also to presence of other components, such as phenolic acids and polyphenols. The content of antioxidants in oils is also affected by the processing, and the amounts of antioxidants can be lowered even by 50% when particular processing conditions are applied (15).

The frying performance of camelina oil was compared with soybean oil and assessed under deep frying conditions. Oil deterioration was monitored by assessing changes in viscosities, free fatty acids, *p*-anisidine values, and the formation of oxidized triacylglycerols (76). During the first 5 days of frying, camelina oil deterioration was similar to that of soybean oil. After that time of frying, camelina oil deteriorated much faster than soybean oil, probably because its antioxidants were depleted. In fact, after 7 days of frying, the levels of oxidized triacylglycerols in camelina oil reached the level permitted in Europe, 25%, and in soybean the amount of these components was at 14% (76). Similarly, viscosity of camelina oil increased 100% by the end of the heating period, whereas in soybean oil, it increased only by 30%. Total carbonyl level, measured by *p*-anisidine values, was three times higher in camelina oil than in soybean oil. In addition, deterioration of camelina oil during 5 days of potato frying caused formation of the strong and objectionable paint-like flavor (76).

Refined camelina oil was blended into fat phase to produce margarines and spreads enriched in omega-3 fatty acids. The resulting spreads had physical properties similar to a product based on typical commercial oils. The stability of the new product was satisfactory, and off-flavors were not detected after 6 months of storage (76).

Camelina oil was also included in formulation of salad dressings. Produced dressings showed a similar stability to conventional products during several months of storage at ambient temperature without off-flavor formation (76).

Taking into consideration that camelina oil production will be less expensive and the oil is more stable than fish oil, this oil can be an excellent ingredient to enrich spreads, margarines, and other fat-containing food products, in omega-3 fatty acids, and by this way change the ratio of omega-3 to omega-6 fatty acids.

## 5. CHIA

### 5.1. Origin

Chia (*Salvia hispanica* L.) is an annual herbaceous plant from the mint family, Labiatae, and it is native to southern Mexico, northern Guatemala, and can be grown in South America and the Southwestern United States (52). This plant was used by the Aztec and other tribes of Central America as an important crop not only for food, but also for medicine and paint. Chia oil is a century-old ingredient that has been rediscovered today as a potential ingredient for cosmetic and food industries (52). Although chia has been cultivated for several centuries, presently it is cultivated only in some states in Mexico. The total area cultivated is less than 450 hectares per year. Trials to adopt this cultivar to other regions of America have been done with positive results (52). Chia seeds and oil are available on the American continent in health food stores.

### 5.2. Oil and Seed Composition

Chia seed contains 25–40% oil and 18–30% protein. The chia meal is high in protein and fiber similar to flaxseed and soybean (52, 53). Chia seed, oil, and meal can be used as ingredients with high nutritional value for human food and animal feed. Chia seed contains mucilage and water-soluble fiber, may possibly contain lignans, and is similar to flax (53). Trials conducted in 1995 and 1996 showed yield and oil contents to be affected by growing conditions and harvested yields were up to 1500 kg/ha (52).

Chia oil is high in polyunsaturated fatty acids, particularly  $\alpha$ -linolenic acid; the content of this fatty acid is higher than flax oil (Table 4). Linoleic acid is the second-most abundant acid in chia with a contribution of 17–26%, which gives PUFA content of 83%, the highest amount among edible oils. Additionally, chia oil has the lowest content of saturated fatty acids (Tables 2 and 4).

The physical properties of chia oil are similar to perilla and camelina with the same effect of PUFA discussed above. Lipid class composition in chia oil is also typical for vegetable oils where triacylglycerols are the main components (Table 4)(52).

## REFERENCES

1. J. M. Hagemann, F. R. Earle, and I. A. Wolff, *Lipids*, **2**, 371 (1967).
2. Q. Pan, *Flax Production, Utilization and Research in China*, **53**, 59–63 (1990).
3. T. P. Freeman, in S. C. Cunnane and L. U. Thompson, eds., *Flaxseed in Human Nutrition*, AOCS Press, Champaign, Illinois, 1995, pp. 11–21.
4. T. L. Krawczyk, *Inform*, **10**, 1029 (1999).
5. Canada Grains Council, *Statistical Handbook*, 2001.
6. A. G. Green and D. R. Marshall, *Aust. J. Agric. Res.*, **32**, 599 (1981).

7. N. A. M. Eskin, B. E. McDonald, R. Przybylski, L. J. Malcolmson, R. Scarth, T. Mag, K. Ward, and D. Adolph, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Wiley, New York, 1996, p. 1.
8. E. N. Frankel, *Trends Food Sci. Technol.*, **4**, 220 (1993).
9. A. G. Green, and P. J. C. Dribnenki, *Lipid Technol.*, **6**, 29 (1994).
10. F. D. Gunstone, J. L. Harwood, and F. B. Padley, eds., *The Lipid Handbook*, Chapman and Hall, London, 1994.
11. D. R. Declercq, J. K. Daun, and K. H. Tipples, in Crop Bulletin, Canadian Grain Commission, Winnipeg, Manitoba, Canada, No. 202, 1992, p. 1.
12. N. S. Hettiarachy, G. A. Hareland, A. Ostenson, and G. Baldner-Shank, in Proceedings of the 53rd Annual Flax Institute of the US Meeting, Fargo, North Dakota, 1990, p. 36.
13. D. G. Dorrell, *Fette Seifen Anstrichm.*, **77**, 258 (1975).
14. A. G. Green, *Crop Sci.*, **26**, 961 (1986).
15. D. Olejnik, M. Gogolewski, and M. Nogala-Kalucka, *Nahrung*, **41**, 101–104 (1997).
16. N. A. M. Eskin and R. Przybylski, in N. A. M. Eskin and D. S. Robinson, eds., *Food Shelf Life Stability*, CRC Press, Boca Raton, Florida, 2001, p. 175.
17. R. B. Tarandjiiska, I. N. Mrekov, B. M. N. Damyanova, and B. S. Amidzhin, *J. Sci Food Agric.*, **72**, 403 (1996).
18. L. U. Thompson, P. Robb, M. Serraino, and F. Cheung, *Nutr. Cancer*, **16**, 43 (1991).
19. M. Axelson, J. Sjøvall, B. E. Gustafsson, and K. D. R. Setchell, *Nature*, **298**, 659 (1982).
20. K. D. R. Setchell, in S. C. Cunnane and L. U. Thompson, eds., *Flaxseed in Human Nutrition*, AOCS Press, Champaign, Illinois, 1995, p. 82.
21. L. U. Thompson, S. E. Rickard, L. J. Orcheson, and M. M. Seidl, *Carcinogenesis*, **17**, 1373 (1996).
22. L. U. Thompson, M. M. Seidl, S. Rickard, L. Orcheson, and H. H. S. Fong, *Nutr. Cancer*, **26**, 159 (1996).
23. W. R. Phipps, M. C. Martini, J. W. Lampe, J. L. Slavin, and M. S. Kurzer, *J. Clin. Endocrinol.*, **77**, 1215 (1993).
24. Y. Mousavi and H. Adlercreutz, *J. Steroid. Biochem. Mol. Biol.*, **41**, 615 (1992).
25. M. E. Martin, M. Haourigui, C. Pelissero, C. Benassayag, and E. A. Nunez, *Life Sci.*, **58**, 429 (1996).
26. T. Fotsis, M. Pepper, and H. Adlercreutz, *Proc. Natl. Acad. Sci. USA*, **90**, 2690 (1993).
27. T. Hirano, K. Fukuoka, K. Oka, and Y. Matsumoto, *Cancer Invest.*, **9**, 145 (1991).
28. K. Prasad, *Mol. Cell Biochem.*, **168**, 17 (1997).
29. G. Block, B. Patterson, and A. Subar, *Nutr. Cancer*, **50**, 207 (1992).
30. D. M. Parkin, C. S. Muir, S. L. Whelan, Y. Gao, J. Ferlay, and J. Powell, in *Cancer Incidence in Five Continents*, Vol VI, International Agency for Research on Cancer, Lyon, France, 1992, p. 865.
31. H. Adlercreutz, T. Fotsis, and J. Lampe, *Scand. J. Clin. Lab. Invest.*, **53**, 5 (1993).
32. H. Adlercreutz, H. R. Heikkinen, and M. Woods, *Lancet*, **2**, 1295 (1982).
33. K. D. R. Setchell and H. Adlercreutz, in I. R. Rowland, ed., *Role of the Gut Flora in Toxicity and Cancer*, Academic Press, London, 1988, pp. 315–345.
34. J. E. Vanderveen, in S. C. Cunnane and L. U. Thompson, eds., *Flaxseed in Human Nutrition*, AOCS Press, Champaign, Illinois, 1995, p. 363.

35. B. F. Haumann, *Inform*, **1**, 934 (1990).
36. E. A. Weiss, *Oils & Fat Int.*, **9**, 23 (1993).
37. *Inform*, **9**, 628 (1998).
38. P. P. Kolodziejczyk and P. Fedec, in S. C. Cunnane and L. U. Thompson, eds., *Flaxseed in Human Nutrition*, AOCS Press, Champaign, Illinois, 1995, p. 261.
39. B. J. Wilson, J. E. Garst, R. D. Linnabary, and R. B. Channell, *Britton. Sci.*, **197**, 573 (1977).
40. J. E. Garst, W. C. Wilson, N. C. Kristensen, P. C. Harrison, J. E. Corbin, J. Simon, R. M. Philpot, and R. R. Szabo, *Anim. Sci.*, **60**, 248 (1985).
41. N. Okazaki, M. Matsunaka, M. Kondo, and K. Okamoto, *Skin Res.*, **24**, 250 (1982).
42. H. A. Gardener, *U.S. Cir.*, **52**, 1 (1917).
43. Rabak, F. and M. S. Lowman, in Perilla, USDA, Agriculture Research Administration, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland, 1945.
44. R. O. Weibel and W. L. Burlison, *Soybean Dig.*, **8**, 14 (1948).
45. I. S. Choi, S. Y. Son, and O. H. Kwon, *Korean Soc. Hort. Sci.*, **25**, 68 (1980).
46. Imperial Institute, *Bul. Imp. Inst.*, **18**, 479 (1920).
47. Imperial Institute, *Bul. Imp. Inst.*, **24**, 205 (1926).
48. Yu, I. S. and S. K. Oh, *Res. Rpt. Off. Rural Dev.*, **18**, 187 (1976).
49. H. S. Shin and S. W. Kim, *J. Amer. Oil Chem. Soc.*, **71**, 619 (1994).
50. E. C. Leonard, *Inform*, **9**, 831 (1998).
51. V. K. S. Shukla, P. C. Dutta, and W. E. Artz, *J. Amer. Oil Chem. Soc.*, **79**, 965 (2002).
52. W. Coates, and R. Ayerza, *J. Amer. Oil Chem. Soc.*, **75**, 1417 (1998).
53. A. A. Bushway, A. M. Wilson, L. Houston, and R. J. Bushway, *J. Food Sci.*, **49**, 555 (1984).
54. R. Ayerza, *J. Amer. Oil Chem. Soc.*, **72**, 1079 (1995).
55. H. S. Park, J. G. Kim, and M. J. Cho, *J. Korean Agric. Chem. Soc.*, **24**, 224 (1981).
56. Publications and Information Directorate, in *The Wealth of India*, Vol.7, New Delhi, India, 1966.
57. S. J. Kim, H. N. Yoon, and J. S. Rhee, *J. Amer. Oil Chem. Soc.*, **77**, 451 (2000).
58. S. J. Kim, S. H. Song, H. N. Yoon, and U. Y. Kong, *Inform*, **6**, 514 (1995).
59. National Research Council (NRC), in *Alternative Agriculture/Committee on the Role of Alternative Farming Methods in Modern Production Agriculture*, National Academy Press, Washington, D.C., 1989.
60. D. H. Putnam, J. T. Budin, L. A. Field, and W. M. Breene, in J. Janick and J. E. Simon, eds., *New Crops*, Wiley, New York, 1993, pp. 314–322.
61. J. Schultze-Motel, *Archaeo-Physika*, **8**, 267 (1979).
62. North Central Regional Technical Committee NC-121, *Weeds of the North Central States*, North Central Regional Res. Pub. 281, Bul. 772, Agr. Expt. Sta., Univ. Illinois, Urbana-Champaign, Illinois, 1981.
63. R. G. Robinson, *Minnesota Agr. Expt. Sta. Bull.*, **579** (1987).
64. K. H. Knorz, *Berichte der Deutschen Botanischen Gesellschaft*, **91**, 187 (1978).
65. R. Seehuber and M. Dambroth, *Landbauforschung Voelkenrode*, **37**, 219 (1987).
66. G. Enge and G. Olsson, *Sveriges Utsadesforenings Tidskrift*, **96**, 220 (1986).
67. B. G. Kartamyshev, *Seleksiya i Semenovodstvo*, **6**, 9 (1985).

68. R. Seehuber and M. Dambroth, *Landbauforschung Volkenrode*, **33**, 183 (1983).
69. R. Seehuber and M. Dambroth, *Landbauforschung Volkenrode*, **34**, 174 (1984).
70. R. K. Downey, *J. Amer. Oil Chem. Soc.*, **48**, 718 (1971).
71. R. Seehuber, *Fette-Seifen-Anstrichmittel*, **86**, 177 (1984).
72. R. Marquard and H. Kuhlmann, *Fette Seifen Anstrichmittel*, **88**, 245 (1986).
73. G. O. Korsrud, M. O. Keith, and J. M. Bell, *Can. J. Anim. Sci.*, **58**, 493 (1978).
74. J. Zubr, *Indust. Crops Products*, **6**, 113 (1997).
75. R. Lange, W. Schumann, M. Petzika, H. Busch, and R. Marquard, *Fat Sci. Technol.*, **97**, 146 (1995).
76. J. G. Crowley and A. Frohlich. (2002, November 29). Factors Affecting the Composition and Use of Camelina. Technical Report, Crops Research Centre, Oak Park, Carlow, Ireland. Available: <http://www.teagasc.ie/research/reports/crops/4319/eopr-4319.htm>.
77. J. T. Budin, W. M. Breene, and D. H. Putnam, *J. Amer. Oil Chem. Soc.*, **72**, 309 (1995).

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## *Olive Oil*

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### **1. INTRODUCTION AND HISTORY**

Olive oil, an important component in the diet of Mediterranean people, is obtained by mechanical extraction from the fruit of the *Olea europaea* L. tree, which belongs to the Olive family, comprises some 400 species, and thrives in temperate and tropical climates (1). Of the 35 species in the genus *Olea*, mainly of African, Indian, and Australian origin, *O. europaea* is the only Mediterranean species. Although its origin is not known, one theory is that it originated in ancient Iran and Turkestan, spreading westward to Anatolia, Syria, and Israel along commercial and migratory routes (2).

Olives appeared in Israel about 45,000 years ago (1). Charred pieces of olive wood have been found in excavations at Lower Boker-Har Hanegev in layers dating to 42,980 B.C. Both charred wood and carbonized stones have been found in many archeological sites in Israel dating from 8000 B.C. onward, and indirect evidence suggests the use of wild olives (*O. oleaster*) by humans as early as the seventh millennium B.C. (3). It is not known whether the carbonized stones and charred wood obtained from Chalcolithic (fourth millennium B.C.) and Early Bronze Age (2900–2700 B.C.) sites represented cultivated or wild olives.

Olive farming and an olive oil industry appear to have been well established throughout the region bordering the Mediterranean from Palestine and Syria to Greece in the middle and late Bronze Age (4). Olive farming in Palestine and Syria

increased dramatically at the turn of the first millennium B.C. (2). An olive oil industry became well established in Palestine, and the export of olive oil from Palestine to Egypt is documented in Old Kingdom Egypt. Olive cultivation provided materials useful as a lamp fuel, lubricants, and body ointments; the fruit was easily cured by salting, and the wood was used for carpentry and fuel. Later, the olive fruit became a source of edible oil.

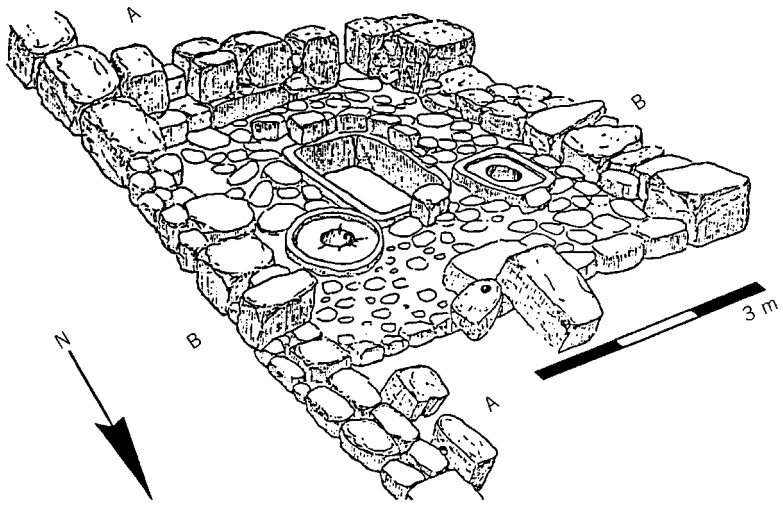
The manufacturer of olive oil became a mass production industry during the Israelite period when processing methods improved (3). In Judea, oil presses generally consisted of large stone beds with a collecting vat in the center of the pressing surface. A beam, which acted as a lever and was weighted down by several stones, was used for pressing. The end of the beam was anchored to a wall behind the press (niche wall) with a special niche stone. Olives were crushed in a rectangular basin by a roller, which an operator set into forward and backward motion by means of an attached shaft.

A typical Iron Age industrial site is that of the seventh century B.C. biblical town of Timnah (Tel Batach), which was a center of olive oil production along with other towns in the Tel Aviv area (5). The oil presses of the town were constructed similarly to those of other Iron Age sites in the area. Each press consisted of a crushing basin with two pressing vats on either side. Olives were crushed in the basin with stone rollers, each of which had wooden handles fitted into sunken depressions at the sides. The crushing basin was a shallow trough made of one large chalk stone. Each pressing vat contained a large stone with a flat top and an inner hollowed space for collecting the oil. Because there was no means of draining the oil from the vat, pottery jugs were used to withdraw the oil. Baskets with crushed olives were pressed by wooden beams anchored at one end to niches in the wall; the other end of each beam was pressed down with three heavy stone weights (Figure 1).

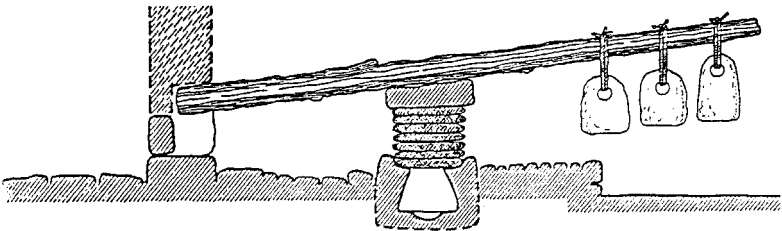
Olive growing reached Cyprus and the Aegean area around the sixteenth century B.C. As Renfrew (6) pointed out, the olive was one of three important constituents, along with the vine and domesticated wheat, that contributed to the emergence of civilization in the Aegean region. Oil production and trade played important roles in the Minoan–Mycenaean economy of Crete and main-land Greece in the second millennium B.C. (7). Olive oil was used in the manufacture of scented perfumes and unguents in the palace industries of Crete and Mycenae. Wild rather than cultivated olives were apparently preferred for Aegean perfume and unguents because of the low fat content of the wild olive.

Initially, olives were harvested by beating the trees with flails (6). After harvesting, the olives were drenched in hot water and pressed to extract the oil. The oil was separated from the water in a vat from which the water was drawn off, and then stored in jars similar to those used to hold wine. Oil was used locally for lighting, hygienic purposes (to clean the body), and as food, especially for cooking. Mycenaean documents suggest that scented olive oil was used for religious purposes and as a body ointment for the rich (8).

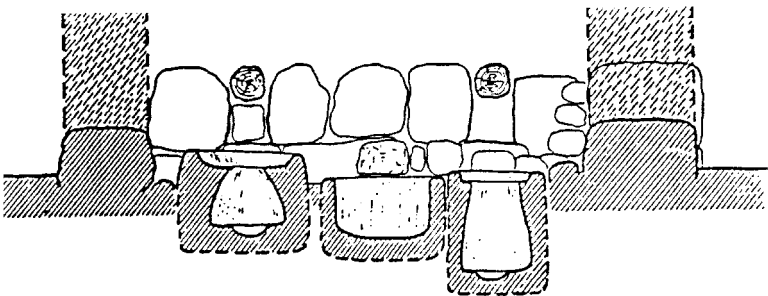
The earliest evidence of olive oil extraction in Cyprus dates to about 1300 B.C. (9). (Wild olives grew on the island at least as early as 4000 B.C.) An olive press (probably a lever and weight press) found at a Maroni excavation site consisted



(a)



(b)



(c)

**Figure 1.** Oil press at Tel Batach (biblical Timnah), 7 km west of Beth-Shemesh, Israel. Isometric plan and sections (5). [Reprinted with kind permission of the editors of *Olive oil in Antiquity* (5).]



of a large rectangular trough (pressing bed) set on a mudbrick platform and sloping downward. The trough is a flat stone with channels cut to meet at a small projection, permitting the liquid to pour off into a jar standing on the floor below the press. Other presses of the late Roman period found on the island were of the lever and screw type in which the horizontal beam is immobile while the screw presses down on the pressing bed. The screw was already used for pressing in Italy in the first century B.C., initially as a lever and screw press and then in a direct frame press (10).

Olive orchards continued to be extensively cultivated in Palestine throughout the Byzantine and Arab periods (11). The chain of mountains from the upper Galilee to Hebron were covered with olive trees. Olive oil was used regularly for food and cooking as well as for lighting and manufacture of soap by boiling the oil with ashes. During the early period under the Umayyides (661–750 A.D.) and Abbasides (750–1258 A.D.), oil surpluses were exported from Palestine by land to neighboring countries. With revival of maritime commerce under the Fatimids (909–1171 A.D.), oil was transported to Egypt and other countries by boat.

Phoenician settlements in the Mediterranean basin introduced olive farming into Sicily, Sardinia, southern France, and Spain (2). The Greeks later spread farming independently of the Phoenicians, reintroducing the olive into Sicily. The Romans spread olive farming throughout their territories and used the olive tree in their land reclamation projects, particularly in North Africa where they instituted olive farming and other projects to reclaim desert areas. Although of variable quality, olive oil was a staple food and an important industrial product in Roman times.

Olive growing continued to prosper in the Mediterranean region until the fifth century A.D., when the Roman Empire was invaded from the north and maritime routes were closed (2). Olive farming was later revived with commercial development of Venice and other maritime republics during the Renaissance. In 1709, olive growing entered a new modern age when all of Europe was hit with a deep cold spell and new orchards were planted to replace those destroyed by the cold weather. As modern farming techniques evolved, large-scale state enterprises were begun and olive farming reached a peak in the first half of the nineteenth century.

## 2. STATISTICS AND DEFINITIONS

Currently, more than 95% of the world's olive trees grow in the Mediterranean Basin. About 81% of total olive production comes from the European Community (EC) (Spain, Italy, Greece, Portugal, and France), with the Near East contributing, ca 7% and North Africa supplying about 11%. The remaining 1% is of American origin, chiefly from Argentina, Mexico, Peru, and the United States (Table 1). Olive oil consumption is growing in the developed countries that produce little or no olive oil (Table 2).

The fruit of the olive tree is an egg-shaped drupe, consisting of a pericarp and an endocarp. The pericarp includes an epicarp (skin) of variable thickness according to the variety, and a mesocarp (pulp) surrounding the endocarp (woody pit) in which

**TABLE 1. World Production of Olive Oil (Thousand Metric Tons).<sup>a</sup>**

Country	1997/98	1998/99	1999/00	2000/01	2001/02	2002/03 (prov.)	2003/04 (est.)
Algeria	15.0	54.5	33.5	26.5	25.5	16.5	40.0
Argentina	8.0	6.5	11.0	4.0	10.0	11.0	22.0
Cyprus	1.5	2.5	3.5	5.5	6.5	7.0	7.0
EC	2,116.5	1,707.0	1,878.5	1,940.5	2,463.5	1,942.5	2,307.0
Croatia		5.0	9.0	5.5	5.0	7.0	3.0
Israel	3.0	4.5	2.5	7.0	3.5	9.0	2.5
Jordan	14.0	21.5	6.5	27.0	14.0	28.0	11.5
Lebanon	3.5	7.0	5.0	6.0	5.0	6.0	4.0
Morocco	70.0	65.0	40.0	35.0	60.0	45.0	80.0
Palestine	9.0	5.5	2.0	20.0	18.0	21.5	5.0
Syria	70.0	115.0	81.0	165.0	92.0	165.0	110.0
Tunisia	93.0	215.0	210.0	130.0	35.0	70.0	180.0
Turkey	40.0	170.0	70.0	175.0	65.0	160.0	60.0
Australia		0.5	0.5	1.0	1.0	2.0	3.0
Egypt	1.0	0.5	2.5	0.5	1.5	5.0	2.0
USA	1.0	1.0	1.0	0.5	0.5	1.0	1.0
Iran	3.0	2.5	2.5	3.0	2.5	1.5	4.0
Libya	6.0	8.0	7.0	4.0	7.0	6.5	6.5
Mexico	2.0	2.5	1.0	1.5	2.0	2.5	2.5
Yugoslavia	0.5	1.0	1.0	0.5	0.5	0.5	0.5
Serbia and Montenegro	8.5	7.5	6.5	7.5	7.5	7.5	7.5
World Total	2,465.5	2,402.5	2,374.5	2,565.5	2,825.5	2,515.0	2,859.0

<sup>a</sup> Source: International Olive Oil Council (IOOC).

the seed is enclosed. The yield per hectare is about 2.45 tons. Oil yield per 100 kg of fruit is 19.6 kg (based on yields in Italy during the past 10 years).

In addition to oil, the pulp and epicarp contain a variety of natural components soluble in the oil. As will be seen later, the oil is obtained from the olive by a variety of techniques, always physical, leaving a residue (pomace) that contains up to 8% oil, which is then extracted by solvent (usually hexane) and named pomace oil.

Because of the behavior of the solvent, solvent-extracted oil contains more minor components at higher levels than those found in physically extracted oil. This provides the basis for designating pomace oil as a commercial product distinct from virgin oil (obtained only by mechanical means) or refined (lower grade) virgin oil mixed with virgin oil (olive oil, Riviera type).

The following internationally recognized definitions of oils derived from olives and available on the market were promulgated by the International Olive Oil Council (IOOC) (12):

1. Olive oil is that oil produced by extraction of the fruit of the olive tree (*Olea Europaea Sativa* Hoffman et Link) to the exclusion of oils obtained using solvents or reesterification processes and of any mixture with oils of other

TABLE 2. Olive Oil Consumption (Thousand Metric Tons).<sup>a</sup>

Country	1997/98	1998/99	1999/00	2000/01	2001/02	2002/03 (prov.)	2003/04 (est.)
Algeria	31.5	44.0	42.0	26.0	25.0	16.0	39.0
Argentina	8.0	8.0	7.0	6.0	5.5	5.5	6.0
Cyprus	2.0	2.5	4.0	5.0	5.5	6.0	6.0
EC	1,705.5	1,709.0	1,728.0	1,835.0	1,894.0	1,904.5	1,932.0
Croatia		4.0	8.5	6.5	5.0	6.0	3.0
Israel	6.5	9.5	12.5	13.5	14.5	14.5	13.5
Jordan	19.0	19.0	9.0	17.0	20.0	25.0	15.5
Lebanon	8.0	9.0	8.0	8.0	7.0	7.0	7.0
Morocco	55.0	55.0	55.0	45.0	60.0	55.0	70.0
Palestine	5.5	4.0	4.0	8.0	10.0	12.0	12.0
Syria	95.0	88.0	90.0	110.0	86.0	100.5	115.0
Tunisia	52.0	49.0	60.0	58.0	28.0	30.0	60.0
Turkey	85.5	85.0	60.0	72.5	55.0	55.0	40.0
	2,073.5	2,086.0	2,088.0	2,210.5	2,215.5	2,237.0	2,319.0
Australia	17.5	24.0	25.5	31.0	27.5	31.0	31.0
Brazil	29.0	23.5	25.0	25.0	22.5	20.0	21.0
Chile							
Egypt	1.0	1.0	1.5	1.0	1.5	3.5	2.5
USA	142.5	151.0	169.5	194.5	188.5	190.0	195.0
Iran	4.0	2.5	2.5	3.0	2.0	2.0	3.5
Libya	7.0	16.0	11.0	7.0	8.0	8.5	8.5
Mexico	4.5	5.0	5.0	6.5	8.0	10.0	10.0
Yugoslavia/Serbia and Montenegro	0.5	1.0	1.0	0.5	0.5	0.5	0.5
Other producing countries	13.5	12.5	13.0	13.0	14.0	14.5	14.5
	219.5	236.5	254.0	281.5	272.5	280.0	286.5
Saudi Arabia	5.0	5.5	4.5	4.0	5.0	7.0	7.5
Canada	17.5	18.5	23.0	24.5	24.0	24.0	24.5
Japan	34.0	28.5	27.0	30.0	31.5	32.5	33.0
USSR/Russia	1.5	2.0	3.0	4.0	4.0	6.0	7.0
Switzerland	5.5	6.0	8.0	8.0	9.0	10.0	10.0
Taiwan	4.5	7.0	6.0	8.0	6.5	5.5	6.0
Other nonproducing countries	20.5	23.0	29.0	20.0	38.0	38.5	38.5
	88.5	90.5	100.5	98.5	118.0	123.5	126.5
Total World	2,381.5	2,413.0	2,442.5	2,590.5	2,606.0	2,640.5	2,732.0

<sup>a</sup>Source International Olive Oil Council (IOOC).

kinds. In no case shall the designation "olive oil" be used to refer to olive-pomace oils.

- A. Virgin olive oil is the oil obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which has not undergone any treatment other than washing, decantation, centrifugation, and filtration.

Virgin olive oil fit for consumption as is (and can be designated as “natural”) includes:

- a. Extra virgin olive oil: virgin olive oil that has an organoleptic rating of 6.5 or more as determined by the IOOC method (13) and a free acidity, expressed as oleic acid, of not more than 1 g per 100 g.
  - b. Fine virgin olive oil: virgin olive oil that has an organoleptic rating of 5.5 or more and a free acidity, expressed as oleic acid, of not more than 1.5 g per 100 g.
  - c. Semifine virgin olive oil (or ordinary virgin olive oil): virgin olive oil that has an organoleptic rating of 3.5 or more and a free acidity, expressed as oleic acid, of not more than 3.3 g per 100 g. (This class of olive oil is normally traded in bulk for blending purposes.)
- B. Virgin olive oil not fit for human consumption, also designated as lampante virgin olive oil: virgin olive oil that has an organoleptic rating of less than 3.5 and/or a free acidity, expressed as oleic acid, of more than 3.3 g per 100 g. This class of olive oil is used to produce refined olive oil or is intended for technical (nonfood purposes).
  - C. Refined olive oil: olive oil obtained from virgin olive oils by refining methods that do not lead to alterations in the original triglyceride structure.
  - D. Olive oil: the oil consisting of a blend of refined olive oil and virgin olive oil in various proportions.
2. Olive–pomace oil: the oil obtained by solvent extraction of olive–pomace and not including any oil obtained by a reesterification procedure or any mixture with other kinds of oils. (The various categories of olive–pomace oil are described below.)
    - A. Crude olive–pomace oil: olive–pomace oil intended for refining to produce a product (as B, below) suitable for human consumption, or intended for technical purposes.
    - B. Refined olive–pomace oil: the oil obtained from crude olive–pomace oil by refining methods that do not lead to alterations in the original triglyceride structure.
    - C. Olive–pomace oil: a blend of refined olive–pomace oil and virgin olive oil (any A, B, or C). In no case may this be called “olive oil.”

Because the yearly production of olive oil is variable, low-production years can follow years of high production. Therefore, it is customary to record average values (Table 1).

### 3. EXTRACTION TECHNOLOGY

Ripe olives contain a variety of components, including water, oil, sugars, proteins, organic acids, and cellulose. Olive cultivars with medium-size fruits generally provide the best oil yields. The pulp-to-kernel ratio of olives for oil production ranges from 4:1 to 8:1.

The epicarp contains a number of components of relatively high polarity that are not removed by mechanical extraction and remain in the pomace. Removal of these components along with the oil by solvent extraction of the pomace accounts for the higher unsaponifiable content of olive-pomace oil.

Most of the oil (96–98%) is in the pulp along with most of the water “vegetation water” (VW), which accounts for 40–60% of the weight of the fruit.

The woody pit inside the mesocarp holds a seed whose oil is more unsaturated than the mesocarp (pulp) oil because of a higher content of linoleic acid. The ratio of fruit oil to seed oil is 50:1.

The approximate chemical composition of olive fruit is as follows: water 52.4%; oil 19.6%; proteins 1.6%; sugars 19.1%; cellulose 6.8%; and ash 1.5%. Oil yield and quality depend on the cultivar of olive tree, ratio of the various anatomical parts, and levels of minor components as well as growing conditions and health of the trees. Soil moisture is very important during fruit development.

Harvesting of fruit for oil production begins in the middle of autumn and lasts until the end of February. In some regions, it begins earlier, and in other locales, it lasts until March. Accordingly, differences in oil quality and composition can be expected along with variations caused by climatic and soil conditions. Variations in quality are chiefly related to the levels of minor components and flavor compounds, acidity, and the presence of mono- and diglycerides (14–16).

Analytical and organoleptic data show that oil content is lower at the beginning than at the end of the harvesting period, but it is of higher quality (15). Harvesting technology is very important for production of high-quality oil. Olives should be collected as soon as they reach optimal maturity; however, it is difficult to have mechanical collection devices available where and when needed. In addition, because of the conformation of the tree branches, strong adherence of the fruit to the tree, and limited accessibility, most olives are picked by hand.

Another harvesting procedure is to wait until the olives drop naturally and then collect the fruit with a system of nets. When the ripening period is delayed, both this procedure and handpicking are used. Although attempts have been made in the past to use chemicals to influence dropping time, chemicals are seldom used. Mechanical devices must be used with caution so that neither the tree nor the branches are damaged. When mechanical devices are used, the olives are caught in nets to avoid contact with the ground and damage to the fruit.

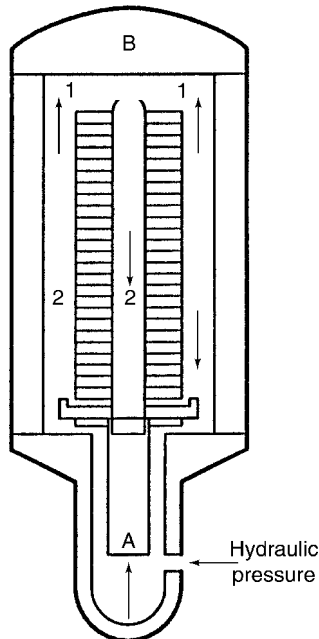
Under optimum conditions, the olives are transferred from the nets to cages (usually plastic), forming layers not higher than 30 cm each, and the olives are sent promptly to the extraction plant. In most regions of Italy and Greece, cages are stored no more than 3 to 5 days before extraction. This procedure ensures

high-quality oil if climatic conditions were good, the trees received proper care, and the fruit was not damaged by pests.

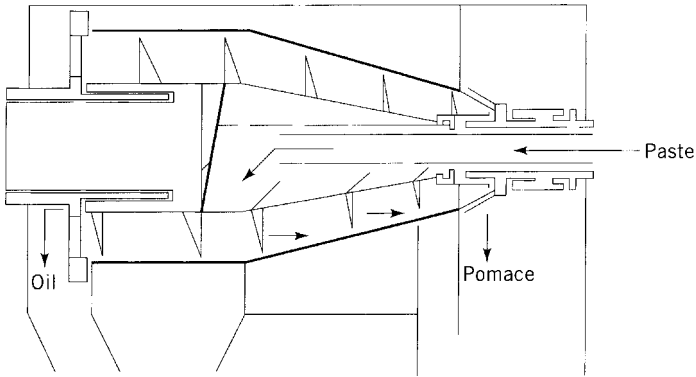
If proper precautions are not taken and the olives are collected in large batches and held in piles several meters high, the fruit may be damaged. The enzymes released will cause hydrolytic and oxidative transformations resulting in off-flavors that affect the quality of the oil. Even with low acidity, such oils will have an unpleasant taste not acceptable for virgin oil and will have to be refined. Because of the difference in price between virgin and refined oils, economic losses to the farmer can be high.

Three systems are used for mechanical extraction of oil from the olive fruit: pressure processing (Figure 2); centrifugation (Figure 3); and adhesion filtering (Figure 4) (17). Pressing is the oldest and most often used method for olive oil extraction. High-speed rotating machines are used for centrifugation extraction. With adhesion filtration, a series of steel plates or blades are dipped into olive paste; when withdrawn, the oil drips off the blades.

Several processing steps are required before extraction. The fruit must first be cleaned to eliminate branches and leaves and any extraneous materials that might damage plant equipment. The fruit is then washed to remove dirt and agricultural contaminants, and finally crushed and milled to a coarse paste (Figure 5). During the last step, enzymatic action breaks up the bitter components and reduces the level of peppery constituents while increasing the amount of minor polar components

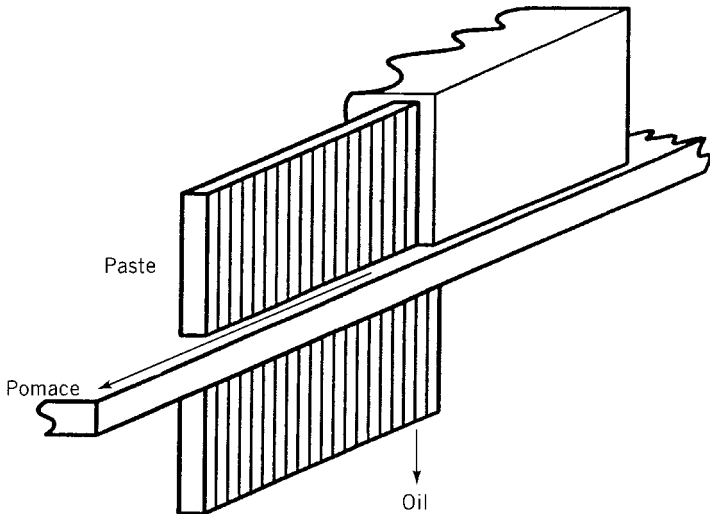


**Figure 2.** Pressure extraction of oil. 1, movement of the rack; 2, movement of the oil; A, mobile head; B, fixed head.



**Figure 3.** Centrifuge for oil extraction from olive-pomace.

and tocopherols in the oil. If enzymatic action is prolonged, the minor polar components break down into water-soluble compounds that are removed from the oil, causing the loss of much of the antioxidant strength of the oil. Milling releases the oil from the oil-bearing cells and helps smaller droplets of oil to merge into larger drops, thus preparing the fruit for the following extraction step. A solid residue and vegetation water are produced during extraction in addition to oil (Figure 6). The vegetation water must be purified before discharge into a municipal sewer. Waste water has been used to grow yeast, to produce butanol using microorganisms, to isolate anthocyanin compounds for use in the food industry, and to produce steam.



**Figure 4.** Diagram of adhesion extraction of oil.

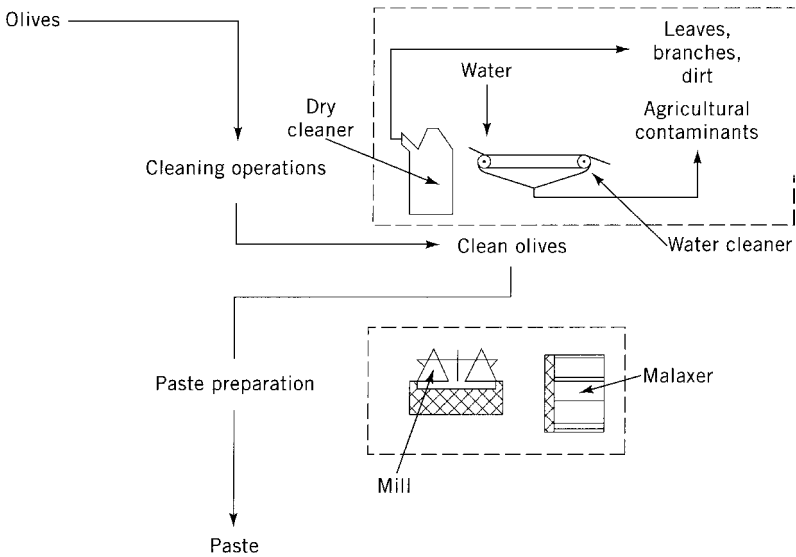


Figure 5. Flow diagram of steps to prepare olives for extraction of oil.

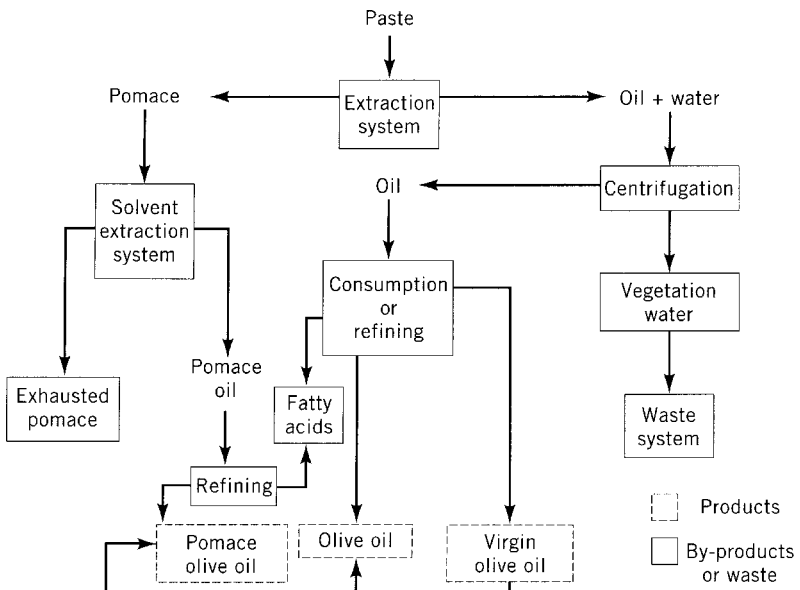


Figure 6. Flow diagram of olive oil extraction and processing to yield olive oil products and byproducts.



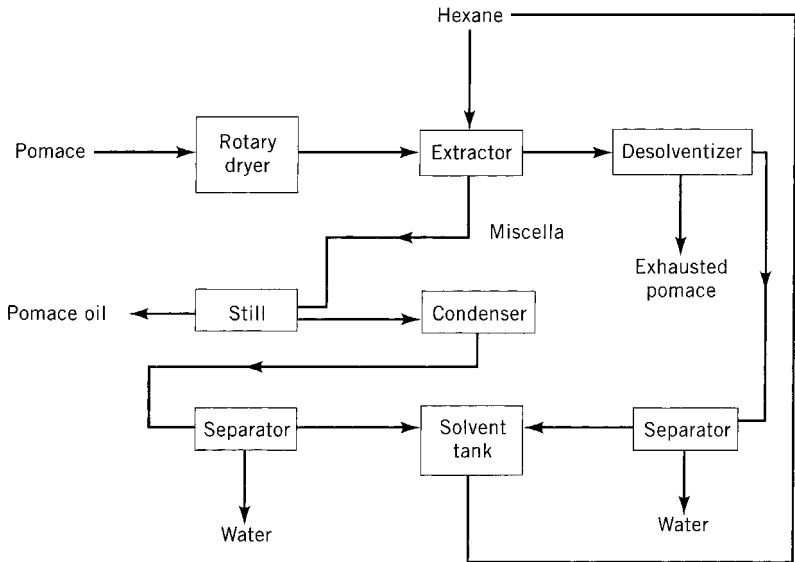


Figure 7. Flow diagram of solvent extraction of pomace.

Efforts are being made to reduce waste water by recycling in the milling process and to decrease its environmental pollution by treatment with biological or physical processes prior to its discharge (22). A number of alternative technologies are available for waste water purification (18–21); however, they are costly and difficult to apply.

If suitable for consumption, the oil is centrifuged after extraction to eliminate solid impurities and residual water. If the free fatty acid content is too high or organoleptic properties are unsatisfactory, the oil is refined.

At the solvent extraction plant, the cake (pomace) containing up to 8% residual oil is dried in a rotary kiln before proceeding to the solvent extraction unit, usually a semicontinuous system (Figure 7). The extracted pomace oil is always refined. Spent cake is used as fuel or is separated into two fractions, the pulp (including skin) and the pit. In addition to use as fuel, the pit is occasionally used to produce fiberboard (23).

#### 4. REFINING OF OLIVE OILS

Olive oil refining is carried out in either of two ways: by alkali refining, generally used for animal and vegetable oils and fats; or by physical refining, a technology not usually used for seed oils. Flow diagrams of the two procedures are shown in Figures 8 and 9.

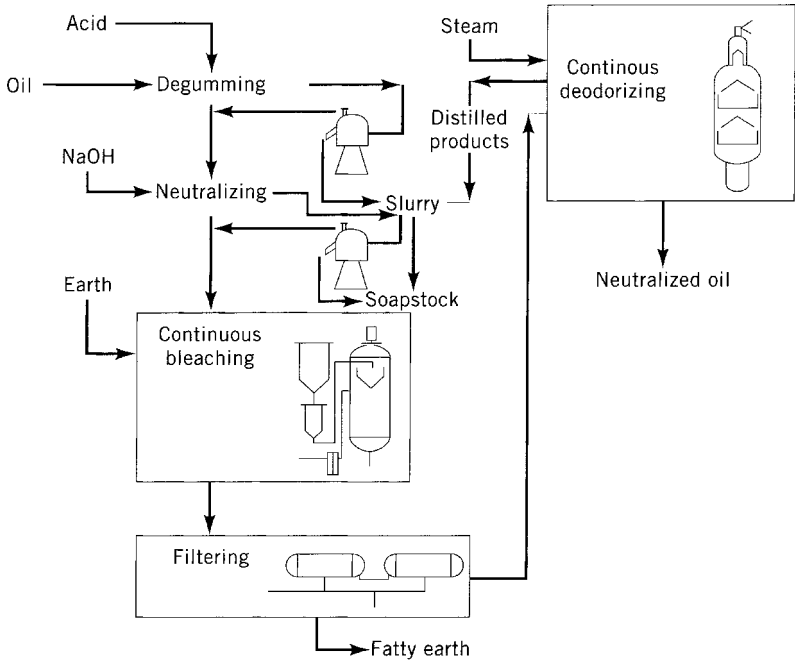


Figure 8. Flow diagram of alkali refining.

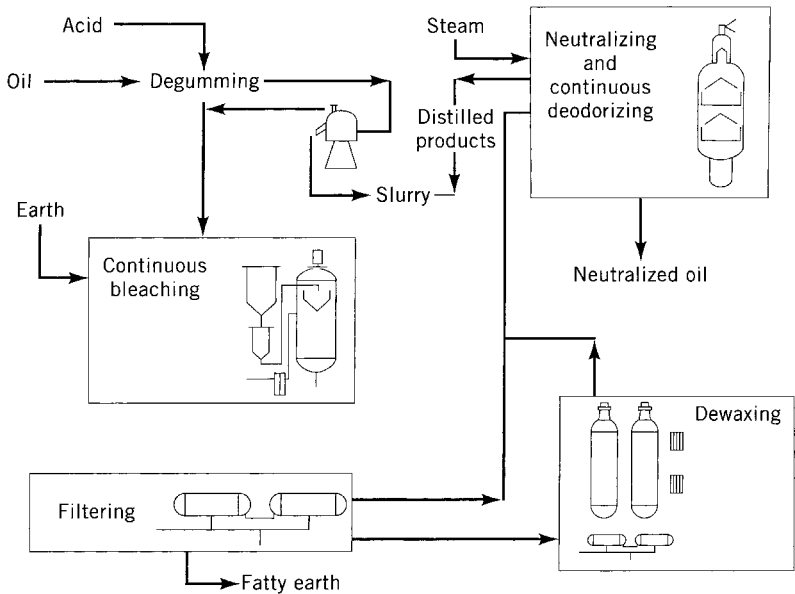


Figure 9. Flow diagram of physical refining.

In the first procedure, the oil is treated with dilute acid to precipitate the gums (phosphatides and proteinaceous material), which are separated by settling or centrifugation. Phosphoric acid and citric acid are the two most common degumming agents. After degumming, the oil is neutralized (alkali refined) either in a batched or continuous system. Batch neutralization is currently preferred because centrifuging of only the settled soap fraction lowers the neutralization coefficient values, thereby shortening the washing time of the oil. The separated soap solution is acidified with sulfuric acid to recover the free fatty acids (containing 30–40% triglycerides) for industrial applications.

The alkali-refined oil is then bleached under vacuum with mixtures of various adsorbents (bleaching earth or clay and sometimes small amounts of activated carbon) and filtered by any of a number of available filter presses occasionally equipped with a solvent system for recovering oil entrained in the bleaching earth.

The bleached oil is deodorized in a semicontinuous or continuous deodorizer operating at a vacuum of less than 2 mm Hg. The final step involves mixing refined oil with virgin oil to improve the organoleptic and keeping properties of the oil. A good olive oil will contain at least 20% virgin oil, but the product must, of course, meet consumer preference, which sometimes requires a very light flavor and taste.

With physical refining, the oil is first degummed and bleached and then fed to a continuous distillation (deodorization) unit, which removes the free fatty acids (92–95%) and volatiles. The refined oil is blended as above. Frequently, distillation is stopped before removal of all of the free fatty acids, and the oil is alkali refined to remove the remainder of the free fatty acids. This procedure has the advantage of eliminating oxidation byproducts and pro-oxidant metals, thus improving product stability.

## 5. REFINING OF POMACE OIL

The technologies adopted for refining pomace oil are based primarily on physical refining because the acidity of pomace oils is about 10% (expressed as oleic acid). Because degumming of pomace oil requires more drastic conditions than those for pulp oil, larger amounts of acidulant are used (phosphoric acid is preferred), and occasionally, the precipitate (gum) that entrains a high proportion of oil is centrifuged to recover the oil. Larger amounts of bleaching earths are required to remove the intense green color of the oil. Additional processing of the bleached oil usually follows the same procedures described for physical refining of olive oil, including incomplete distillation (deodorization) followed by alkali refining of the partially deodorized oil.

Dewaxing (winterization) of pomace oil is mandatory because of its high content of waxes (olive oil may also be winterized, especially if it is used to produce margarine or mayonnaise). Higher melting point triglycerides are also removed. Winterization can be carried out after bleaching or following partial deodorization

and alkali neutralization (alkali refining). If alkali neutralization is performed at a low temperature, winterization can be carried out simultaneously. A continuous apparatus is generally used for winterization (Figure 9) coupled with continuous filtering units. The winterization oil is then blended with virgin oil to restore the oil's antioxidant properties.

## 6. OLIVE OIL COMPONENTS

Glycerides account for at least 97% of a virgin oil if the acidity is disregarded. The free fatty acid content is used to distinguish the various classes of virgin oil, from extra virgin to lampant. It must be emphasized that virgin olive oil is a natural product and therefore subject to variations in composition, both qualitative and quantitative. The origin, cultivar, extraction technology, state of ripening of the fruit, climatic conditions, and rainfall all influence biosynthesis within the fruit and, therefore, the composition and quality of the oil. The fatty acid composition of olive oil is shown in Table 3, which lists typical compositions of European, Turkish, and African (Tunisian) oil as well as IOOC limits (12). Differences in composition are due chiefly to linoleic, linolenic, and palmitic acid content. Olive oils from Argentina resemble those from Tunisia. The triglyceride composition of European, Turkish, and Tunisian olive oils is shown in Table 4 (main glycerides are shown). Fatty acid distribution in the triglycerides follows the 1,3-random, 2-random rule (24–26).

Several classes of minor components are present in virgin olive oil. The structure, concentration, and number of these substances are characteristic of virgin oils. Some are minor glyceridic components (MGCs); others fall into other categories as listed below.

**TABLE 3. Fatty Acid Composition of Olive Oil (%).**

Acid	CAN <sup>a</sup>	European	Turkish	Tunisian <sup>b</sup>	Limits (12)
Palmitic	16:0	8.4	12.1	15.3	7.5–20.0
Palmitoleic	16:1	0.7	0.7	1.6	0.3–3.5
Heptadecanoic	17:0	0.1	0.2	0.1	0.0–0.3
Heptadecenoic	17:1	0.1	0.2	0.1	0.0–0.3
Stearic	18:0	2.5	3.1	2.1	0.5–5.0
Oleic	18:1	78.0	71.3	62.5	55.0–83.0
Linoleic	18:2	8.3	10.6	16.5	3.5–21.0
Linolenic	18:3	0.8	0.7	0.8	0.3–0.9
Arachidic	20:0	0.5	0.4	0.5	0.2–0.6
Eicosenoic	20:1	0.3	0.3	0.3	0.1–0.4
Behenic	22:0	0.1	0.2	0.1	0.0–0.2
Lignoceric	24:0	0.2	0.2	0.1	0.0–0.2

<sup>a</sup>CAN = Carbon atom number.

<sup>b</sup>Typical values for Tunisian olive oil analyzed during 1994.

**TABLE 4. Main Triglycerides of Olive Oil (%).**

ECN <sup>a</sup>	Triglyceride <sup>b</sup>	European	Turkish	Tunisian <sup>c</sup>
42	LLL, TLO <sup>d</sup> , TLP <sup>d</sup>	0.5	0.8	1.6
44	LLO <sup>d</sup>	2.4	3.2	10.6
	TOO <sup>d</sup> , LLP	2.6	2.9	1.7
46	LOO <sup>d</sup>	13.3	13.8	16.0
	LOP <sup>d</sup> , PLP	8.0	9.7	16.2
48	OOO	39.9	34.0	23.2
	POO	26.0	24.4	22.0
	POP	—	—	5.1
50	SOO	5.1	5.1	4.3
	SOP	1.0	1.4	1.2
52	OSS, PSS	0.8	—	0.5

<sup>a</sup>ECN = equivalent carbon number.

<sup>b</sup>L = C18 : 2; T = 18 : 3; O = C18 : 1; P = C16 : 0; S = C18 : 0.

<sup>c</sup>Typical values for Tunisian olive oil analyzed in 1994.

<sup>d</sup>Mixture of isomers.

Hydrocarbons

Tocopherols

Linear short chain alcohols and their esters

Linear long chain alcohols and their esters

Sterols and their esters

$\alpha$ -Methyl sterols

Monohydroxytriterpenes

Dihydroxytriterpenes

Triterpenic acids

Phytol

Geranylgeraniol

Phenols and related compounds

Flavor components

Methyl and ethyl esters

Other components

## 6.1. Minor Glyceridic Components

Monoglycerides (MGs) and diglycerides (DGs) in the olive fruit are caused by enzymatic hydrolysis of the triglycerides and incomplete triglyceride biosynthesis (16). In general, DGs are more abundant than MGs. Determination of DG concentration is useful for evaluating oil freshness and time of fruit harvesting because the DG level is strongly related to climatic influences. DG concentration can even be used to determine the source of an oil, even a refined oil, because the DG content of edible virgin olive oils differs from that of high acidity oils or solvent-extracted oils. Phospholipids are essentially absent from olive oil.

## 6.2. Nonglyceridic Minor Components

*Hydrocarbons.* Both even- and odd-chain *n*-paraffins, including branched-chain (iso and anteiso) compounds, which are minor components of the hydrocarbon fraction, are present in virgin olive oil. The polyunsaturated triterpenic hydrocarbon squalene, and biochemical precursor of sterols, is the main component of the hydrocarbon fraction. The squalene content of olive oil ranges from 150 to 700 mg per 100 g (27–30).  $\beta$ -Carotene is also present in olive oil as are aromatic hydrocarbons, including benzenoid, naphthalenic, and more complex aromatic hydrocarbons (30–37).

*Linear Short Chain Alcohols and Their Esters.* Methanol and ethanol esters of the fatty acids present in olive and in the same proportions as in the olive are present among the volatile compounds in virgin olive oil (31–37).

*Straight Long Chain Alcohols.* Linear long-chain alcohols with carbon numbers between C22 and C32 are present in olive oil both free and esterified (waxes). The components are abundant in the epicarp of the fruit and concentrate in solvent extracted oil. Phytol, probably derived from biodegradation of chlorophyll, is also present along with geranyl (38).

*Cyclic Monohydroxy Compounds.* Triterpenic tetra- and pentacyclic monohydroxy compounds are characteristic of olive oils (34–49). The following compounds have been shown to be present, accompanied by small amounts of lanosterol and obtusifoliosol:

Tetracyclic: cycloartenol  
 24-methylene cycloartanol  
 Pentacyclic:  $\alpha$ -amyrin  
 $\beta$ -amyrin

Methylsterols (4-desmethyl triterpenes) and sterols (4,4-di-desmethyl triterpenes) present in olive oils are derived from the tetracyclic alcohols. The following methyl sterols (4 $\alpha$ -methyl-7-cholesten-3 $\beta$ -ol compounds) are present: 24-methylene, 24-methyl-, 24-ethylidene, and 24-ethyl.

The main sterols of olive oil are (40, 43, 45–66) campesterol, stigmasterol, clerosterol,  $\beta$ -sitosterol, sitostanol, and  $\delta$ -5-avenasterol.

These are accompanied by small amounts of cholesterol (max. 0.5%), brassicasterol (max. 0.1%), 24-methylenecholesterol (max. 0.5%), campestanol (max. 0.5%),  $\delta$ -5,24,-stigmastadienol (max. 1%),  $\delta$ -7-stigmastenol (max. 0.5%), and  $\delta$ -7-avenasterol (max. 1.1%).

Analysis of the sterol fraction isolated from the unsaponifiable fraction is very important, as will be seen later, for determining the authenticity of the oil. The triterpenes and sterols are present both as free alcohols and as fatty acid esters (46, 47).

*Cyclic Dihydroxy Compounds.* Pentacyclic triterpenes in olive oil include 3 $\beta$ ,17 $\beta$ -dihydroxy-12-oleanene (erythrodiol) and its parent compound uvaol, obtained largely from the epicarp and therefore characteristic of solvent extracted oils (42, 65).

*Triterpenic Acids.* The following pentacyclic mono- and dihydroxy triterpenic acids are present in virgin olive oil (35, 43, 44): 3 $\beta$ -hydroxy-17-carboxy- $\delta$ -12-oleanene (oleanolic acid); 3 $\beta$ ,2 $\alpha$ -dihydroxy-17-carboxy- $\delta$ -12-oleanene (maslinic acid); 3 $\beta$ -hydroxy-17-carboxy- $\delta$ -12-ursene (ursolic acid); 2 $\alpha$ ,3 $\beta$ -dihydroxy-17-carboxy- $\delta$ -12-ursene (2 $\alpha$ -hydroxyursolic acid); and deoxyursolic acid (structure not fully elucidated).

*Chlorophylls.* Both chlorophyll  $\alpha$  and chlorophyll  $\beta$  are present in olives and are partially extracted into the oils.

*Flavor Components.* Olive oil volatiles contain at least 100 compounds (33–37) in several categories: hydrocarbons (5 compounds), aliphatic alcohols (13 compounds), terpenic alcohols (4 compounds), aldehydes (27 compounds), ketones (8 compounds), ethers (2 compounds), furans (3 compounds), thiophenes (6 compounds), and esters (29 compounds).

### 6.3. Minor Polar Components

The olive mesocarp contains a number of phenolic and polyphenolic compounds and their esters, small amounts of which are present in olive oil (35, 43, 44). These include monohydroxy- and dihydroxy-phenylethanol, including tyrosol and other phenols and a series of carboxy-phenols, including caffeic, *o*-coumaric, *p*-coumaric, cinnamic, ferulic, gallic, *p*-hydroxybenzoic, protocatechuic, sinapic, syringic, and vanillic acids. Benzoic and cinnamic acids are produced by hydrolysis of flavonoids. The hydroxyphenyl-ethanols arise from hydrolysis of oleuropein. Their esters are responsible for the bitterness and pepperlike sensation occasionally dominant in the taste of olive oils.

Olive oil contains  $\alpha$ -tocopherol in the range of 12–190 mg/kg. According to one report (43), olive oil tocopherols were found to consist of 88.5%  $\alpha$ -tocopherol, 9.9%  $\beta$ - +  $\gamma$ -tocopherol, and 1.6%  $\delta$ -tocopherol. Tocopherol content can be used to detect adulteration of olive oil with seed oils.

## 7. ANALYSIS OF OLIVE OILS

Olive oil is initially examined to determine purity, then to place it in the proper category, and finally to establish its quality.

### 7.1. Determination of Purity

*Sterol Composition.* Sterol analysis involves preparation of the unsaponifiable fraction, fractionation by thin-layer chromatography (TLC), and gas chromatographic analysis of the TMS derivatives (66). The following limits apply to all types of olive oil (12):

Sterol	Sterol Fraction (%)
Cholesterol	Max. 0.5
Brassicasterol	Max. 0.1
Campesterol	Max. 4.0
Stigmasterol	Less than 4.0
$\delta$ -7-Stigmastenol	Max. 0.5
The sum of the following sterols must be more than 93.0% of the sterol function:	
$\beta$ -Sitosterol	
$\delta$ -5-Avenasterol	
$\delta$ -5,23-Stigmastadienol	
Clerosterol	
Sitostanol	
$\delta$ -5,24-Stigmastadienol	

*Total Sterol Content.* The gas liquid chromatographic method for sterol determination using an internal standard (cholestanol) is used to calculate the absolute (total) sterol content of an oil (68, 69). Gravimetric, enzymatic, colorimetric, and liquid chromatographic methods have also been reported (69). Limits (mg/100 g) are as follows (12): virgin olive oil, refined olive oil, and olive oil (mixture of refined and virgin) >100; crude olive-pomace oil >250; and refined olive-pomace oil, olive oil and olive-pomace oil (mixture) >180.

*Fatty Acid Composition.* Olive oil triglycerides are converted into methyl esters, and the methyl esters are analyzed by gas-liquid chromatography (GLC) (70, 71). The limits of genuine olive oil are as follows (% m/m) (12):

Acid	CAN <sup>a</sup>	Minimum	Maximum
Myristic	14:0	—	0.05
Palmitic	16:0	7.50	20.00
Palmitoleic	16:1	0.30	3.50
Heptadecanoic	17:0	—	0.30
Heptadecenoic	17:1	—	0.30
Stearic	18:0	0.50	5.00
Oleic	18:1	55.00	83.00
Linoleic	18:2	3.50	21.00
Linolenic	18:3	—	0.90
Arachidic	20:0	—	0.60
Eicosenoic	20:1	—	0.40
Behenic	22:0	—	0.20
Lignoceric	24:0	—	0.20

<sup>a</sup>CAN = carbon atom number.

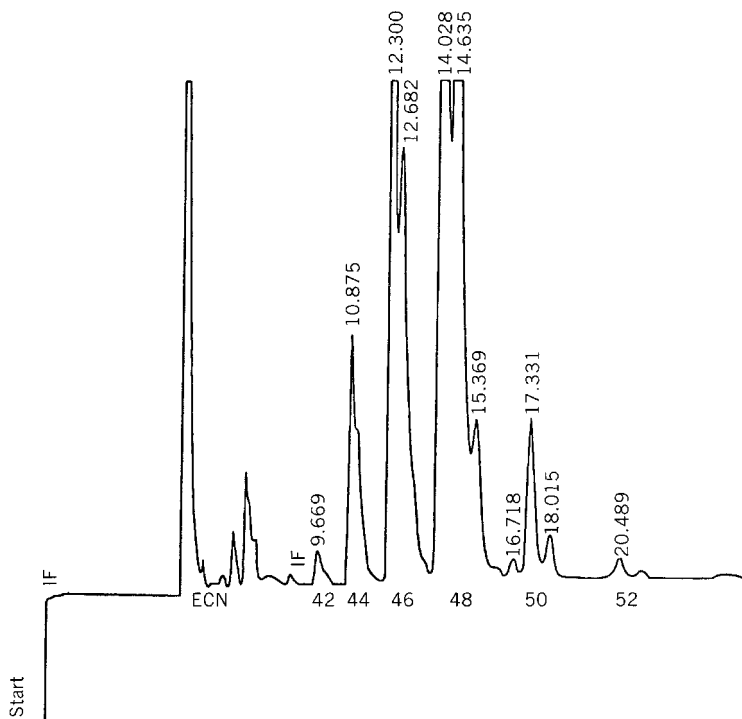
*Saturated Fatty Acids in Position 2 of the Triglycerides.* Hydrolysis with pancreatic lipase is followed by thin-layer chromatographic isolation of the monoglyceride fraction, which is converted to methyl esters. The methyl esters are analyzed



by GLC (72, 73). Maximum acceptable level is the sum of palmitic and stearic acid (% m/m) (12):

Virgin olive oil	≤1.5
Refined olive oil	≤1.8
Olive oil (mixture of refined and virgin)	≤1.8
Crude olive-pomace oil	≤2.2
Refined olive-pomace oil	≤2.2

*Absolute Difference Between Found and Theoretical Equivalent Carbon Number (ECN) 42 (Trilinolein) Values.* The triglyceride composition of the oil is determined by high-performance liquid chromatography (HPLC) (74). (A chromatogram of an olive oil sample (ECN 42, 0.8%) is shown in Figure 10.) The theoretical triglyceride composition is calculated with a Lotus 123 program provided by the IOOC. The maximum difference of theoretical ECN 42 vs. ECN 42 found is calculated. ( $ECN = CN - 2n$ , where CN is the carbon number and  $n$  is the number of double bonds.) The maximum difference between the real and theoretical ECN content



**Figure 10.** HPLC chromatogram of olive oil triglycerides. Column: LC-18, 200 × 4.6 mm i.d.; mobile phase: acetone:acetonitrile (60:40, v/v); flow rate: 0.75 mL/min; refractive index detector; oven and detector temperature: 40° C. IUPAC Method 2.324 (72) with injection of 10- $\mu$ L test sample diluted 1:20 with acetone. ECN 42, 0.8% of total glycerides.

of olive oils and olive–pomace oils should be 0.3 and 0.5, respectively. This procedure avoids errors because of miscalculation of trilinolein alone (75).

*Trans-Fatty Acid Content.* Trans-fatty acids arise during refining of vegetable oils as well as during hydrogenation, or from attempts to eliminate the sterol fraction of seed oils with a fatty acid composition similar to that of olive oil. Methyl esters are analyzed by capillary column GLC (76, 77). The following limits (% m/m) are mandatory (12):

Oil	18:1 trans	18:2 trans + 18:3 trans
Virgin olive oil	<0.03	<0.03
Lampant olive oil	≤0.10	≤0.10
Refined olive oil	≤0.20	≤0.30
Olive oil (mixture of refined and virgin)	≤0.20	≤0.30
Crude olive–pomace oil	≤0.20	≤0.10
Refined olive–pomace oil	≤0.40	≤0.35
Olive–pomace oil and olive oil mixture	≤0.40	≤0.35

## 7.2. Differentiation Between Olive Oil and Olive–Pomace Oil

*Wax Content.* Olive oil fatty acid esters of straight chain alcohols (wax esters present in solvent extracted olive–pomace oil are isolated by column chromatography on silica gel (LC) and quantitated by GLC to determine if olive–pomace oil is present in olive oil (78). LC separation of the wax esters can be replaced with HPLC to automate the separation step and improve reliability and repeatability (79). Limits for content of C40 + C42 + C44 + C46 wax esters (mg/kg) are as follows (12):

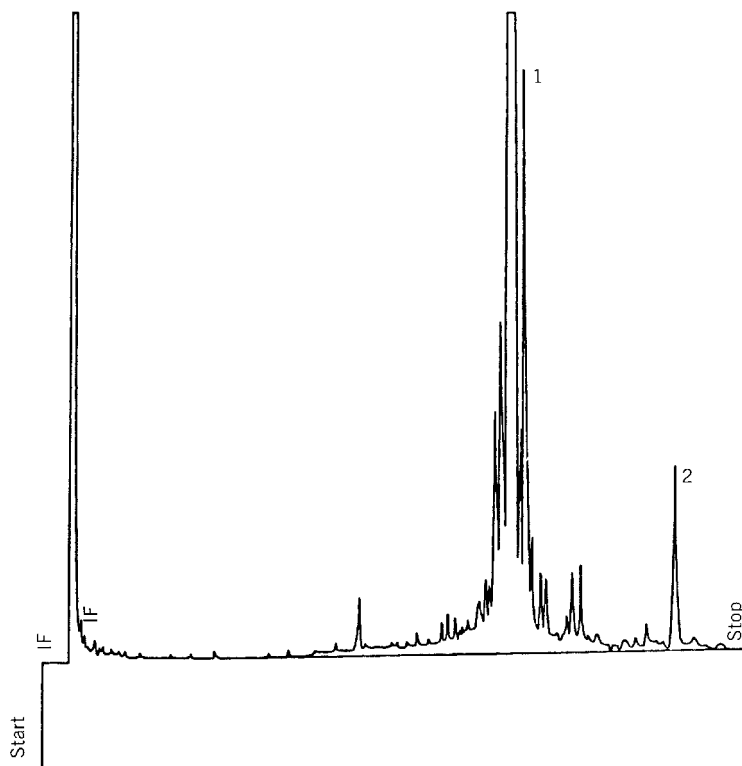
Virgin olive oil	≤250
Lampant olive oil	≤350
Refined olive oil	≤350
Olive oil (mixture of refined and virgin)	≤350

*Dihydroxyterpene Alcohol Content.* Olive–pomace oil contains relatively high levels of erythrodiol, uvaol, and wax esters. Erythrodiol and uvaol (total diol) content is determined by the same procedure as that used for sterol analysis (80, 81). Limits for total diol content (as % of total sterols) are as follows:

Virgin olive oil	≤4.5
Lampant olive oil	≤4.5
Refined olive oil	≤4.5
Olive oil (mixture of refined and virgin)	≤4.5

### 7.3. Differentiation Between Virgin and Refined Olive Oil and Detection of Refined Olive Oil and Seed Oils in Virgin Olive Oil

*Concentration of Stigmasta-3,5-Diene.* When olive oil and seed oils are refined, stigmasta-3,5-diene is produced by dehydration of  $\beta$ -sitosterol, the parent sterol (82). Refined olive oils contain significant amounts of stigmasta-3,5-diene (3–100 mg/kg) not present in any significant amount in virgin olive oils. Refined seed oils also contain significant amounts of steroidal hydrocarbons, including campesta-3,5-diene and stigmasta-3,5,22-triene in addition to stigmasta-3,5-diene. The relative amounts of these steroidal hydrocarbons can be used to detect refined seed oils or seed oils desterolized for the purpose of adulterating olive oil. Isolation of the hydrocarbon fraction from the unsaponifiables by column chromatography on silica gel followed by GLC is used to determine the concentration of stigmasta-3,5-diene and accompanying hydrocarbons (83, 84). A chromatogram of the hydrocarbon fraction from an olive oil is shown in Figure 11.



**Figure 11.** Capillary GLC of the hydrocarbon fraction of olive oil (blend of refined and virgin olive oil). Column: DB-5, 25 m  $\times$  0.25 mm i.d., 0.2- $\mu$ m film thickness; split ratio; 1 : 15; temperature program: 235 $^{\circ}$  C, 6 min; 20 $^{\circ}$  C/min; 285 $^{\circ}$  C final temperature; injector: 300 $^{\circ}$  C; detector; 320 $^{\circ}$  C, 1, cholesta-3,5-diene (internal standard); 2, stigmasta-3,5-diene.

A chromatogram of the hydrocarbon fraction from an olive oil admixed with des-terolized, refined seed oil is shown in Figure 12. Ratios of stigmasta-3,5-diene to campesta-3,5-diene (R1) and stigmasta-3,5-diene to stigmasta-3,5,22-triene (R2) are determined when the level of stigmasta-3,5-diene exceeds 4 ppm (12).

Oil	Stigmasta-3,5-diene (ppm)	R1 <sup>a</sup>	R2 <sup>b,c</sup>
Virgin olive oil	≤0.1	—	—
Lampant olive oil	≤0.5	—	—
Refined olive oil	≤50.0	≥15	≥15
Olive oil	≤50.0	≥15	≥15
Crude olive–pomace oil	≤0.5	≥15	≥15
Refined olive–pomace oil	≤120.0	≥15	≥15
Pomace and olive oil mixture	≤120.0	≥15	≥15

<sup>a</sup>R1 = ratio of stigmasta-3,5-diene to campesta-3,5-diene.

<sup>b</sup>R2 = ratio of stigmasta-3,5-diene to campesta-3,5,22-triene.

<sup>c</sup>Provisional limits.

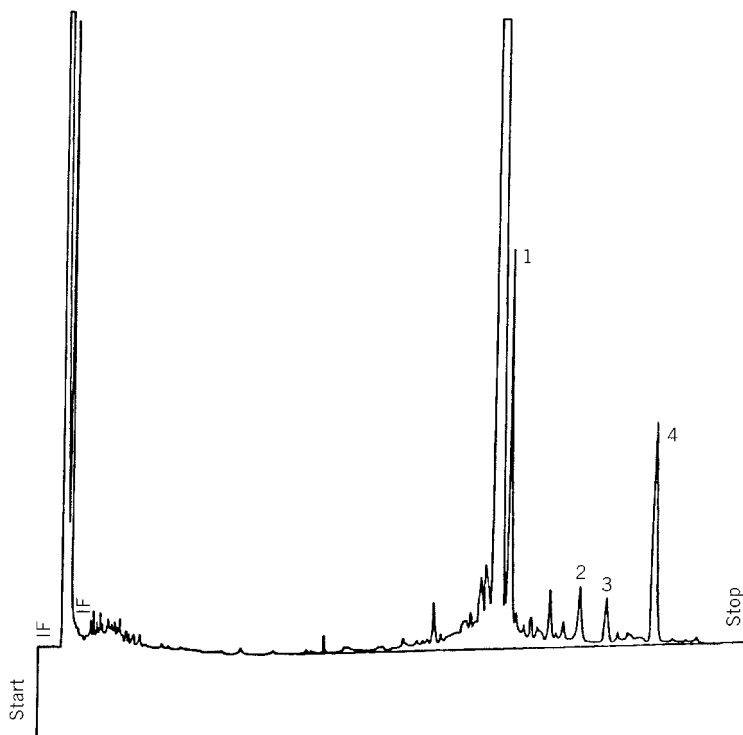
However, a July 1994 IOOC report (84) noted that the R1 and R2 values of many Italian and Greek olive oils were considerably lower than those proposed by the IOOC (12) and that the composition of steroidal hydrocarbons should be identical to that of the sterols from which they are derived when the R1 and R2 ratios are used to identify extraneous oils in refined olive oil.

*UV Absorption at 268 nm.*  $K$  (1%, 1 cm) and related value,  $\delta-K$ , are useful for readily classifying olive oil quality according to the following values (12, 85):

Oil	$K_{270}$ nm	$\delta-K^a$
Extra virgin olive oil	≤0.25	≤0.01
Virgin olive oil (fine)	≤0.25	≤0.01
Virgin olive oil (semifine)	≤0.30	≤0.01
Lampant olive oil	No limits	No limits
Refined olive oil	≤1.10	≤0.16
Olive oil	≤0.90	≤0.15
Crude olive–pomace oil	No limits	No limits
Refined olive–pomace oil	≤2.00	≤0.20
Pomace and olive oil mixture	≤1.70	≤0.18

$$^a\delta-K = K_{268} - ([K_{262} + K_{274}]/2).$$

Both  $K$  and  $\delta-K$  are altered when oxidation products are present. In this case, the oil is dissolved in hexane and passed through an alumina column before measurement of  $K$  and  $\delta-K$ .



**Figure 12.** Capillary GLC of the hydrocarbon fraction of olive oil admixed with a deesterolized seed oil (GLC column and operating conditions as described for Figure 11). 1, Cholesta-3,5-diene (internal standard); 2, campesta-3,5-diene; 3, stigmasta-3,5,22-triene; 4, stigmasta-3,5-diene.

#### 7.4. Quality Parameters

*Organoleptic Characteristics.* Organoleptic properties of virgin oil can be determined by a “panel test” (13, 86), which gives results that are often controversial. Organoleptic testing is currently undergoing revision. Currently the IOOC is preparing a draft method for the organoleptic assessment of virgin olive oil using a designation of origin (DO) code. It is intended for use by DO authorities to ensure that the oil meets requirements (87). The panel test method is based on examination of virgin oil by a panel of 8 to 12 trained personnel who grade various characteristics and defects that are then converted into a number score. The following scores apply to various grades of virgin olive oil:

Extra virgin olive oil	>6.5
Fine virgin olive oil	>5.5
Semifine virgin olive oil	>3.5
Lampant virgin olive oil	<3.5

*Free Fatty Acid Content.* Free fatty acid content (expressed as % oleic acid) (88) is used to define the various grades of virgin olive oil (12):

Extra virgin olive oil	<1.0
Fine virgin olive oil	<1.5
Semifine virgin olive oil	<3.3
Lampant virgin olive oil	>3.3

Refined olive oil and mixtures have the following limits (12):

Refined olive oil	≤0.3
Olive oil	≤1.5
Refined olive–pomace oil	≤0.3
Olive–pomace and olive oil	≤1.5

Olive oil and mixtures of olive–pomace and olive oil have higher free fatty acid contents because they are generally mixed with virgin olive oils of high acidity.

*Peroxide Value (PV).* PV (expressed in meq per kg oil) (89) allowed for various grades of olive oil is as follows (12):

Extra virgin, fine, and semifine virgin olive oil	≤20
Refined olive oil	≤10
Olive oil	≤15
Refined olive–pomace oil	≤10
Pomace oil and olive oil mixture	≤15

Virgin olive oil contains components that interfere with conventional PV determination. Even freshly expressed olive oil has PV values of about 10, and under certain climatic conditions (dry weather), the PV value can be higher than 10.

*Tocopherol Content.* Tocopherols can be determined by colorimetry or GLC (90), or by HPLC (91, 92). Added tocopherols are not permitted in virgin olive oils and crude olive–pomace oils (12). Added  $\alpha$ -tocopherol is allowed in refined olive oil, olive oil, refined olive–pomace oil, and olive–pomace oil to restore natural tocopherol lost during refining with a maximum level of 200 mg/kg of total  $\alpha$ -tocopherol in the final product (12).

*Impurities.* Water content (93) of virgin olive oil should not exceed 0.2% (m/m); for refined oil and mixtures (olive oil, olive–pomace and olive oil), the maximum value is 0.1%; for lampant olive oil, 0.3%; for crude olive–pomace oil, 1.5% (12). Allowable hydrocarbon (hexane, petroleum ether) residues (94) are as follows (% m/m):

Extra virgin, fine, and semifine virgin olive oil	≤0.10
Refined olive oil, olive oil	≤0.05
Refined olive–pomace oil, olive–pomace and olive oil	≤0.05

The occurrence of  $\mu\text{g}/\text{kg}$  to  $\text{mg}/\text{kg}$  amounts of tetrachloroethylene in some olive oils (95) led to an EEC regulation limiting the tetrachloroethylene content of olive oil and products containing olive oil to not more than 0.1  $\text{mg}/\text{kg}$ , as determined by a head space/electron capture GLC method (96).

Maximum allowable contents of iron and copper (97) are 3 ppm and 0.1 ppm, respectively.

Smoke point (98) is a function of acidity level in the oil. The smoke point for olive oil generally ranges from 150°C to 163°C.

## 7.5. Combined Gas Chromatography–Mass Spectrometry (GC/MS)

GC/MS is a powerful tool for identification and confirmation of the various components of olive oil. With GC/MS in the selective ion mode, unresolved GC peaks can be identified and accurately quantitated. For example, an apparent  $\beta$ -sitosterol peak in the sterol fraction was resolved into clerosterol ( $m/z$  218) and  $\Delta$ -5-avenasterol ( $m/z$  314), and both sterols measured regardless of inadequate GC resolution.

Italian and Spanish olive oil from the 1991–1992 crop year contained a very high level of 9,19-cyclolanosterol ( $>400$   $\text{mg}/\text{kg}$ ), which was not found with the standard method for sterol analysis. Two isomers of this sterol were identified by GC/MS of the unsaponifiable fraction, and their levels were found to be inversely proportional to the levels of  $\beta$ -sitosterol in the oils. GC/MS of the unsaponifiable fraction with high-resolution GC capillary columns provides a relatively rapid means of checking product purity and the identity of individual components. Thus, triterpene diols were identifiable at  $m/z$  203,  $\alpha$ -tocopherol at  $m/z$  165, squalene at  $m/z$  69, cholesterol at  $m/z$  386, and brassicasterol, characteristic of canola oil and other Brassica oils, at  $m/z$  398.

## REFERENCES

1. N. Lipshchitz in M. Heltzer and D. Eitam, eds., *Olive Oil in Antiquity*, University of Haifa, Haifa, Israel, 1987, pp. 139–145 (Sorgon Publications, Padova, Italy, 1994).
2. P. Fiorino and F. Nizzi Griffi, *Olivae*, (44), 9–12 Dec. 1992).
3. D. Eitam, in Ref. 1, pp. 16–36.
4. D. Zohary and P. Spiegel-Roy, *Science* **187**, 319 (1975).
5. G. I. Kelm and A. Mazar, in Ref. 1, pp. 121–125.
6. C. Renfrew, *The Emergence of Civilization, The Cyclades and the Aegean in the Third Millennium BC*, Methuen & Co., Ltd., London, 1972.
7. S. Bunimovitz, in Ref. 1, pp. 11–15.
8. J. Chadwick in W. A. McDonald and G. R. Rapp, Jr., eds., *The Minnesota Messenia Expedition. Reconstructing a Bronze Age Regional Environment*, The University of Minnesota Press, Minneapolis, 1972, p. 115.
9. S. Hadjisavvas, in Ref. 1, pp. 98–105.
10. R. Frankel, in Ref. 1, pp. 63–80.

11. J. Frenkel, in Ref. 1, pp. 57–62.
12. International Olive Oil Council (IOOC), Doc. IOOC/T.15/NC no. 1/Rev. 6, June 10, 1993; see also *Codex Alimentarius Commission Doc. CL 1993/15-FO*, May 1993 (proposed draft revised standard for olive oils).
13. IOOC, *Organoleptic Assessment of Virgin Olive Oil*, IOOC/T.20/Doc. 3/Rev. 2, May 28, 1992; see also *Official Journal of the European Communities*, No. L 248/49, Annex, XII, 1991.
14. E. Fedeli in *Proceedings of the Meeting of the Association of Italian Oils and Fats Producers*, June 6–7, 1991.
15. E. Fedeli and N. Cortesi, *Riv. Ital. Sostanze Grasse* **70**, 419 (1993).
16. N. Cortesi, P. Rovellini, and E. Fedeli, *Riv. Ital. Sostanze Grasse* **69**, 305 (1992).
17. L. Di Giovacchino, *Olivae*, (36), 14–41 (Apr. 1991).
18. P. Bondioli, A. Lanzani, E. Fedeli, M. Sala, and G. Gerali, *Riv. Ital. Sostanze Grasse* **69**, 487 (1992).
19. C. Carola, A. Arpino, and A. Lanzani, *Riv. Ital. Sostanze Grasse* **52**, 335 (1975).
20. A. Lanzani, P. Bondioli, E. Fedeli, A. Ponzetti, and G. Perialisi, *Riv. Ital. Sostanze Grasse*, 117 (1988).
21. P. Bondioli, A. Lanzani, and E. Fedeli, *Riv. Ital. Sostanze Grasse* **68**, 1 (1991).
22. I. M. Ordovas, “in F. J. Francis,” *Encyclopedia of Food Science and Technology*, Vol. 3, John Wiley & Sons, Inc., New York, 2000, p. 1775.
23. P. Bondioli, A. Lanzani, E. Fedeli, and L. Casarotto, *Riv. Ital. Sostanze Grasse* **66**, 623, 661 (1989).
24. N. Cortesi, P. Rovellini, and E. Fedeli, *Riv. Ital. Sostanze Grasse* **69**, 1 (1992).
25. C. Mariani and E. Fedeli, *Riv. Ital. Sostanze Grasse* **62**, 3 (1985).
26. R. V. Flor, L. T. Hecking, and B. D. Martin, *J. Amer. Oil Chem. Soc.* **70**, 199 (1993).
27. P. Capella, E. Fedeli, M. Cirimele, and G. Jacini, *Riv. Ital. Sostanze Grasse* **41**, 635 (1964).
28. E. Fedeli, *Rev. Franc. Corps Gras* **15**, 281 (1968).
29. C. Mariani, S. Venturini, and E. Fedeli, *Riv. Ital. Sostanze Grasse* **70**, 321 (1993).
30. C. Mariani and E. Fedeli, *Olivae* (45), 34–39 (Feb. 1993).
31. R. Vazquez, A. J. DelValle, and J. L. M. DelValle, *Grasas y Ace* **27**, 185 (1976).
32. E. Fedeli, D. Baroni, and G. Jacini, *Riv. Ital. Sostanze Grasse* **50**, 38 (1973).
33. E. Fedeli, G. Favini, D. Baroni and G. Jacini, *La Chimica e l'Industria* **55**, 681 (1973).
34. R. A. Flath, R. R. Forrey, and D. G. Guadagni, *J. Agric. Food Chem.* **21**, 948 (1973).
35. E. Tiscornia, M. Forina, and F. Evangelisti, *Riv. Ital. Sostanze Grasse* **59**, 519 (1982).
36. G. Montedoro, M. Bertuccioli, and F. Anichini in G. Charalampous and G. Inglett, eds., *Flavor of Foods and Beverages. Chemistry and Technology*, Academic Press, Inc., New York, 1978.
37. H. Guth and W. Grosch, *Fat Sci. Technol.* **93**, 335 (1991).
38. E. Fedeli, A. Lanzani, P. Capella, and G. Jacini, *J. Lipid Res.* **7**, 437 (1966).
39. J. Eisner, J. L. Iverson, A. K. Mazingo, and D. Firestone, *J. Assoc. Off. Agric. Chem.* **48**, 417 (1965).
40. P. Capella, E. Fedeli, M. Cirimele, A. Lanzani, and G. Jacini, *Riv. Ital. Sostanze Grasse* **40**, 645 (1963).



41. A. Fiecchi, P. Capella, E. Fedeli, A. Lanzani, and G. Jacini, *La Ricerca Scientifica* **36**, 1316 (1966).
42. E. Fedeli and G. Jacini, *Advan. Lipid Res.* **9**, 335 (1972).
43. E. Fedeli, *Prog. Chem. Fats Other Lipids* **15**, 57 (1977).
44. E. Fedeli, *Rev. Franc. des Corps Gras* **30**, 51 (1983).
45. P. Capella, E. Fedeli, M. Cirimele, and G. Jacini, *Riv. Ital. Sostanze Grasse* **40**, 296 (1963).
46. P. Capella, E. Fedeli, M. Cirimele, A. Lanzani, and G. Jacini, *Riv. Ital. Sostanze Grasse* **40**, 660 (1963).
47. C. Mariani, E. Fedeli, K. Grob, and A. Artho, *Riv. Ital. Sostanze Grasse* **68**, 179 (1991).
48. C. Mariani, S. Venturini, P. Bondioli, E. Fedeli, and K. Grob, *Riv. Ital. Sostanze Grasse* **69**, 393 (1992).
49. G. Bianchi and G. Vlahov, *Fat Sci. Technol* **96**, 72 (1994).
50. P. Capella, E. Fedeli, M. Cirimele, A. Lanzani, and G. Jacini, *Fette Seifen Anstrichm.* **66**, 997 (1964).
51. E. Fedeli, A. Lanzani, P. Capella, and G. Jacini, *J. Amer. Oil Chem. Soc.* **43**, 254 (1966).
52. G. Jacini, E. Fedeli, and A. Lanzani, *J. Assoc. Off. Anal. Chem.* **50**, 84 (1967).
53. E. Fedeli in *Proceedings of the Second Meeting on Mass Spectrometry*, Ispra, Italy 1971, 391.
54. E. Fedeli and C. Mariani, *Riv. Ital. Sostanze Grasse* **51**, 129 (1974).
55. G. Jacini and E. Fedeli, *Fette Seifen Anstrichm.* **77**, 1 (1975).
56. E. Fedeli, N. Cortesi, C. Mariani, A. Govoni, and D. Baroni, *Riv. Ital. Sostanze Grasse* **49**, 105 (1972).
57. E. Fedeli, A. Daghetta, D. Baroni, and N. Cortesi, *Riv. Ital. Sostanze Grasse* **49**, 159 (1972).
58. N. Cortesi, C. Mariani, and E. Fedeli, *Riv. Ital. Sostanze Grasse* **50**, 411 (1973).
59. E. Fedeli, N. Cortesi, C. Mariani, D. Baroni, and G. Jacini, *Sci. Tecnol. degli Alimenti* **3**, 143 (1974).
60. E. Fedeli, *Annali di Chimica* **64**, 213 (1974).
61. E. Fedeli, N. Cortesi, and C. Mariani, *Riv. Ital. Sostanze Grasse* **53**, 130 (1976).
62. N. Cortesi, E. Fedeli, A. Gasparoli, and E. Tiscornia, *Riv. Ital. Sostanze Grasse* **54**, 16 (1977).
63. N. Cortesi, E. Fedeli, and E. Tiscornia, *Riv. Ital. Sostanze Grasse* **55**, 168 (1978).
64. A. M. Leone, E. La Notte, and F. Lamparelli, *Riv. Ital. Sostanze Grasse* **53**, 259 (1976).
65. T. Itoh, K. Yoshida, T. Tamura, and T. Matsumoto, and G. F. Spencer, *J. Amer. Oil. Chem. Soc.* **58**, 545 (1981).
66. C. Mariani, E. Fedeli, and K. Grob, *Riv. Ital. Sostanze Grasse* **68**, 233 (1991).
67. E. Fedeli and C. Mariani, *Riv. Ital. Sostanze Grasse* **50**, 164 (1973).
68. *Official Journal of the European Communities, Determination of the Composition and Content of Sterols by Capillary-Column Gas Chromatography*, no. L 248/15, Annex V, May 9, 1991.
69. G. Morchio, R. De Andreis, and E. Fedeli, *Riv. Ital. Sostanze Grasse* **64**, 185 (1987).
70. International Standards Organization, *Animal and Vegetable Fats and Oils—Analysis by Gas Chromatography of Methyl Esters of Fatty Acids*, International Standard ISO 5508, Geneva, 1990.

71. D. Firestone, ed., *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th ed., American Oil Chemists' Society, Champaign, Illinois, Method Ch 2-91.
72. C. Paquot and A. Hautfenne, eds, *Standard Methods for the Analysis of Oils, Fats and Derivatives*, (IUPAC), 7th ed., Blackwell Scientific Publications, Boston, 1987, Section 2.210.
73. D. Firestone, ed., in Ref. 71, Method Ch 3-91.
74. A. Dieffenbacher and W. D. Pocklington, eds., *Standard Methods for the Analysis of Oils, Fats and Derivatives*, (IUPAC), 1st Supple. to the 7th ed. Blackwell Scientific Publications, Boston, 1992, Section 2.324.
75. IOOC, IOOC/T.20/Doc. No. 9, 1991; IOOC/T.20/Doc. No. 18.3, Annex 2; IOOC/T.20/Doc. No. 19-2, Annex 1, Madrid, June 1990.
76. *Riv. Ital. Sostanze Grasse* **68**, 646 (1991).
77. M. Amelio and co-workers, *Riv. Ital. Sostanze Grasse* **70**, 561 (1993).
78. *Riv. Ital. Sostanze Grasse* **66**, 368 (1989).
79. M. Amelio, R. Rizzo, and F. Varazini, *J. Amer. Oil Chem. Soc.* **70**, 793 (1993).
80. C. Paquot and A. Hautfeene, eds., *Standard Methods for the Analysis of Oils, Fats and Derivatives*, (IUPAC) 7th ed., Blackwell Scientific Publications, Boston, 1987, Section 2.431.
81. J. Gracian Tous, W. D. Pocklington, and A. Hautfenne, *Rev. Franc. Corps Gras* **34**, 465 (1987).
82. K. Grob, A. Artho, and C. Mariani, *Fat Sci. Technol.* **94**, 394 (1992).
83. International Union of Pure and Applied Chemistry, Commission on Oils, Fats and Derivatives, Minutes of the Meeting in Budapest, Sept. 9–11, 1992, pp. A9–A11; meeting at Lisbon, working report, July 1993, pp. A1–A4; meeting at Bruges, Belgium, working report, July 1994, pp. A1–A2 and M1–M11.
84. IOOC, Spain, IOOC/T.20/Doc.9 (1991), Doc.11/Rev. 1 (1994), Doc. 12 (1993), Doc. 22-2, Annex 3 (1992), Doc. 28-4, Annex 2 (1993) Madrid; report on the collaborative trial run by the Italian Technical Board on the determination of steroidal hydrocarbons in olive oils, July 1994.
85. Italian Method NGD C40 (1976); see also D. Firestone, ed. in Ref. 71, Method Ch 5-91.
86. E. Fedeli, *Riv. Ital. Sostanze Grasse* **70**, 81 (1993).
87. "Technical methods," IOOC, [www.internationaloliveoil.org](http://www.internationaloliveoil.org), accessed July 2004.
88. C. Paquot and A. Hautfenne, eds., in Ref. 80, Section 2.201.
89. C. Paquot and A. Hautfenne, eds., in Ref. 80, Section 2.501.
90. C. Paquot and A. Hautfenne, eds., in Ref. 80, Section 2.411.
91. A. Dieffenbacher and W. D. Pocklington, eds., in Ref. 74, *Standard Methods* Section 2.432.
92. D. Firestone, ed., in Ref. 71, Method Ce8-89.
93. C. Paquot and A. Hautfenne, eds., in Ref. 80, Section 2.601 or 2.603.
94. A. Dieffenbacher and W. D. Pocklington, eds., in Ref. 74, Section 2.607.
95. M. Matta, G. Gaetano, and M. A. Giannandrea, *Riv. Ital. Sostanze Grasse* **66**, 581 (1989).
96. *Official Journal of the European Communities*, No. L 166/12, (July 1, 1988).
97. A. Dieffenbacher and W. D. Picklington, eds., in Ref. 74, Section 2.631.
98. D. Firestone, ed., in Ref. 71, Method Cc 9a-48.

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## *Palm Oil*

Yusof Basiron

### 1. INTRODUCTION

#### 1.1 Scope

The rapid expansion in world production of palm oil over the last three decades has attracted the attention of the oils and fats industry. Many are interested to know how palm oil has been able to compete successfully to gain an increasing share of the international oils and fats markets. The increasing importance of palm oil has naturally led to a steady buildup of scientific, technical, and trade data and information. Many palm oil producing countries have established dedicated organizations and research institutes that generate data and information to add to the body of knowledge on oil palm cultivation, palm oil processing, and applications. It would be impossible to include the voluminous body of existing and new information as space constraint necessitates the coverage to be selectively confined to describing only the essential aspects of the palm oil industry. Specialists in the field could refer to numerous books and journals or databases that are now available on the subject.

This chapter will provide the reader with an understanding of the role of the palm oil industry in the international oils and fats market, the technology involved in extracting and processing of the oil, and the various quality parameters useful in understanding the applications of palm oil products. Some of the common applications of palm oil in food and nonfood end products are described.

## 1.2. General Considerations

Palm oil is an edible oil referred to by the FAO/WHO Codex Alimentarius (1) as being derived from the fleshy mesocarp of the oil palm fruit. In the unprocessed form palm oil is reddish brown in color, and it has a semisolid consistency at ambient temperature. Readers should not confuse palm oil with palm kernel oil, which is another product obtained from the kernel of the oil palm fruit while palm oil is derived from the mesocarp or fruit flesh. The two oils have different chemical composition and physical characteristics, and they are used and marketed separately according to their own supply and demand situations.

## 1.3. Production

World production of palm oil had increased tremendously during the last 30 years as a result of rapid expansion of oil palm planting in South East Asian countries spearheaded by Malaysia and Indonesia. Papua New Guinea is also a significant producer. Significant amounts of palm oil continue to be produced by the traditional producer countries in West Africa but the growth was much slower. Nevertheless, toward the end of the 1980s, Cote d'Ivoire (Ivory Coast) has emerged as a leading palm oil producer and exporter in Africa with projection of further expansion in its production in the future. Countries of South America are also striving to expand their cultivation of oil palm, and increasing output of palm oil is projected for Columbia, the leading producer from the region.

The continuing investment in oil palm cultivation in South East Asia, Africa, and Central and Latin America especially in the late 1980s and early 1990s contributed further to the growth in the future share of palm oil in the world supply of oils and fats.

Many countries plant oil palm to produce the oil to fulfill their local consumption. In contrast, Malaysia and to a certain extent Indonesia are unique in that the production of palm oil is meant for export. For these countries, palm oil production for export purposes is found to be highly viable, and oil palm has become a favorite cash crop to replace other traditional crops such as rubber. The viability of palm oil for export is determined by the ability of the oil palm to be grown successfully in the country concerned. High yield of the palm throughout the year is essential to achieve viability for the export market.

Oil palm grows well in the tropical climate within 5° north and south of the equator. Ideal growing conditions include adequate rainfall of over 2000 mm per year spread evenly through the year, adequate sunshine of over 2000 h per annum, and moderately high temperature of 25–33°C. Many countries keen to grow oil palm unfortunately experience a few months of drought during each year, and this will severely affect the yield of the palm. Monsoon rains that can cause flooding and problems of fruit evacuation may also affect crop yield. Countries not having the ideal conditions for growing oil palms are reported (2) to have high cost of production to the extent that exporting of the product would not be viable. World oilseed production, vegetable oils production, and protein meal production are listed in Tables 1–2.

**TABLE 1. World Oilseed Production,  $\times 10^6$  t 1995/96 to Date (2).**

Item	Years							
	1995/96	1996/97	1997/98	1998/99	1999/2000	2000/01	2001/02	2002/03 <sup>a</sup>
Production								
Soybeans	124.90	132.22	158.07	159.82	159.90	175.10	183.78	184.49
Cottonseed	35.15	33.61	34.35	32.62	32.93	33.53	36.61	33.37
Peanuts	27.47	28.96	27.29	29.77	28.99	31.12	33.11	31.84
Sunflowerseed	25.72	23.80	23.21	26.63	27.22	23.29	21.25	23.33
Rapeseed	34.44	31.53	33.23	35.89	42.47	37.52	35.87	32.17
Copra	5.13	6.05	5.33	4.38	5.46	5.90	5.26	5.30
Palm kernel	4.87	5.21	5.05	5.62	6.41	6.91	7.24	7.40
Total	257.67	261.38	286.53	294.72	303.37	313.36	323.10	317.89

<sup>a</sup>Forecast.

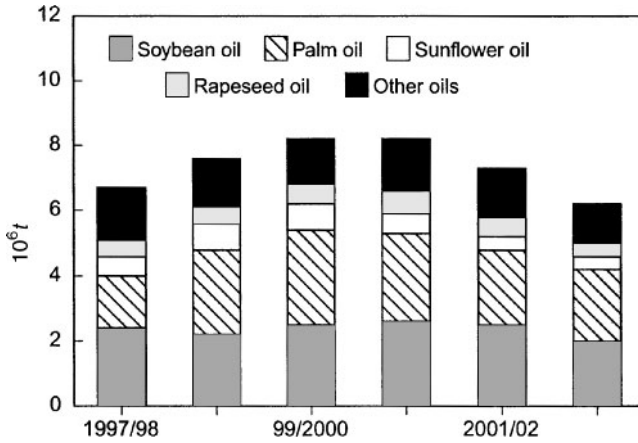
Source: Foreign Agricultural Service, USDA.

**TABLE 2. World Vegetable Oils and Protein Meal Production,  $\times 10^6$  t 1995/96 to Date (2).**

Item	Years							
	1995/96	1996/97	1997/98	1998/99	1999/2000	2000/01	2001/02	2002/03 <sup>a</sup>
Production, Vegetable oil								
Soybeans	20.17	20.53	22.57	24.65	24.74	26.80	28.72	29.85
Palm	16.26	17.64	16.97	19.25	21.80	23.93	24.88	25.37
Sunflowerseed	9.01	8.61	8.29	9.18	9.63	8.41	7.57	8.32
Rapeseed	11.24	10.52	11.43	11.81	13.64	12.96	12.20	11.41
Cottonseed	4.15	3.70	3.70	3.57	3.57	3.52	3.82	3.56
Peanut	4.15	4.38	4.18	4.44	4.15	4.30	4.75	4.51
Coconut	3.16	3.69	3.29	2.71	3.34	3.63	3.26	3.23
Olive	1.45	2.46	2.53	2.50	2.37	2.48	2.53	2.35
Palm Kernel	2.10	2.22	2.20	2.43	2.75	2.95	3.11	3.17
Total	73.08	73.76	75.16	80.54	85.97	88.98	90.85	91.79
Production, Protein meal								
Soybeans	89.08	90.82	98.84	107.54	107.74	116.47	124.71	129.58
Cottonseed	13.11	11.89	11.79	11.36	11.45	11.30	12.10	11.31
Rapeseed	18.58	17.53	18.85	19.12	22.27	21.18	19.99	18.64
Sunflowerseed	10.21	10.06	9.51	10.51	10.72	9.43	8.45	9.25
Fish	6.52	6.64	5.08	5.80	6.29	5.75	5.43	5.61
Peanut	5.73	6.01	5.41	5.76	5.27	5.52	6.13	5.79
Copra	1.74	1.97	1.74	1.44	1.77	1.90	1.68	1.70
Palm Kernel	2.54	2.70	2.67	2.93	3.32	3.56	3.75	3.82
Total	147.49	147.62	153.88	164.47	168.82	175.12	182.23	185.69

<sup>a</sup>Forecast.

Source: Foreign Agricultural Service, USDA.



**Figure 1.** Global vegetable oil stocks, 2002/03 preliminary. (Source: Foreign Agricultural Service, USDA.)

Global palm oil production edged up just 1.0 million tons in 2001/02 to 24.9 million. After several years of large gains in production, the rate of increase in new oil palm area in Southeast Asia was slowing. Last year, Malaysia implemented a replanting program for older trees that covered nearly 200,000 hectares. Malaysian oil yields were lower as palm trees showed signs of stress. Reduced fertilizer application and very dry conditions beginning in February also hurt productivity. Waning yields from Malaysian plantations trimmed 2001/02 production to 11.7 million tons from 11.9 million in 2000/01. Indonesia’s younger plantations helped its growth in palm oil production to exceed Malaysia’s, which rose from 7.9 million to 8.8 million tons. Global vegetable oil stocks for 1997–2002 are represented in Figure 1 (2).

Although Malaysian palm oil output in 2001/02 failed to keep up with the previous year’s level, relatively large beginning stocks sustained a stable export pace. Both Malaysian and Indonesian exports benefited from Argentina’s difficulties in exporting soybeans and soybean oil in 2002. Malaysian palm oil exports for 2001/02 steadied around 10.35 million tons while Indonesian exports expanded to 5.5 million tons. Like vegetable oil stocks in the United States and EU, palm oil stock in Malaysian and Indonesia gradually declined. Malaysian palm oil stocks were 1.1 million tons at the end of September 2002 compared to 1.5 million in early 2001. Tightening stocks buoyed the Malaysian palm olein price to \$388 per ton by September 2002. This was the highest price level since early 1999 and much higher than the September 2001 value of \$274 per ton (2).

### 1.4. Palm Oil Trade

Although many countries are involved in the production of palm oil, only a few are net exporters of the commodity. The net exporting countries are those where oil

palm can grow well to make it viable to produce the oil for export. Only Malaysia and Indonesia are the major net exporters of palm oil, while the other exporters have only a small share, each accounting for not more than 3% of the total export. Thus, for some years, the sources of palm oil have been confined mostly to the two major exporting countries as they account for more than 90% of the total exports of palm oil.

Crude palm oil used to be the main form of export in the past. With the establishment of refineries especially in Malaysia during the mid-1970s and 1980s, refined palm oil products have replaced the crude as the main form of palm oil export. A wide range of processed or semiprocessed products are exported, and these include the different fractions of processed palm oil known as palm olein (liquid) and palm stearin (solid). The availability of refineries also led to the production of specialty fats products aimed at the confectionery markets. A similar trend has been seen in the export of palm kernel oil. Palm kernel oil is a coproduct to palm oil produced at a ratio of 10–13 tons of palm kernel oil for every 100 tons of palm oil. Even the export of refined palm kernel oil has begun to decline as more is being used locally by the oleochemical industry that has been established in recent years.

Most major buyers of palm oil products use the NIOP or FOSFA contracts to secure their palm oil supplies. Other major buyers such as India, Pakistan, and China have their own trading specifications. Palm oil prices are quoted in the terminal markets such as Rotterdam, New York, and Kuala Lumpur. There is a futures market for palm oil in Kuala Lumpur, and this is actively used as the reference point for price determination. A network of brokers and dealers are involved in facilitating trade in palm oil products. In addition, some major multinational buyers have established their buying offices in the producing countries. Sellers also participate in responding to tenders called by a number of importing countries for the supply of palm oil. In this way, palm oil has been exported through many different channels and mechanisms to suit consumer needs.

Trade is facilitated by the existence of bulking installations at the major ports of loading for the export of the palm oil products. Codes of practice for the handling and shipment of palm oil have been formulated by the international trade associations to ensure the quality of the oil is protected. For example, the trading contracts such as FOSFA and NIOP stipulate that the previous cargoes of the ship carrying palm oil must not be any from the list of banned substances. Efforts are made to continuously upgrade the quality of palm oil products through improvements in standards, and these are discussed at international forum such as the Codex Alimentarius meetings. Many mills and refineries are also adopting the ISO 9000 to provide quality assurance for the products that they export.

The major importers of palm oil used to be the developed countries of the European Economic Community (EEC), the United States, and Japan. They accounted for about 75% of the imports of palm oil in the early 1970s. With the increasing exports of refined palm oil products, many developing countries, which did not have refining capacities, were able to import processed palm oil for direct consumption with minimal or no further refining. This helped to expand the market for palm

oil in the developing countries. By the end of the 1980s, the developing countries have become the major consumers of palm oil accounting for 75% of the import trade. While the import share of palm oil by the developed countries has declined to about 25%, the actual volume consumed by them has continued to expand. This reflects the competitiveness of palm oil in terms of price and technical suitability for all types of end uses both in the developed and developing countries.

Many countries are facing chronic shortages of oils and fats due to shortfall in the domestic production in the face of increasing population and income. For these countries, the relative availability of palm oil provides a convenient source of supply. Because of the rapidly expanding supply of palm oil, its price has been very competitive. For the last 30 years, palm oil has been selling at a discount compared to the other major oils in the world market, but prices of palm oil are highly correlated with those of the other oils. This suggests that the market acknowledges the high degree of substitutability of other oils and fats by palm oil.

India's vegetable oil consumption still rose steadily in 2001/02, but moderated from a robust 2000/01 growth rate of 11 percent. A larger domestic oilseed harvest and a paring of stocks dampened import requirements. Total vegetable oil imports by India (which surged by nearly one-fourth in 2000/01) declined to 5.2 million tons in 2001/02 from 6.0 million. Imports of palm oil and soybean oil dipped to 3.4 million and 1.65 million tons, respectively. Negligible quantities of rapeseed oil and sunflowerseed oil were imported, as they became less price-competitive because of an import duty structure that favored soybean oil and crude palm oil. India has not materially changed vegetable oil tariffs since October 2001, when it cut the rate on crude palm oil from 75 percent to 65 percent.

Palm oil exporters had hoped that China would replace lagging Indian sales by raising its import quota. China officially entered the WTO on December 11, 2001. China's accession agreement stipulated that its 2002 tariff-rate quota (TRQ) on soybean oil increase to 2.518 million tons and the within-quota tariff fall from 13 percent to 9 percent. Tariffs on soybeans and soybean meal were bound at their previous rates. But ample domestic production of soybean and rapeseed oils continued to limit China's need for vegetable oil imports.

China had originally set issuance of its vegetable oil import licenses by March 5 but had only begun distributing them in early April. Two-thirds of the annual 2.4-million ton quota was to be allocated to private importers. Nevertheless, palm oil imports by China surged in March. Before the quota, China had already imported about 300,000 tons this year, some of which were waiting at ports in bonded warehouses for the licenses to be distributed. About half of the palm oil imports were allowed to clear customs before April because importers could deposit a 52-percent over-quota tariff for them. When the importers received their quotas, the differences against the 9-percent-within-quota tariff was refunded.

China did not require foreign exporters to obtain separate safety certificates for each cargo of soybean oil produced from biotech varieties. However, soybean oil imports were temporarily handicapped by a requirement that safety certificates be approved for biotech soybeans before the same applications for soybean oil can be accepted.



A tightening of China's soybean and rapeseed supplies by mid-2002 created opportunities for vegetable oil imports. Palm oil was the most favorably priced and imports were unfettered by the country's requirements for safety certificates, inspections, and labeling of biotech oilseeds. Therefore, China's importers tried to fill their increased 2002 palm oil TRQ (2.4 million tons) first. Palm oil imports by China rose to 2.0 million tons from 1.6 million in 2000/01. For soybean oil, rising world prices narrowed the differential to China's domestic prices, which limited its import needs. China's soybean oil imports were 375,000 tons in 2001/02, still well below the TRQ but substantially above the 80,000 tons imported the previous year (2).

The versatility of palm oil in terms of its presentation of various subproducts and the wide range of technical properties increase the competitiveness of palm oil to the consumers. Palm oil has become the major oil among the imported oils in most countries. Even countries that are net exporters of oils and fats, such as the United States, are importing palm oil in substantial quantities. For these countries palm oil can provide certain technical advantages in some end uses, and palm oil usage gives better margins of profit compared to the use of locally available oils and fats.

## 2. CHEMICAL AND PHYSICAL PROPERTIES OF PALM OIL

Palm oil, like all oils and fats, is made up mostly of glyceridic materials with some nonglyceridic materials in small or trace quantities. It is this chemical composition that defines the chemical and physical characteristics of palm oil, which in turn will determine the suitability of the oil in various processes and applications.

### 2.1. Chemical Properties of Palm Oil

*Triglyceride and Fatty Acid Composition* Triglycerides form the major component and bulk of the glyceridic material present in palm oil with small amounts of monoglycerides and diglycerides, which are artifacts of the extraction process. The fatty acid chains present in the palm oil triglycerides could vary in the number of carbons present in the chain (chain length) and in structure (presence of double bonds, i.e., unsaturation). It is the variations in the structure and number of carbons in these fatty acid chains that largely define the chemical and physical properties of palm oil.

The chain lengths of the fatty acids present in the triglycerides of palm oil fall within a very narrow range from 12 to 20 carbons as shown in Table 3.

It can be seen that about 50% of the fatty acids present in palm oil are saturated and about 50% are unsaturated. This even balance between saturation and unsaturation determines the iodine value of the oil (about 53) and confers some stability against oxidation to the oil as compared to other vegetable oils. The three fatty acids in the triglycerides could be represented by the multitude of fatty acids listed in Table 3. The different placement of the fatty acids attached to the glycerol molecule can lead to a large number of different triglycerides. Subjecting lipolysis data to statistical computer analysis, more details about the triglyceride composition of

**TABLE 3. Fatty Acid Composition of Malaysian Palm Oil (3).**

Fatty Acids Chain Lengths	% of Total	
	Mean	Range
12:0	0.23	0.1–1.0
14:0	1.09	0.9–1.5
16:0	44.02	41.8–46.8
16:1	0.12	0.1–0.3
18:0	4.54	4.2–5.1
18:1	39.15	37.3–40.8
18:2	10.12	9.1–11.0
18:3	0.37	0–0.6
20:0	0.38	0.2–0.7

palm oil could be obtained as shown in Table 4. These are in terms of the actual acid chains present in the three carbon positions of the triglyceride molecule.

From the computation data, it can be seen that the triglyceride molecules could be divided according to the number of saturated (S) and unsaturated (U) groups that they contain. The computational results were found to be very close to the analytical data obtained by Tan (6) shown in Table 5.

Carbon number analysis by high-temperature gas-liquid chromatography provides a rapid but less detailed analysis of the triglyceride composition in terms of the total number of carbon atoms present in the three fatty acid chains of the triglyceride molecule. Carbon number analysis data of palm oil are shown in Table 6.

Knowledge about the detailed structures of the triglycerides present in palm oil is important because they define some of the physical characteristics of the oil. The melting points of triglycerides are dependent on the structures and position of the component acids present. They also affect the crystallization behavior of the oil. The semisolid nature of palm oil at room temperature has been attributed to the presence of the oleo-disaturated fraction.

As mentioned previously, partial glycerides are artifacts of the extraction process, especially the stages prior to sterilization. Oil obtained from unbruised sterilized fruits shows trace levels of partial glycerides. Random analyses of samples of refined palm oil, palm olein, and palm stearin have shown the presence of about 2% of 1,2-diglycerides and about 4% of 1,3-diglycerides with trace amounts of mono-glycerides. These partial glycerides are important as they are known to affect the crystallization behavior of the oil.

*Minor Components.* The carotenoids, tocopherols, sterols, phosphatides, triterpenic, and aliphatic alcohols form the minor constituents of palm oil. Though present in less than 1% altogether in palm oil, nevertheless they play a significant role in the stability and refinability of the oil, in addition to increasing the nutritive value of the oil.

Crude palm oil contains between 500 and 700 ppm of carotenoids mainly in the forms of  $\alpha$ - and  $\beta$ -carotenes, the precursor of vitamin A. Unless extracted prior to

**TABLE 4. Triglyceride Composition of Malaysian Tenera Palm Oil.<sup>a</sup>**

	No Double Bond		1 Double Bond		2 Double Bond		3 Double Bond		4 or More Double Bonds					
	A	B	A	B	A	B	A	B	A	B				
MPP	0.29	0.5	MOP	0.83	1.4	MLP	0.26	MLO	0.14	0.2	PLL	1.08	0.8	
PMP	0.22	0.2	MPO	0.15	0.2	MOO	0.43	0.7	PLO	6.59	6.0	OLO	1.71	1.4
			POP	20.02	23.7	PLP	6.36	6.3	POL	3.39	3.1	OOL	1.76	1.5
PPP	6.91	7.2	POS	3.50	3.1	PLS	1.11	0.8	SLO	0.60	0.4	OLL	0.56	
PPS	1.21	1.0	PMO	0.22		PPL	1.17	1.0	SOL	0.30	0.2	LOL	0.14	0.1
PSS	0.12	0.1	PPO	7.16	6.9	OSL	0.11		OOO	5.38	5.1			
PSP		0.7	PSO	0.68	0.6	SPL	0.10	0.1	OPL	0.61	0.5			
			SOS	0.15		POO	20.54	21.5	MOL		0.1			
			SPO	0.63	0.5	SOO	1.81	1.4						
						OPO	1.86	1.6						
						OSO	0.18	0.2						
						PSL		0.1						
Others	0.16			0.34	0.3		0.19	0.6		0.15			0.22	
Total	9.57	9.7		33.68	35.8		34.12	34.6		17.16	15.6		5.47	3.8

<sup>a</sup>A: based on Kan-Ichi Hayakawa Computation: see Ref. (4); B: based on Vander Wal's method: see Ref. (5).

**TABLE 5. Triglyceride Analysis of Tenera Palm Oil (6) (based on saturation–unsaturation criterion).**

Triglyceride Type	Composition (%)
Trisaturated (GS <sub>3</sub> )	10.2
Disaturated (GS <sub>2</sub> U)	48.0
Monosaturated (GSU <sub>2</sub> )	34.6
Triunsaturated (GU <sub>3</sub> )	6.8

**TABLE 6. Carbon Number Analysis of Malaysian Palm Oil (3).**

Carbon Number	Mean	Range
C46	0.8	0.4–1.2
C48	7.4	4.7–10.8
C50	42.6	40.0–45.2 (POP, PPO)
C52	40.5	38.2–43.8 (POO)
C54	8.8	6.4–11.4

refining, these carotenoids are thermally destroyed during the deodorization stage in order to produce the desired color for a refined oil. In crude palm oil, the presence of these carotenoids appears to offer some oxidative protection to the oil through a mechanism where they are oxidized prior to the triglycerides. Table 7 lists the carotenoid types that are present in crude palm oil.

Crude palm oil contains tocopherols and tocotrienols in the range of 600–1000 ppm. Refined palm oil retains about 50% of these products. Tocopherols and tocotrienols are antioxidants and provide some natural oxidative protection to the oil. Table 8 shows the types of tocopherols and tocotrienols present in palm oil.

**TABLE 7. Carotenoid Composition of Palm Oil (7).**

Carotenoid	Percentage
Phytoene	1.27
<i>cis</i> -β-Carotene	0.68
Phytofluene	0.06
β-Carotene	56.02
α-Carotene	35.16
<i>cis</i> -α-Carotene	2.49
ξ-Carotene	0.69
γ-Carotene	0.33
δ-Carotene	0.83
Neurosporene	0.29
β-Zeacarotene	0.74
α-Zeacarotene	0.23
Lycopene	1.30
Total carotene (ppm)	673

**TABLE 8. Tocopherols and Tocotrienols in Crude Palm Oil (8).**

Type	Percentage
$\alpha$ -Tocopherols	21.5
$\beta$ -Tocopherols	3.7
$\gamma$ -Tocopherols	3.2
$\delta$ -Tocopherols	1.6
$\alpha$ -Tocotrienols	7.3
$\beta$ -Tocotrienols	7.3
$\gamma$ -Tocotrienols	43.7
$\delta$ -Tocotrienols	11.7

From Table 8, it can be seen that  $\alpha$ -tocopherols and  $\gamma$ -tocotrienols account for the major portion of the total tocopherols and tocotrienols present in palm oil. Gapor (9) confirms the presence of the above-listed tocopherols and tocotrienols by high-performance liquid chromatography (HPLC) and also indicated the probable presence of the esterified forms.

The combined effects of the properties of the carotenoids, tocopherols, tocotrienols and the 50% unsaturation of the acids confer on palm oil a higher oxidative stability as compared to a lot of other vegetable oils.

In terms of sterols, palm oil contains far less cholesterol than many other vegetable oils as shown in Table 9. Table 10 gives the sterol composition of crude and

**TABLE 9. Cholesterol Levels in Crude Oils and Fats (10).**

Oil Type	Average (ppm)	Range (ppm)
Coconut oil	14	5–24
Cocoa butter	59	n.a.
Palm kernel oil	17	9–40
Palm oil	18	13–19
Sunflower oil	17	8–44
Soybean oil	28	20–35
Rapeseed oil	49	25–80
Maize oil	50	18–95

**TABLE 10. Sterol Composition of Crude and Refined Palm Oil and Their Products (ppm) (11).**

Sample	Cholesterol	Campesterol	Stigmasterol	Sitosterol	Unknown
Crude palm oil	7–13	90–151	44–66	218–370	2–18
Degummed, bleached RBD	5–10	49–116	22–51	113–286	Trace–8
Crude palm olein	1–5	15–16	8–30	45–167	Trace
Degummed, bleached RBD	6–8	57–104	30–51	149–253	24–28
Crude palm oil	3–4	36–43	21–25	99–123	Trace–5
Degummed, bleached RBD	2	26–30	12–23	68–114	—

**TABLE 11. Inherent Chemical Properties of Malaysian Palm Oil (12).**

Chemical Characteristics	Mean	Range
Saponification value (mg KOH/g oil)	195.7	190.1–201.7
Unsaponifiable matter (%)	0.51	0.15–0.99
Iodine value (Wijs)	52.9	50.6–55.1
Slip melting point (°C)	34.2	30.8–37.6

refined palm oil and their products. From Table 10, it can be seen that the low-cholesterol levels in crude palm oil and crude palm olein are further reduced to even lower levels upon refining. This low-cholesterol level, together with the antithrombotic and anticarcinogenic properties of some of the carotenoids, tocopherols, and tocotrienols present add further to the nutritive value of palm oil and palm oil fractions.

*Inherent Chemical Properties of Palm Oil.* Table 11 summarizes some of the inherent chemical properties of Malaysian palm oil.

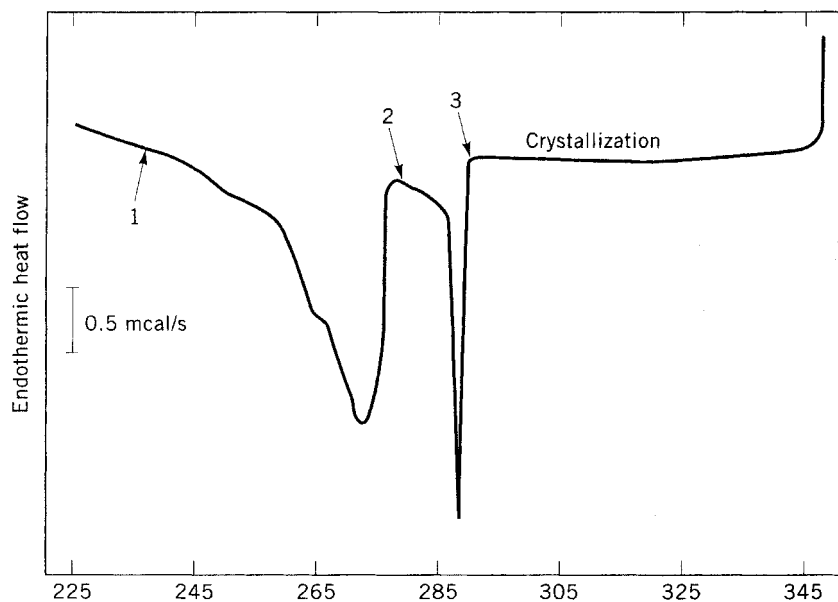
## 2.2. Physical Properties of Palm Oil

Table 12 shows some of the physical properties of palm oil. The apparent density is an important parameter from the commercial point of view since it is used for volume to weight conversions. It can also be used as a purity indicator.

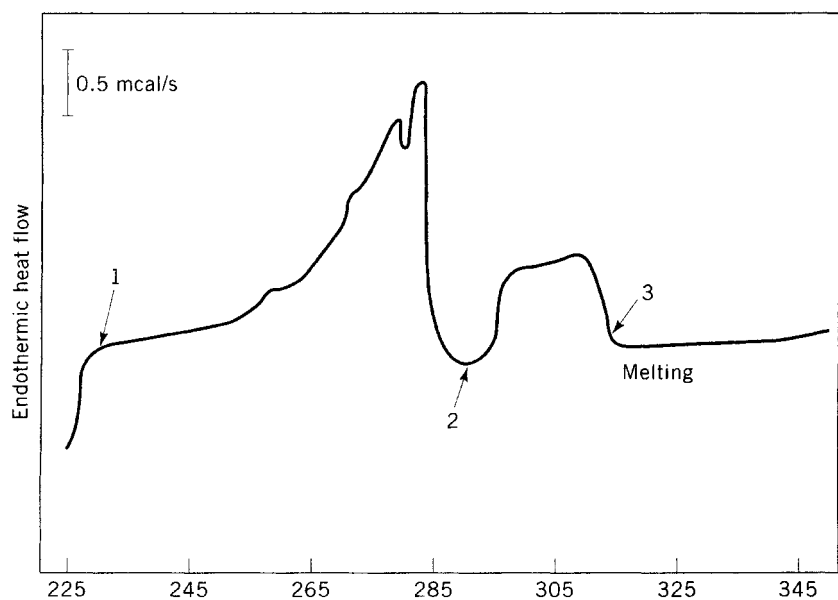
The solid fat content of an oil is a measure (in percent) of the amount of solid fat present in the oil at any one temperature. It is measured by means of wide-line nuclear magnetic resonance (NMR) spectrometry after a standard tempering procedure for the samples.

**TABLE 12. Major Physical Properties of Palm Oil (13,14).**

Property	Mean (of 215 Samples)	Range
Apparent density at 50°C (g/mL)	0.889	0.888–0.889
Refractive index at 50°C	1.455	1.455–1.456
Solid fat content		
5°C	60.5	50.7–68.0
10°C	49.6	40.0–55.2
15°C	34.7	27.2–39.7
20°C	22.5	14.7–27.9
25°C	13.5	6.5–18.5
30°C	9.2	4.5–14.1
35°C	6.6	1.8–11.7
40°C	4.0	0.0–7.5
45°C	0.7	
Slip melting point (°C) (+)	34.2	31.1–37.6



**Figure 2.** Crystallization thermogram of Malaysian palm oil (15).



**Figure 3.** Melting thermogram of Malaysian palm oil (15).

The solid present in the oil at any one temperature is due to the process of crystallization occurring in the oil as a consequence of its chemical properties. The different molecular triglyceride structures with their differing chemical characteristics manifest their physical states at different temperatures, thus imparting certain crystallization and melting behavior to the oil. These thermally associated processes can be followed by means of differential scanning calorimetry (DSC). Figures 2 and 3 show the crystallization and melting thermograms of palm oil, respectively. In the crystallization thermograms, points 1 to 2 define the olein crystallization peak while points 2 to 3 define the stearin crystallization peak. These are defined by points 1 to 2 and 2 to 3, respectively, in the melting thermogram.

From the thermal characteristics considered above, it can be seen that palm oil can be separated under controlled thermal conditions into two components, i.e., a solid (stearin) and a liquid (olein) fraction. This fractionation process can be affected either in the dry form in the presence of a detergent or solvent. The method employed, to a certain extent, determines some of the chemical and physical properties of the oleins and stearins produced, especially the stearins. By varying the fractionation methods and conditions used, a range of stearins with differing chemical and physical properties could be produced, yet keeping the chemical and physical properties of the oleins to within a very narrow range of values as shown in Tables 13, 14, and 15.

**TABLE 13. Fatty Acid Compositions (%) (16).**

Fatty Acids	Oleins		Stearin	
	Range Observed	Mean	Range Observed	
12:0	0.1–0.5	0.2	0.1–0.6	
14:0	0.9–1.4	1.0	1.1–1.9	
16:0	37.9–41.7	39.8	47.2–73.8	
16:1	0.1–0.4	0.2	0.05–0.2	
18:0	4.0–4.8	4.4	4.4–5.6	
18:1	40.7–43.9	42.5	15.6–37.0	
18:2	10.4–13.4	11.2	3.2–9.8	
18:3	0.1–0.6	0.4	0.1–0.6	
20:0	0.2–0.5	0.4	0.1–0.6	
Iodine value (Wijs)	56.1–60.6	58.0	21.6–49.4	

**TABLE 14. Triglyceride Composition by Carbon Number (16).**

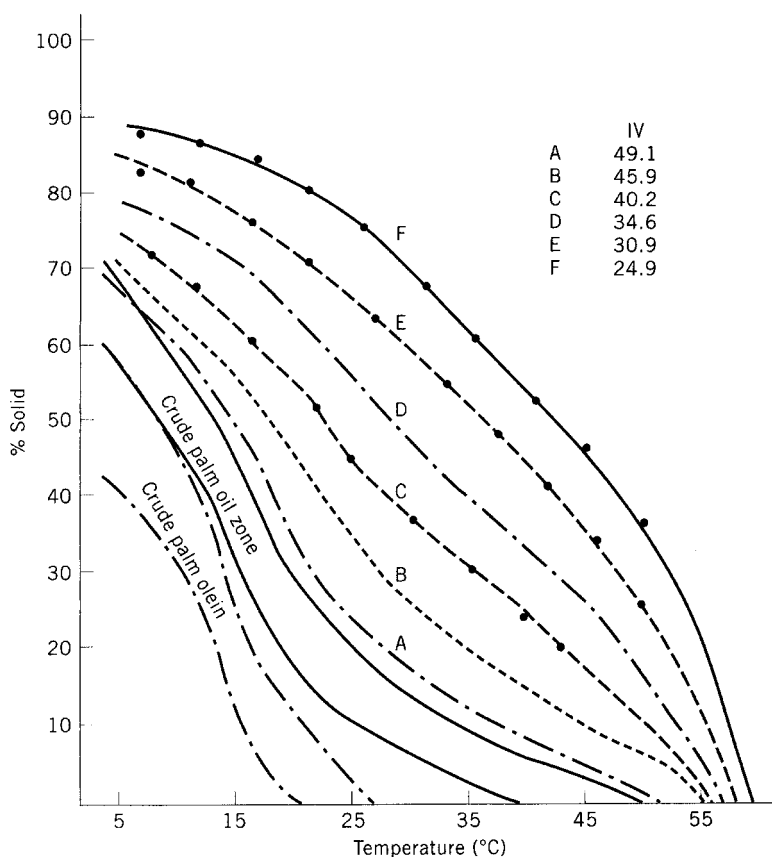
Carbon Number	Oleins		Stearin	
	Range	Mean	Range	
C46	—	—	0.5–3.3	
C48	1.3–4.0	2.3	12.2–55.8	
C50	37.7–45.4	42.0	33.6–49.8	
C52	43.3–51.3	45.7	5.1–37.3	
C54	7.0–12.6	9.9	Trace–8.4	



**TABLE 15. Melting and Solidification Characteristics (16).**

Tests	Oleins		Stearin
	Range	Mean	Range
Slip melting point (°C)	19.4–23.5	21.6	44.5–56.2
Cloud point (°C. crude)	6.6–14.3	10.4	—
Neutralized	5.4–11.9	8.1	—
Refined	6.0–11.5	8.8	—

Under normal fractionation conditions, soft stearins and oleins with cloud points in the range of 8–10°C are produced. Where required, fractionation conditions could be specifically altered to produce stearin or olein of a desired specification for specialized application, but within the domain of the composition of palm oil. For example, stearins of differing iodine values (IV) ranging from the hard (IV of about 20) to soft (IV of about 50) could be produced, each with their characteristic solid fat content curve as shown in Figure 4.

**Figure 4. Solid fat content of crude stearin (15).**

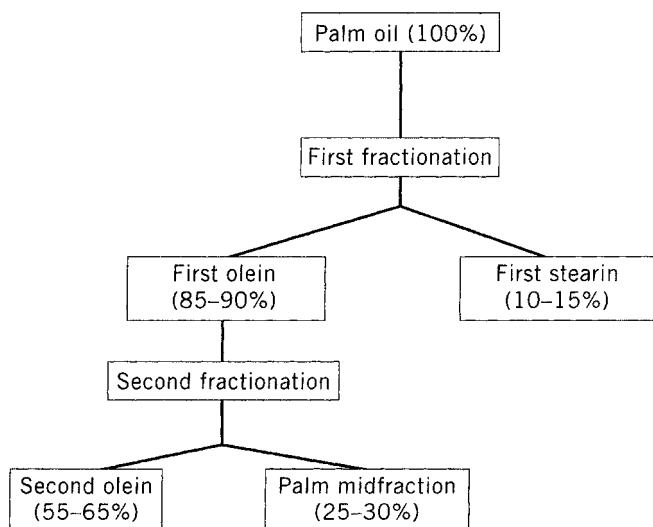


Figure 5. Fractionation and palm midfraction.

TABLE 16. Palm Midfractions Solids Content by NMR.

Code	Temperature (°C)	Palm Oil	1	2	3	4	6	7	8	5A	5B	5C
	10	50.3	81.3	58.4	60.1	61.5	65.6	66.4	53.8	71.9	51.8	71.1
	15	35.2	71.1	37.1	48.5	45.2	48.3	55.2	41.6	63.2	32.9	61.8
	20	23.2	59.5	18.5	34.3	26.8	30.5	42.0	27.1	43.3	17.5	45.0
	25	13.7	29.7	1.7	22.8	8.7	8.2	28.7	15.8	22.1	9.3	28.0
	30	8.5	8.6		14.2	2.4	2.9	19.6	9.6	11.4	4.6	16.8
	35	5.8	3.6		10.4	1.2		15.1	5.6	6.6		12.0
	40	3.5			7.1			10.9	2.6	2.5		7.3
	45				3.4			5.7				3.0
	50											

For more specialized usage such as in the confectionery industry, a more specific type of stearin is required or desired. This is catered to by using a double fractionation process as shown in the scheme in Figure 5. Table 16 shows the solid fat content profiles of the more common palm midfractions produced in Malaysia at present.

Other characteristics of palm oleins and palm stearin are shown in Table 17. The different types of palm oil products available for export are illustrated in Table 18.

### 2.3. Test Methods for Palm Oil and Products Analysis

In the palm oil trade, test methods for palm oil product analysis were traditionally based on the American Oil Chemists Society (AOCS) test methods. Where test methods are not available under the AOCS, other test methods were used, drawn

**TABLE 17. Additional Analytical Characteristics (16).**

Tests	Oleins	Stearins
Apparént density at 40°C (g/mL) 60°C (g/mL)	0.8965–0.8992 ( $n=21$ )	0.8659–0.8756 ( $n=40$ )
Refractive index $n_D$ 40°C $n_D$ 60°C	1.4586–1.4592 ( $n=21$ )	1.4472–1.4511 ( $n=41$ )
Saponification value mg KOH/g oil	194–202 ( $n=21$ )	193–206 ( $n=41$ )

**TABLE 18. PORAM Standard Specification for Processed Palm Oil.**

Type of Palm Oil	Specification <sup>a</sup>	Values
1. Neutralized palm oil	*FFA (as palmitic)	0.25% Max.
	Moisture and Impurities	0.1% Max.
	IV (Wijs)	
	+Melting point (°C)	50–55
2. Neutralized and bleached palm oil	(AOCS Cc 3–25)	33–39
	*FFA (as palmitic)	0.25% Max.
	Moisture and Impurities	0.1% Max.
	IV (Wijs)+Melting point (°C)	50–55
3. Refined, bleached, and deodorized (RBD)/neutralized, bleached, and deodorized (NBD) palm oil	(AOCS Cc 3–25)	33–39
	Color (5¼-inch Lovibond cell)	20 Red max.
	*FFA (as palmitic)	0.1% Max.
	Moisture and Impurities	0.1% Max.
4. Crude palm olein	IV (Wijs)+Melting point (°C)	50–55
	(AOCS Cc 3–25)	33–39
	Color (5¼-inch Lovibond cell)	3 or 6 Red max.
	*FFA (as palmitic)	5.0% Max.
5. Neutralized palm olein	Moisture and Impurities	0.25% Max.
	IV (Wijs)+Melting point (°C)	56 Min.
	(AOCS Cc 3–25)	24 Max.
	*FFA (as palmitic)	0.25% Max.
6. Neutralized and bleached palm olein	Moisture and Impurities	0.1% Max.
	IV (Wijs)+Melting point (°C)	56 Min.
	(AOCS Cc 3–25)	24 Max.
	Color (5¼-inch Lovibond cell)	20 Red max.
7. Refined, bleached, and deodorized (RBD)/neutralized, bleached, and deodorized (NBD) palm olein	Color (5¼-inch Lovibond cell)	20 Red max.
	*FFA (as palmitic)	0.1% Max.
	Moisture and Impurities	0.1% Max.
	IV (Wijs)+Melting point (°C)	56 Min.
	(AOCS Cc 3–25)	24 Max.
	Color (5¼-inch Lovibond cell)	3 or 6 Red max.

TABLE 18. (Continued)

Type of Palm Oil	Specification <sup>a</sup>	Values
8. Double fractionated palm olein	*FFA (as palmitic)	0.1% Max.
	Moisture and Impurities	0.1% Max.
	IV (Wijs)+Melting point (°C)	60 Min.
	(AOCS Cc 3-25)	19 Max.
	Color (5 $\frac{1}{4}$ -inch Lovibond cell)	3 Red max.
9. Crude palm stearin	*FFA (as palmitic)	5.0% Max.
	Moisture and Impurities	0.25% Max.
	IV (Wijs)+Melting point (°C)	48 Max.
	(AOCS Cc 3-25)	44 Min.
10. Neutralized palm stearin	*FFA (as palmitic)	0.25% Max.
	Moisture and Impurities	0.15% Max.
	IV (Wijs)+Melting point (°C)	48 Max.
	(AOCS Cc 3-25)	44 Min.
11. Neutralized and bleached palm stearin	*FFA (as palmitic)	0.25% Max.
	Moisture and Impurities	0.15% Max.
	IV (Wijs)+Melting point (°C)	48 Max.
	(AOCS Cc 3-25)	44 Min.
	Color (5 $\frac{1}{4}$ -inch Lovibond cell)	20 Red max.
12. Refined, bleached, and deodorized	(RBD)/neutralized, bleached, and deodorized (NBD) palm stearin	0.2% Max.
	*FFA (as palmitic)	
	Moisture and Impurities	0.15% Max.
	IV (Wijs)+Melting point (°C)	48 Max.
	(AOCS Cc 3-25)	44 Min.
	Color (5 $\frac{1}{4}$ -inch Lovibond cell)	3 or 6 Red max.
13. Palm acid oil	*Total fatty matter	95% Min. (basis 97%)
	Moisture and Impurities	3% Max.
14. Palm fatty acid distillate	*FFA (as palmitic)	50% Min.
	Saponifiable matter	95% Min. (basis 97%)
	Moisture and Impurities	1.0% Max.
	*FFA (as palmitic)	70% Min.

<sup>a</sup>+, Slip point, softening point, or rising point; \*, molecular weight of palmitic acid is taken as 256.

Source: PORAM Technical Brochure, 1989.

from the British Standards Institute (BSI), International Union of Pure and Applied Chemistry (IUPAC) methods, or the International Standards Organization (ISO). However, the recent trend has been toward the adoption and standardization of ISO methods. In early 1994, FOSFA have called for a harmonization of test methods between the various test method organizations such as the American Official & Analytical Chemistry (AOAC), AOCS, IUPAC, BSI, and other national standards organizations. The aim of this exercise is to standardize the test methods for oils and fats from all these organizations for trade purposes, to be placed under the ISO.

### 3. PRODUCTION PROCESS

#### 3.1. Origin of the Oil Palm

The oil palm, *Elaeis guineensis* Jacq., is grown commercially in Africa, South America, Southeast Asia, and the South Pacific, and on a small scale in other tropical areas. Until recent centuries the palm has been confined to West and Central Africa where it existed in a wild, semiwild, and cultivated state. In Africa it remained a domestic plant, supplying a need for oil and vitamin A in the diet, and it was not until the end of the eighteenth and the beginning of the nineteenth centuries that oil palm cultivation expanded to the Southeast Asian regions and strengthened the entry of palm oil into the world oils and fats trade.

#### 3.2. Oil Palm Plantations

The early development of the oil palm industry is well described by Hartley (17). The export of palm oil and kernels from Africa began in the nineteenth century. At this stage the only source of supply was the palm groves, the oil being extracted by primitive and inefficient methods. The palm groves were sometimes developed into "peasant plantations" (18) by deliberate planting of seedlings, but the first large plantations were established in Sumatra and Malaysia in the early years of this century. These were followed in the 1920s by plantations in the Belgian Congo (now Zaire) and then in other parts of West Africa. In recent years very considerable further expansion of the industry has occurred, and oil palm products are now an important component of world vegetable oil supplies.

Although the cultivation of the oil palm in plantations started in the Far East, strangely there was no direct connection between the African groves and the establishment of this new industry. The earliest record of the introduction of oil palms to the East Indies is of four seedlings, two from Bourbon (Reunion) or Mauritius and two from Amsterdam, which were planted in the Botanic Gardens at Buitenzorg, now Bogor, in Java in 1848. The foundation of the industry is generally attributed to M. Adrien Hallet, a Belgian with some knowledge of oil palms in Africa, who planted palms of Deli origin in 1911 in the first large commercial plantations in Sumatra. Hallet recognized that the avenue palms growing in Deli were not only more productive than palms in Africa but had a fruit composition superior to the ordinary *dura* palms of the African west coast. Open pollinated selected tenera seed was used on commercial plantations as early as 1924 (19). In the meantime, however, M.H. Fauconnier, who had been associated with M. Hallet, had established during 1911 and 1912 some palms of Deli origin at Rantau Panjang in the Kuala Selangor district of Malaysia. These palms were in full bearing by 1917, and in that year the first seedlings were planted on an area later to be known as Tennamaran Estate.

The industry grew rapidly in Sumatra, but did not gain its full momentum in the Far East until the 1930s. In 1925 there were 31,600 hectares planted in Sumatra and only 3,348 in Malaysia (20) but by 1938 the areas had risen to 92,300 and 29,196

TABLE 19. Planted Oil Palm Area (1000 ha).

	1960	1970	1980	1990	1993
Malaysia	54.6	291.3	1,023.3	2,029.5	2,281.0
Indonesia	n.a.	134.0	294.1	1,126.7	1,603.7
Papua New Guinea <sup>a</sup>	n.a.	n.a.	12.0	37	55
Ivory Coast	5.2	68.2	100.3	128 <sup>a</sup>	158 <sup>a</sup>
Nigeria <sup>a</sup>	n.a.	n.a.	230.0	270	298
Columbia	0.4	19.8	36.7	81 <sup>a</sup>	111 <sup>a</sup>

<sup>a</sup>Denotes mature area only.

Source: *Oil World Weekly*, April 4, 1985. *Oil World Annual*, 1993. H. A. J. Moll, *Economics of Oil Palm* (Ref. 2), Pusat Penelitian Marihat, Indonesia.

hectares, respectively. With over 120,000 hectares, an industry of considerable importance capable of producing more oil than was being exported from Africa had in the span of about 20 years been established. The rate of planting in Malaysia was rapid in the 1960s and 1970s. In 1960 there were only 55,000 hectares, but by 1975 half a million hectares had been added and a total of over 2 million hectares was reached by 1990 (21). A major part of the planting was done by federal and state land development authorities, government-sponsored settlement schemes, which by the early 1980s accounted for half the total planted area in Malaysia.

In Indonesia, oil palm cultivation has expanded rapidly especially during the 1980s, where government estate enterprises, foreign private estates, private national estates, and nucleus estates have been established. The expansion in planted area for oil palm for this historical period is shown in Table 19.

### 3.3. Yield of Palm Oil

The oil palm is a highly efficient producer of vegetable oil. On per unit area basis the oil palm is considerably higher yielding than any other vegetable oil crops. Record yields for other crops such as soybean are about 2 tons of oil per hectare, 3 tons for rapeseed and olive, and 4 for coconut and sunflower. In contrast, thousands of hectares of oil palm plantations in Southeast Asia regularly yield 5 tons of oil per hectare per year, and record yields are appreciably higher. The figures shown in Table 20 represent some of the highest oil yields recorded in experiments; higher yields may well have been obtained by other workers. Individual palms with over 30% oil to bunch ratio exist, with a fruit yield of 40 t/ha, this would give an oil yield of 12 t/ha.

*Oil Yield Components.* Palm oil and palm kernel oil are obtained from the oil palm fruit. Yield of oil can be considered in terms of various components; the two main components are yield of fruit bunches and oil—bunch weight ratio (or extraction ratio). Fruit yield can be considered in terms of the component bunch number and mean bunch weight. Bunch weight increases with palm age while bunch number decreases. The first yield of fruit bunches normally ripen during

TABLE 20. Yield Records Obtained from Some Trials in Malaysia.

Type of Trial	Yield of		Yield of Oil (t/ha)
	Fruit Bunches (t/ha)	Oil/Bunch (%)	
Progeny trial (best progeny, 1 year)	30.4	27.7	8.42
Spacing trial (best treatment, 1 year)	28.5	23.7	6.76
Spacing trial (best plot, 1 year)	30.9	23.7	7.32
Fertilizer trial (best plot, 1 year)	37.2	16.9 (dura)	6.28
Estate planting (best plot, 3rd year of production)	32.6	26.2	8.55

the third year after field planting. Yield rises to a maximum in the first few years and thereafter usually tends to decline slowly.

*Oil—Bunch Ratio.* The yield of oil depends on yield of fruit bunches and a further component, oil—bunch ratio. The oil—bunch ratio is the product of a number of components: these are fruit—bunch ratio, mesocarp—fruit ratio, and oil—mesocarp ratio. The oil content of the fruit of young palms is low: Hartley (17) stated that it increases steadily until the fourth or fifth year of bearing. However, Corley (22) reported that with tenera palms, an oil—bunch ratio of over 28% may be reached as early as 40 months after field planting.

Mesocarp—fruit ratio is largely genetically determined and is little affected by environmental factors. Fruit—bunch ratio depends mainly on the efficiency of pollination. Oil—mesocarp ratio depends in part on the ripeness of the fruit, since oil is only synthesized during the later stages of fruit development. There is also considerable variation in oil—mesocarp of bunches from the same progeny harvested at different times of the year (23). It has also been shown that application of potassium fertilizer leads to a reduction in the oil—bunch ratio (24); but the increase in fruit yield in response to potassium was more than enough to compensate for the reduced oil—bunch ratio.

*Yield Variation.* In seasonal climates the annual yield of oil palm usually has only one peak, the time of the peak depending on the age and leaf production of the palms and, in the mature plantation palm, on climatic conditions about 28 months before fruit ripening. In nonseasonal climates, there are occasionally two peaks of production in the year, though one tends to be much more prominent; there is considerable variation in the magnitude of the peaks.

### 3.4. Production Costs

Production cost of crude palm oil is made up of cost of production of fresh fruit bunches (FFB) and the cost to mill the FFB. Refining costs are incurred when crude palm oil undergoes refining to produce processed palm products.

*Cost of FFB Production.* To produce FFB, the costs covered involve felling and land preparation, lining, terracing, drainage, planting of palms and leguminous covers, preparing of roads, pathways, and bridges, application of fertilizers, and pest and disease control. The cost of establishing oil palm also depends on whether

the area is undergoing replanting or new planting. Replanting refers to planting on land that was formerly developed with other crops while new planting refers to establishing an area formerly under jungle. In Malaysia, the cost of establishing a hectare of oil palm area for the first three years is about MR 5013.26, or \$1856.75 (all dollar amounts are in U.S. dollars unless specified) for areas under replanting. For new plantings, the cost of establishment would be 20–30% higher because new plantings would require more intensive land preparations such as new terraces, new drainage systems, and new roads and pathways.

After three years, the palms are already mature and fresh fruit bunches could be harvested monthly for the next 25 years or more. The direct items involved in the production of FFB during the mature period include costs of fertilizers and their applications, harvesting, control of pests, weeds, and diseases, maintenance of infrastructures such as roads, harvesting paths, and bridges, soil and foliar analysis, and agricultural equipment. On the whole, the direct cost of FFB production ranges from \$238 to \$520 per hectare per year while the cost of producing a ton of FFB ranges from \$14.80 to \$44.40 (Table 21). The range in cost is very much dependent upon FFB yield, type of soil, drainage, FFB quality, and other factors. Variations in costs are also due to the management systems of the oil palm, viz. plantations, unorganized small holdings, or organized small holdings.

The indirect costs to produce FFB or to maintain mature oil palm hectares are categorized into two areas, personal emoluments and services and supplies. Personal emoluments include staff salaries, staff costs and benefits, wages, workers' costs and benefits, and other costs and benefits. Services and supplies covers traveling, office expenses, maintenance, professional expenses, utilities, and sundries. The

**TABLE 21. FFB Production for Estate Sector, Group Smallholders, Independent Smallholders (U.S. \$/hectare).**

Sector Costs	Estate	Group Smallholders	Independent Smallholders	
			U.S. \$/ha	Ave.
<i>Direct costs</i>				
Manuring	51.9 (22.5)	100.0 (32.1)	175.7	109.2
Weeding	26.0 (10.8)	1.9 (0.6)	3.3	10.4
Supply of damaged palms	26.4 (11.0)	15.6 (5.0)	27.4	23.1
Pest/disease	7.4 (3.1)	9.0 (2.9)	15.9	10.8
Pruning	10.6 (4.4)	14.0 (4.5)		12.3
Harvesting and collection	96.9 (40.3)	69.5 (22.3)	228.1	131.5
Transport of FFB	17.6 (7.3)	95.7 (30.7)	62.9	58.7
Miscellaneous	1.4 (0.6)	5.9 (1.9)	7.4	4.9
Total	238.2 (100.0)	311.6 (100.0)	520.7	356.8
<i>Indirect costs</i>				
Salaries and wages	138.1 (59)	117.4 (46)		85.17
Supply	95.9 (41)	135.6 (54)	11.1	80.9
Total	234.0 (100)	253.0 (100)	11.1	166
Total direct and indirect	472.2	564.6	531.8	522.9



**TABLE 22. Milling Costs.**

	Cost/Ton FFB		
	RM	U.S.	Percent
Mill management	1.51	0.56	5.04
Mill process staff	1.46	0.54	4.85
Mill process labor	3.11	1.15	10.37
Machinery upkeep	5.25	1.94	17.50
Building upkeep	0.11	0.04	0.36
Utilities	2.68	0.99	8.93
Kernel bagging	0.58	0.21	1.93
Head office costs	3.21	1.19	10.71
Depreciation	10.71	3.97	35.71
Insurance	0.38	0.14	1.25
Palm oil cess	1.00	0.37	3.33
Total	30.00	11.1	100

indirect cost per year per mature hectare of oil palm in Malaysia ranges between \$234.00 and \$253.00. From studies of estates, schemes, and small holdings in Malaysia, the indirect cost per ton of FFB averages at \$8.30.

*Milling Cost.* After the fresh fruit bunches are harvested they are sent to palm oil mills where the oil is extracted and the nuts separated. The amount of crude palm oil (CPO) obtained from the bunch is in the ratio of 18–24% depending on the planting materials.

As the mill receives and processes fresh fruit bunches, it is logical to base milling cost on per ton of FFB received. Presently, the average cost of milling a ton of FFB is in the region of MR 30.00, or \$11.10 (Table 22). Table 22 illustrates that the largest cost is depreciation, some 35.71%, followed by machinery upkeep, which is 17.5%, and mill process labor being just a little above 10%.

*Production Cost of CPO.* The costs outlined previously are summarized in Table 23. The establishment cost was amortized over 25 years and allocated to the cost of producing FFB. An average yield of 20 tons of FFB per hectare per year and an oil extraction rate of 20% were utilized for data conversions.

**TABLE 23. Product Cost of CPO in Malaysia (U.S. \$).**

	Per ha/year	Per ton FFB
Establishment cost amortized ( $1856 \div 25$ )	74.24	3.71
Average direct cost	256.80	17.84
Average indirect cost	166.00	8.30
Total cost of FFB production	597.04	29.85
Milling cost		11.10
Cost of FFB production and milling		40.95
Cost/ton of CPO	204.75	

It can be seen that the cost to produce a ton of CPO is about \$204.75. The largest component in the cost of production of CPO is the cost of FFB at 73% while the contribution of milling cost is at 27%.

Cost data on production of CPO is not available for most countries, but studies conducted on Malaysia and Indonesia indicated that the cost of production is lower than the market price of palm oil. However, cost of production in some countries is higher than the international market prices of palm oil (2), and for these countries exporting palm oil would not be viable.

*Refining Cost.* Crude palm oil can be further processed by refining. Presently, most of the palm products obtained in the market are processed using physical or steam refining. Crude or processed palm products may also undergo fractionation where the solid and the liquid portions are separated. The total cost of refining a ton of palm oil is about \$25.92 while the cost of fractionation is about \$5.55 per ton.

### 3.5. Palm Oil and Palm Kernel Extraction

*Fruit Reception.* In order to obtain good-quality palm oil, it is essential that the damage to the fruit is minimal and therefore the handling of the bunches from the field to the sterilizers must be carried out with the utmost care. At the mill the FFB is generally discharged from the lorries, or trailers, onto a loading ramp for the filling of sterilizer cages, which have a nominal capacity of 2.5 tons. A flow diagram of the palm oil milling process is shown in Figure 6.

*Sterilization.* Sterilization is carried out by placing the sterilizer cages in horizontal vessels at a steam pressure of 3 kg/cm<sup>2</sup> (143°C) and the time “under steam” is approximately 60 min. The objectives of sterilization are:

1. Prevention of further rises in the free fatty acid (FFA) of the oil due to enzymatic reaction.
2. Facilitation of mechanical stripping.
3. Preparation of the pericarp for subsequent processing.
4. Preconditioning of the nuts to minimize kernel breakage.

*Stripping.* The objective of stripping is the separation of the sterilized fruit from the bunch stalks. There are two basic actions involved in separating the fruits: (1) a small vigorous shaking and (2) beating. Although many machines have been evolved over the years, only the “drum” type is in general use.

The drum stripper consists of a long horizontal drum made up of small channel section or T bars spaced far enough apart to permit the escape of the fruit yet close enough to prevent the passage of the stalks or spikelets. Drum diameters vary from 1.8 to 2 m and lengths from 3 to 5 m and they usually rotate at about 20–25 rpm. The cage is fitted with lifting bars, and as the cage rotates the bunches are lifted up and then drop back under the action of gravity, and by this action the fruits are shaken out. As this action is repeated many times over, with the bunches turning round and round as they pass along the drum, a good stripping is obtained.

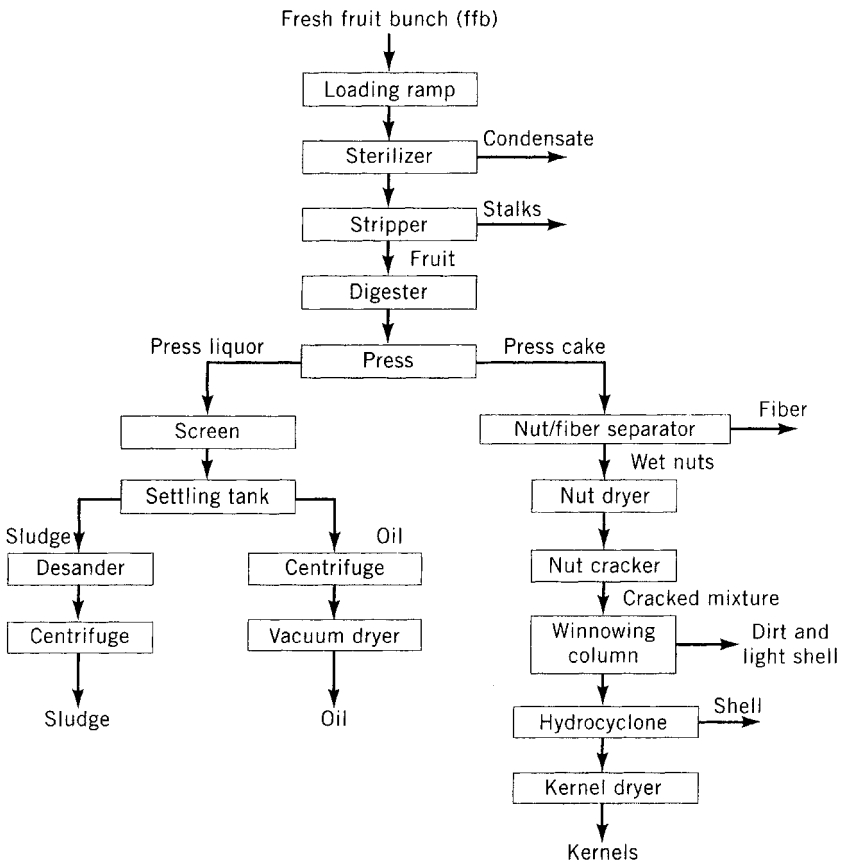


Figure 6. Process flow diagram of a conventional palm oil mill.

**Digestion.** The object of digestion is to reheat the sterilized fruits and to loosen the pericarp from the nuts and to break the oil cells before passing to the oil extraction unit. The best digestion conditions are obtained by mixing the fruits at a temperature between 95 and 100°C for approximately 20 min. The digester is generally a vertically arranged cylindrical vessel fitted with a central shaft carrying a number of radial arms. Heating may be from a steam jacket or direct steam injection.

**Oil Extraction.** Oil extraction is generally carried out using continuous screw presses comprising a perforated horizontal cage of a figure 8 cross section in which two screws or worms run. A cone at the discharge end of the cage controls the pressure to ensure a minimum of residue oil in the press cake with an acceptable amount of broken nuts.

There are two products from the press: (1) a mixture of oil, water, and solids, and (2) a press cake containing fibers and nuts.

**Clarification.** The crude oil from the press has an average composition of 66% oil, 24% water, and 10% nonoily solids (NOS). Because of the high proportion of

solids, it has to be diluted with water to obtain satisfactory settling. After dilution, the crude oil is screened to remove fibrous materials and then pumped to a continuous settling tank where it separates into two parts, i.e., oil and sludge. The "top" oil is skimmed off and passed to a centrifugal purifier followed by a vacuum dryer and finally a cooler before being pumped to the storage tanks. The sludge has an oil content of approximately 10%, and this is reclaimed and fed back to the main settling tank. The oil fed to the storage tanks has a moisture content between 0.1 and 0.12% and impurities less than 0.02%.

*Oil Storage.* It is recommended that storage tanks are internally coated with epoxy materials to prevent iron pickup. To prevent damage by overheating of the oil, the temperature of the oil during storage and transit is closely controlled between 32 and 40°C. The unloading or loading temperature is between 50 and 55°C, and for heating to this temperature the maximum rate is 5°C per 24 hr.

*Nut and Fiber Separation.* When the oil is extracted from the digested fruit, a cake of nuts and fiber is produced. This is fed, via a "breaking" conveyor, to a vertical column having an upward airflow at a velocity of 6 m per second. At this velocity all the fiber is moved upward or held in suspension, and the nuts drop to the bottom of the column. The fiber is led to a cyclone for use as a boiler fuel while the nuts pass to a rotating polishing drum installed at the bottom of the column. This drum can also be used to remove any large pieces of stalks, stones, or metal that have gotten into the system.

*Nut and Kernel Treatment.* This treatment covers four distinct operations: (1) nut conditioning, (2) nut cracking, (3) kernel and shell separation, and (4) kernel drying.

**Nut Conditioning.** Nuts coming directly from the nut fiber separator are still usually warm, and the kernels are still adhering to the shell. If an attempt is made to crack them in this condition, many of the kernels will be damaged and pieces of kernels will still be adhering to the shell. For ideal nut cracking it is necessary to dry the nuts sufficiently to loosen the kernels and then cool the nuts to harden the shell before cracking. This process is usually referred to as nut conditioning.

**Nut Cracking.** When a nut has been properly conditioned, its shell will crack cleanly in two or more pieces, and the kernel will be released. Nut cracking machines are almost invariably of the centrifugal type in which the nuts are given velocity by being fed through a rotor and are caused to crack by being flung against a stator ring.

However, the latest development has been the introduction of the ripple mill, which consists of a balanced rotor of the squirrel cage design and two pieces of semicircular ripple plates with ripple configurations. The performance of the ripple mill is determined by the speed and clearance of the rotor, and the rotor provides the velocity to force the nuts between the stationary ripple plate and the rotor. The main advantage claimed for the ripple mill is that nut conditioning may not be necessary.

**Kernel and Shell Separation.** This is normally achieved in two operations. First, a winnowing system is used to remove the small pieces of shell and dirt followed by hydrocyclones or claybaths.

The action of a hydrocyclone is somewhat similar to that of an air cyclone. By imparting a circular motion to the fluid by means of the tangential entry, heavy particles are thrown by centrifugal force to the wall of the cylinder. After tracing a helical path these particles find their way out through the bottom of the cyclone while the lighter particles after taking part in an initial downward circular movement gradually move toward the center of the cylinder and start moving upward leaving the cyclone via the overflow tube. Although it is mainly the difference in densities that enables the hydrocyclones to act as a shell and kernel separator, the size and shape of the particles have some effect, i.e., flow resistance.

The specific gravity of undried kernels is about 1.07 and that of shell about 1.17. Therefore in a clay and water mixture of specific gravity 1.12 (about 24 Twaddell), the kernels will float and the shell will sink; this is the principle on which the clay-bath separator works. Many models were developed from manually operated to completely automatic versions. As suitable clays were not always readily available, salt solutions and even dilute molasses were tried for the suspension. The claybath is quite an efficient separator as long as the density of the suspension is maintained at the correct value.

**Kernel Drying.** The moisture content of fresh kernels is about 20%. If bagged or stored in this condition fresh kernels would soon become moldy. In addition there would be a rapid increase in the FFA of palm kernel oil (the oil content of dried kernels can be over 50%). Tests have shown that if the moisture content is reduced to about 7%, kernels can be safely stored and transported without deterioration due to mold growth.

*Bagging and/or Storage of Kernels.* After drying, the kernels are usually bagged, approximately 12 bags to the ton, and stored in sheds awaiting transportation to the kernel crushing plants. However, with the increase in the costs of bags and handling, there is a tendency to go over to bulk storage and transportation.

### 3.6. Mill Construction and Design

*Crop Projection.* To establish the mill-rated throughput, it is first necessary to have details of the projected crop over say a 10-year period depending on the planting program. The general practice is to consider the peak month crop at 12.5% of the annual crop and that the mill will operate at 20 hr per day for 25 days during the peak month.

*Siting of Mill.* The major points to be considered when siting the mill are:

1. The transport costs to bring the FFB to the mill: Ideally the mill should be at the center of the planted area.
2. The costs to evacuate the produce: This depends on the distance from the mill to the nearest main road.
3. The distance of the nearest reliable water supply.
4. The ground conditions: Poor ground conditions may involve piling, undulating areas requiring excavation and/or filling, etc.

Therefore, a compromise has to be made between these factors in deciding the mill siting.

*Mill Design.* From the crop projections it is possible to calculate the final capacity of the mill and whether phasing of the mill capacities is necessary. The availability of fuel has to be considered as nowadays there is only just sufficient fuel for mills operating at over 10 tons of FFB per hour.

A typical layout of a palm oil mill is shown in Figure 7 where the main stations of the process are also indicated. The equipment selected has to be carried out with care in order to obtain the correct balance for throughput, steam consumption, energy demands, and economics of the supply of stand-by equipment. Standardization of equipment is an important point to consider when selecting machinery on the basis of “within” mill and “between” mills when applicable. When a set range and/or make of equipment can be chosen (e.g., valves, gearboxes, electric motors and starts, etc.), considerable benefits can accrue by way of a reduction in the amount of spare parts to be carried.

Once the main items of equipment have been selected, it is possible to proceed with the design of the layout of the mill, design of the buildings, and some initial work on the layout of the machinery. Final work on the machinery layout will have to await working drawings from the suppliers, which will not be forthcoming until after the orders have been placed for the equipment.

During the course of the design for the layout of the mill and its machinery, thought must be given to the matter of safety and safe working practices.

For projects of this magnitude and complexity it is essential that a critical path network (CPN) is established.

*Mill Construction.* Sufficient time should be allocated to carry out the actual construction work for the mill; otherwise it could result in a host of contractors and workers descending on the site, which makes good supervision virtually impossible.

In a normal situation the civil works (foundations, building, etc.) are usually completed before work commences on the erection of machinery.

The erection of the machines should follow a logical sequence of commencing from “in to out,” i.e., threshing station erected first in the main bay, with the pressing, kernel, and clarification stations to follow. This sequence can only be implemented if deliveries are planned accordingly. This, of course, depends on the correct timing for orders, and this is where the CPN is invaluable. In most cases the erection of the machinery is carried out by contractors. However, there are many advantages in having the future mill engineer on site during the erection period. Besides being good training for the engineer for obvious reasons, it should ensure that a better standard of work is carried out.

### 3.7. Treatment of Palm Oil Mill Effluent

*Sources of Wastewater Production.* Large quantities of water are required in the palm oil milling operations. It is estimated that about 1 ton of water (including boiler feedwater) is required to process 1 ton of fresh fruit bunches. Obviously, a great

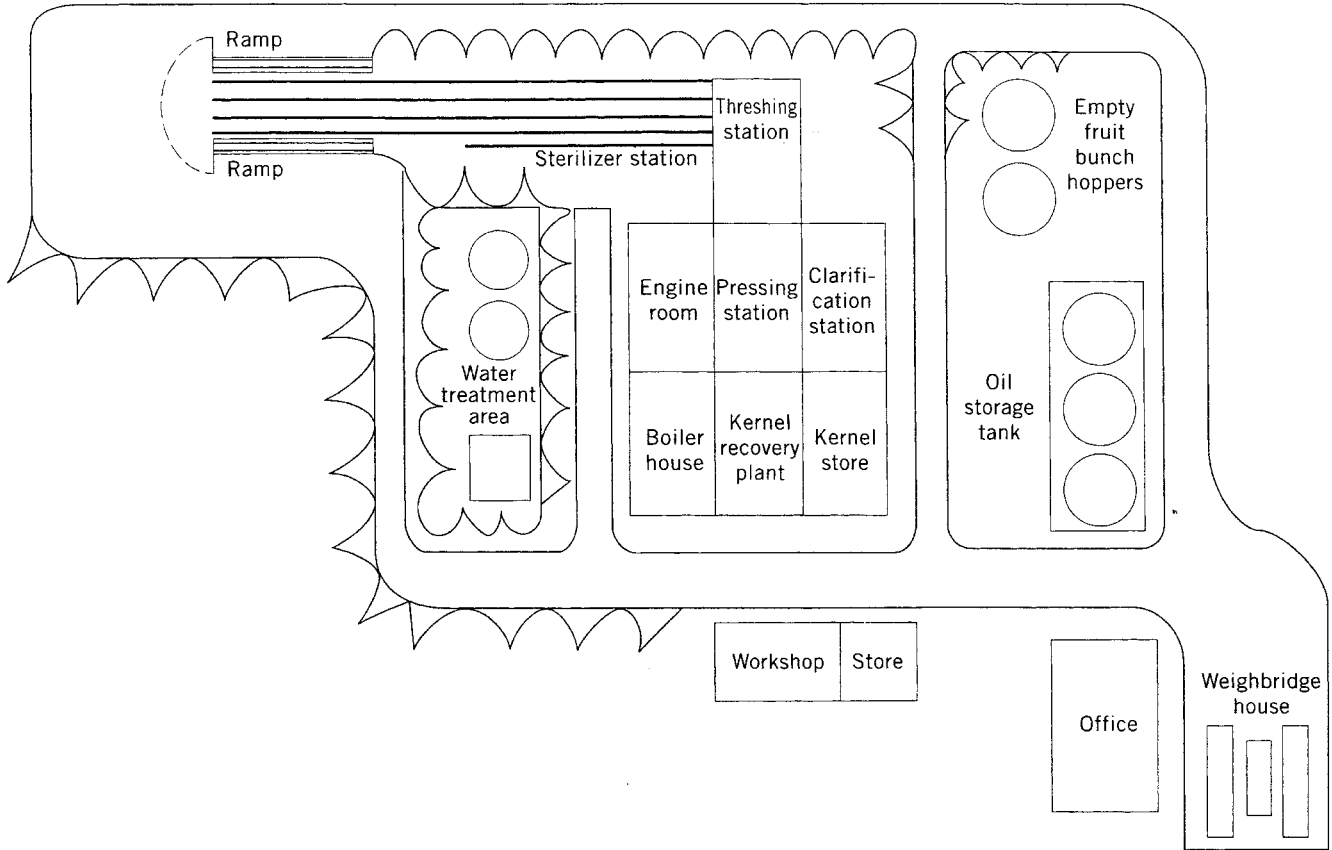


Figure 7. Typical layout of a palm oil mill.

proportion of the water will be discharged as wastewater, commonly known as palm oil mill effluent (POME). Some water is lost as vapor (steam).

POME is mainly generated from sterilization and oil clarification processes in which large quantities of steam/hot water are used. Another waste stream originates from hydrocyclone operation where water (with clay or salt) is used as a medium to separate shell and kernel. For a well-operated mill with good housekeeping practices, the amount of wastewater generated from the sterilization process (sterilizer condensate), oil clarification process (separator sludge), and hydrocyclone are 0.9, 1.5, and 0.1 ton per ton of oil produced, respectively (25). Thus about 2.5 tons of POME are generated for every ton of palm oil produced. Obviously water consumption and wastewater production can be minimized through good housekeeping and process control.

The sterilizer condensate and separator sludge are segregated into separate oil pits for residual oil recovery before they are mixed again for treatment. The hydrocyclone waste contains very little residual oil and is discharged directly into the treatment plant. The final mixed wastewaters are commonly known as palm oil mill effluent.

The residual oil recovered from the oil pits are of poor quality. It is drummed and sold as technical oil for nonedible applications. It is not advisable to recycle or mix this poor-quality oil with the normal production oil as such practice will negatively affect the normal production oil quality.

*Characteristics of POME.* POME, when fresh, is a thick brownish colloidal slurry of water, oil, and fine suspended solids. It is hot (80–90°C) and acidic (pH 4–5) and contains very high organic matter as indicated by its high biochemical oxygen demand (BOD<sub>3</sub>, sample incubated at 30°C for 3 days) (Table 24). In terms of BOD, POME is 100 times as polluting as domestic sewage. It also contains very high suspended solids (SS), which are mainly oil-bearing cellulosic materials from the fruits. The POME is nontoxic as no chemical is added to the oil extraction process. The characteristics of the sterilizer condensate, separator sludge, and hydrocyclone wastewater are also shown in Table 24.

**TABLE 24. Characteristics of Palm Oil Mill Effluent (25).**

Parameter <sup>a</sup>	Sterilizer Condensate	Separator Sludge	Hydrocyclone Water	POME
pH	5.0	4.5	300	4.7
Oil and grease	4,000	7,000	300	6,000
BOD	23,000	29,000	5,000	22,000
COD	47,000	64,000	15,000	61,000
SS	5,000	23,000	7,100	13,000
DS	34,000	22,000	100	21,000
TN	500	1,200	100	800
AN	20	40		35

<sup>a</sup>All in mg/L except pH: BOD, biochemical oxygen demand; COD, chemical oxygen demand; SS, suspended solid; DS, dissolved soli; TN, total nitrogen; AN, ammoniacal nitrogen.



**TABLE 25. Metal Content in Palm Oil Mill Effluent (26).**

Metal	Conc. (mg/L)
P	180
K	2,270
Mg	615
Ca	439
C	25,440
B	7.6
Fe	46.5
Mn	1.98
Cu	0.89
Zn	2.30

In addition, POME also contains high metal content, which can be of importance for other application like recycling as plant nutrients. Some of the essential metal contents are given in Table 25.

*Treatment Technology for POME.* As palm oil milling processes require large quantities of water, it is common to find palm oil mills located near rivers where (free) water is readily available. Because of its high organic content (high BOD), if discharged untreated into the watercourse, POME will soon undergo biological oxidation that depletes the dissolved oxygen in the water system. Oxygen depletion destroys aquatic life in the water and consequently the surrounding environment. Therefore the industry has both the social and ethical obligations to reduce the environmental impact caused by POME.

There are several options available to reduce the pollution problem created by palm oil mills. These include complete treatment and disposal of POME or systematic utilization of POME for beneficial purposes. The choice depends very much on the local environment.

POME, because of its high organic content (BOD) is easily amenable to biodegradation (27). Therefore biological oxidation is the most suitable process to breakdown the organic pollutants in POME. Biological treatment of wastewater is a process in which a mixed population of microorganisms utilize as nutrients substrates contaminating the water. Wastewater containing polluting substances is brought into intimate contact with a dense population of microorganisms for a duration sufficient for the microbes to break down and remove the pollutants to the desired level.

The oxygen required for the microbial activities is supplied through dissolved oxygen in water. Invariably the biological treatment system consists of a train of anaerobic (absence of oxygen), aerobic (presence of oxygen), and/or facultative (anaerobic and aerobic) processes to meet the required treatment efficiency. The end products for anaerobic process are biogas [a mixture of methane (60–70%), carbon dioxide (30–40%), and a small amount of hydrogen sulfide] and biosolids. The end products for aerobic process are mainly carbon dioxide and some amount of biosolids that need to be disposed of separately.

The three most common and effective treatment systems developed for the POME published so far are shown in Figures 9, 10, and 11. It has been shown over the last decade that all these systems if operated according to design and maintained properly could meet the local discharge standards for POME as shown in Table 26.

*Ponding System.* This is by far the most popular treatment system used by the palm oil mills in Malaysia. It is the most economic system provided suitable land is available at very low or no cost to the palm oil mill. Large land area is required for a ponding system. Various designs and configurations of ponding systems are used. Figure 8 shows a typical system used in Malaysia. It consists of essentially a number of ponds for different functions. Ponding systems are reliable, stable, and capable of producing good-quality final discharge with a BOD of less than 100 mg/L.

The anaerobic ponds are usually 5–7 m deep each and the facultative ponds are 1–1.5 m each. The hydraulic retention times (HRT) for the deoiling tank, acidification, anaerobic, and facultative ponds are 1, 4, 45, and 16 days, respectively.

A ponding system is normally operated at low rate, with organic loadings ranging from 0.2 to 0.35 kg per BOD per cubic meter per day. Because of the size and configuration of the ponds, they are quite difficult to control and monitor. Furthermore, there is very little mixing. Mixing is only achieved through bubbling of the biogas generated by the anaerobic process. It is hardly adequate to mix the digester content. Also the rising biogas brings along very fine solids to the top of the ponds.

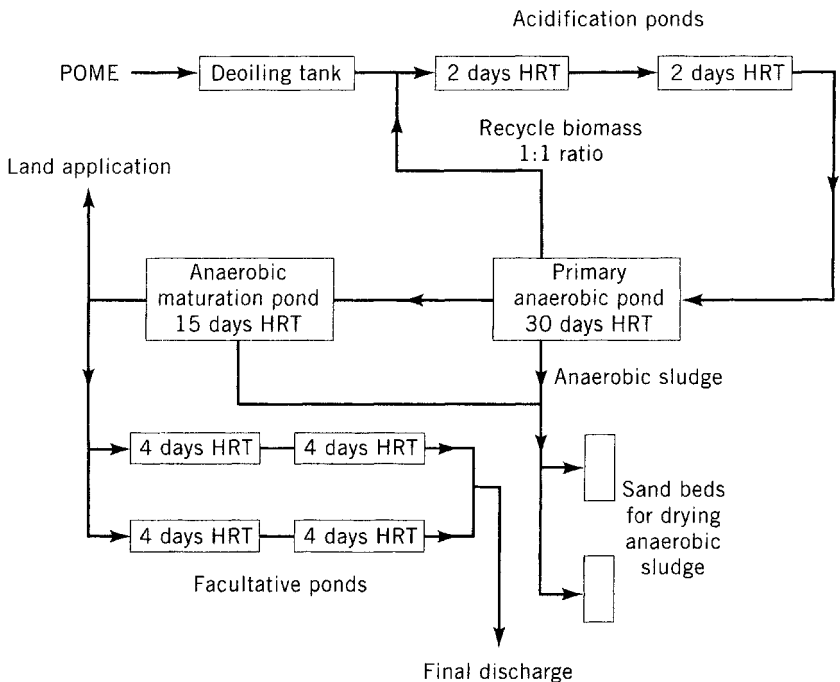
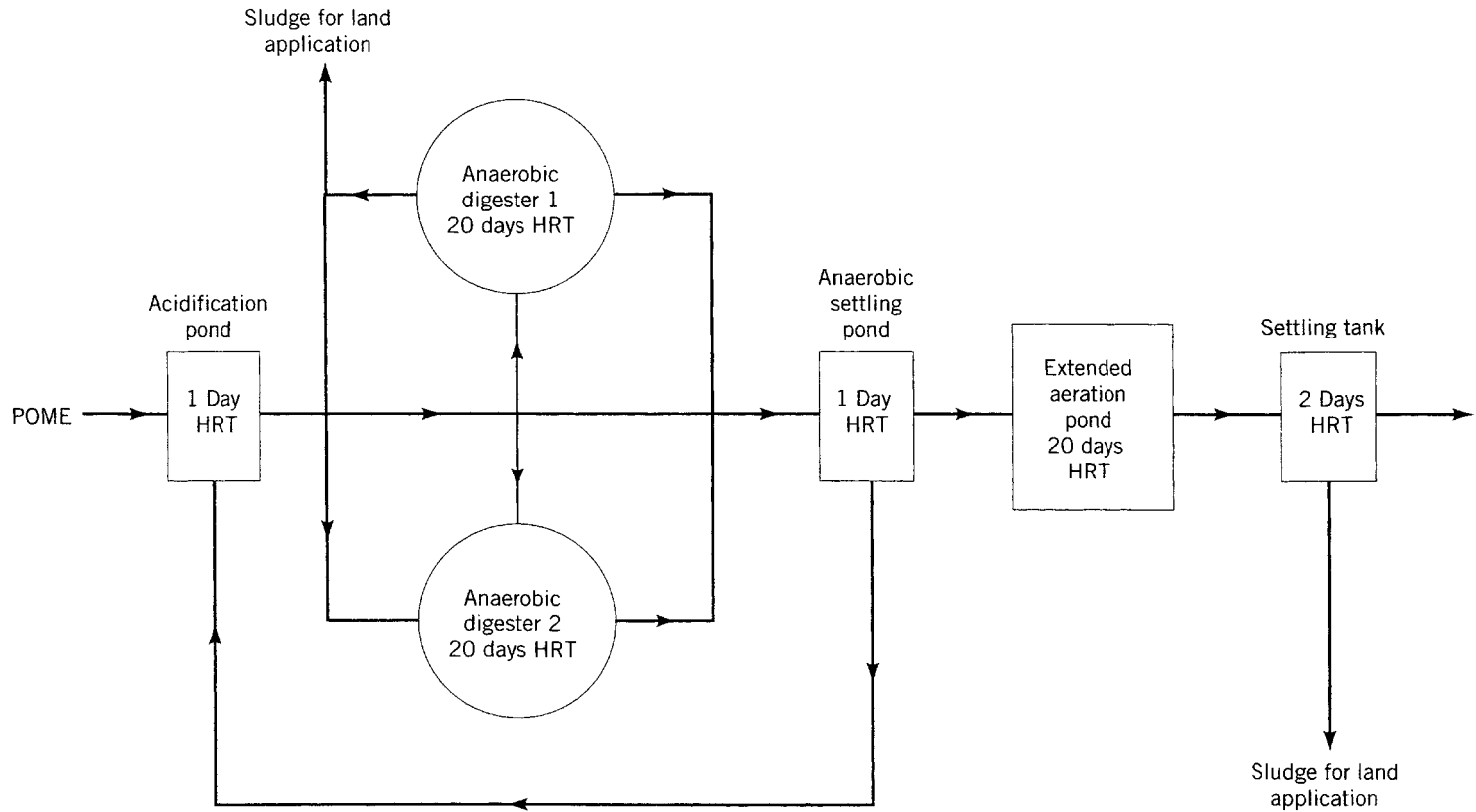


Figure 8. Schematic flow diagram for ponding system (28).



**Figure 9.** Schematic flow diagram of opened tank digester and extended aeration system (29).

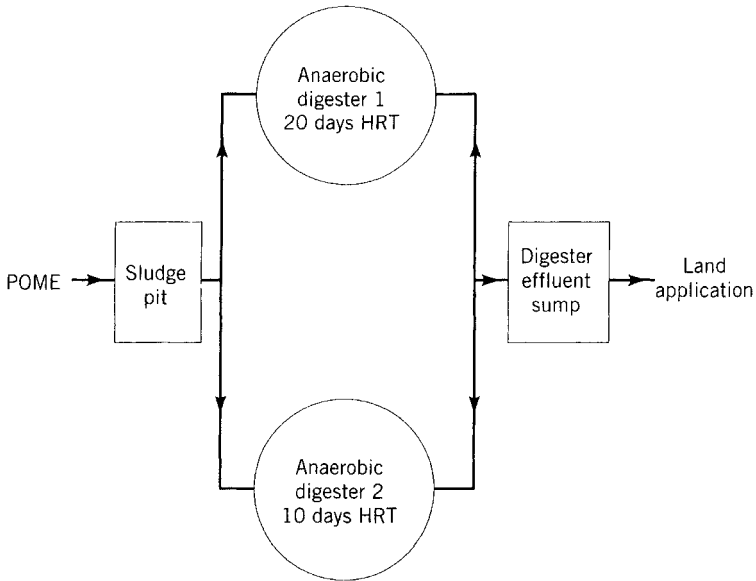


Figure 10. Schematic flow diagram of closed anaerobic digester and land application system (30).

Therefore it is very common to find islands of solids floating in the anaerobic ponds. This often results in dead spots and short-circuiting in the ponds, which reduces the treatment efficiency of the system. Obviously it is very labor intensive and expensive to maintain the ponding system in very satisfactory conditions. It is also imperative to ensure that as little oil as possible is allowed to get into the anaerobic pond. Otherwise the oil will agglomerate with the rising solids brought up by the biogas and form a sticky scum that is difficult to remove. It is not advisable to allow excessive accumulation of the scum so that the effectiveness of the system is not adversely affected.

TABLE 26. Discharge Standards for Palm Oil Industrial Effluents for Malaysia.

Parameter <sup>a</sup>	POME	PORE and OIE Standard	
		A	B
Temperature(°C)	45	40	40
pH	5.0–9.0	6.0–9.0	5.5–9.0
BOD <sub>5</sub> (mg/L) (3 days 30°C)	100	20	50
COD (mg/L) <sup>h</sup>	—	250	250
SS (mg/L)	400	50	100
O & G (mg/L)	50	—	—
AN (mg/L)	150	—	—
TN (mg/L)	200	—	—

<sup>a</sup>BOD<sub>5</sub>, biochemical oxygen demand; COD, chemical oxygen demand; SS, suspended solids; O & G, oil and grease; AN, ammoniacal nitrogen; TN, total nitrogen.

<sup>h</sup>Ungazetted. POME, palm oil mill effluent; PORE, palm oil refinery effluent; OIE, oleochemical industrial effluent. Standard A or B depends on locations.

Due to inadequate mixing by biogas, solid buildup at the bottom of the anaerobic pond poses another maintenance problem to the palm oil mills. Excessive solid buildup at the bottom of the ponds will reduce the effective digester capacity and consequently shorten the hydraulic retention time. Thus the treatment efficiency will be adversely affected.

Regular desludging of the ponds is recommended. Submersible slurry pumps can be used for this purpose. The solids are removed at regular intervals to a series of drying beds constructed besides the ponds. The dried solids, which contain high plant nutrients, are used as fertilizers in the estates.

*Open Tank Digester and Extended Aeration.* In this system, after the oil recovery pit, POME is treated in a two-phase anaerobic digestion process followed by extended aeration in a pond. The digesters are open-top and unstirred. Figure 9 shows a schematic flow diagram of the system. The HRT for the acidification, anaerobic, and aerobic process are 1, 20, and 20 days, respectively. The organic loading of the anaerobic digester is in the range of 0.8–1.0 kg BOD per cubic meter per day (27).

Similar to the ponding system, limited mixing is provided by the biogas generated. Hence such a system faces the same problem of solid buildup at the bottom of the digester. In order to maintain sufficient HRT for effective digestion, the solids have to be removed at regular intervals as in the ponding system. This can be easily done by means of slurry pumps. The solids are carted away for land application in the estates.

As compared to the ponding system, the scum and solid buildup in the digester can be readily monitored and controlled.

Mechanical surface aerator is used to supply air/oxygen to the aerobic pond. In aerobic systems it is important to ensure that enough oxygen is supplied to the aerobic microorganisms to do the job.

*Close Tank Digester with Biogas Recovery and Land Application.* The digesters (see Figure 10) are operated as conventional high rate systems with organic loading of 4.8 kg volatile solids (VS) per cubic meter per day. The HRT for anaerobic digestion is about 10 days operating at a slightly elevated temperature of 42–50°C. Good mixing is ensured by recycling the compressed biogas through an emitter and a draught tube. From the emitter, the biogas rises through the draught tube in large bubbles. Thus the digester liquor is drawn into the bottom of the draught tube and discharged from the top, causing effective circulation and hence mixing of the digester content. As the content of the digester is well mixed, there is no problem of solid buildup at the bottom of the digester.

In this system, the digester liquor, having a BOD of about 2000 mg/L, is applied to the plantation nearby as fertilizer (31). Several systems have been developed for land application of POME. The biogas generated can be harnessed for heat and electricity generation. Excess biogas is flared off. About 0.59 m<sup>3</sup> of biogas is produced per kilogram of VS added. Thus for a 60-ton FFB per hour mill operating 20 hr per day, about 20,000 m<sup>3</sup> of biogas is obtainable. The biogas contains about 65% methane, 35% carbon dioxide, and less than 2000 ppm of hydrogen sulfide. It has a calorific value of about 5300 kcal/m<sup>3</sup>. It can be used as an energy source to supply

heat or electricity to supplement the energy requirement if necessary (31,32). Substantial saving in energy bill can be realized.

### 3.8. Oil Palm By-products

Apart from the production of CPO and palm kernel oil (PKO), the oil palm industry also generates large quantities of by-products in the form of biomass. The bulk of the by-products derived from the palm oil industry are basically lignocellulosic and organic in nature and with a high plant nutrient content. With proper handling and management, these by-products could be utilized and converted into value-added products.

*Biomass Production and Availability.* The biomass production from the palm oil industry is derived mainly from two sources, i.e., the plantations and the palm oil mills. From the plantation the biomass produced per hectare of oil palm has been estimated and shown in Figure 11. On an annual basis, about 0.4 tons of palm

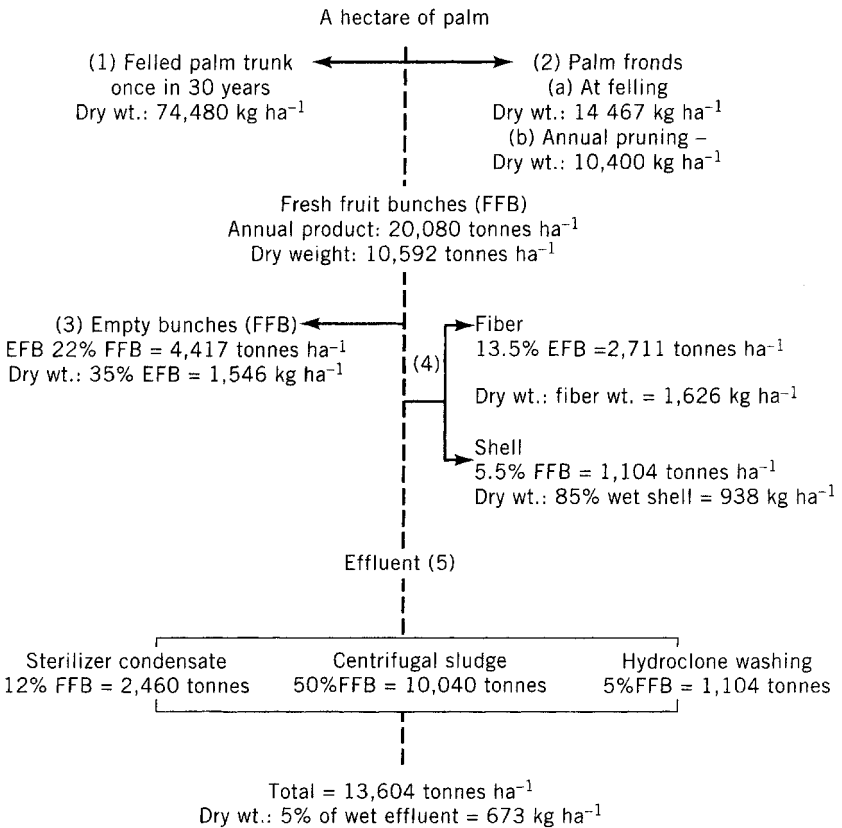


Figure 11. Diagrammatic representations of actual quantity of oil palm wastes (33).

fronds per hectare are available through routine maintenance pruning and harvesting. However, at the end of its economic life span (about 25 years), another 14.4 tons of palm fronds per hectare are available during replanting. In addition a total of 74.4 tons of palm trunks per hectare are also available during the replanting program.

At the palm oil mills, about 20% CPO and 1.6% of PKO are recovered from the FFB thus leaving about 78.4% biomass, including palm kernel meal.

As shown in Figure 11 the processing of fresh fruit bunches generated about 1.5 tons of empty fruit bunches (EFB), 1.6 tons of palm press fibers, 0.9 ton of palm kernel shell, 2.4 tons sterilizer condensate, and 0.7 ton of dry mill effluent per hectare of oil palm annually.

*Utilization of By-product. Oil Palm Trunks and Fronds.* Under normal plantation practices, the pruned fronds are placed along the palm interrows and act as mulch. Besides conserving soil moisture and reducing soil surface erosion, the fronds on decomposition return organic matter and slowly release plant nutrients to the soil (Table 27).

During the replanting program, the oil palm trunks and fronds are chipped into small pieces and pulverized using a special pulverizer. The biomass residues are left in the field to allow for decomposition processing, which could then yield organic matter and release of plant nutrients. The placement of trunk residues on field terraces could also reduce soil erosion.

**TABLE 27. Content of N, P, K, Mg, and Ca Obtained from Wastes of a Hectare of Oil Palm (33).**

Tissues and Dry Weight		Weight in kg ha <sup>1</sup>				
		N	P	K	Mg	Ca
Felled palm trunk						
75,460 kg ha <sup>-1</sup>	368.2	35.5	527.4	88.3	146.4	
Palm fronds						
(a) At felling:	Pinnae	114.0	7.5	109.4	8.4	7.1
14,467 kg ha <sup>-1</sup>	Rachis	<u>36.1</u>	<u>6.4</u>	<u>84.5</u>	<u>15.6</u>	<u>28.6</u>
		-150.1	13.9	193.9	24.0	35.7
(b) Annual pruning	Pinnae	819	5.4	78.7	6.0	5.1
10,400 kg ha <sup>-1</sup>	Rachis	<u>26.0</u>	<u>4.6</u>	<u>60.7</u>	<u>11.2</u>	<u>20.5</u>
		107.9	10.0	139.4	17.2	25.6
Empty bunches						
1,546 kg ha <sup>-1</sup>		5.4	0.4	35.3	2.7	2.3
Fiber						
1,626 kg ha <sup>-1</sup>		5.2	1.3	7.6	2.0	1.8
Shell						
938 kg ha <sup>-1</sup>		3.0	0.1	0.8	0.2	0.2
Effluent						
13,604 kg ha <sup>-1</sup>	Raw	12.9	2.1	26.6	4.7	5.4
	Digested	4.4	0.9	20.7	3.9	3.1

*Empty Fruit Bunches and Fibers and Shells.* Traditionally, the empty fruit bunches generated at the palm oil mill are mostly incinerated to produce bunch ash. Bunch ash is considered a good source of potassic fertilizer and is also useful as liming materials because of its high alkalinity (pH 12). However, incineration of EFB could cause air pollution, and this practice is not encouraged by the Department of Environment.

Alternative uses of EFB have been investigated and results have shown that they are suitable as a mulching material for oil palm. The EFB are fibrous in nature and have a high moisture content (about 60%). The application of EFB in the interrows of palm avenues has been shown to improve oil palm growth and yield performance. When applied onto the soil surface, the EFB undergoes a degradation process that will yield organic matter and slowly release plant nutrients for crop uptake.

Some of the oil palm fibers and shells are usually used as a surface mulching material for oil palm seedlings at the nursery stage. These materials are beneficial in conserving soil moisture and reducing fertilizer leaching in the polybags, and thus they enhance the growth of the seedlings.

*Palm Oil Mill Effluent (POME).* Palm oil mill effluent is essentially organic in nature and nontoxic but has a high polluting potential. In its raw state, POME has an extremely high concentration of biochemical and chemical oxygen demand (BOD and COD) and high in plant nutrient contents, particularly in nitrogen and potassium. After treatment processes various types of POME are available, and their chemical composition are shown in Table 28.

POME has proven to be a good source of organic fertilizer and is available in large volume (Table 29). Applied at rates corresponding to the nutrient requirement of crops, it will not have detrimental impact on the environment. The beneficial effects of POME application on crop yield and performance have been investigated. Several methods of land application systems are available.

*Energy Potential from Oil Palm By-products.* Apart from crude palm oil and palm kernel, a palm oil mill also produces a large quantity of biomass as by-products. In general, an FFB contains about 20% palm oil, 6–7% palm kernel,

**TABLE 28. Types of POME Available and Their Chemical Compositions (34–36).**

Type of POME	Chemical Composition (mg/L.)				
	BOD	Total			
		N	P	K	Mg
Raw	25,000	948	154	1,958	345
Digested (anaerobic)					
Stirred tank	1,300	900	120	1,800	300
Supernatant	450	450	70	1,200	280
Supernatant +10% slurry	191	320	42	1,495	258
Bottom slurry	1,000–3,000	3,552	1,180	2,387	1,509
Digested (aerobic)					
Supernatant	100	52	12	2,300	539
Bottom slurry	150–300	1,495	461	2,378	1,004



**TABLE 29. Annual Production of Raw Effluents for Mills with Capacity Ranging from 10 to 60 tons FFB Per hour.**

Annual Rate (ton)	Mill Capacity (Ton FFB/Hr)				
	10	20	30	40	60
Total FFB processed (capacity × 16 hr × 300 days)	48,000	96,000	144,000	192,000	288,000
Effluent production (FFB × 0.67%)	32,160	64,320	96,480	128,640	192,960

**TABLE 30. Heat Value of Biomass (37).**

Biomass	Moisture Content (%)	Oil Content (%)	Heat Value kcal/kg (dry)
Empty fruit bunches	65	5	3700
Fiber	42	5	4420
Shell	7	1	4950

14–15% fiber, 6–7% shell, and 23% EFB. The heat value of each biomass is shown in Table 30.

**Fiber and Shell.** The palm oil mill uses fiber and shell as boiler fuel to produce steam for electricity generation and palm oil and kernel production processes. The fiber and shell alone can supply more than enough electricity to meet the energy demand of a palm oil mill. It is estimated that about 20 kWh (lower for higher-capacity mill) of electrical energy is required to process 1 ton of FFB.

**Empty Fruit Bunch.** Apart from fiber and shell, EFB is another biomass that can be readily converted into energy. However, this material has only been utilized to a limited extent. This is because there is already enough energy available from fiber and shell. Also due to its physical nature and high moisture content (50–65%), the EFB has to be pretreated to reduce its bulkiness and moisture content to below 50% in order to render it more easily combustible (37, 38).

**Biogas from Palm Oil Mill Effluent.** Biogas is generated from anaerobic treatment of POME. It contains about 65% methane (CH<sub>4</sub>), 35% carbon dioxide (CO<sub>2</sub>), and trace amounts of hydrogen sulfide (H<sub>2</sub>S). It has a calorific value of about 4740–6150 kcal/N m<sup>3</sup>. About 28 m<sup>3</sup> of biogas are generated for every cubic meter of POME digested. In a gas engine, about 1.8 kWh of electricity can be generated from every cubic meter of biogas.

## 4. REFINING AND FRACTIONATION

### 4.1. Physical and Chemical Refining

Crude palm oil extracted commercially from the fresh fruit bunches contains a small but variable amount of undesirable components and impurities. These include some mesocarp fibers, moisture and insolubles, free fatty acids, phos-pholipids,

trace metals, oxidation products, and odoriferous substances. As a result, palm oil is normally refined to a bland, stable product before it is used for direct consumption or for formulation of edible product. In Africa, however, crude palm oil is often consumed in the crude form.

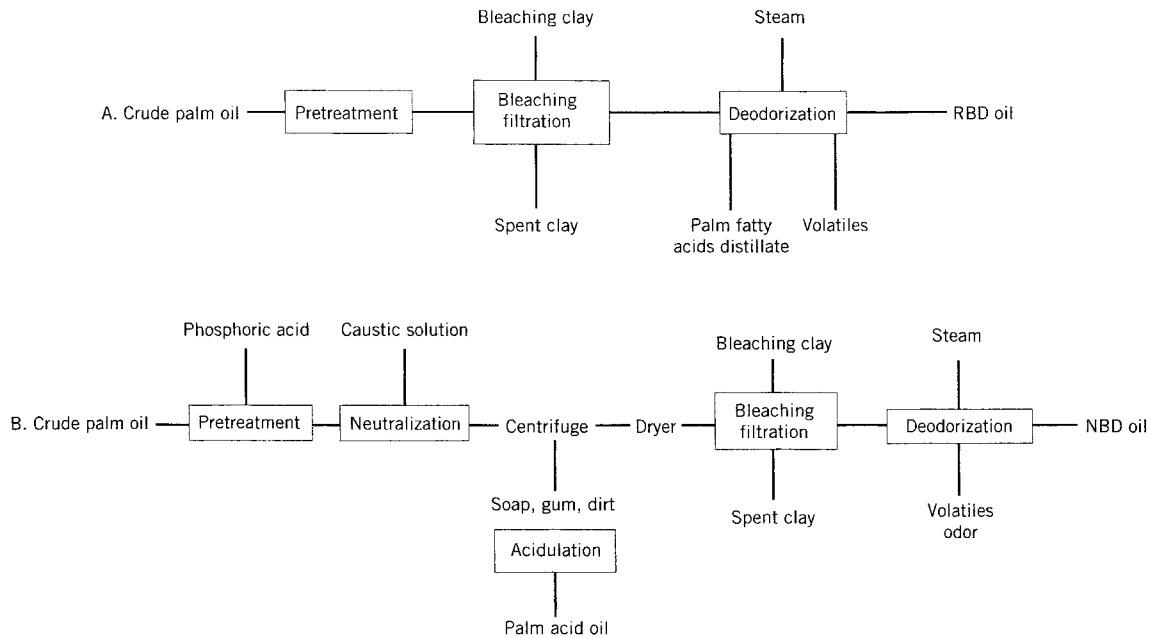
Two methods, namely physical refining and chemical refining, are available for refining crude palm oil. They differ basically in the manner in which the free fatty acids are removed. Physical refining has become the major processing route because of its cost effectiveness, efficiency, and simple effluent treatment (39). Both processes are able to produce refined, bleached, and deodorized (RBD) palm oil of desirable quality and stability suitable for edible purposes (40). The unit operations involved in these two processes and the components removed are shown in Figure 12 and Table 31, respectively.

*Physical Refining.* Physical refining was introduced to palm oil processing in 1973 (41). Its unique feature is that the deacidification, deodorization, and thermal decomposition of carotenoids are accomplished in one process in a stainless steel deodorizer. It is a continuous processing consisting of a two-step operation of pretreatment followed by steam distillation (42).

**Pretreatment.** Pretreatment refers to the initial degumming of crude palm oil with concentrated phosphoric acid and the subsequent adsorptive cleansing with bleaching clay. Crude palm oil is dosed with phosphoric acid (80–85% concentration) at a rate of 0.05–0.2% (of the feed oil), heated to 90–110°C, and given a residence time of 15–30 min before passing to the bleacher where bleaching earth is added as a slurry. The earth required ranges from 0.8 to 2.0%, depending on the quality of the crude oil.

The purpose of the phosphoric acid is to precipitate the nonhydratable phosphatides while the function of the earth is fourfold: (1) to adsorb the undesirable impurities such as trace metals, moisture, insolubles, and part of the carotenoids and other pigments (43). (2) to reduce the oxidation products, (3) to adsorb the phospholipids precipitated by the phosphoric acid, and (4) to remove any excess phosphoric acid present in the oil after degumming. The final residual color of the pretreated oil alone is unimportant as the role of the bleaching earth is not so much of color removal but more critically in its ability to act as an adsorptive cleansing agent (44). Complete removal of residual phosphoric acid in the bleaching stage is also critical as any “slip through” can result in the rapid rise of free fatty acid content and color of the final RBD oil (39,42,45). As a further assurance, a suitable quantity of calcium carbonate is often added after dosing of the bleaching earth to the degummed oil, to help neutralize the residual phosphoric acid (46).

Bleaching is carried out under a vacuum of 20–25 mmHg and at a temperature of 95–110°C with retention time of 30–45 min (47). The slurry containing the oil and earth is then filtered to recover a clear, light orange color pretreated oil. Usually a small amount of diatomaceous earth is used to precoat the filter leaves to improve the filtration process. As a quality precaution, the filtered oil is polished through another security filter bag in series, to trap any earth particles that escape through the first filter. This is essential as the presence of spent earth particles in the pretreated oil reduces the oxidative stability of the final RBD oil (46). The spent



**Figure 12.** Flow diagrams of (A) physical refining and (B) chemical refining of crude palm oil.

**TABLE 31. Refining Crude Palm Oil: Unit Processes.**

Stage	Principal Impurities Reduced or Removed
Degumming	Phospholipids, trace metals, pigments
Neutralization	Fatty acids, phospholipids, pigments, oil insolubles, water solubles
Washing	Soap
Drying	Water
Bleaching	Pigments, oxidation products, trace metal, traces of soap
Filtration	Spent bleaching earth
Deodorization	Fatty acids, mono- and diglycerides, oxidation products, pigment decomposition products
Physical Refining	Fatty acids, mono- and diglycerides, oxidation products, pigment decomposition products
Polishing	Removal of trace oil insolubles

earth from the filter normally contains about 20–40% oil, and this is the major source of oil loss in the refining process.

The pretreatment process can be carried out in batch, semicontinuous, or continuous equipment, and the filters used are either plate and frame presses or verticle or horizontal pressure filters with verticle stainless steel filter screens.

**Deodorization.** The pretreated oil is then ready for deacidification and deodorization. The pretreated oil is first deaerated followed by heating to 240–270°C in an external heat exchanger before pumping into the deodorizer, which is kept under a vacuum of 2–5 mm Hg. Traditionally thermal fluids are commonly used as the heating medium. However, to eliminate the risk of possible contamination of refined oil with thermal fluid, superheated high-pressure steam is now commonly being used, especially in new plants. Temperatures above 270°C are to be avoided to minimize loss of neutral oil, tocopherols/tocotrienols, and also the possibilities of isomerization and undesirable thermochemical reactions (48). Under such conditions and with the help of stripping steam, the free fatty acids, which were still present in the pretreated oil, are distilled together with the more volatile odoriferous and oxidation products such as aldehydes and ketones, which otherwise would impart undesirable odor and taste to the oil. At the same time, the residual carotenoids present are also thermally decomposed (Figure 13), and the end result is the production of a light-colored, bland RBD palm oil. To maximize the recovery of thermal energy, the hot deodorized oil is heat exchanged against incoming pretreated oil to be cooled down to a temperature of 120–150°C. Further cooling is effected by water down to 55–65°C prior to storage. Antioxidant and citric acid, if required, are dosed into the RBD palm oil at this stage.

The desirable qualities of the pretreated and RBD palm oil are given in Table 32 (46.50).

**Development in Palm Oil Deodorization.** The main operation in the deodorizer is the stripping of volatile materials and thermal action due to the combined effects of superheated steam, high temperature, and efficient vacuum. The older deodorizers (prior to 1985) use bubble caps or sieve-tray designs to effect the

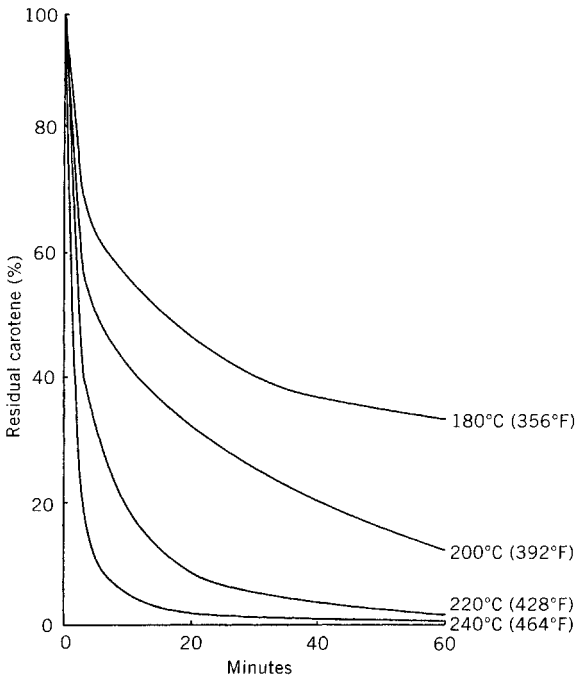


Figure 13. Thermal destruction of  $\beta$ -carotene (49).

countercurrent mixing action between the stripping steam and oil flow. However, technological innovations have resulted in many plants changing over to new deodorizers of packed column or the falling film types (51–53). These new features reduce pressure drop and improve the contact between the oil film and stripping steam, thus enhancing mass-transfer efficiency. Incorporation of this new design has resulted in deodorizers of larger capacities, faster throughput, lower loss of neutral oil and thus lower steam consumption on a per ton basis (54).

*Chemical Refining.* Also called caustic refining, chemical refining involves three stages: (1) gum conditioning and neutralization, (2) bleaching and filtration, and (3) deodorization.

TABLE 32. Desirable Quality of Pretreated and RBD Palm Oil from the Factory.

Parameter	Pretreat Palm Oil (Degummed/Bleached)	Refined Bleached and Deodorized Palm Oil
Free fatty acids (as C16:0) (%)	Same as crude feed	0.10% max.
Peroxide value (mEq/kg)	0	0
Moisture and impurities (wt %), max.	0.1	0.1
Iron (mg/kg), max.	0.12	0.12
Copper (mg/kg), max.	0.05	0.05
Phosphorus, (mg/kg), max.	4	4
Lovibond color, max. ( $5\frac{1}{4}$ -inch cell)	—	3.0R

**Gum Conditioning and Neutralization.** The crude oil is heated to a temperature of 80–90°C. Phosphoric acid of 80–85% concentration is then dosed in at a rate of 0.05–0.2% (of the feed oil). This serves to precipitate the phospholipids. After this, the degummed oil is further treated with a caustic soda solution of about 4 *N* (or 20 Bé) concentration with a calculated excess (based on free fatty acid content of the crude oil) of about 20%. The reaction between caustic soda and the free fatty acids in the degummed oil results in the formation of sodium soap, which is readily removed by a centrifugal separator. The lighter phase discharged consists mainly of neutralized oil containing 500–1000 mg/kg of soap and moisture while the heavy phase is mainly soap, insoluble impurities, gums, phosphatides, excess alkali, and a small quantity of oil loss through emulsification. As an excess of alkali is used, it is unavoidable that a slight loss of neutral oil through saponification also occurs.

The neutralized palm oil (NPO) is then washed with 10–20% hot water to remove traces of soap still present. After another stage of centrifugal separation, the washed oil is then dried under vacuum to a moisture level below 0.05%.

**Bleaching and Filtration.** The neutralized palm oil is treated with bleaching earth in a similar manner as that described in physical refining. However, in this case, the earth also removes traces of soap that are present.

**Deodorization.** The neutralized and bleached oil is then channeled to the deodorizer in a similar manner to that in the physical refinery. The oil is subjected to distillation at a temperature of 240–260°C and a vacuum of 2–5 mm Hg with direct steam injection. Under such conditions, residual free fatty acids, volatile oxidation products, and odoriferous materials are removed together with thermal decomposition of carotenoids (Figure 14). The final product, called neutralized, bleached, and deodorized (NBD) palm oil is then cooled down to 60°C and passed through polishing filter bags before pumping to the storage tanks. The desirable quality characteristics of intermediate and final products are given in Table 33.

**Refining Factor.** The efficiency of the refining process is estimated by the use of a refining factor (RF):

$$RF = \frac{\% \text{ Total oil loss}}{\% \text{ FFA in crude oil}}$$

**TABLE 33. Desirable Quality of Freshly Produced Intermediate and Final Products in Alkaline Refining of Palm Oil (48,50).**

Parameter	Neutralized Palm Oil	Neutralized and Bleached Palm Oil	Neutralized, Bleached, and Deodorized Palm Oil
Free fatty acids (as C16:0) (%) max.	0.15	0.15	0.10
Peroxide values (mEq/kg)	—	0	0
Moisture and impurities (wt %) max.	0.1	0.1	0.1
Iron (mg/kg)	—	0.15	0.12
Copper (mg/kg)	—	0.06	0.05
Phosphorus (mg/kg)	—	4	4
Soap content (mg/kg)	20	0	0

**TABLE 34. Desirable Quality of RBD/NBD Palm Kernel Oil.**

Parameter	Value
Free fatty acid (as % C12:0), max.	0.05
Moisture and impurities (wt %), max.	0.1
Lovibond color (5 $\frac{1}{4}$ -inch cell), max.	1.5R
Iron (mg/kg), max.	0.5
Copper (mg/kg), max.	0.1
Phosphorus (mg/kg), max.	1.0

Both figures are corrected for moisture and insoluble impurities in the crude oil. In alkaline refining, the term acid oil factor (AOF) is sometimes used:

$$\text{AOF} = \frac{\% \text{Acid oil}}{\% \text{FFA}}$$

The AOF is used for monitoring losses of neutral oil in the neutralization process.

Values of RF for chemical refining range from 1.5 to 2.0, while lower figures of 1.2–1.4 are usually recorded in physical refining.

**Refining of Other Palm Products.** Beside crude palm oil, crude palm olein, crude palm stearin, crude kernel oil, crude palm kernel olein, and crude palm kernel stearin can also be refined by either chemical or physical processes described before. The basic unit operations and processing conditions for crude palm olein and stearin are similar to those of palm oil. However, in refining palm kernel products, due to the virtual absence of carotenoids, the earth dosage required in the bleaching stage is lower, usually less than 1.0%. Furthermore, due to the presence of shorter chain (C8–C14) fatty acids, the deodorization temperature required is about 230–250°C. Typical achievable quality of RBD/NBD palm kernel oil is given in Table 34.

**By-products. Chemical Refining.** The neutralization of free fatty acid in the crude palm oil with caustic soda results in the formation of soapstock, which is treated with dilute sulfuric acid of pH 2.0–3.5 at 110–130°C for 30 min. A by-product called palm acid oil is then separated from the aqueous phase by centrifugation followed by hot-water washing. It consists mainly of free fatty acids, neutral oil, and partial glycerides. A small amount of unsaponifiable matter is also present. Characteristics and properties of palm acid oil (derived from chemical refining of crude palm oil, stearin, and olein) are given in Table 35 (55).

**Physical Retining.** The by-product in the physical refining of crude palm oil is the palm fatty acid distillate (PFAD). It is obtained as a condensate of the volatile matters carried over from the deodorizer by the action of the stripping steam. It consists of 80–90% of free fatty acid. It has often been used as a raw material for soap making, feed compounding, and oleochemical feedstock. An important and valuable constituent of PFAD is vitamin E in the form of tocopherols and

**TABLE 35. Characteristics of Malaysian Palm Acid Oil.**

Parameter	Range	Mean
Free fatty acid (% as C16)	66.9–88.7	72.8
Moisture content (wt %)	0.10–0.68	0.28
Iodine value (Wijs)	41.8–64.4	53.2
Titer (°C)	38.8–47.1	44.6
Unsaponifiable matter (wt %)	0.40–1.95	0.79
Fatty acid composition (wt %)		
C12:0	0.1–0.5	0.1
C14:0	1.0–1.6	1.2
C16:0	31.8–56.0	47.1
C18:0	4.1–5.2	4.6
C18:1	29.9–48.9	36.3
C18:2	6.3–12.0	9.6
C18:3	0.3–0.8	0.7

**TABLE 36. Characteristics of Palm Fatty Distillates from the Physical Refining of Palm Oil Products (55).**

Parameter	PFAD from Palm Oil		PFAD from Palm Olein		PFAD from Palm Stearin	
	Range	Mean	Range	Mean	Range	Mean
Free fatty acid (% as C16:0)	72.3–89.4	83.3	71.8–98.6	85.5	77.7–89.5	85.9
Moisture content (wt %)	0.03–0.15	0.08	0.03–0.12	0.07	0.04–0.16	0.09
Iodine value (Wijs)	51.2–57.4	55.3	45.6–59.1	57.4	44.3–52.6	44.8
Titer (°C)	40.7–49.0	46.3	36.9–47.8	45.5	44.3–52.6	49.8
Unsaponifiable matter (wt %)	1.5–3.4	2.5	1.6–3.7	2.3	1.3–2.5	1.9
Fatty acid composition (wt %)						
C12:0	0.1–0.3	0.2	0.01–0.6	0.2	0.1–0.3	0.1
C12:0	0.9–1.5	1.2	0.3–1.5	1.2	1.2–1.6	1.3
C16:0	42.9–51.0	47.1	39.1–49.0	44.1	47.6–61.3	57.0
C18:0	4.1–4.9	4.5	3.8–5.1	4.5	4.2–5.4	5.1
C18:1	32.8–39.8	36.6	29.3–42.6	39.0	25.2–36.3	29.0
C18:2	8.6–11.3	9.6	7.1–12.8	10.2	6.0–7.4	6.8
C18:3	0.2–0.6	0.5	0.3–0.9	0.5	0.2–0.5	0.4

tocotrienols (56). A process for the economical recovery of vitamin E from PFAD has been developed by the Palm Oil Research Institute of Malaysia (PORIM) (57). Characteristics and properties of PFAD from the physical refining of palm oil are given in Table 36.

## 4.2. Fractionation

The triglycerides of palm oil consist of a combination of fatty acids with different chain length as well as degrees of unsaturation. This results in the presence of substantial quantity of both low- and high-melting triglycerides. Crystallization of the



oil under controlled cooling followed by separation will yield a low-melting liquid phase (olein) and a high-melting solid phase (stearin). Factors affecting the crystallization process are oil composition, polymorphism, and cooling condition (58,59).

*Oil Composition.* Palm oil contains about 4–8% of diglycerides (50), which can form a eutectic mixture with the triglycerides resulting in lower solid content. This can slow down the rate of crystallization. The monoglycerides, present at less than 1% in palm oil, have no significant effect on the crystallization.

*Polymorphism.* Palm oil triglycerides are polymorphic and thus can crystallize in several forms. The polymorphic forms are designated as  $\alpha$ ,  $\beta'$ , and  $\beta$  in the order of increasing stability and melting points. Upon cooling, palm oil initially crystallizes in the  $\alpha$  form, which gradually transforms in the order of  $\alpha \rightarrow \beta' \rightarrow \beta$  form. To have good separation, it is desirable to have  $\beta'$ -form crystals because  $\beta'$  crystals agglomerate into large, firm clusters resulting in good subsequent filtration.

*Cooling Rate.* Cooling rate affects the nucleation and crystal growth of the oil. As the oil is cooled, it becomes supersaturated. When the temperature is sufficiently low (about 32–36°C), saturated glycerides will crystallize, and these crystals act as nuclei for further crystallization of the lower melting glycerides, resulting in formation of larger clusters of crystals. Slow cooling rate and proper stirring speed is essential for the formation of the desired crystal form.

*Process Description.* There are three commercial methods for fractionating palm oil: dry, detergent, and solvent process.

*Dry Fractionation.* This is usually carried out semicontinuously using neutralized, neutralized and bleached, or fully refined palm oil. It does not require the use of any chemicals or additives. The oil is kept homogenized at about 70°C to destroy any presence of crystal in order to induce crystallization in a controlled manner during subsequent cooling. Crystal formation and growth occur as the oil is agitated and cooled using chilled-water circulation. The cooling program is controlled by setting the temperature differential between the oil and chilled water, and also the time of cooling. When the temperature reaches the desired temperature, which is dependent on the quality of olein required (but usually about 20°C), the cooling is stopped and the thick partially crystallized mass is ready for filtration. The different filtration systems now used in the industry are drum rotary filters, stainless steel belt florentine filters, and “membrane filters.” Over the last decade, the membrane filter, which actually is a filter press equipped with a membrane plate, is increasingly used because it gives a higher yield of olein (about 70–75%) and a harder stearin compared to that of about 65% obtainable from florentine or rotary drum filters (60).

*Detergent Fractionation.* Also known as the Lanza or Lipfrac process, detergent fractionation is normally carried out on crude palm oil. The oil is first cooled in the crystallizer with chilled water to allow the crystallization of the higher melting glycerides. When the desired temperature is reached (usually about 20°C), the crystallized mass is mixed with an aqueous detergent solution containing about 0.5% of sodium lauryl sulfate and magnesium sulfate as an electrolyte. The stearin crystals are wetted by the detergent solution and separate out into a suspension in the aqueous phase. On centrifuging, the olein is discharged as the lighter phase, and the

stearin forms part of the aqueous phase. The olein phase is then washed with hot water to remove excess detergent and vacuum dried before storage. The aqueous phase is heated to 95–100°C to break the emulsion for recovering the stearin, which is again washed with hot water and dried under vacuum before storage. Yield of olein is about 80%.

*Solvent Fractionation.* This process is the most expensive because of solvent loss, solvent recovery equipment, much lower temperature requirement, and stringent safety features. The process involves the use of solvents such as hexane or acetone. The oil is first dissolved in the solvent followed by cooling to the desired temperatures to obtain the desired crystals. Cooling is effected by brine if very low temperature is required. The miscella containing the partially crystallized oil and solvent is then filtered under vacuum suction in an enclosed drum filter. The olein miscella and stearin miscella are then separately distilled to remove the solvent and recover the fractions. Yield of olein is about 80%. The solvent process nowadays is only viable in the production of high value products such as cocoa butter equivalent or other specialty fats.

*Double Fractionation.* Double fractionation is carried out for the production of palm olein with higher iodine value of above 60 or for the production of palm-midfraction (PMF), which contains a high proportion of oleodipalmitin used for production of palm-based cocoa butter equivalent (60, 61). Usually the first olein obtained is recycled back to the plant for further cooling, crystallization, and filtration. The second stearin obtained is termed palmmidfraction. Special and skillful control of the crystallization of both stages is critical in achieving the desired quality of the products.

*Fractionation of Palm Kernel Oil.* As in palm oil, palm kernel oil can also be fractionated via the dry, detergent, and solvent processes (62). The principles applied are quite similar. The conditions of operation, however, are quite different because of the different triglyceride composition and crystallization behavior of palm kernel oil. In the dry fraction process, the separation of palm kernel olein from the palm kernel stearin is effected by hydraulic pressing under high pressure. In this case, the palm kernel stearin, which is an important material for production of lauric-basic cocoa butter substitute, is the premium product. Its yield ranges from 25 to 40% depending on the process used.

### 4.3. Quality Assurance

*General.* Palm oil is one of the most stable vegetable oils, and this can be attributed to the presence of natural antioxidants, and also to the balanced ratio of saturated to unsaturated fatty acids. Nevertheless palm oil, whether crude or refined, is still susceptible to quality deteriorations. Stringent preventive measures are necessary to ensure the production of refined palm oil products of superior quality and acceptability.

*Quality Chain.* The processing chain of palm oil begins with the harvesting of fresh fruit bunches (FFB) from the estates followed by processing of the FFB into

crude palm oil. Thereafter, the crude palm oil is sent to the refinery for processing into various grades of refined products that are then transported to the bulking installations for export. Because any quality problem that may arise at any point in the chain will affect the other stages down the line, it is necessary that the right quality be attained right from the beginning of the process chain. The two main quality problems associated with palm oil are hydrolysis, leading to formation of fatty acids, and oxidation, leading to rancidity.

*Hydrolysis.* The hydrolysis of palm oil is promoted by the presence of free moisture and heat and also by lipolytic enzymes endogenous to the plant tissue (63,64). This mode of deterioration occurs during the bruising of fruits in the harvesting and transportation of the FFB to the mill and also their extended storage under unfavorable conditions. In the case of oil during storage, the hydrolysis is attributed to a chemical reaction that is autocatalytic (65). The presence of high FFA in crude palm oil is undesirable as it (1) reduces the yield of RBD palm oil through higher loss of PFAD by-product, (2) reduces the capacity of refining, and (3) results in poor bleachability of crude oil and poor stability in the final product (50). Crude palm oil with high FFA content invariably also contains a high amount of partial glycerides, especially diglycerides. Interactions between the diglycerides and the triglycerides often lead to formation of eutectics resulting in poor crystal formation during fractionation, difficulty in separation of olein and stearin by filtration, and also in production of olein with poor cold stability (66, 67).

*Oxidation.* Oxidation of oils and fats is due to prolonged exposure to air. By virtue of the low polyunsaturated fatty acid content, palm oil is relatively more stable to oxidative deterioration than the polyunsaturated vegetable oils. However, in the presence of trace metals such as iron and copper, excessive oxidation at the olefin bonds of the oleic and linoleic acids can occur, resulting in rancidity. Highly oxidized crude palm oil is known to have poor bleachability and thus requires more bleaching earth and more severe refining conditions, and the final product will likely be of poor stability (44, 45, 68).

*Quality Assurance Measures in Plantation and Milling.* Availability of good-quality crude palm oil is a prerequisite for the production of good-quality refined palm oil products. The criteria for good-quality crude palm oil are:

- Low free fatty acid content
- Low in oxidation characteristics
- Good bleachability
- Low in trace metals and insoluble impurities
- Moisture content of about 0.15–0.20%
- High in deterioration of bleachability index (DOBI)

[DOBI, which is defined as the ratio of the uncorrected absorbance values at 446 nm to that at 269 nm, was introduced as a quality parameter to differentiate the refinability of good- and poor-quality crude palm oil. The relation to quality is DOBI >3, good; 2.4–2.9, fair, and <2.3, poor (45, 69).]

**TABLE 37. Crude Palm Oil Quality.**

Component	Ripe, Fresh, Unbruised Fruit	Average Traded PO
Triglycerides (%)	98	<98
Diglycerides (%)	2–3	4–8
Monoglycerides (%)	0.1	0.2
FFA (% <sub>as C16:0</sub> )	0.1	3.5 (max 5)
Phosphorus (ppm)	2–3	20–30
Tocopherols (ppm)	800	600–800
Carotene (ppm)	550	550
Totox	1	>5
Iron (mg/kg)	0.1–0.3	5–10
Copper (mg/kg)	0.01	0.05

In the palm oil industry, it is often said that “good quality is made in the field, not in the mill.” This statement clearly emphasizes the importance of maintaining good harvesting practices of fruit bunches in the plantation. A good harvesting practice is one that gives the best compromise between oil yield, oil quality, and harvesting cost. Field factors that determine quality of the palm oil include the degree of ripeness of the fruit bunches, the severity of bruising of the harvested crop, delays between harvesting and sterilization, and contamination of FFB by sand, dirt, or stones (70). Data in Table 37 serve to indicate that the oil extracted from fresh unbruised fruits can have very low FFA and oxidative characteristics compared with that normally traded (50). Precautionary measures taken by mills to minimize hydrolysis, oxidation, and contamination of the crude palm oil are summarized below:

*FFB handling:* minimize bruising and sterilize as soon as possible ( $\leq 24$  hr)

*Sterilization:* optimize conditions, avoid overheating, do not mix boiler condensate with crude palm oil

*Clarification:* eliminate water and impurities; use hermetic system

*Drying:* reduce moisture to 0.17–0.2% before storage

*Processing of Crude Palm Oil.* The ultimate aim of the processing of crude palm oil is to obtain various products such as RBD palm oil, RBD palm olein, or RBD palm stearin that meet the requirement of the end users. An effective and efficient quality assurance program in a processing plant is essential and should consist of the following monitoring activities:

*Raw material:* Each and every delivery must be carefully inspected to ensure that specifications are met and that the shipment is free from contamination. Good raw material is a prerequisite to good-quality product.

*In-process materials:* Regular analyses of important quality parameters will serve as a check that proper processing has been achieved. Good commu-

nication between laboratory and production personnel is of great importance to ensure success of the quality assurance program.

*Finished products:* Regular checks on the finished products must be carried out to ensure compliance to quality specifications before the products are permitted for discharge to storage or shipment.

*Process control:* Proper processing conditions (dosage of processing aids, temperature, pressure/vacuum, flow rate, etc.) must be closely adhered to and monitored to ensure the oil is processed correctly and to minimize undesirable side reactions. In order to assess process efficiency, the oil losses through spent clay or soapstock (as in the case of alkaline refining only) must also be monitored.

#### 4.4. Palm Oil Refinery Effluent Treatment

The characteristics of palm oil refinery effluent vary according to the type of refinery operation (chemical or physical refining, fractionation process, etc.), process control, and housekeeping program. It is quite difficult to derive general characteristics for raw effluent. Therefore the choice of treatment system will depend very much on the complexity of the raw effluent, i.e., its flow and characteristics.

However, there has been a trend among refiners over the last two decades to reduce effluent and other forms of pollution by:

1. Changing from chemical refining to physical refining
2. Automation and strict process control to prevent spillage and product loss
3. Installation of new equipment that is based on low energy and water consumption

In Europe and the United States, there are two basic ways of providing effluent treatment facilities for the edible oil refining industry. One way is for the industry to construct a treatment system at the manufacturing plant site to treat its effluent to an acceptable level for discharge directly to rivers or other public water courses. The second is to discharge the untreated or partially treated effluent to sewers of a local government agency providing wastewater conveyance and treatment services or publicly owned treatment works (71–73). The latter practice is generally termed “joint treatment.” The industry practicing joint treatment is required to provide control and pretreatment to various degrees in order to use the publicly owned facilities.

The main reason for the joint treatment practice is that it costs less than the alternative. The other treatment costs are fairly shared among the users and beneficiaries of the system. Another important advantage is minimizing the space necessary for the treatment facilities. This saving of space is particularly important for crowded industrial estates. Obviously, the joint treatment plant is expected to operate to achieve better efficiency because its operation is carried out by specialized, full-time, and well-trained professionals. Understandably, in any commercial operation,

an effluent treatment plant is way down on the priority list. The industry is quite reluctant to spend money on pollution control equipment. Therefore joint treatment is the most economical and practical choice unless such facility is not available.

**Treatment Method. Oils and Fats Recovery.** The oils and fats recovery system adopted obviously depends on the local circumstances. Typically, the first stage of pretreatment is the use of a physical process to recover the free oils and fats. The most commonly used physical separation process for the removal of free oils and fats are fat traps, tilted-plate separators, and dissolved air flotation units. In addition, centrifuge and electroflotation systems are occasionally used (73).

**pH Control and Chemical Treatment.** pH adjustment is often necessary to prepare the effluent for subsequent treatment processes, especially the biological ones. Chemical treatment involves the use of coagulants and flocculants such as ferric chloride, aluminum sulfate, lime, and polyelectrolytes to reduce the total fatty matters prior to the separation by flotation and sedimentation processes. pH adjustment is often required for optimum results. Chemical treatment is usually required for effluent from a chemical refining process.

**Aerobic Treatment Process.** Effluent from edible oil refineries has been shown to be amenable to biological treatment, both anaerobic and aerobic processes (74,75). The application of activated sludge process or aerated lagoon in this context is well established in the edible oil industry (71, 73, 76). Figure 14 shows the process flow of a typical activated sludge process.

Aerated lagoon treatment was very popular in the United States (73). The main disadvantage of the aerated lagoon as compared to the activated sludge process was that it required large land area and long hydraulic retention time (5–20 days). The long retention time was required because of the low mixed-liquor suspended solids (MLSS) concentration in the aerated lagoon. There was no recycle of the MLSS from the discharge to the aerated lagoon. On the other hand the MLSS in the activated sludge process is maintained at optimum level by recycling the MLSS, which is a standard feature of the process. The MLSS is normally maintained between 2000 and 5000 mg/L depending on the process requirement. Thus the retention time can be very short, a fraction of a day.

The effluent from edible oil refinery tends to be deficient in nitrogen for aerobic biological treatment. Nitrogen has to be added to fulfill the nutrient requirement. In general, a ratio of BOD:N of 100:5 is required for biological treatment. Phosphorus is generally present in adequate amounts in the effluent. This is because phosphoric acid is used in the refining pretreatment process. A ratio of BOD:P of 100:1 is adequate for the same purpose.

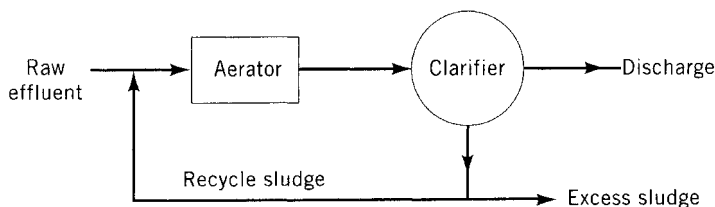


Figure 14. Conventional completely mixed.

Total fatty matter (TFM) concentrations in the raw effluent can lead to poor performance of the treatment process. Therefore, care should be taken to ensure efficient removal of TFM in the pretreatment (physical/chemical) process. TFM is one of the main contributors to BOD.

Another important design criterion that affects the treatment process performance is the organic loading rate. Typically, good treatment efficiency can be achieved when the organic loading rate is less than 0.15 kg BOD per kilogram MLSS per day. Under good maintenance and operation conditions, the discharge BOD and suspended solids (SS) concentration of 20 and 30 mg/L, respectively, can readily be obtained.

The treatment system for those refineries employing the chemical refining processes consists of a train of processes with balancing tanks with pH adjustment, chemical and physical treatment facilities (coagulation and flocculation as well as air flotation), and the activated sludge process (40). The main problems encountered in the operation of the activated sludge plant are the high fluctuations of the loading rates (both hydraulic and organic) and the requirement of close system monitoring and supervision by skilled operators. This was much lacking in the industry. Thus the process seldom achieved the expected treatment efficiency.

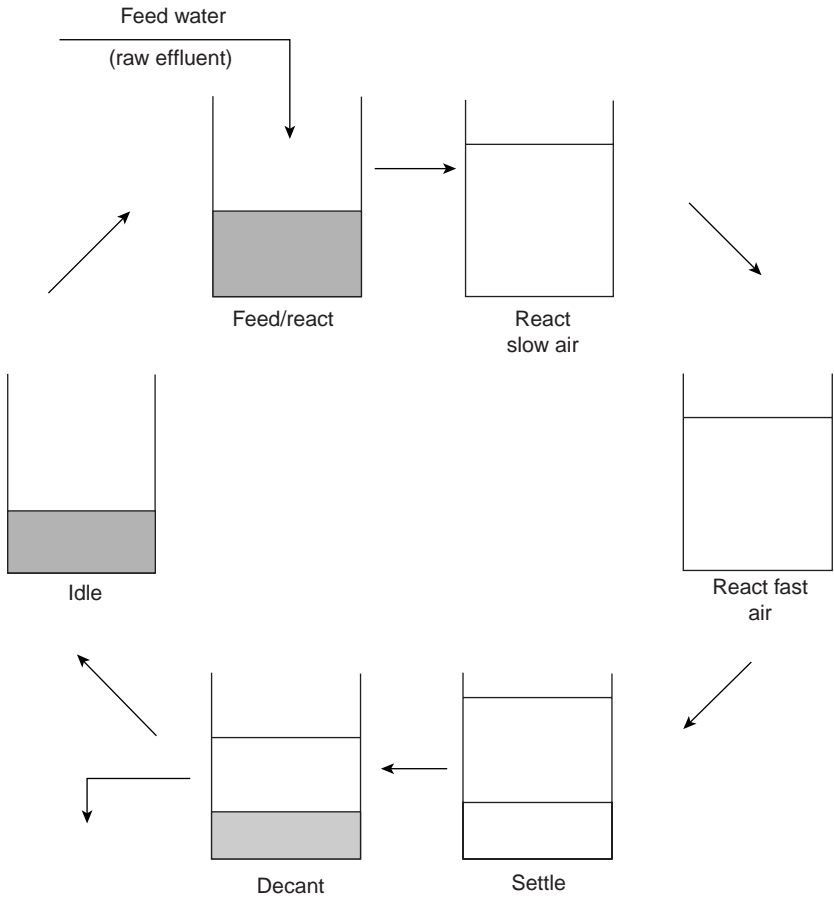
There has been very little publication/information on the treatment of palm oil refinery effluent. Osenga (41) introduced a treatment process consisting of a cross flow interceptor (CFI) for oil separation, physical and chemical treatment, and air flotation units to remove the flocs followed by a batchwise activated sludge process for the liquid effluent treatment. This process also requires close supervision in order to achieve the desired treatment efficiency.

Chin and Wong (74) attempted to treat palm oil refinery effluent by conventional activated sludge process with limited success. The treated effluent was highly colored with over 800 Hazen units.

*Sequencing Batch Reactor—A New Treatment Process for Palm Oil Refinery Effluent.* Since the early 1970s, an alternative aerobic process called the sequencing batch reactor (SBR) has gained much popularity in the treatment of various types of wastewaters. It is very similar to the old fill and draw (batch) system. There are essentially five modes of operation in the SBR process: fill, react, settle, draw, and idle.

All these operations take place in a single reactor instead of two as in the conventional AS process (Figure 15). These operations have been made simple by the advent of reliable and inexpensive microprocessor-based controllers. The process has been found to be very efficient in the treatment of a variety of wastewaters.

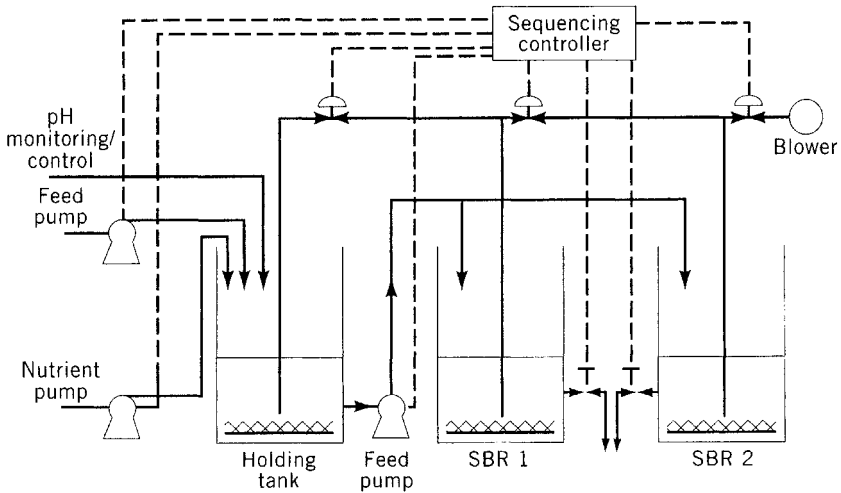
Arora and co-workers (77) have reported that SBR has many advantages over the conventional AS process. These advantages include equalization, ideal settling, simple operation, compact layout, and cost saving (capital and running costs). Irvine and co-workers (78–80), Palis and Irvine (81), and Melcer and co-workers (82) have used SBR to treat wastewater from small communities. Excellent removal efficiency of BOD and nitrogen were recorded. Lo and co-workers (83,84) and Ng (85) have also successfully applied the laboratory-scale SBR treatment to milking parlor wastewater and piggery wastewater, respectively.



**Figure 15.** Schematic representation of SBR cycle.

**SBR Treatment Plant Characteristics.** The flow diagram of the SBR treatment plant used is shown in Figure 16. It consists of one holding tank and two reactors. The effluent, consisting of circuit bleed water from the barometric condenser, cooling water, floor washing and cleaning water, is collected in a sump where any oil and fatty matter is recovered. It is then pumped to the holding tank where nutrient and pH adjustments are carried out, if necessary, before it is fed to the one of the reactors by a centrifugal pump. Air is supplied by a compressor through cone-shaped diffusers installed at the bottom of the reactor. Discharge of the treated effluent is controlled by the operation of a valve. Time for each mode of operation is predetermined. All the operations including the switching on and off of the pumps and compressor, opening of discharging valve, etc., are controlled by a microprocessor-based sequencing controller as shown in Figure 16. The program can be easily changed on site to meet the operation requirements.





**Figure 16.** Schematic diagram of full-scale SBR process.

**Characteristics of Palm Oil Refinery Effluent.** Table 38 shows the characteristics of the effluent from a typical palm oil refinery employing a physical refining process. The effluent is slightly milky and is acidic in nature. It can be seen that the characteristics of the effluent vary quite widely. Nutrient (N and P) contents seem to be sufficient for biological process according to BOD : N : P of 100 : 5 : 1 ratio. It contains low suspended solids and fatty matter, which are mainly dirt and free oil from washwater and oil spillage.

**TABLE 38. Characteristics of Palm Oil Refinery Wastewater (physical refining and dry fractionation process).**

Parameter <sup>a</sup>	Range	Mean
Temperature (°C)	30–45	35
pH	3.8–7.0	5.3
BOD	50–1500	530
COD	150–3000	900
TS	100–2000	580
SS	50–100	80
TN	0–20	10
P	1.0–10	4
O&G	25–600	200

<sup>a</sup>All parameters in milligrams per liter (mg/L) except pH and temperature. BOD, biochemical oxygen demand; COD, chemical oxygen demand; TS, total solids; SS, suspended solids; TN, total nitrogen; P, phosphorus; O&G, Oil and grease.

**Process Performance.** The performance of the SBR process is shown in Figure 17. It can be seen that very stable and consistent performance can be achieved. The process could sustain high fluctuations in feed chemical oxygen demand (COD). With the feed COD varying between 240 and 1000 mg/L, the SBR could produce highly purified final discharge. The COD and SS were consistently less than 100 and 50 mg/L, respectively. It has been established that when the COD is less than 250 mg/L, the BOD is consistently less than 50 mg/L.

Very good settling characteristics of the sludge were also observed. The MLSS settled very well in less than 30 min (Figure 18). The SVI of the sludge was less than 50 mL/g. Random checking on the viability of the sludge showed that the MLSS consisted of over 80% of MLVSS.

## 5. END USES

Palm oil is used in both edible and nonedible applications (Figure 19). Ninety percent of palm oil and its products are used for edible purposes. Currently palm oil is used in food preparation or food manufacture worldwide. The remaining 10% of palm oil and its products are used for nonedible applications, mainly in the soap industry and in the manufacture of oleochemicals.

### 5.1. Food Applications

The use of palm oil in food dates back 5000 years. For edible and nonedible uses, palm oil is normally refined. However, even today, unrefined palm oil is still used for cooking in certain African villages much the same way as it used to be. Examples demonstrating the range of palm oil applications in food are shortening, margarine, vanaspati, deep frying fat, and specialty fats.

*Shortening.* At 20°C, palm oil has 22–25% solid fat content and is a valuable ingredient for shortening formulations. Unlike margarine, which is an emulsion of about 80% oil and 20% water, shortening is pure (100%) oil and fat.

There are many types of shortenings, each tailor-made for a particular application. There are also general-purpose shortenings that are used in the preparation of many foods: in cooking and frying and in the manufacture of bakery products such as cakes, cookies, rusks, wafer, pastries, and bread (86, 87).

Other related bakery products include cream fillings and icings. One important function of a shortening is to incorporate and hold air, whether beaten in a cake batter or creamed with sugar (87, 88). This ability to trap air enables the formation of a porous structure and increases the volume of the cream or the baked cake. This in turn influences texture: shortening contributes to tenderness of various baked products. For optimum creaming ability and to be functional in cakes, the shortening must be stable in the  $\beta'$  form. The  $\beta'$  form refers to tiny fat crystals that are responsible for the smooth texture of the shortening and aid in incorporation of numerous air bubbles during the creaming process. In this respect palm oil shortening is at an advantage because the crystals exist in the  $\beta'$  form (87, 89).

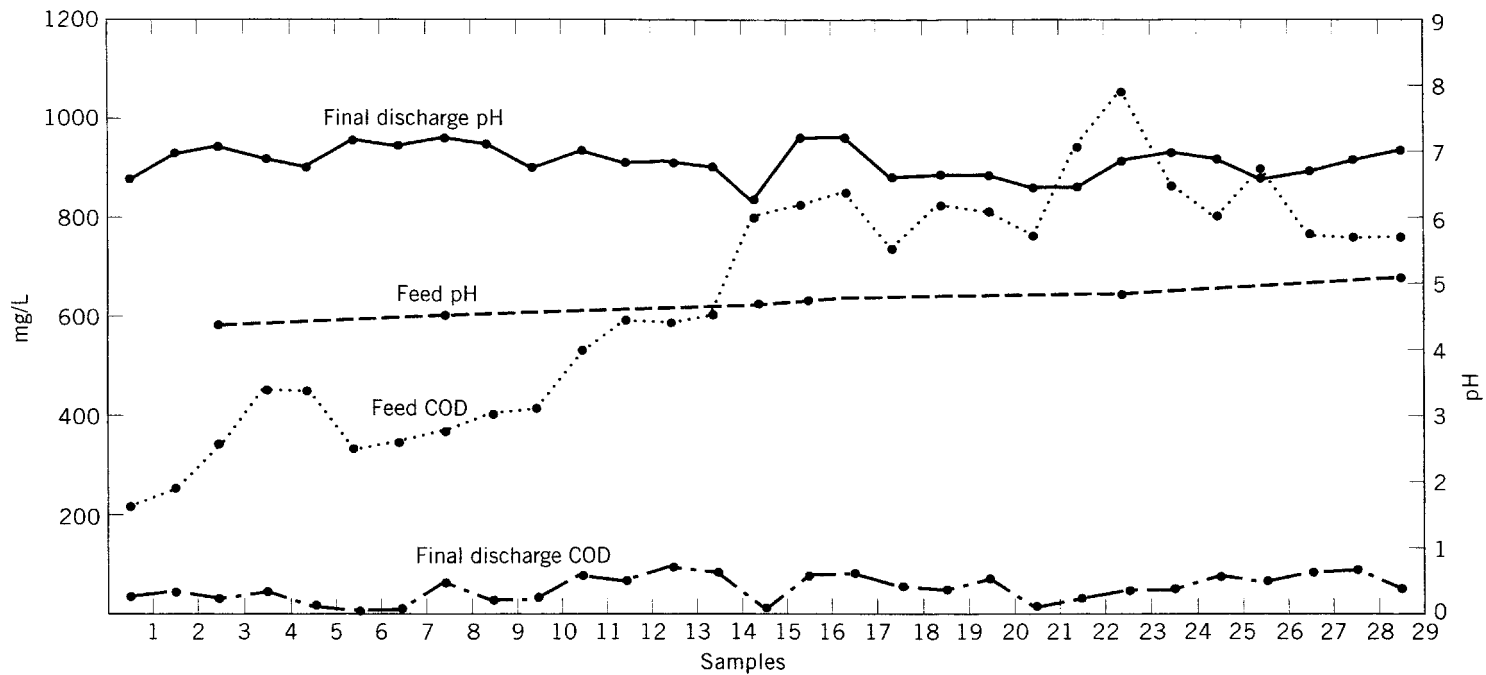


Figure 17. Performance of sequencing batch reactor.

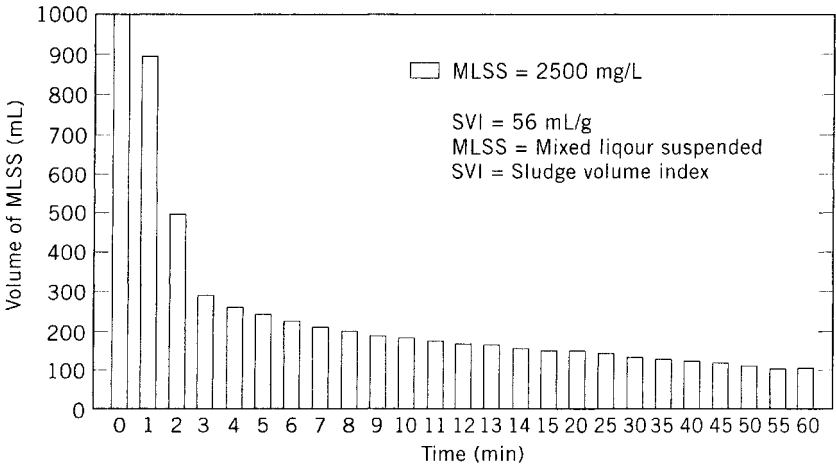


Figure 18. Setting characteristics of MLSS.

In a study on some shortening formulations based on palm products in combination with other vegetable oils, Idris and co-workers (88) reported that a high-palmitic-acid content was good for aeration of fat/sugar mixtures. The results of the study indicated that palm stearin—cottonseed oil (3 : 2) shortening was best for aerated cream filling. Table 39 shows baking performance of some palm-based shortenings. A blend of palm stearin and low-erucic-acid rapeseed oil was very economical and performed excellently in cakes. For application both in cream fillings and baking, interesterified palm olein was the suitable material (88).

Palm oil can be blended with butterfat for use as a biscuit shortening or alternatively diacetyl flavoring can be added to palm oil shortening to give the desirable buttery taste (90). Idris and co-workers (90) reported that biscuits made with shortenings containing palm oil and butterfat were not significantly different in flavor from those made with 100% butterfat. In another study, Idris and co-workers (91) reported the texture characteristics of pressed cookies made with oil, hydrogenated palm oil, and interesterified palm oil shortenings. With higher solid fat content and firmer consistency, shortening based on hydrogenated palm oil produced firmer dough and harder as well as crispier cookies.

*Margarine.* Margarine is a type of emulsion consisting of fat and water. Although the original purpose in developing margarine was to imitate butter, there has since been a considerable diversification of margarine products, which now include:

- Table margarine in tubs
- Table margarine in block form
- Cream/cake margarine
- Margarine for tropical climates
- Puff pastry margarine

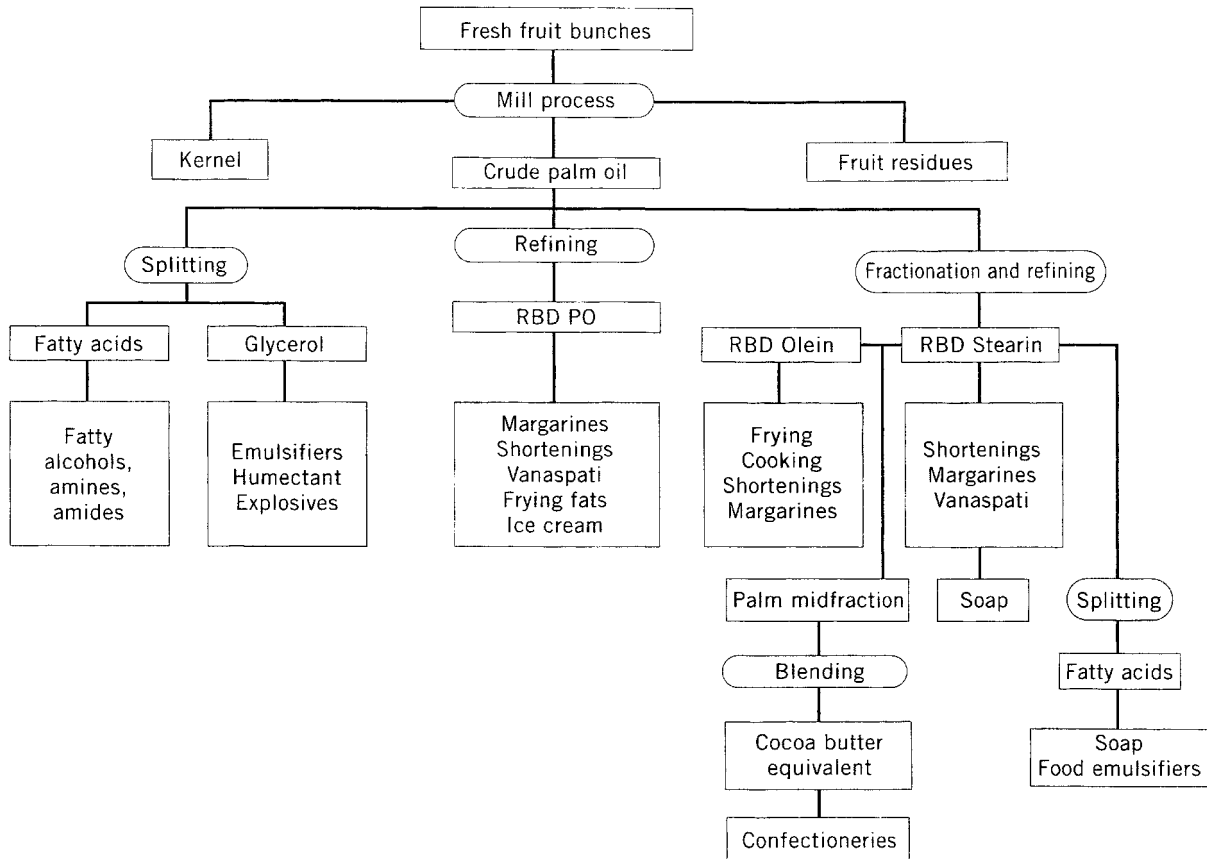


Figure 19. Palm oil utilization chart.

**TABLE 39. Baking Performance of Shortenings Based on Palm Products in Combination with Other Vegetable Oils (88).**

Shortening Composition	Specific Cake Volume Experimental/Control×100 (%)
18% Hydrogenated palm oil (MP 41.5°C) 42% Palm stearin (IV 44) 40% Soybean oil	95
18% Hydrogenated palm oil (MP 41.5°C) 42% Palm stearin (IV 44) 40% Cottonseed oil	90
18% Hydrogenated palm oil (MP 41.5°C) 42% Palm stearin (IV 44) 40% Low-erucic-acid rapeseed (LEAR) oil	101
50% Palm stearin (IV 44) 50% Soybean oil	96
50% Palm stearin (IV 44) 50% Cottonseed oil	96
50% Palm stearin (IV 44) 50% Low-erucic-acid rapeseed (LEAR) oil	101
60% Palm stearin (IV 44) 40% Soybean oil	96
60% Palm stearin (IV 44) 40% Cottonseed oil	95
50% Palm stearin (IV 44) 50% Low-erucic-acid rapeseed (LEAR) oil	97
100% Interesterified palm olein	99

There are also low-calorie spreads that are similar to margarine in their physical behavior but have a much higher water content. The physical properties of margarines are largely determined by the fat component, and these properties vary with the type of product. Thus tub margarines are soft and are spreadable straight from the refrigerator. Table margarines in packets are not as soft but are spreadable at room temperature, while cake or cream margarines are a little firmer than table margarines. At the extreme end, pastry margarines are much firmer, in order to give the flaky texture to the end product. Palm olein is suitable as the liquid component of margarine blends, while palm stearin or hardened palm oil can be used as the solid component (92). Ward (93) recommended that at least 10% palm oil be incorporated in canola-based margarines. Palm oil and palm oil products have also been found to be very good ingredients for puff pastry margarine (94).

*Vanaspati*. Vanaspati, or vegetable ghee, is a major commodity in countries such as India, Pakistan, Egypt, Saudi Arabia, Iraq, and Iran. In India and Pakistan, consumers prefer products with a granular texture. In Iraq and Iran a smooth texture is preferred. Kheiri (95) reported that vanaspati from India contained between 5 and 20% of palm oil products. A higher percentage, more than 50% of palm oil products, has been reported in Pakistan vanaspati formulations (95).

**TABLE 40. Summaries of Published Work on Frying in Palm Oil.**

Authors	Country	Conclusion
Von Zeddelman and Wurziger (96)	Germany	Hardened groundnut oil and palm oil products best
Faur (97)	France	Palm olein and palm oil excellent for catering
Toregard and Eriksson (98)	Sweden	Palm oil and palm olein superior to hardened soybean oil
Herendi and Bethke (99)	Germany	Palm olein performed as well as groundnut oil

*Deep Frying Fat.* Palm oil is the most widely used industrial frying fat because it has no unpleasant room odor, has high resistance to oxidation, does not polymerize to gums, and has a nutritionally good fatty acid composition (50% unsaturated and no trans acids). A number of published reports show palm oil products in a favorable light when compared with alternative frying media (Table 40). The good frying properties of palm oil are due to its moderate degree of unsaturation, the absence of linolenic acid, and the presence of tocopherol. The tocopherol (380–890 ppm) acts as an effective natural antioxidant (100). For industrial frying of instant noodles, palm oil is very suitable (101).

The liquid fraction of palm oil, palm olein, is also widely used for frying. In fact, in Malaysia, it is now the main cooking oil used in most households. During fractionation the tocopherols are somewhat concentrated in the palm olein, so that refined palm olein typically has 500–600 ppm total. The longer frying life and its reduced tendency to foam and polymerize make it a better frying oil than corn or soybean oils (102). In another frying study, Augustine and co-workers (103) found that palm olein was comparable in terms of oxidative stability during frying with the hydrogenated vegetable oils, namely hydrogenated soybean, hydrogenated sunflower, and hydrogenated cottonseed oils. The ability of palm olein to produce fried foods of acceptable quality without the need for hydrogenation can be considered an advantage.

## 5.2. Specialty Fats

Palm oil and palm kernel oil are also ideal raw materials for the production of specialty fats. Specialty fats are particularly suitable for confectionery products, especially chocolates. Specialty fats can be classified according to their chemical composition into three types: (1) symmetrical, (2) lauric, and (3) high trans.

*Symmetrical-type* specialty fats contain predominantly SOS-type triglycerides. The major triglycerides in cocoa butter are POST, StOSt and POP. These triglycerides, comprising about 75% of the total, are often summarized as SOS or symmetrical triglycerides. Solvent fractionation (104) of palm oil (Figure 20) produces a midfraction with a high content of the POP triglyceride but deficient in StOSt and POST. This deficiency can be corrected by adding Illipe fat (or Borneo tallow), which contains these glycerides. Therefore the physico—chemical characteristics

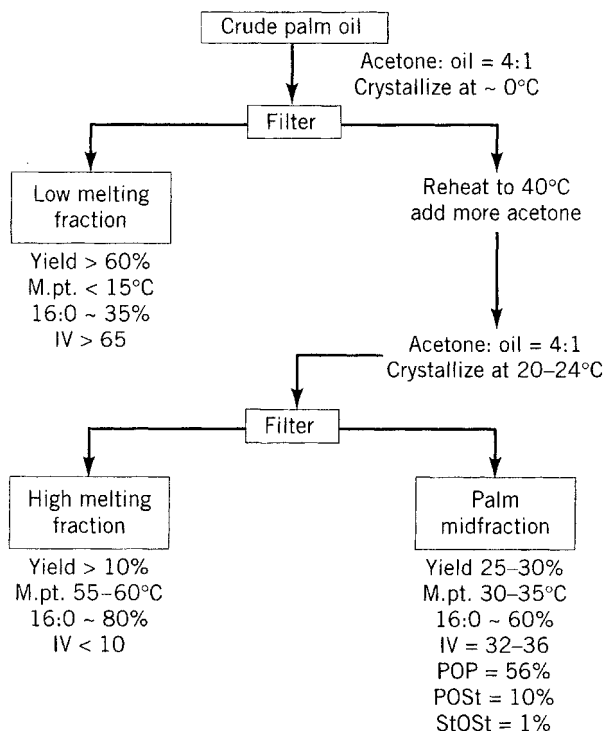


Figure 20. Acetone fractionation of palm oil.

of cocoa butter can be closely matched by correctly blending palm midfraction with Illipe fat.

Because of the similarity in the chemical compositions of the symmetrical-type fats and cocoa butter, they are compatible with each other in almost any proportions, and for this reason these specialty fats are usually called *cocoa butter equivalents* (105). In certain countries, legislation allows up to about 15% of the cocoa butter in chocolate to be replaced by symmetrical-type specialty fat and the product may still be described as "chocolate." In terms of texture and flavor these products are very close to real (cocoa butter) chocolate.

*Lauric-type* specialty fats are produced from oils containing mainly lauric and myristic acids. The simplest lauric-type fats can be made by hardening palm kernel oil to slip melting points between 32 and 41°C. Palm kernel oil can also be fractionated to give a stearin with much better melting properties than the hardened palm kernel fats. The palm kernel stearin with physical properties resembling that of cocoa butter, is called *cocoa butter substitute*, or hard butter. It is usually hydrogenated to further improve its meeting profile.

Hydrogenated palm kernel oil or olein is used as a cheaper alternative toffee fat to replace the more expensive dairy butter, either completely or in combination with butter. Hydrogenated palm kernel oil is also a good *general-purpose coating fat*.



*High-trans-type* specialty fats can be produced by a combination of selective hydrogenation and fractionation from liquid oils. These high-trans-type fats can be produced by selectively hydrogenating blends of soybean oil and palm olein or palm olein alone. They are more compatible with cocoa butter than the lauric-type cocoa butter substitutes, thus they are sometimes called cocoa butter partial replacers.

### 5.3. Recent Food Applications

The newer applications of palm oil in foods include its use in emulsion-based, powdered, and convenience food products. Butterfat has been traditionally used in ice cream, but palm oil and palm kernel oil are now used commercially to replace it. Similarly, palm oil can also replace butterfat in the manufacture of milk, to give a product known as “filled milk.”

Palm oil is used because it is more economical than other oils and is easily available. In addition, it is more stable to oxidation than butterfat. Filled-milk powder can be made from skimmed-milk powder recombined with refined palm oil.

Another use of a palm oil product is in infant food formulations. The low-melting olein has been found to be very suitable for use in infant food formulations when blended with other vegetable oils. Low-melting olein contains 10–15% palmitic acid in the 2-position of the glycerol chain. This contributes to the high digestibility of the product (106).

Apart from the products mentioned, there are many other foods that contain palm oil and palm kernel oil products. These include soup mixes, cake and dessert mixes, “rendang” or curry mixes, sardines, baked beans, breakfast cereals, shrimp-paste powder, bouillon, peanut butter, and beverages. Palm oil products have also been used as a spray oil on biscuits.

### 5.4. New Potential Food Applications

An important future application of palm oil in food is the use of refined red palm oil in cooking. Refined red palm oil is a highly nutritious oil rich in vitamin E and  $\beta$ -carotene. Nor Aini (107) reported that the deep red color of the oil blends well with ingredients such as chili and curry, making the dishes more attractive and appealing. The use of refined red palm oil is a possible alternative means of combatting vitamin A deficiency, which is prevalent in many countries.

Another promising application of a palm oil product is the use of RBD palm olein of high IV as salad oil. The use of palm olein as salad oil can be made possible by blending it with other vegetable oils (108,109). Yet another potential use of RBD palm oil is as a barbecue oil. Its high stability and bland taste makes it a good choice for this application. The oil acts as a flavor carrier, and it also prevents the barbecued meat from drying out, so that one gets a juicy and tasty end product. Palm oil is indeed a versatile oil. Its applications are varied and it can be used in almost any food.

### 5.5. Nonedible Applications

For simplicity the nonfood uses of palm oil and palm kernel oil and their products will be divided into two categories, i.e., those where products are made directly from the oils (direct route) and those where they are obtained via the oleochemicals route (Figure 21).

*Direct Route. Soaps.* Soaps are derived from oils or fats by reacting them with caustic soda at 80–100°C in the process known as saponification. The use of soap as a laundering agent and for cleansing the skin is many centuries old. Although modern detergents have almost eliminated the use of soap for home laundry purposes, soap is still the main ingredient in toilet bars for personal use. In 1990 the world consumed 8.9 million tons of soaps, and consumption is expected to grow at 2.2%

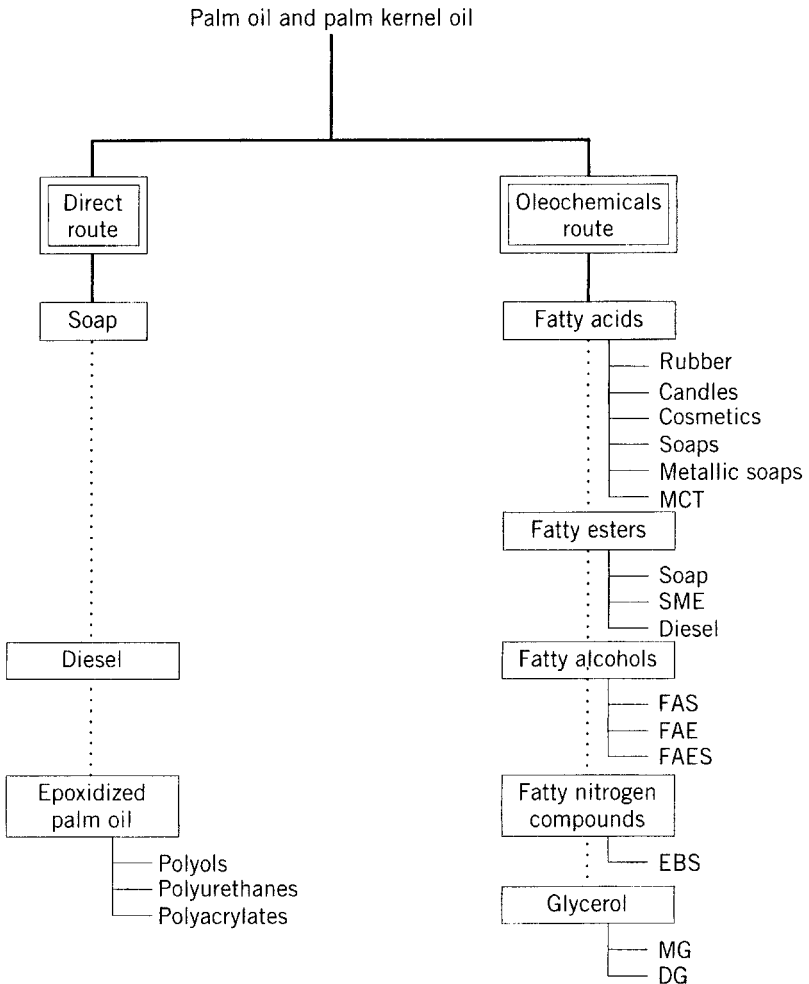


Figure 21. Nonfood applications of palm oil and palm kernel oils.

TABLE 41. Fatty Acids Compositions of Selected Oils/Fats.

Fatty Acids	Weight Percentage							
	Palm Oil	Palm Stearin	Tallow	Palm Kernel Oil	Palm Kernel Olein	Coconut	Palm Olein	Soybean Oil
C6	—	—	—	0.3	0.4	0.2	—	—
C8	—	—	—	4.4	5.4	8.0	—	—
C10	—	—	—	3.7	3.9	7.0	—	—
C12	0.2	0.3	—	48.3	41.5	48.2	0.2	—
C14	1.1	1.3	2.5	15.6	11.8	18.0	1.0	—
C16	44.0	55.0	26.6	7.8	8.4	8.5	39.8	6.5
C18	4.5	5.1	21.8	2.0	2.4	2.3	4.4	4.2
C18:1	39.2	29.5	42.8	15.1	22.8	5.7	42.5	28.0
C18:2	10.1	7.4	2.3	2.7	3.3	2.1	11.2	52.6
Other	0.8	0.7	4.0	0.1	0.1	—	0.9	8.0
IV	53.3	35.5	35–48	17.8	25.5	9.5	58.4	133
SAP. V	196	199	195	245	—	256	198	192

per annum, slightly faster than the world population (110). The developing countries are expected to show higher growth rates in soap consumption than the developed countries.

The incorporation of C16–C18 and C12–C14 fatty acids in soaps is important as they provide the cleaning, solubility, and foaming properties required. Tallow and coconut oil have been the traditional sources of these fatty acids. A comparison between the fatty acid compositions of palm oil, palm stearin, tallow, palm kernel oil, palm kernel oleins, and coconut oil (Table 41) indicates that the first three are rich in C16–C18 fatty acids while palm kernel and coconut oils are rich in C12–C14 fatty acids. However, for palm products to establish a niche in the market as raw materials, soap manufacturers have to be convinced that apart from price competitiveness, they will yield soaps with properties and performance comparable if not superior to those from tallow and coconut oil.

Palm stearin (POs) and palm kernel olein (PKOo) are produced, along with palm olein (POo) and palm kernel stearin (PKOs), when palm oil and palm kernel oil (PKO) are fractionated. While palm olein and palm kernel stearin have higher added value because of their specific food applications, POs and PKOo are normally sold at discount prices. Several studies carried out by Kifli and co-workers (111) revealed that POs and tallow can be formulated together with PKO to give soaps that are comparable with tallow-PKO blends (Tables 42, 43, and 44). Since POs is cheaper than tallow, the resulting soaps are expected to be cheaper. Perfume retention of palm-based soaps has also been found to be better than that of soaps made from tallow (112, 113). More interesting are the observations of Kifli and co-workers (111) on POs and PKOo blends: Soaps based on these were found to have comparable foaming power and better color.

**TABLE 42. Characteristics of Soaps Based on Tallow-Palm Stearin-Palm Kernel Oil Blends.**

Parameters	Blends				
	00 : 80 : 20	60 : 20 : 20	40 : 40 : 20	20 : 60 : 20	80 : 00 : 20
Acid value	223	222	220	219	216
Titer (°C)	49	44	41	39	39
IV	29	29	27	31	34
Free caustic (%)	0.3	0.3	0.2	0.2	0.1
Moisture	9	10	8	8	9
Hardness	23	22	22	23	21
Foamability	255	255	245	265	260
Whiteness (Hunter)	81	82	84	81	80

**TABLE 43. Characteristics of Palm Oil-Palm Stearin-Palm Kernel Fatty Acids Blends in Soap.**

Parameters	Blends				
	80 : 00 : 20	60 : 20 : 20	40 : 40 : 20	20 : 60 : 20	00 : 80 : 20
Acid value	214	215	217	219	219
Titer (°C)	42	44	46	47	48
IV	38	34	33	30	34
Free caustic (%)	2.1	0.1	0.8	6.7	2.3
Moisture	7	7.6	8.6	8.2	7.8
Hardness	13.5	12.0	12.3	10.5	16.2
Foamability	320	355	340	150	295

**TABLE 44. Characteristics of Soaps Based on Palm Stearin-Palm Kernel Olein Blends.**

Parameters	Blends				
	90 : 10	80 : 20	70 : 30	60 : 40	50 : 50
Acid value	179	185	188	191	196
Titer (°C)	47	44	42	41	40
IV	28	30	28	28	25
Free caustic (%)	0.12	0.05	0.2	0.09	0.1
Moisture	16.3	22.7	25.2	17.2	27.6
Hardness	10	12	11	16	7
Foamability	340	270	315	340	365
Whiteness (Hunter)	94	93	92	90	93

Poor color and discoloration are common complaints expressed by soap manufacturers attempting to use palm products for the production of white soaps. Saponification color value (SCV), which represents the color of the saponified oil, will, to a limited extent, indicate the whiteness of the soap produced from the oil. For white soap the SCV of the oil has to be lower than 3R. Palm products have SCVs greater

TABLE 45. Saponification Color Values of NBD and RBD Palm Oil and Palm Oil Products.

Samples	No.	Original Color		Saponification Color	
		Ave.	Range	Ave.	Range
RBD palm stearin	11	2.1R	1.6R–2.5R	6.4R	5.2R–6.5R
NBD palm stearin	5	1.6R	1.5R	5.6R	5.4R–6.1R
RBD palm oil	16	2.0R	1.2R–2.8R	6.8R	5.5R–9.0R
NBD palm oil	2	1.7R	1.7R	6.9R	6.7R–7.2R
RBD palm olein	10	2.5R	2.1R–2.9R	8.1R	6.7R–8.9R
NBD palm olein	4	2.1R	2.0R–2.3R	8.5R	7.5R–8.9R

than 5R (Table 45) and are therefore unsuitable for the production of white soaps. However, bleaching the oil with hydrogen peroxide has been found effective in reducing the SCV and producing stable white soaps (114). Since the reaction with hydrogen peroxide is exothermic, extra care must be exercised. Endogeneous minor components present in palm oil could be one of the factors causing the color and discoloration. Preliminary effort by Ooi and co-workers (115) identified hydroxy- $\alpha$ -carotene-5,8-epoxide and chrysanthemaxanthin as two of the possible minor components causing the yellow color in palm oil soaps. Besides minor components, soap manufacturers know that the presence of trace metals and synthetic antioxidants contribute to the discoloration.

**Diesel Substitute.** Vegetable oils were used as motor fuel by Rudolf Diesel in 1900 when demonstrating his compression engines (116). Since then many publications referring to similar usage of cracked products of oils and fats have been published.

Recent research (117) has demonstrated that crude palm oil can be used directly as a fuel to run cars fitted with suitable diesel Elsbett engines. The exhaust fumes from crude palm oil engines were found to be cleaner than those from diesel engines, with essentially no sulfur or nitrogen oxides. It is also cheaper and safer to transport crude palm oil than diesel because of the higher flash point (crude palm oil at 240°C vs. diesel at 52°C).

Cost is always the main factor that determines large-scale utilization. However, the initial results suggest that the use of crude palm oil as an engine fuel would be 30% more costly compared with petroleum diesel under Malaysian conditions.

#### **Epoxidized Palm Oil and Products, Polyols, Polyurethanes, and Polyacrylates.**

Epoxidized palm oil and palm oil products (EPOP) can be produced by reacting palm oil, palm stearin, or palm olein with peracids. Preformed peroxyacetic and peroxyformic acids, as well as peroxyacetic acid and peroxyformic acid generated *in situ*, were studied by Ahmad and co-workers (118,119) to find suitable methods for the production of EPOP. The best procedures were found to be preformed peroxyacetic acid and peroxyformic acid generated *in situ* (Table 46).

Epoxidized oils, especially epoxidized soybean oil (ESBO), are used extensively as plasticizers and stabilizers for plastics, particularly polyvinyl chloride (PVC). A plasticizer increases the workability of a plastic while a stabilizer reduces the rate

**TABLE 46. Reaction Conditions for the Production of Epoxidized Crude Palm Oil.**

Reaction Parameters	Preformed Peroxyacetic Acid	Peroxyacetic Generated <i>in situ</i>	Preformed Peroxyformic Acid	Peroxyformic Generated <i>in situ</i>
Mole ratio acid : H <sub>2</sub> O <sub>2</sub>	2.06 : 1	1.1 : 1	4.6 : 1	0.35 : 1
Catalyst (%)	1.52	0.02	—	—
Temperature (°C)	40–45	—	RT	—
% Peracid formed	13.7	—	11.0	—
Epoxid temperature (°C)	60–65	60–70	50–60	50–60
Oxirane oxygen content (%)	2.62	1.87	Low	1.82
				2.08 (toluene)
				1.86 (CH <sub>2</sub> Cl <sub>2</sub> )

of degradation of a plastic by heat, light, or microorganisms. Epoxidized oils can fulfill both functions, and their compatibility with a plastic increases with their epoxide content.

Because palm oil and its products have lower iodine values, the epoxide contents of EPOP are lower than that of ESBO. As plasticizers and stabilizers, EPOP are therefore not expected to perform better than ESBO, but their performance could be made comparable by slight modifications of the formulations. PVC jungle and rain boots plasticized and/or stabilized with EPOP have been produced that are comparable in performance to those plasticized and/or stabilized with ESBO (120).

The value of epoxidized oils lies in the versatility of the epoxide rings. Being labile, they can easily be converted to other useful functional groups, thus diversifying end uses. EPOP can be converted to various polyols by reacting them with short-chain polyhydric alcohols in the presence of catalysts. By changing the ratio of EPOP to polyhydric alcohols and the types of polyhydric alcohols, polyols with a range of hydroxyl values and viscosities can be produced (121–123) (Table 47). Polyols when reacted with isocyanates produce polyurethane foams. The water formed in the reaction acts as an internal blowing agent, thus avoiding the need to use environmentally unfriendly blowing agents such as chlorofluorocarbons.

Polyols from EPOP react with isocyanates at a slower rate than do polyols based on petrochemicals. The resulting foams, however, have regular cell structures and exhibit good hydrophobicity. With suitable formulations these properties could be fully exploited to give rise to interesting products (124).

**TABLE 47. Properties of Palm-Based Polyols.**

Parameters	Ratio of EPOO-Polyhydric Alcohol		
	1 : 1	2 : 1	4 : 1
OH value	350–450	200–300	150–200
Viscosity (mPa S)	980–1300	1300–2100	3500–4700

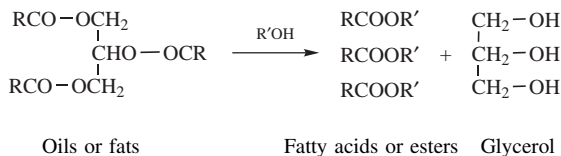
**TABLE 48. Characteristics of Some Polyacrylates Coating Based on Epoxidized Palm Oil/Products After uv Curing<sup>a</sup>**

Additives	Formulations				
	1	2	3	4	5
EPOLA	60	60	60	60	80
PUA	—	—	—	—	5
HPA	10	10	10	10	10
NPGDA	—	—	—	15	—
TMMTA	25	—	—	—	—
TMPTA	—	25	—	10	—
PETA	—	—	25	—	—
Viscosity (cps/25°C)	614	423	705	228	947
Pencil hardness	3H	4H	4H	4H	<2B
	<2B	2B	<2B	<2B	—
Gel fraction (%)	86	87	86	88	84

<sup>a</sup>In all the formulations 5% benzophenone was used as photoinitiator. EPOLA, epoxi-dized palm olein acrylates; PUA, polyurethanes acrylates; HPA, hydroxypropyl acrylate; NPGDA, neopentyl glycol diacrylates; TMMTA, trimethylmethane triacrylates; TMPTA, trimethylpropane triacrylates; PETA, pentacrythritol triacrylates.

Polyacrylate resins can be produced from EPOP by reacting them with acrylic acids. These resins can be applied on solid surfaces and when they are cured by ultraviolet irradiation, clear and glossy finishes result. The hardness and the tackiness can be increased or reduced by varying the amount and types of crosslinkers and the strength of the irradiation used (125–127). Characteristics of some polyacrylates based on epoxidized palm olein are listed in Table 48.

**Oleochemical Route. Oleochemicals.** Oleochemicals are chemicals derived from oils or fats. They are analogous to petrochemicals, which are chemicals derived from petroleum. The hydrolysis or alcoholysis of oils or fats form the basis of the oleochemical industry. The hydrolysis of the triglycerides composing oils and fats produces fatty acids and glycerol. If oils or fats are made to react with an alcohol instead of with water, the process is alcoholysis, and the products are fatty acid esters and glycerol.



In hydrolysis, R' = H.

In alcoholysis, R' = alkyl group.

Fatty acids or their esters can be used as the starting materials for making fatty alcohols and fatty nitrogen compounds. These products can be further modified to produce various derivatives. Hence oleochemicals are often divided into at least two categories: basic oleochemicals and derivatives. The five basic oleochemicals are

**TABLE 49. Production of Basic Oleochemicals from Pacific Regions Compared to the World Production.**

Countries	1990	1995	2000
Malaysia	262,200	806,950	1,200,000
Philippines	172,470	285,000	480,000
Indonesia	62,700	199,500	400,000
Thailand	11,000	22,000	44,000
Total	508,370	1,313,450	1,124,000
World (%)	4,417,000	5,264,000	6,098,000
	12%	25%	35%

fatty acids, esters, alcohols, nitrogen compounds, and glycerol. There are various types of derivatives that can be produced from these through different chemical modifications. Figure 22 shows some of them.

**Raw Materials for Oleochemicals.** Oleochemicals or derivatives based on C12–C14 and C16–C18 chain lengths have a variety of uses. Tallow and coconut oil have been the traditional raw materials used for the production of C16–C18 and C12–C14 chain lengths, respectively. While tallow is produced by the developed countries such as the United States, the world has to rely on the Pacific region for the supply of lauric oils (C12–C14 source). The Philippines has been the main supplier of lauric oils.

**Palm-Based Oleochemicals.** Palm kernel oil, like coconut oil, is a lauric oil. Its fatty acid composition is in fact very similar to that of coconut oil (Table 41).

Table 49 shows the present volume of production of basic oleochemicals in the Pacific region and forecasts of production up to the year 2000 (127). The oleochemicals produced by Malaysia and Indonesia will be based mostly on palm and palm kernel oils, while those from the Philippines will be based mostly on coconut oil. According to this forecast, the ASEAN region will be producing 35% of the world's basic oleochemicals by the year 2000. Malaysia alone will account for nearly 20%.

*Uses of Oleochemicals Based on Palm Oil and Palm Kernel Oil. (a) Fatty Acids.* The most common method for the production of fatty acids adopted by the oleochemicals industry is high-temperature and high-pressure fat splitting. The fatty acid mixture produced is separated into broad cuts or pure fatty acids by simple or fractional distillations. Tables 50 and 51 list examples of fatty acids derived from palm products. The exact specifications of the various fatty acids produced vary slightly depending on the exact raw materials and process used. The specifications could also change due to continuous upgrading of processes.

One of the traditional raw materials used for the production of stearic acid is tallow, and very often, consumers or customers will ask for products equivalent to stearic acid from tallow. Single, double, and triple pressed stearic acids from palm oil are in fact produced via distillation processes, but similar terminologies were used to indicate their similar characteristics. Besides their light color, fatty acids derived from palm products have a low content of unsaponifiables, indicating excellent purity.



TABLE 50. Palm-Based Fatty Acids.

Fatty Acids	Fatty Acid Compositions							
	C8	C10	C12	C14	C16C18	C18:1	C18:2	C18:3
Distilled PKO	3	3	50	15	9	16	2	—
Stripped PKO	—	—	52	17	10	16	3	—
Distilled PO	—	—	0.5	1.5	45.5	37.5	9.5	0.5
Distilled POs	—	—	0.5	1.5	61.6	25	6	0.5
Single pressed	—	—	0.5	1.5	50.0	10	—	—
Double pressed	—	—	0.5	1.5	53.0	3	—	—
Triple pressed	—	—	0.5	1.5	51.0	—	—	—
Lauric 70%	—	0.2	>70	>22	2	—	—	—
Lauric 92%	—	2–5	>92	>2	—	—	—	—
Lauric 98%	—	0–1	>98	—	—	—	—	—

TABLE 51. Palm-Based Fatty Acids.

Fatty Acids	Quality Parameters						
	Acid Value	Sap. Value	Iodine Value	Unsap. Matter	Titer (°C)	Color	
						R	Y
Lauric 70%	270–275	270–275	0.1–1	0.5	33–35	0.5	2
Lauric 92%	277–281	277–281	0.1–0.5	0.5	41–42	0.2	2
Lauric 98%	278–282	278–282	0.1–0.2	0.2	43–44	0.2	1
Rubber g. stearic	>195	>196	<10	—	>52	5.0	50
Candle g. stearic	208–214	209–215	1.0	0.5	53–56	0.4	4
Cosmetic g. stearic	206–211	207–212	0.5	0.5	55–56	0.3	3

Without further chemical modification, fatty acids are used in rubber processing and in the manufacture of candles and cosmetic products.

(b) *Fatty Acids for Rubber Processing.* In rubber processing, fatty acids are added as processing aids with softening effect, as external lubricants and as vulcanization accelerators. The chain lengths of the fatty acids have no effect on the performance of the fatty acids. However, a high degree of unsaturation will interfere with the process (128).

(c) *Fatty Acids for Candles.* Fatty acids or mixtures of fatty acids and petroleum waxes can be used for the production of candles. For maximum shrinkage in order to ensure easy removal from the mold, about 7:2 ratio of C16:C18 fatty acids is required (129). This ratio favors fatty acids from PP since they have higher palmitic acids content.

(d) *Fatty Acids for Cosmetics Products.* Only good grades of fatty acids can be used for the production of cosmetic products. The normal types of fatty acids used are myristic, palmitic, and stearic acids. They are used for various purposes such as lather improver, conditioners, and to provide luster and sheen (128).

(e) *Fatty Acids for Soaps*. The most important application of fatty acids is for the production of soaps via a neutralization process. As discussed, white soaps cannot be prepared directly from PP due to their high SCVs. When palm fatty acids are distilled, part of the impurities are removed and the SCVs of palm fatty acids are usually lower than 3R. Good-quality soaps can therefore be derived from palm fatty acids. Besides the ease in production, the use of fatty acids allow soap formulators to blend their own ratio of fatty acids, thus allowing greater flexibility.

Lately, purely for aesthetic reasons, transparent and translucent soaps are gaining popularity, especially in South America. Stearic acid and triple pressed stearic acids can be used to produce soaps having good transparency. High palmitic acid content, however, appears to reduce transparency (130). The crystals of transparent/translucent soaps based on palm kernel oil and palm fatty acids are found to be in the  $\beta$  form (131).

(f) *Fatty Acids for the Production of Metallic Soaps*. Another important application of palm fatty acids is for the production of metallic soaps other than sodium soaps. The most common are the Ca and Zn palmitates or stearates. They can be prepared either via the fusion or the precipitation method. During the processing of rubber, the processability is improved regardless of the fatty acids used. However, Zn soaps were found to provide better internal lubrication (132).

(g) *Fatty Acids for Medium-Chain Triglycerides*. When palm kernel oil is used as the starting raw material, the medium-chain fatty acids, i.e., C6–C10, present are normally stripped off since these acids are known to cause skin irritation. Originally considered as waste products, these medium-chain fatty acids can be resynthesized into a new class of oil known as medium-chain triglycerides (MCTs). MCTs have many applications such as in the flavor and fragrance industries, in surface treatment of confectionery products, as release agent in the baking industry, and for the lubrication of machines (133).

(h) *Fatty Esters*. Esterification of fatty acids with alcohols and alcoholysis of triglycerides are two of the most common methods used for the production of fatty esters based on palm products. Table 52 lists some of the properties of the various types of palm methyl esters in comparison to fatty acids. Fatty esters are found in several industries such as textiles, cosmetics, pharmaceuticals, plastics, and lubricants. As synthetic lubricants, fatty esters are getting closer attention (128, 134, 135) due to their good lubricity, minimum viscosity change with temperature, low-temperature fluidity, and high thermal and oxidative stability (128).

(i) *Fatty Esters for Soaps Production*. Fatty esters are increasingly being used for the production of pure white soaps (136). In contrast to fatty acids, soaps produced from fatty esters are normally better in quality since the fatty esters can be better purified. In the process, alcohols (usually methanol) will be produced. Complete removal of alcohol is necessary before the soaps can be certified fit for use.

(j)  *$\alpha$ -Sulfonated Methyl Esters*.  $\alpha$ -Sulfonated methyl ester (SME) is a new class of anionic surfactant. SME has received a lot of attention as an active ingredient for

**TABLE 52. Comparison Between Fatty Acids and Fatty Methyl Esters.**

Product	Acid Value	Sap. Value	Iodine Value	Unsap. Matter	Titer (°C)	Color		C12	C14	C16	C18	C18:1	C18:2
						R	Y						
PKO FA	225	256	15	1	22	1	10	47–53	15–19	8–11	1–3	12–19	2–4
	265	266	20	—	26	—	—	—	—	—	—	—	—
PKO FME	1	238	14	0.5	–9	0.3	3	45	14	7	1	12	2
	—	248	19	—	—	—	—	50	18	10	3	19	—
POs FA	206	207	28	1	48	3	30	0	0	55	3	20	4
	216	217	38	—	54	—	—	1	3	70	7	30	—
POs FME	0.5	196	22	1	21	0.5	5	0	0	55	3	20	5
	—	208	45	—	—	—	—	1	3	70	7	30	10
Stearic A	188	188	1	1	65	1	10	—	—	6	92	0	5
98%	195	196	3	—	66	—	—	—	—	8	94	1	10
Methyl stearate 92%	1	187	1	1	36	0.2	2	—	—	6	92	—	—
	195	191	—	—	—	—	—	—	—	8	94	—	—

the production of washing and cleaning products due to several factors that include (136, 137):

1. Easy production procedures
2. Good detergency especially in hard water and in the absence of phosphates
3. C16–C18 have been found to exhibit good detergency
4. Good biodegradation characteristics

Distilled fatty methyl esters with low iodine values are used as the starting raw material for the production of SME. The fatty methyl ester is first reacted with sulfur trioxide at 80–90°C in a falling-film reactor. The dark product obtained from this process is bleached using hydrogen peroxide. After bleaching, the lighter color product is neutralized with alkali to produce an  $\alpha$ -sulphonated methyl ester.

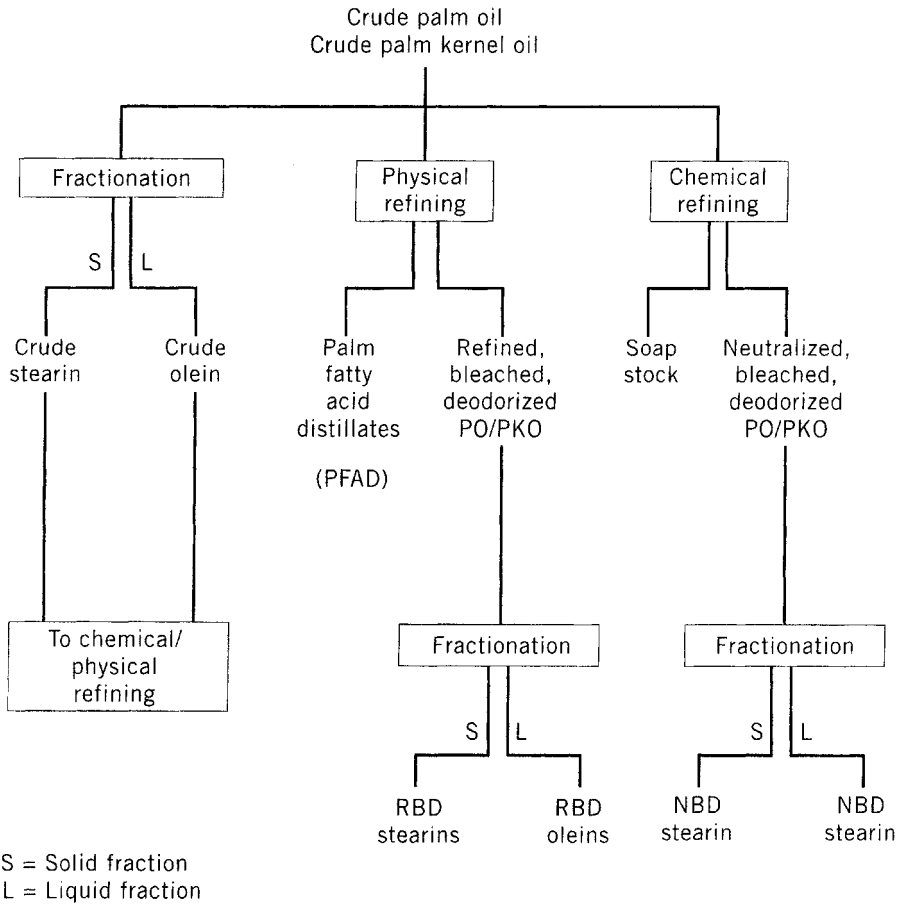
Due to the good detergency properties of C16–C18 fatty methyl esters, palm stearin provides a suitable and cheap source of raw material for the production of SME (138). The detergency properties of SMEs derived from palm stearins have been found to be comparable to linear alkyl benzene sulfonates (LAS), the “workhorse” of the detergent industry. It was interesting to note that SME based on palm fatty acids distillates (PFAD), a by-product of the physical refining industry (Figure 22), performs as well as those derived from fractionated esters. These findings indicate that SME could very well be an important anionic surfactant for the future, and palm fatty acids distillates can be a cheap source of raw materials.

(k) *Diesel Substitute*. Palm-based methyl esters have been extensively tested as diesel substitutes in taxis, buses, lorries, tractors, and stationary engines (139, 140). Methyl esters from crude palm oil and crude palm stearin have very similar fuel properties as petroleum diesel (Table 53). Data available to date indicate that cold starting is easy, engines run smoothly with less unburnt hydrocarbon, CO, SO<sub>2</sub>, and black smoke in the exhaust fumes. No dilution of the lubricating oil was observed and ignition lag was shortened (139). In contrast to crude palm oil, the use of palm methyl esters as diesel substitute does not require any modification of the engines. The economic viability of palm methyl esters as diesel substitute will depend on the cost of diesel, crude palm oil, and glycerol.

(l) *Fatty Alcohols*. The most important application of fatty methyl esters is for the production of fatty alcohols. For economic reasons, three technologies for the production of fatty alcohols have gained worldwide acceptance:

1. High temperature and pressure hydrogenation of oils/fats
2. High temperature and pressure hydrogenation of fatty methyl esters
3. High temperature and pressure hydrogenation of fatty acids

To date the most common method for the production of fatty alcohols is via high-temperature and high-pressure hydrogenation of fatty methyl esters using a



**Figure 22.** Processing of palm oil/palm kernel oils.

copper-chromite catalyst in a fixed-bed reactor. If the unsaturation present in the molecule needs to be preserved, then a Zn-containing catalyst is used.

Novel catalysts (141) such as Ru-Sn and Re-Sn can be used to hydrogenate fatty acids or methylesters to fatty alcohols. Using these catalysts, the hydrogenation can be carried out at the same temperature (250°C) but lower pressure (50 bars), and the unsaturations present remain unaffected. The presence of tin has been found to be instrumental in the preservation of the unsaturation during hydrogenation. Ru-Sn supported on alumina was found to be most selective when they were prepared via the sol-gel method.

Fatty alcohols as such find limited uses. Cetyl and stearyl alcohols are used as suppressors of water evaporation in dry areas. Unsaturated alcohols are used as emulsifiers and textiles auxiliaries. More than 90% of the fatty alcohols produced worldwide are used for the production of anionic (fatty alcohol sulfates and fatty

**TABLE 53. Fuel Characteristics of Malaysian Diesel, Methyl Esters from Crude Palm Oil and Crude Palm Stearin.**

Product Test Conducted	Malaysian Diesel	Methyl Esters of CPO <sup>a</sup>	Methyl Esters of CPS <sup>a</sup>
Specific gravity ASTM D-1298 (°F)	0.8330 at 60.0	0.8700 at 74.5	0.871 at 78.0
Sulfur content (% wt) IP 242	0.10	0.04	0.002
Viscosity at 40°C (cST)			
ASTM D-97	4.0	4.5	4.6
Pour point (°C) ASTM D-97	15.0	16.0	17.0
Distillation D 86 (°C)			
I.B.P.	228.0	324.0	320.0
10%	558.0	330.0	331.0
20%	270.0	331.0	332.0
50%	298.0	334.0	335.0
90%	376.0	343.0	343.0
F.B.P.	400.0	363.0	349.0
Final recovery (%)		98.0	98.5
Cetane index ASTM D-976	53	50	52
Gross heat of combustion (kJ/kg)	45,800	40,135	39,826
Flash point (°C) ASTM D-93	98	174	165
Conradson carbon residue (% wt) ASTM D-189	0.14	0.02	0.02

<sup>a</sup>CPO, crude palm oil; CPS, crude palm stearin.

alcohol ether sulfates) and nonionic (fatty alcohol ethoxylates) surfactants. These derivatives are extensively used in the production of washing and cleaning products (128).

(*m*) *Fatty Nitrogen Compounds*. The most common fatty nitrogen compounds are fatty amides, nitriles, amines, and quarternary ammonium. The most important of these are the quarternary ammonium compounds, better known as quats. Lately manufacturers in the developed countries are voluntarily reducing or stopping the use of quats and imidazoline derivatives in softeners and conditioners in view of the findings that they may not be completely biodegradable and may involve the possible formation of nitrosamine (142) in products containing them.

(*n*) *Glycerol*. Glycerol is a valuable coproduct of the oleochemical industry. Although glycerol can be produced synthetically, natural glycerol (i.e., glycerol derived from oils or fats) are preferred by the customers or consumers of today.

During the production of fatty acids via fat splitting or fatty ester via alcoholysis, mixtures of 10–30% glycerol and water, known as sweet waters are produced. These sweet waters can be processed to pure glycerol via distillation or ion-exchange methods (116). To achieve the pharmaceutical grade further treatment with activated carbon is carried out. Table 54 gives some of the important characteristics of glycerols produced from palm oil.

**TABLE 54. Types of Glycerol Derived from Palm Oil and Products.**

Quality Parameters	Glycerol		
	Crude	99.5%	99.8%
Ref. Density 20/20C	—	1.2623	1.2631
Ref. index $n_D^{20}$	—	1.4731	1.4737
Glycerol %	88	99.5	99.8
$\alpha$ Color	—	5	5
Acid content	—	0.1	0.1
Saponification eqv.	—	1.0	1.0
Ash (%)	1.0	0.01	0.01
Cl (organic cl) (ppm)	—	2(5)	2(5)
Heavy metals (ppm)	2	1	1

Glycerol is a polyhydric alcohol that finds wide uses in several areas of applications. These include; as solvent or drugs carrier in pharmaceutical products; as humectants in cosmetics and tobacco; as ingredients for the production of explosives; as plasticizer/stabilizer for less polar polymers; as antifreeze or heat transfer agent; as hydraulic fluid; for the production of polyesters that can be used in grease and/or lubricants; and for polyols and polyurethanes and mono and diglycerides, which are useful food emulsifiers.

Ability to reduce the surface or interfacial tension of water and oil is one of the properties required of an emulsifier. Different chain length fatty acids in monoglycerides were found to have little effect on the interfacial tension between palm oil and water, in contrast C18:2 (linoleic acid) monoglyceride (143) reduces the interfacial tension to greater than 50% (Table 55).

*Prospects.* The amount of palm oil/palm oil products used in the production of soaps is expected to increase in the near future especially in the developing countries. Besides being price competitive and exhibiting good performances, soaps from palm oil/palm oil products are acceptable by all religions.

There is a strong competition between products derived from oleochemicals and those derived from petrochemicals. With the current awareness on environmental issues and preference for environmentally friendly products, the utilization of palm oil/palm oil products for nonfood applications via the oleochemicals route is also expected to increase. Due to the ready availability of raw materials, technology, capital, and market demand, the nonfood applications of palm oil/palm oil products are expected to have a bright future.

**TABLE 55. Interfacial Tensions of RBD Palm Oil and Water at 1% Concentration of Monoglyceride of Various Fatty Acids.**

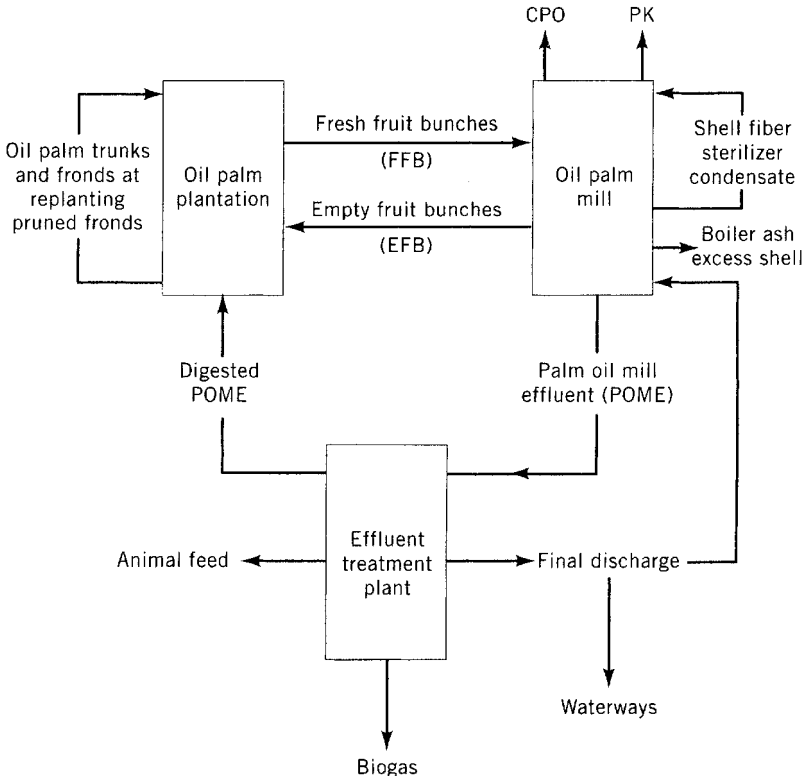
Fatty Acids	C12	C14	C16	C18	C18:1	C18:2
Interfacial tension (mN/m)	11.3	12.2	12.1	13.1	14.8	6.5

## 6. POTENTIAL DEVELOPMENTS

### 6.1. Environmental Trends: Toward Zero Waste Operation

With the growing awareness for the protection of the environment, there is a greater need for producers to improve the environmental profile of their products. Consumers and regulatory bodies expect more information on the effects of products on the environment during their manufacture, use, and disposal. The Life Cycle Assessment (LCA) is used as a holistic approach to assess the impact of a product throughout its life cycle. An important aspect of LCA is recycling and waste management.

In oil palm plantations, the main residues that must be disposed of are the fronds that are pruned regularly and the biomass comprising the palm trunk and fronds at the end of the crop's economic cycle (about 25 years). In commercial practice, pruned fronds are redistributed to the fields while the biomass, senescent palms, are recycled with the zero burning technique of replanting. Without burning the palm residues, this approach does not pollute the air and it enhances soil fertility through recycling of organic matter and plant nutrients.



**Figure 23.** Current use and recycling of by-products and wastes from oil palm plantings and conventional palm oil milling (144).



The main by-products and wastes produced from the processing of palm oil are the empty fruit bunches (EFB), palm oil mill effluent (POME), palm fiber, and palm kernel shell. EFB and POME have been used extensively as mulch and organic fertilizers in oil palm plantations while palm fiber and shell are used as fuel, making the palm oil mill self-sufficient in energy (Figure 23). Excess shell has been used for road surfacing in estates.

The underutilized wastes from the oil mill are biogas generated by anaerobic digestion of POME and clinker and boiler ash; however, the quantities of the latter two are insignificant to cause any environmental impact. In segments of the palm oil industry, biogas is being used to generate heat and electricity to supplement the energy demand by the subsidiary factories.

Although most of the by-products and "wastes" from oil palm planting and palm oil processing are recycled or utilized, there is still room for improvement, the ultimate aim being toward zero waste. Concerted efforts by all sectors are being directed toward improving the efficiency of oil palm cultivation and processing with a view to optimizing the use of inputs and energy and reducing the production of wastes/effluents. Currently, the by-products from the palm oil industry are used mainly as organic fertilizers, soil ameliorants, and fuel. Research and development effort has shown that these resources can be made into value-added products such as fiberboards, furniture, and single-cell proteins. Table 56 shows the potential utilization of oil palm by-products.

## 7. NUTRITIONAL EFFECTS OF PALM OIL

### 7.1. General Nutritional Properties

Archeological evidence shows that palm oil has been consumed by humans for more than 5000 years (145). Its digestion and absorption rates in the human body are in excess of 97%, which is very similar to other common edible oils and fats. In many communities palm oil is an important source of dietary energy and provides sufficient quantities of the essential fatty acid, linoleic acid (18:2, *n*-6) for normal healthy metabolic functions. Like all other edible oils of vegetable origin, palm oil is considered cholesterol free.

### 7.2. Effect of Palm Oil on Blood Lipids

Being arbitrarily classified as a saturated fat, palm oil has been postulated to increase serum cholesterol levels and hence enhance the risk of coronary heart disease (CHD). Recent findings, besides earlier published literature, however, indicate that this hypothesis is not uniformly true. For instance, Kris-Etherton and co-workers (146) demonstrated that feeding a palm oil diet to rats did not raise plasma cholesterol in comparison to a highly polyunsaturated corn oil diet. Similarly, Sugano and co-workers (147) were unable to establish significant differences in plasma cholesterol in rats fed a palm olein diet compared to other polyunsaturated oils. Sundram and co-workers (148) compared the effect of palm oil and its

**TABLE 56. Utilization of Oil Palm/Palm Oil by-Products and Wastes (144).**

Waste/By-product	Quantity <sup>a</sup>	Where Utilized	Present Level of Utilization	Status	Potential New Uses
<i>Oil Palm Plantations</i>					
Pruned fronds	10.4 tons/ha	Recycled in plantation	Very high	By-product	Vitamin E extraction, fiber-board, etc.
Palm trunks and fronds at replanting	89.9 tons/ha	(i) Recycled in plantation	Very high	By-product	Wood product, pulp, paper, animal feed, palm heart, glucose, cellulase, fuel etc.
		(ii) Furniture	Very low	By-product	
<i>Palm Oil Mill</i>					
EFB	20–23%	Mulching in plantation	Very high	By-product	Fiber board: MDF
Fiber	12–13%	Fuel to boiler	Very high	By-product	Fiberboards
Shell	6–8%	Fuel to boiler	Very high	By-product	Activated carbon; potting medium
Decanter solid	2–3%	Land application as fertilizer	Moderate-high	By-product	Animal feed
Boiler ash	0.4–0.6%	Surface landfill in plantation/fertilizer	Low	By-product/waste	Fertilizer/soil ameliorant
Clinker	Small quantity	Landfill/disposal	Very low-low	Mainly waste	Surface landfill
Sterilizer condensate	12–20%	(i) Feed to ETP	Very high	Waste/by-product <sup>b</sup>	Cellulase, single-cell protein
		(ii) Recycle for dilution	Low	By-product	Crude oil dilution
Centrifuge waste	40–50%	Feed to ETP	Very high	Waste/by-product <sup>b</sup>	Oil recovery for acid oil production
Decanter effluent	30–40%	Feed to ETP	Very high	Waste/by-product <sup>b</sup>	
Hydrocyclone/claybath water	5–11%	Feed to ETP	Very high	Waste/by-product <sup>b</sup>	Recycling to reduce quantity
Factory washing tion	4–8%	Feed to ETP	High	Waste/by-product <sup>b</sup>	De-oiling-oil recovery for acid oil production
<i>Effluent Treatment Plant</i>					
Sludge cake		(i) Land application as fertilizer	Moderate-high	By-product	
		(ii) Animal feed	Very low-low	By-product	
Anaerobic solid	5–10%	Land application as fertilizer	Very high	By-product	
Aerobic solid	<5%	Land application as fertilizer	High	By-product	
Biogas	28 m <sup>3/1</sup> EFB	Biogas engine	Very low	Mainly	Heat and power generation

<sup>a</sup>Figures in percentages refer to % to FFB.

<sup>b</sup>These are mainly waste products in the mill but as they are reused immediately after treatment in the Effluent Treatment Plant (ETP), they are considered by-products.

fractions with two commonly used polyunsaturated oils, namely soybean and corn oil. It was demonstrated that palm oil feeding did not elevate plasma cholesterol whereas high-density lipoprotein cholesterol (HDL-C) tended to be raised on the palm oil diet relative to the corn oil diet. Similar observations have also been recorded in other animal models including the rabbit, chicken, and hamsters (149–151).

### 7.3. Human Studies Evaluating the Effect of Palm Oil on Blood Lipids

The effects of palm oil on serum lipids and lipoproteins recorded in animal studies have similarly been observed in several human studies. In some early human studies (152, 153), it was reported that subjects on a palm oil diet had elevated plasma and low-density lipoprotein cholesterol LDL-C levels compared to a diet containing a polyunsaturated fat. However, on a critical reassessment of these and other relevant studies (154), it was found that plasma cholesterol levels after the palm oil period were actually lower than at the point of entry of the experiments when the subjects were on their habitual diets.

Sundram and co-workers (155) performed a dietary intervention trial in a free living European (Dutch) population, consuming a diet that was traditionally high in fat content. The habitual fat intake of this population was maximally replaced with palm oil (up to 70% replacement). The consequence of this fat replacement was carefully monitored over an experimental duration of 6 weeks using a double-blind crossover design. It was found that compared to a Western-type diet, the palm oil diet did not raise serum total cholesterol (TC) and LDL-C. Maximum substitution with palm oil, however, resulted in an elevation of the beneficial HDL2-C while significantly lowering the triglyceride content in the atherogenic LDL fraction. The apolipoproteins (apo AI and apo B), which are increasingly being recognized as better indicators of atherogenic risk, were also regulated by the diet wherein a net beneficial effect (lower Apo B/Apo AI ratio) was evident on consumption of palm oil. Thus palm oil, when used to replace the habitual fat content in a Western-type diet, had no deleterious effects on serum or lipoprotein cholesterol and triglyceride levels. In fact, as demonstrated in this study, the use of palm oil caused a slight improvement of the cardiovascular risk indicators associated with lipoproteins and apolipoproteins.

A human study by Marzuki and co-workers (156) using young volunteers evaluated the effect of consuming foods containing either palm olein or soybean oil. In normal healthy volunteers the level of blood cholesterol was not changed by the palm olein or soybean oil diets. Similarly both LDL-C and HDL-C levels were unaffected by these diets. When the same diets were fed to volunteers having high blood cholesterol levels (hypercholesterolemia), the soybean oil diet was found to induce higher cholesterol levels than the palm olein diet. Similarly LDL-C was also raised by the soybean oil diet.

In a similar experiment conducted on a Malaysian population (157), diets containing palm olein, corn oil, and coconut oil were evaluated for their potential to modulate serum lipids. A reduction in serum cholesterol was observed on administering a palm olein or corn oil diet relative to a coconut oil diet. A second study (158) evaluated the effects of palm olein and olive oil on serum lipids and lipopro-

teins in comparison to a coconut oil diet. Each test oil was served as the sole cooking fat and contributed 23% of the total dietary energy or two thirds of the total fat intake. The coconut oil diet significantly raised all the serum lipid and lipoprotein parameters measured, i.e., TC, LDL-C, and HDL-C. However, the one-to-one exchange between palm olein (rich in 16 : 0) and olive oil (rich in 18 : 1) resulted in identical TC (192. 193 mg/dL). LDL-C (130, 131 mg/dL), and HDL-C (41, 42 mg/dL). This indicates that in healthy normocholesterolemic humans, palm olein can be exchanged for olive oil without affecting the serum lipoprotein concentration or distribution.

In a study of 30 middle aged men, six different fats were used as ingredients of a normal American diet, forming 50% of the total fat intake (159). When palm oil was the test fat, there was no significant effect on TC but HDL-C and apolipoprotein AI was increased while apolipoprotein B was decreased as compared with the baseline diet.

Heber and co-workers (160) evaluated diets enriched in palm oil, coconut oil, or hydrogenated soybean oil for three 3-week test periods in healthy American males. No significant changes in TC, LDL-C, or apolipoprotein AI or B were apparent following consumption of the palm oil diet. They therefore concluded that enrichment of the diet of normal healthy individuals with palm oil does not increase cardiovascular risk factors related to lipids and lipoproteins. Truswell and co-workers (161) compared the effect of palm olein and canola oil on plasma lipids and reported that the mean 3% rise in TC on palm olein compared with a normal Australian diet was predominantly due to a 10% rise of HDL-C.

#### **7.4. Possible Mechanism for the Cholesterol-Lowering Potential of Palm Oil**

It has long been recognized that the cholesterol-raising potential of the saturated fatty acids is variable. Thus, it has been shown that stearic acid (C18 : 0) does not raise serum cholesterol (162). The major saturated fatty acid in the human diet as well as in palm oil is, however, palmitic acid. This, together with lauric (C12 : 0) and myristic (C14 : 0) acids, is considered hypercholesterolaemic (163). Hayes and co-workers (164) recently reexamined this hypothesis in nonhuman primates (monkeys), using dietary fats containing predominantly lauric and myristic acids (coconut oil) or palmitic acid (palm oil). They showed that compared to diets rich in lauric and myristic acids, diets containing palmitic acid were actually neutral in their effect on both total serum and LDL-C. Hayes and Khosla (165) have advanced a hypothesis postulating that the LDL receptor activity is modulated by an "energy threshold effect" of the different saturated fatty acids in the presence of linoleic acid (18 : 2). Above a threshold of 6.5% energy as 18 : 2, saturated fatty acids of any kind have minimal effects. Between 3 and 6.5% energy as 18 : 2, myristic acid (14 : 0) is the only fatty acid to increase LDL-C while below 3% energy as 18 : 2, 14 : 0 is highly hypercholesterolemic and 16 : 0 only moderately so.

These observations have been validated in a recent human study (166) in which 5% energy was exchanged between 16 : 0 and 12 : 0 + 14 : 0, whereas all other fatty acids were held constant. Resident male volunteers received diets (30% as fat) on

4-week rotations. Compared with the 12 : 0 + 14:0-rich diet, the 16:0 diet produced a significant 9% lower serum cholesterol concentration reflected primarily by a lower (11%) LDL-C concentration.

In a follow-up study (167), diets enriched by 16:0 (palm olein), 18:1 (rapeseed oil), or the American Heart Association (AHA) step-one diet were compared by feeding these diets in rotation to 23 volunteers. TC and LDL-C levels were found to be unaffected by these diets, despite the exchange of key fatty acids common in human diets. The AHA diet, however, significantly increased HDL-C while lowering the LDL/HDL cholesterol ratio. There was hardly any difference in the lipid and lipoprotein concentrations of subjects following consumption of the 16 : 0 and 18 : 1 enriched diets.

These human and animal studies provide strong evidence that the lipemic effects of the different saturated fatty acids are not equal. 16 : 0 is hypothesized to behave as a neutral fatty acid (does not raise cholesterol) in normocholesterolemic individuals (<5.2 mmol/L) and when dietary cholesterol intake is low (<300 mg/day). In such situations 14 : 0 appears to be the unique cholesterolraising fatty acid. The lack of 14 : 0 in palm oil and the hypothesized neutrality of 16 : 0 gives credence for the use of palm oil as a dietary oil suitable for the majority of the world's populations.

## 7.5. Nutritional Properties of Minor Components in Palm Oil

Palm oil, both crude and refined, is a rich source of vitamin E, which consists of a mixture of tocotrienols and tocopherols. A technology for the preparation of a locotrinol-rich fraction (TRF) from palm fatty acid distillate has been developed. Palm Vitee (TRF in superolein and encapsulated) has been evaluated in a number of nutritional studies in both animals and humans. The nutritional properties of TRF are as follows:

Qureshi (168) first isolated tocotrienols from barley and proved that they could suppress the hepatic production of cholesterol through their ability to suppress the activity of the enzyme HMG-CoA reductase, which regulates cholesterol synthesis in the liver.

Subsequently, Qureshi (169) extended his investigations to TRF (Palm Vitee) from palm oil in both animal and human models. In a double-blind crossover study involving 20 hypercholesterolaemic human subjects (serum cholesterol >294 mg/dL), Palm Vitee supplementation was found to cause a significant drop in serum TC and LDL-C. The LDL-associated apolipoprotein Apo B was also decreased by 9–11%. Moreover, Palm Vitee supplementation resulted in a significant decrease (25%) in serum thromboxane and platelet factor PF4 by 16%. Similar cholesterol-lowering effects of Palm Vitee have also been indicated in genetically hypercholesterolemic swine (170).

In a similar study Tan and co-workers (171) fed volunteers one Palm Vitee capsule per day for 30 consecutive days. Each capsule contained 18 mg tocopherol and 42 mg tocotrienols. In these volunteers, Palm Vitee lowered both serum TC and LDL-C. The magnitude of reduction for serum cholesterol was up to 36% while reduction in LDL-C ranged from 0.9 to 37% when compared to their respective starting values.

These studies indicate that Palm Vitee is most effective in reducing cholesterol when subjects have elevated blood cholesterol levels. However, these observations have not been uniformly reproduced by different workers. For example, Wahlqvist and co-workers (172) have reported that Palm Vitee has no effect on blood cholesterol levels in their hypercholesterolemic subjects. As a result, it has been suggested that certain population groups behave as “responders” and others as “nonresponders” when given Palm Vitee to manage their hypercholesterolemia. Studies are presently in progress to evaluate the underlying mechanisms associated with these observations.

The structural differences between *d*- $\alpha$ -tocopherol and tocotrienol, viz, the unsaturated side chains in the latter, account for some differences in their physiological activities. Serbinova and co-workers (173) have reported that in membranes palm oil tocotrienols had 40–60 times higher antioxidant potency than  $\alpha$ -tocopherol largely due to a higher recycling efficiency and uniform distribution in membrane bilayers. Under oxidative stress, tocotrienols protected human LDL against oxidation and their protective potency was greater than that of  $\alpha$ -tocopherol in the presence of ascorbate. This may be a key factor in protection against the onset of degenerative atherosclerotic disease. A tocopherol-tocotrienol mixture in a ratio similar to that present in palm oil has also been shown to depress the systolic blood pressure and increase the aortic production of prostacyclin in spontaneously hypertensive rats (174).

## 7.6. Effect of Palm Oil on Experimental Carcinogenesis

Sundram and co-workers (175), using a rat model treated with the chemical carcinogen DMBA, evaluated the effect of palm oil on the progression of mammary tumors. Both crude and refined palm oils were evaluated against the polyunsaturated corn oil and soybean oils. Rats fed either 20% soybean oil or corn oil developed tumors first at 9 weeks following DMBA administration. The appearance of tumors was more rapid and enhanced in the polyunsaturated corn and soybean oil fed rats compared to the palm oil groups. At the time of sacrifice tumor incidence was 90% in the soybean fed rats, 85% in with corn oil, and only 65% with crude palm oil maintained rats. At the same level of fat intake, tumor incidence in animals fed polyunsaturated oils was significantly higher than in rats fed palm oil diets. Tumor yield in the palm oil groups was significantly lower than that in the corn or soybean oil diet.

In an earlier study Sylvester and co-workers (176) found that palm oil induced lower tumor numbers and tumor load per rat comparable to a low-fat control (5% corn oil). In comparison to a palm oil diet (20% by weight), diets containing 20% corn oil, beef tallow, or lard all resulted in significantly higher tumor numbers and tumor load.

These studies indicate that palm oil exerts an inhibitory effect on the progression of chemically induced carcinogenesis.

The 10% level of linoleic acid in palm oil seems ideal in meeting the nutritional requirements of this essential fatty acid, without eliciting growth responses in the tumor cells in comparison to more polyunsaturated oils. However, when compared

to animal fats such as lard, which contain similar levels of the linoleic acid, it appears that the inhibitory effect of palm oil cannot be attributed to its fatty acid composition alone. The answer may lie in the various minor components present in both crude and refined palm oil. It is postulated that these minor components, more so the tocotrienols, which have antioxidant activity, may be involved in exerting the inhibitory effect on tumor development.

The effect of tocotrienols on cancer progression was evaluated by Komi-yama and Yamaoka (177). The antitumor activity of tocotrienols was evaluated in terms of the increase in the lifespan of mice inoculated with tumor cells.  $\alpha$ -Tocotrienols and  $\gamma$ -tocotrienols were effective against the sarcoma cancer cell lines and Ehrlich carcinoma. When human lung carcinomas were challenged with these tocotrienols, a cytotoxic activity due to the tocotrienols was exhibited. Similarly, DMBA-treated rats responded with lower tumor numbers when their diets were supplemented with palm tocotrienols (178). Recently,  $\alpha$ -carotene isolated from palm oil has been shown to have antitumor activity against mouse lung cancer and against skin cancer (179).

## 8. PROSPECTS OF PALM OIL AND MARKET REQUIREMENTS

In the past decade, palm oil has become internationally well known as a vegetable oil suitable for various applications—both edible and nonedible. This is brought about by it being a versatile oil for the production of various products, with technical and economic advantages over other oils and fats. Its price competitiveness and readily available supply is able to serve the needs of oils and fats consumers worldwide.

### 8.1. Versatility of Palm Products

Palm oil has the flexibility to be used as it is or in fractionated forms to produce a very wide range of products. Interesterification can further significantly modify its properties including crystallization behavior. It has good oxidative stability. It has long been known as a good heavy-duty frying medium because of its relatively low polyunsaturation and the slip melting point, which is low enough to avoid excessive waxiness in most applications (180).

Margarine blends can be developed containing higher levels of palm products and having solids content profiles close to those of popular commercial brands. Standard quality table margarine can contain palm products as high as 70% (50% palm oil and 20% hardened palm oil). Table margarines (packet) can contain as high as 63% palm oil, 30% palm kernel oil, and 7% palm stearin. Blends for random interesterification could utilize as much as 60% of hardened palm oil or palm stearin. Tub margarines could be formulated containing 50% palm oil.

Another major area of use for palm oil due to it being a semisolid fat is in the manufacture of shortenings and vanaspati. Shortenings include a variety of products such as the fats used domestically for cooking, frying, and flour confectionery and those used industrially in cake baking and in large-scale frying operations for products such as potato crisps and doughnuts.

In ambient temperature range of 20–35°C there is a close similarity between butterfat, palm oil, and European shortening. It has, so to speak, been hydrogenated by nature to be very close to many specifications for shortenings and vegetable ghee.

The world consumption of oils and fats can be classified into two equally important categories, i.e., 50% of solid fats and 50% of liquid oil markets. The supply pattern of vegetable oils and fats is not equally divided between the solid and liquid oils and fats. Only palm oil is semisolid accounting for 20% of the vegetable oils market. Under current pattern of consumer demand, there is a shortage in the supply of solid fats and oversupply in the liquid oil market. Most of the liquid oils have to be hydrogenated to turn them into solid fats as shortenings, margarines, and ghee. Palm oil is naturally placed in an advantageous position with respect to the pattern of large consumer demand for solid fats. With skillful formulation, no hydrogenation is required for palm products. Interesterification methods may be used to improve the ability of different components of palm oil to be used to meet customer specifications in the fat products.

Palm oil can also yield liquid fractions for the liquid oil market, i.e., palm olein and stearin can be obtained through fractionation of palm oil. Palm olein, the liquid fraction of palm oil, behaves as a liquid oil in hot climates. It is a very stable frying medium that is comparable to any other frying fat for resistance to breakdown. However, in temperate climates or during cold nights where temperatures are below 18–20°C, palm olein begins to solidify. This problem can be overcome by blending palm olein with a more unsaturated oil. In Japan, a blend of 50% palm olein and 50% rapeseed oil is being marketed successfully.

Palm stearin, the solid fraction of palm oil, has the edge over tallow because of the assurance of expanding supply, while world production of tallow has stagnated. Applications where stearin could replace tallow are in shortenings, frying fats, and soaps. Lard could also be substituted by palm stearin or RBD palm oil in most of its applications.

Palm stearin can be the cheapest source of C16–C18 fatty acids for soap. Palm stearin alone has a very high titer value (47–50°C) such that when a high proportion is incorporated into toilet soap formulation, the soap becomes hard and cracks easily. Experiments conducted indicated that 30–50% of palm stearin could be incorporated with tallow and 20% of lauric (palm kernel) fatty acid to obtain titer of the finished product of between 40.5 and 44°C (181).

## 8.2. Technical and Economic Advantages

Hence, the utilization of palm oil in products requiring a proportion of solid fats in their formulation would offer technical and economic advantages. Substantial cost savings can be achieved when palm oil is used in place of other oils and fats in various applications. Savings are made through the lower costs of raw materials, through the reduction in use of chemicals to process the oil, reduction in the cost of hydrogenation, reduction in costs due to minimal process losses, and savings resulting from the long life of palm oil during the frying applications.

The amount of chemicals required to process oils and fats depends on the level of FFA, color, and other impurities in the oils. Normally about 2 kg of phosphoric



acid, 2.5 kg of caustic soda, and 30 kg of bleaching earth are required to refine 1 ton of crude palm oil. Since processed palm products are available from the markets, importers need not have to refine crude palm oil but can instead import and use processed palm products directly. If unrefined vegetable oils were to be imported, these oils need to be processed by the importers. In addition, liquid oils would require a catalyst, usually nickel, for hydrogenation. Hence, the cost of chemicals would be minimal when RBD palm oil is used.

Palm oil, being a semisolid or consistent fat, results in a reduction in cost if utilized in products requiring a proportion of solid fats in their formulation because hydrogenation is not necessary. If liquid oils are used, they have to be hydrogenated to obtain the solid consistency, which in turn leads to the formation of both *cis* and *trans* isomers (182). Hydrogen is produced by electrolysis, and with the increasing cost of energy, hydrogenation is increasingly becoming expensive. To hydrogenate 1 ton of liquid oil from an IV of 130 to 70 would require 348-kW of electricity. The additional cost incurred by using liquid oil depends on the electricity rates of the countries involved.

The availability of semiprocessed to fully processed palm oil products for trade provides benefits especially to countries that are lacking in refining capacities. If further refining is still needed, losses incurred would be relatively negligible. Refining of crude oils results in losses ranging from 4.2 to 5.5%, depending on whether the oils undergo continuous or batch processes.

Another advantage in the use of imported refined palm oil is the avoidance of having to deal with effluent treatment. When palm oil is refined at the source, such as in Malaysia, physical refining is used instead of alkali refining, and the amount of effluent generated is much lower and can be easily treated.

### **8.3. Meeting World Oils and Fats Demand**

Numerous forecasts on the demand for vegetable oils indicated that the world would require at least 105 million tons of oils and fats from 83 million tons by 1992. This was equivalent to an additional demand of 2.75 million tons per year. In the long run, production of by-products from oilseeds and meat is not expected to rise more sharply than the demand for the main products. Similarly, production of other oils and fats such as groundnut, sesame, olive, coconut, and fish oils including butter are increasing at a rate that is below the demand growth for all oils and fats. Hence, the additional requirement of the world for oils and fats has to be met by the above average growth of only four oils and fats: sunflower, rape, palm, and palm kernel oils. However, oils of sunflower and rape are subject to meal demand and the per hectare returns of competing commodities (grains, pulses, rice, etc.). These thus leave palm oil to be the main oil to cater for the majority of the additional world requirements.

### **8.4. United States Use of Palm Oil**

Table 57 gives U.S. supply and disappearance of edible oils (2).

**TABLE 57. Edible Fats and Oils: U.S. Supply and Disappearance, 10<sup>6</sup> lb (2).**

Item	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002 <sup>a</sup>	2003 <sup>b</sup>
<b>Stocks October<sup>a</sup></b>													
Coconut	277	188	251	164	163	84	150	393	152	136	260	227	148
Corn	138	196	150	118	241	116	129	102	135	267	117	104	114
Cottonseed	137	78	81	106	82	94	66	79	76	49	93	39	40
Lard	24	27	26	34	24	23	20	40	21	18	14	10	5
Palm	53	44	33	35	15	31	46	35	48	48	61	70	42
Palm kernel	53	49	88	73	55	22	51	64	73	49	155	128	50
Peanut <sup>c</sup>	25	51	50	25	40	65	86	41	40	32	31	32	50
Safflower	28	28	18	31	21	44	27	38	48	36	21	17	19
Soybean	1,786	2,239	1,555	1,103	1,137	2,015	1,520	1,382	1,520	1,993	2,767	2,359	1,486
Sunflower	47	100	56	65	82	147	93	60	121	157	136	23	25
Canola	41	71	67	137	54	77	65	112	169	206	110	52	55
Tallow, edible	41	33	41	36	52	34	48	46	43	40	49	24	35
<b>Imports</b>													
Coconut	841	1,163	999	1,100	874	1,188	1,438	791	926	1,115	1,093	860	970
Corn	5	7	7	10	11	14	28	42	18	27	61	65	65
Cottonseed	18	38	26	0	0	0	0	48	8	0	0	22	0
Lard	2	3	3	2	2	1	2	2	2	3	6	10	10
Olive	216	253	262	260	227	304	333	355	397	455	455	485	540
Palm	220	267	368	218	236	322	282	284	345	399	490	425	440
Palm kernel	342	302	304	280	262	392	359	401	393	351	330	470	475
Peanut <sup>c</sup>	1	0	11	4	5	14	10	73	12	79	39	70	70
Canola	815	861	902	938	1,086	1,075	1,088	1,060	1,139	1,193	1,108	929	1,215
Safflower	22	15	16	26	35	30	51	51	33	34	40	43	45
Soybean	1	10	68	17	95	53	60	83	83	73	46	50	85
Sunflower	9	0	7	1	2	22	8	5	4	8	36	60	5
Tallow, edible	6	10	15	18	8	5	2	3	10	32	7	11	10

## Production

Corn	1,821	1,878	1,906	2,227	2,139	2,231	2,335	2,374	2,501	2,403	2,461	2,453	2,650
Cottonseed	1,280	1,126	1,119	1,312	1,229	1,216	1,224	832	939	847	876	725	865
Lard	1,016	1,011	1,015	1,052	1,013	979	1,065	1,106	1,069	1,050	1,080	1,075	1,100
Peanut <sup>c</sup>	356	286	212	314	321	221	176	145	229	179	230	286	219
Canola	32	49	406	299	355	342	451	548	617	641	585	541	629
Safflower	69	87	111	115	127	103	115	111	91	88	76	89	91
Soybean	14,345	13,778	13,951	15,613	15,240	15,752	18,143	18,078	17,825	18,420	18,898	18,435	17,020
Sunflower	911	730	580	1,165	860	840	959	1,177	1,046	873	673	320	595
Tallow, edible	1,515	1,414	1,535	1,550	1,559	1,407	1,517	1,677	1,792	1,764	1,932	2,075	2,000

## Exports

Coconut	22	0	19	18	12	12	6	11	14	8	7	8	10
Corn	566	712	717	865	977	988	1,118	989	970	951	1,172	890	900
Cottonseed	269	184	248	329	221	232	208	111	141	131	150	110	115
Lard	131	129	119	140	94	103	122	140	189	93	90	105	100
Olive	20	15	11	21	24	21	19	15	12	9	10	12	12
Palm kernel	2	9	4	2	2	2	2	2	2	2	2	2	2
Palm	7	7	7	13	20	9	11	11	11	11	10	11	10
Peanut <sup>c</sup>	151	52	61	97	108	21	13	10	18	14	8	42	19
Canola	15	16	76	153	147	295	349	272	284	187	255	166	157
Safflower	73	65	75	93	122	83	83	92	51	35	37	37	40
Soybean	1,644	1,461	1,531	2,683	992	2,033	3,079	2,372	1,375	1,401	2,519	2,250	850
Sunflower	471	586	450	978	628	709	815	800	630	545	453	110	200
Tallow, edible <sup>d</sup>	333	306	316	277	241	181	236	322	224	338	475	485	490

## Domestic disappearance

Coconut	910	1,084	1,067	1,083	941	1,111	1,189	1,021	927	983	1,119	930	958
Corn	1,202	1,220	1,228	1,250	1,298	1,244	1,271	1,394	1,417	1,630	1,363	1,618	1,804
Cottonseed	1,088	975	873	1,007	996	1,012	1,004	772	833	672	780	636	750
Lard	885	886	890	924	922	880	925	987	886	964	1,000	985	990
Olive	216	253	262	260	227	304	333	355	397	455	455	473	528
Palm	223	271	359	225	201	298	282	260	335	375	471	425	427
Palm kernel	344	254	315	295	293	362	344	390	414	243	355	511	458
Peanut	179	236	187	206	193	194	217	208	233	244	260	296	275

**TABLE 57** (Continued)

Item	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002 <sup>a</sup>	2003 <sup>b</sup>
Canola	801	898	1,162	1,165	1,271	1,134	1,143	1,287	1,435	1,744	1,496	1,301	1,687
Safflower	15	47	40	57	17	67	73	59	86	102	89	93	95
Soybean	12,248	13,012	12,939	12,913	13,465	14,267	15,262	15,652	16,059	16,318	16,833	17,108	16,522
Sunflower	396	188	129	171	168	207	186	320	385	357	370	268	385
Tallow, edible	1,197	1,109	1,239	1,275	1,345	1,218	1,286	1,360	1,581	1,449	1,488	1,590	1,515

<sup>a</sup> Preliminary and estimated.

<sup>b</sup> ERS and WAOB forecast.

<sup>c</sup> August-July year beginning 1982.

<sup>d</sup> Disappearance, as defined by the USDA-ERS, means beginning food stocks, production, and imports minus exports, shipments to U.S. territories, and ending stocks.

Source: Bureau of the Census.

## REFERENCES

1. FAO/WHO. *Fats, Oils and Related Products*, Vol. 8, Codex Alimentarius Commission, Rome, 1993, p. 61.
2. Oil Situation and Outlook Yearbook, Economic Research Service, USDA, accessed Oct. 2004.
3. W. L. Siew, T. S. Tang, C. H. Flingoh Oh, and A. A. Rahman, *PORIM Rep.* PO(172) (1990).
4. H. Kifli, Malaysian Oil Palm Growers Council Technical Bulletin No. 3. 1981.
5. H. P. Sow, Ph.D. Thesis. University Science Malaysia, 1979.
6. B. K. Tan, paper presented at the Conference on Chemical Research in Malaysia, held in Kuala Lumpur Mar. 19–21. 1979, Kuala Lumpur, Malaysian Institute of Chemistry.
7. S. C. Yap, Y. M. Choo, C. K. Ooi, A. S. H. Ong, and S. H. Goh, *Elaeis* 3(2) (12), 375 (1991).
8. F. Mordret and A. M. Laurent, *Rev. Fr. des Corps Gras* 25, 245 (1978).
9. Ab. Gapor and co-workers. International Conference on Palm Oil Product Technology in the Eighties, Kuala Lumpur, June 22–24, 1981.
10. M. J. Downes, *Leatherhead Res. Rep. Nos.* 781, (1982), 411 (1983), 487 and 445 (1984), 518, 516, and 519 (1985).
11. W. L. Siew, private communication, 1982.
12. Anon, Malaysian Standard MS 814: 1983.
13. A. H. G. Chin, C. H. Oh, and W. L. Siew, *MARDI Res. Bull.* 10(1), 80 (1982).
14. B. K. Tan and Flingoh C. H. Oh, *PORIM Tech.* (3), (1981).
15. C. H. Oh, Flingoh, private communication, 1982.
16. B. K. Tan and C. H. Oh, *PORIM Tech.* No. (4), (May 1981).
17. C. W. S. Hartley, *The Oil Palm*, Longman, London, 1967.
18. A. C. Zeven, Thesis, University of Wageningen, 1967.
19. R. Godding, *Bull. Agric. Congo Bedge.* 21, 1263 (1930).
20. R. B. Jagoe, *Malay. Agric. J.* 35, 3 (1952).
21. Anon, *Statistics on Commodities*. Ministry of Primary Industries, Malaysia, 1991.
22. R. H. V. Corley in R. L. Wastie and D. A. Earp, ed., *Advances in Oil Palm Cultivation*, Incorporated Society of Planters, Kuala Lumpur, 1973, pp. 37–49.
23. H. A. M. Van der Vossen, Thesis, University of Wageningen (1974).
24. H. L. Foster, M. Tayeb Dolmat, and Gurmit Singh, *Proceedings of International Oil Palm Conference*, PORIM, Kuala Lumpur, 1987, pp. 294–304.
25. D. Whiting, *Market Development of Palm Oil Products*, Geneva, International Trade Center, UNCTAD/GATT, 1982, pp. 108–113.
26. K. H. Lim, *Proc. of 1987 International O. P. P. O. Conf.-Agriculture*, 1992, p. 595.
27. A. N. Ma, *PORIM Bull.* (4), (May 1982).
28. C. F. Chooi, workshop, *Proc. Palm Oil Res. Inst., Malaysia*, (9), 53–62 (1984).
29. K. H. Lim, workshop, *Proc. Palm Oil Res. Inst., Malaysia*, (9), p. 11–41 (1984).
30. K. H. Lim, S. K. Quah, D. Gillies, and B. I. Wood, workshop, *Proc. Palm Oil Res. Inst. Malaysia*, (9), 42–52 (1984).

31. S. K. Quah and D. Gillies, *Proc. of Nat. Workshop on Palm Oil By-Product Utilization*, 1981, pp. 119–126.
32. N. S. Chua and H. L. Giam, paper presented at National Workshop on Recent Development in Palm Oil Milling and Pollution Control Aug. 5–6, 1986.
33. K. W. Chan, I. A. Watson, and K. C. Lim in E. Pushparajah and S. C. Chin, eds., *Soil Science and Agriculture Development in Malaysia*, 1980, pp. 213–242.
34. J. Kanagaratnam, A. L. Lai, K. H. Lim, and B. J. Wood, *Proc. of Nat. Workshop on Oil Palm By-Product Utilization*, 1981, pp. 16–23.
35. C. H. Lim, T. C. Ping, K. W. Chan, and S. Y. Chooi, *Proc. of Land Application of Palm Oil and Rubber Factory Effluent*, Malaysia Society of Soil Science, 1983, pp. 71–89.
36. K. H. Yeow and Gurmit Singh. MOPGC/TRC Internal Report, 1983.
37. N. S. Chua, paper presented at Seminar on Developments in Palm Oil Milling Technology and on Developments in Palm Oil Milling and Technology and Environment Management, May 16–17, 1981.
38. H. K. Jorgensen, *AOCS*, **60**(2), 282 (1985).
39. M. S. A. Kheiri, *J. Am. Oil Chem. Soc.* **62**, 210 (1985).
40. L. R. Strecker, J. M. Hasman, and A. Mazer, "Proceedings of World Conference in Emerging Technologies in the Fats and Oils Industries," *AOCS*, **51**, (1985).
41. F. E. Sullivan, *J. Am. Oil Chem. Soc.* **53**, 358 (1976).
42. T. S. Tang, K. T. Teoh, and Y. Y. Lee, *Palm Oil Product Technologies in the Eighties*, Incorp. Soc. Planter, Kuala Lumpur, 1983, p. 195.
43. M. S. Fraser and G. Frankl, *J. Am. Oil Chem. Soc.* **58**, 926 (1981).
44. P. A. T. Swoboda, *J. Am. Oil Chem. Soc.* **62**, 287 (1985).
45. K. G. Berger, *PORIM Bull.*, (4), 57 (1982).
46. F. V. K. Young, *PORIM Tech.* (1), 31 (1981).
47. P. D. Howes, T. C. Soon, S. H. Lim, D. B. Shaw, and P. K. Stemp, *Proceedings of PORIM International Conference on Progress, Prospects and Challenges Towards the 21st Century. Module III: Chem. & Tech.*, PORIM, Kuala Lumpur, 1993, p. 55.
48. M. G. A. Wilhems, *J. Am. Oil Chem. Soc.* **62**, 454 (1985).
49. M. Loncin, *Fette Seifen Anstrichm* **64**, 531 (1962); **76**, 104 (1974).
50. B. Jacobsberg, *PORIM Occasional Paper*, No. 12, 11 (1983).
51. P. Sjoberg, *Proceedings of PORIM International Oil Palm Conference, Progress and Prospects Conference II: Technology*, PORIM, 1987, p. 219.
52. A. Athanassiadis, *Ibid*, 440 (1987).
53. M. M. Sein, *Proceedings of PORIM International Palm Oil Conference, Progress, Prospects & Challenges Towards the 21st Century, Module III: Chem. & Tech.*, 1991, p. 78.
54. H. L. Gian and N. S. Chua, *Proceedings of 1987 International Oil Palm/Palm Oil Conference: Progress & Prospects (Technology)*, PORIM/ISP, 1988, p. 177.
55. Hamirin Kifli, *PORIM Rep.* PO(5a), 83 (1983).
56. M. T. Ab, Gapor and co-workers, *Palm Oil Product Technology in the Eighties*, PORIM, Kuala Lumpur, 1983, p. 145.
57. M. T. Ab, Gapor and co-workers Austral. Pat. 618,791 (1993).
58. R. Timms, *Chem. Ind.*, 342 (1991).

59. F. V. K. Young in F. D. Gunstone, *Critical Reports on Applied Chem.*, Vol. 15, John Wiley & Sons, Inc., 1987, p. 62.
60. S. Wong, T. H. Goh, L. C. Tan, and P. P. Kerk, *Proceeding of PORIM International Palm Oil Conference, Module III: Chem. & Tech.*, 1993, p. 90.
61. E. Diffense, *J. Am. Oil Chem. Soc.* **62**, 376 (1985).
62. J. B. Rossell, *J. Am. Oil Chem. Soc.* **62**, 385 (1985).
63. D. R. Abigor, F. I. Opute, A. R. Opoku, and A. U. Osagie, *J. Sc. Food Agric.* **36**, 599 (1985).
64. S. Ravigadevi, C. L. Chong, K. C. Oo, K. H. Yeo, and R. Premavathy, *J. Expt. Bot.* **42**, 1199 (1991).
65. M. Loncin, *Oleagineaux* **7**, 695 (1952).
66. D. A. Okiy, *Oleagineaux* **33**, 625 (1978).
67. K. G. Berger, *Oil Palm News* **22**, 10 (1977).
68. V. K. Lal and A. Gasper, *Proceedings of 1991 PORIM International Palm Oil Conference Module III*, 1993, p. 243.
69. W. L. Siew, *PORIM Bull.*, (24), 41 (1992).
70. S. B. Ng and A. Southworth, *Proceedings of Workshop on Quality in the Palm Oil Industry*, PORIM, Kuala Lumpur, 1985, p. 7.
71. G. N. McDermott, *JAOCs*, **53**, 449 (1976).
72. W. C. Seng, *JAOCs*, 926A (1980).
73. Donnelly, *Wastewater from Edible Oil Refining and Margarine Processing* (1989).
74. K. K. Chin and K. K. Wong, *Water Res.* **15**, 1087 (1981).
75. K. K. Chin, W. J. Ng, and A. N. Ma, *Biological Wastes* **20**, 101 (1987).
76. G. Choffel, *JAOCs* **53**, 446 (1976).
77. M. L. Arora, E. F. Barth, and M. B. Umpbres, *JPWCF* **57**, 867 (1980).
78. R. L. Irvine, L. H. Ketchum, Jr., M. L. Arora, and E. F. Barth, *JWPCF* **59**, 847 (1985).
79. R. L. Irvine, G. Miller, and Ajit Singh Bhamrah, *JWPCF* **51**, 224 (1979).
80. R. L. Irvine, D. V. S. Murthy, M. L. Arora, J. L. Copeman, and J. A. Heidman, *JWPCF* **59**, 132 (1987).
81. J. C. Palisand and R. L. Irvine, *JWPCF* **57**, 82 (1985).
82. H. Melcer, W. K. Bedford, B. H. Topnik, and N. W. Schmidtke, *JWPCF* **59**, 19 (1987).
83. K. V. Lo, N. C. R. Bulley, and E. Kwong, *Agricul. Wastes* **13**, 131 (1985).
84. K. V. Lo, J. P. Tam, and P. H. Liao, *Biolog. Wastes* **25**, 193 (1988).
85. W. J. Ng, *Biolog. Wastes* **22**, 285 (1987).
86. K. C. Okkyung and Y. Pomerang, *J. Am. Oil Chem. Soc.* **60**, 1848 (1983).
87. N. A. Idris, *PORIM Information Series No. 9*, Palm Oil Research Institute of Malaysia (1992).
88. N. A. Idris, K. G. Berger, and A. S. H. Ong, *J. Sci. Food Agric.* **46**, 481 (1989).
89. N. A. Idris, Ph.D. Thesis, Univeriti Kebangsaan Malaysia, 1991.
90. N. A. Idris, A. Abdullah, M. S. Embong, and A. H. Hassan, *Elaeis* **3**, 302 (1991).
91. N. A. Idris, A. Abdullah, and M. S. Embong, *ASEAN Food J.* **7**, 202 (1992).

92. K. G. Berger, *PORIM Occasional Paper* No. 2, Palm Oil Research Institute of Malaysia (1981).
93. J. Ward, *J. Am. Oil Chem. Soc.* **65**, 1731 (1988).
94. Y. K. Teah and A. S. H. Ong, *Palm Oil Develop.*, (4), 17–18 (1986).
95. M. S. A. Kheiri, *PORIM Occasional Paper* No. 5, Palm Oil Research Institute of Malaysia (1982).
96. H. Von Zeddelman and J. Wurziger, *Fette, Seifen Anstrichmineral* **75**, 18 (1973).
97. L. Faur, *Rev. Frane. des Corps Gras* **78**, 77 (1975).
98. B. Toregard and A. Eriksson, Proceedings of 10th Scandinavian Symposium on Lipids, Nyborg, Denmark Sec. A. 1979, p. 54.
99. M. E. Herendi and R. Bethke, *Susswaren* **12**, 410 (1982).
100. A. M. T. Gapor, *Palm Oil Develop.* No. **12**, 25–27, (1990).
101. S. Mashashi, T. Yoshikazu, and S. Masanori, *J. Am. Oil Chem. Soc.* **62**, 449 (1985).
102. M. A. Augustine, L. K. Heng, and I. Nor Aini, *Pertanika* **11**, 393 (1988).
103. M. A. Augustine, L. K. Heng, and I. Nor Aini, *Pertanika* **12**, 167 (1989).
104. S. Torrey, *Edible Oils and Fats—Developments Since 1978*, Royes Data Corp., Park Ridge, N. J., 1983.
105. J. A. Wolfe, *Manufacturing Confectioner*, 53 (Apr. 1977).
106. H. Traither and A. Dieffenbacher, *J. Am. Oil Chem. Soc.* **62**, 417 (1985).
107. I. Nor Aini, *Palmsearch Circular* No. 2, 87–92, (1990).
108. I. Nor Aini, H. Hanirah, F. C. H. Oh, and N. Sudin, *J. Am. Oil Chem. Soc.* **69**, 1206 (1992).
109. I. Nor Aini, H. Hanirah, N. Sudin, F. C. H. Oh, and T. S. Tang, *JAOCS* (1994).
110. H. J. Richtler and J. Knaut, *Seifen-Ole-Fene-Waschse* **117**(15), 545 (1991).
111. H. Kifli and S. Krishnan, *Proceedings of the PORIM International Oil Palm/Palm Oil Conference: Progress and Prospects*, PORIM, Kuala Lumpur, 1987, pp. 304–315.
112. A. Kuntom and H. Kifli, *Proceedings of the PORIM International Oil Palm/Palm Oil Conferences: Progress and Prospects*. PORIM, Kuala Lumpur, 1987, pp. 328–331.
113. A. Kuntom and H. Kifli, in T. H. Applewhite, ed., *Proceedings of the World Conference on Oleochemicals into the 21st Century*, American Oil Chemists' Society Champaign, III., 1990, pp. 298–304.
114. S. Ahmad and H. Kifli, in A. R. Baldwin, ed., *Proceedings of the Second World Conference on Detergents: Looking Toward 90's*. American Oil Chemists' Society, Champaign, III., 1987, pp. 250–256.
115. T. L. Ooi, A. Kuntum, W. L. Siew, Y. A. Tan, and B. Muhammad, in *Proceedings of the 1993 PORIM International Palm Oil Conference*, PORIM, Kuala Lumpur, 1993.
116. G. Dieckelmann and H. J. Heinz, *The Basics of Industrial Oleochemistry*, Oleochemicals Consulting International, D-4330 Mulheim (Ruhr)-Saarn, 1988.
117. Y. Basiron and A. Hitam, *PORIM Information Series* No. 4, 1992.
118. S. Ahmad, Y. C. Leong, I. Mohammad, and E. L. Ong, *Proceedings of the PORIM International Oil Palm/Palm Oil Conferences: Progress and Prospects*. PORIM, Kuala Lumpur, 1987, pp. 291–303.
119. S. Ahmad, A. Rafiei, and Z. Ismail, *PORIM Rep.* PO (125a) 87 General (1987).



120. S. Ahmad, Y. C. Leong, C. S. Wong, and A. Rafiei, *PORIM Rep.* PO (134B)88 General (1988).
121. H. Hassan, S. Ahmad, and A. Ibrahim, *PORIM Information Series* No. 6 (1992).
122. H. Kluth and A. Meffert, U.S. Pat. 4,508,853, (Apr. 2, 1985).
123. H. Kluth, B. Gruber, A. Meffert, and W. Huebner, U.S. Pat. 4,742,087, (May 3, 1988).
124. H. Hassan, S. K. Yeong, and S. Ahmad, *Proceedings of the PORIM International Palm Oil Conference*, PORIM, Kuala Lumpur, 1993.
125. H. M. Nor, M. H. Mahmood, H. Kifli, and M. A. Rahman, in T. H. Applewhite, ed., *Proceedings of the World Conference on Oleochemicals into the 21st Century*, American Oil Chemists' Society, Champaign, Ill., 1990, pp. 311–314.
126. H. M. Nor, M. H. Mahmood, H. Kifli, M. A. Rahman, and A. Rafiei A, *Nuc. Sci. J. Malaysia* **8**, 149 (1990).
127. M. H. Mohammad, H. Nor, H. Kifli, M. A. Rahman, and A. Rafiei, *Nuc. Sci. J. Malaysia* **9**(2), 95 (1991).
128. H. Kifli and S. Ahmad, *PORIM Rep.* (1985).
129. T. L. Ooi and A. S. H. Ong, *Proceedings of the PORIM International Oil Palm/Palm Oil Conferences: Progress and Prospects*. PORIM, Kuala Lumpur, 1987, pp. 322–327.
130. A. Kuntom, H. Kifli, and C. H. Oh, *Transparent Soaps Derived from Palm Oil*, ABISA Congress, Olindo, Brazil, 1992.
131. A. Kuntom, unpublished results, Palm Oil Research Institute of Malaysia, Kuala Lumpur.
132. I. Mohamed, E. L. Ong, H. Kifli, and M. A. Rahman, *PORIM Rep.* 1989.
133. F. Timmermann, *Medium Chain Triglycerides—The Unconventional Oil*, Chemicals Fabrik Grunau GmbH, Germany, 1991.
134. R. M. Speed, *Proceedings of 1989 PORIM International Palm Oil Development Conference*, PORIM, Kuala Lumpur, 1989, pp. 127–129.
135. M. A. Margeson and J. S. Schwartz, *Proceedings of 1991 PORIM International Palm Oil Conference*, PORIM, Kuala Lumpur, 1991, pp. 193–198.
136. T. Ogoshi and Y. Miyawaki, *J. Am. Oil Chem. Soc.* **62**(2), 331 (1985).
137. T. C. Inagaki, in T. H. Applewhite, ed., *Proceedings of the World Conference on Oleochemicals into the 21st Centuries*, American Oil Chemists' Society, Champaign, Ill., 1990, pp. 269–278.
138. S. Ahmad, *Proceedings of the PORIM International Palm Oil Conference*, PORIM, Kuala Lumpur, 1993.
139. A. S. H. Ong, A. H. Hassan, Y. Basiron, Y. M. Choo, and A. Gapor, *Proceedings of the Symposium on New Developments in Palm Oil*, 1989.
140. A. N. Ma, and co-workers, *Proceedings of the PORIM International Palm Oil Conference*, PORIM, Kuala Lumpur, 1993.
141. K. Y. Cheah and co-workers, *J. Am. Oil Chem. Soc.* **69**(5), (1992).
142. G. Eisenbrand, M. Blankart, H. Sommer, and B. Weber, *N-Nitroalkanolamines in Cosmetics*, IARC Science Publication, 1991, pp. 238–241.
143. M.C. Chow and C. C. Ho, *Proceedings of the PORIM International Palm Oil Conference*, PORIM, Kuala Lumpur, 1993.

144. C. H. Teoh and C. S. Chia, paper presented at 1993 PIPOC, Sept. 20–25, 1993, Kuala Lumpur, Malaysia.
145. N. MacFarlane, A. A. Swetman, and D. G. Coursey, *Proceedings of Second African Palm Oil Processing Workshop, Nigeria (1983)*, FAO, Rome, 1984.
146. P. M. Kris-Etherton, C. Y. Ho, and M. A. Fosmire, *J. Nutr.* **114**, 675 (1984).
147. M. Sugano, T. Ishida, and K. Koba, *J. Nutr.* **118**, 548 (1988).
148. K. Sundram, H. T. Khor, and A. S. H. Ong, *Lipids* **25**, 187 (1990).
149. D. Klurfeld, *Proceedings PORIM International Development Conference*, PORIM, Kuala Lumpur, 1989.
150. A. S. H. Ong and co-workers, *FASEB 2: Abst.* 7228 (1988).
151. S. Lindsey, J. Benattar, A. Pronczuk, and K. C. Hayes, *Proc. Soc. Biol. Med.* **195**, 261 (1990).
152. F. M. Mattson and S. M. Grundy, *J. Lipid Res.* **26**, 194 (1985).
153. C. W. Laine and co-workers, *Am. J. Clin. Nutr.* **35**, 683 (1982).
154. Anon, *Nutr. Rev.* **45**, 205 (1987).
155. K. Sundram, G. Hornstra, A. C. Houwelingen, and A. D. M. Kester, *Br. J. Nutr.* **68**, 677 (1992).
156. A. Maozuki, F. Arshad, T. A. Razak, and K. Jaarin, *Am. J. Clin. Nutr.* **53**, 1010S (1991).
157. K. W. Ng and co-workers, *Am. J. Clin. Nutr.* **53**, 1015S (1991).
158. K. W. Ng and co-workers, *J. Am. Coll. Nutr.* **11**, 383 (1992).
159. R. Wood, K. Kubena, R. Crook, G. Martin, and S. Tseng, *J. Nutr. Biochem.* **4**, 1 (1993).
160. D. Heber and co-workers, *Nutr. Res.* **12**, S53 (1992).
161. A. S. Truswell, N. Choudhury, and D. C. K. Roberts, *Nutr. Res.* **12**, S43 (1992).
162. A. Bonanome and S. M. Grundy, *N. Engl. J. Med.* **318**, 1244 (1988).
163. D. M. Hegsted, R. B. McGandy, M. L. Meyers, and F. J. Stare, *Am. J. Clin. Nutr.* **27**, 281 (1965).
164. K. C. Hayes, A. Pronczuk, S. Lindsey, and D. Diersen Schade, *Am. J. Clin. Nutr.* **53**, 491 (1991).
165. K. C. Hayes and P. R. Khosla, *FASEB J.* **6**, 2600 (1992).
166. K. Sundram, K. C. Hayes, and O. H. Siru, *Am. J. Clin. Nutr.* **59**, (1994).
167. K. Sundram, K. C. Hayes, and O. H. Siru, (1994).
168. A. A. Qureshi, W. C. Burger, D. M. Peterson, and C. E. Elson, *J. Biol. Chem.* **261**, 10544 (1986).
169. A. A. Qureshi and co-workers, *Am. J. Clin. Nutr.* **53**, 1021S (1991).
170. D. T. S. Tan and H. T. Khor, *Am. J. Clin. Nutr.* **53**, 1027S (1991).
171. A. A. Qureshi and co-workers. *Proceedings PORIM International Development Conference*, PORIM, Kuala Lumpur, 1989.
172. M. L. Wahlqvist and co-workers, *Nutr. Res.* **12**, S181 (1992).
173. E. Serbinova, V. Kagan, D. Han, and L. Packer, *Free Rad. Biol. Med.* **10**, 263 (1991).
174. K. Kazunori, A. Khoji, K. Ikuo, and M. Sugano, *Biosci. Biotech. Biochem.* **56**, 1420 (1992).
175. K. Sundram, H. T. Khor, A. S. H. Ong, and R. Pathmanathan, *Cancer Res.* **49**, 1447 (1989).

176. P. W. Sylvester, M. Russel, M. M. Ip, and C. Ip, *Cancer Res.* **46**, 757 (1986).
177. K. Komiyama and M. Yamaoka, *Chem. Pharm. Bull.* **37**, 1369 (1989).
178. K. Nesaretnam and co-workers, *Nutr. Res.* **12**, 63 (1992).
179. M. Murakoshi and co-workers, *Cancer Res.* **52**, 6583 (1992).
180. J. Podmore, in R. J. Hamilton and A. Bhati, eds., *Application of Modification Techniques in Recent Advances in Chemistry and Technology of Fats and Oils*, 1987.
181. H. Kifi and S. Krishnan, *Proceedings of the 1987 International Oil Palm/Palm Oil Conference, Progress and Prospects*. 29th June–1st July, 1987.
182. E. Bernardini, *Oilseeds, Oils and Fats, Vol. II, Oils and Fats Processing*, 2nd ed., B.E. Oil Publishing House, Rome.

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## *Peanut Oil*

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### **1. PEANUT ORIGIN AND HISTORY**

In 1753, Linnaeus described the domesticated peanut species as *Arachis* (derived from the Greek “arachis,” meaning a weed) *hypogaea* (meaning a underground chamber) or a weed with fruit produced below the soil. The domesticated peanut (*A. hypogaea*) is believed to have originated in an area covered by southern Bolivia and northern Argentina because of the primitive characteristics associated with the germplasm from this region (1). Subspecies *hypogaea* var. *hypogaea* is the predominant peanut type found in this area, and Krapovickas (2) hypothesized that the var. *hypogaea* may represent the most ancient cultivars because they have the runner habit, branching patterns, similar to related *Arachis* species, and no floral compound spikes.

Additional information now suggests that a second origination event in the area north of Lima on the west coast of Peru could have been involved in the evolution of *A. hypogaea*. Archeological excavations near Casma at Pampa de la Llamas-Moxeke have recovered peanut shells at a level dated to be approximately 1500 B.C. (3–5), and gold carvings found in ancient tombs just to the north of Pampa de las Llamas-Moxeke (6, 7) closely resemble the reticulation of the cultivated types now grown in the Casma area.

As peanut is native to South America, the early Spanish and Portuguese explorers found the Indians cultivating the peanut along with other food crops. It was from the tropical and subtropical areas of this hemisphere that the peanut was disseminated to Europe, to both the coasts of Africa, to Asia, and to the Pacific Islands (8). The Incas of Peru, who achieved one of the world's most highly developed agricultural civilizations, cultivated the peanut throughout the long coastal regions of Peru. Garcilaso de la Vega describes the peanut as "another vegetable which is raised under the ground, called by the Indians *ynchic*. It is very like marrow, and has the taste of almonds." Of its food and medicinal uses: "If the *ynchic* is eaten raw it caused headache, but when toasted it is wholesome, and very good with treacle; and they make an excellent sweetmeat from it. They also obtain an oil from the *ynchic*, which is good for many diseases" (8). Just when the peanut was first purposefully introduced into Europe and into the colonial seaboard of the southeastern United States is not documented. However, from this introduction around the world, the peanut has become a significant agricultural commodity and its oil a primary ingredient in the culinary process in many countries.

## 2. GLOBAL

### 2.1. Peanut Production

The peanut is known by several names throughout the world, such as groundnut and earth nut, because the seeds develop under the ground. Peanuts are produced on a significant basis in more than 30 different countries throughout the world. The worldwide production for 2002 was estimated to be in excess of 31 million metric tons (MMT) (9). India, China, and the United States were the three largest producers of peanuts and accounted for over 70% of the world production in 2002. Peanut production worldwide has undergone significant increases in the last 30 years (Table 1). In 1972, the average production was 14.4 MMT, 1980 16.0 MMT, 1990 21.6 MMT, and 2000–2002 32.0 MMT. (9, 10). Some of the production increase was a result of a 22% increase in the area harvested between 1972 and 2002. However, the major factor was the increase in yield from 0.93 MT/ha in the 1970s to 1.4 MT/ha in 2001/2002. Among the three major producers, India had a 42% increase in production between the 1970s and 1990s but decreased 16% between the 1990s and 2002. China increased production 179% between the 1970s and 1990s and another 136% to 2002. The U.S. production increased 15% in the first 20 years and has remained near 1.9 MMT since the 1990s (9, 10). Total area harvested and production levels in the next tier of eight countries has averaged approximately 4.63 mha harvested and 4.46 MMT produced across the 30 years. From the data given in Table 1, the high total area harvested for this tier of eight countries was 5.38 mha in 1972 with a low of 3.93 mha in 1990. Highest production occurred in 2000 at 5.50 MMT and the lowest in 1990 at 3.46 MMT (Table 1). World exports of peanuts from the producing countries have only increased 22%, from 1178 MMT in 1972 to 1518 MMT in 2002.

**TABLE 1. Major Countries and World Peanut Production and Utilization (mha or MMT) Across 30 Years.<sup>1</sup>**

Year	Country	Area Harvested	Production	Total Supply	Exports	Domestic Consumption				Total Distribution
						Crushed	Food	Feed; Seed; Waste	Total	
1972	China	1878	2092	2092	42	1018	768	264	2050	2092
1972	India	6990	4092	4342	33	3511	532	266	4309	4342
1972	United States	601	1485	1663	236	386	768	78	1232	1663
1972	Argentina	370	440	457	2	331	46	21	398	457
1972	Brazil	506	590	590	78	401	59	52	512	590
1972	Burma	633	390	390	0	228	142	20	390	390
1972	Indonesia	407	483	483	29	91	340	23	454	483
1972	Nigeria	1220	772	797	284	383	100	30	513	797
1972	Senegal	1100	540	540	7	385	45	103	533	540
1972	Sudan	690	568	568	156	91	210	111	412	568
1972	Zaire	451	230	230	0	81	137	12	230	230
1972	World	18121	14421	16263	1178	8569	4873	1270	14712	16263
1980	China	2339	3600	3600	305	1667	1257	371	3295	3600
1980	India	6801	5005	5205	71	4059	325	650	5034	5205
1980	United States	566	1045	1512	228	202	663	232	1097	1512
1980	Argentina	197	243	282	74	147	12	11	170	282
1980	Brazil	235	310	312	37	196	37	42	275	312
1980	Burma	514	431	431	0	319	91	21	431	431
1980	Indonesia	508	791	806	2	47	682	75	804	806
1980	Nigeria	650	530	530	0	204	220	106	530	530
1980	Senegal	1064	521	521	3	258	101	159	518	521
1980	Sudan	894	707	707	133	377	151	46	574	707
1980	Zaire	480	320	320	0	107	185	28	320	320
1980	World	17508	16040	17805	1113	8507	5697	1989	16193	17805

**TABLE 1** (Continued)

Year	Country	Area Harvested	Production	Total Supply	Exports	Domestic Consumption				Total Distribution
						Crushed	Food	Feed; Seed; Waste	Total	
1990	China	2907	6368	6369	448	3250	2209	462	5921	6369
1990	India	8309	7514	7514	45	5999	490	980	7469	7514
1990	United States	732	1634	1964	296	313	916	129	1358	1964
1990	Argentina	198	311	341	110	123	31	30	184	341
1990	Brazil	95	157	172	2	50	95	20	165	172
1990	Burma	554	472	472	10	320	96	46	462	472
1990	Indonesia	600	860	1011	0	45	850	93	988	1011
1990	Nigeria	500	250	260	0	80	100	60	240	260
1990	Senegal	914	703	758	4	480	146	93	719	758
1990	Sudan	540	325	325	20	145	135	25	305	325
1990	Zaire	530	380	380	0	129	229	22	380	380
1990	World	19089	21656	23498	1304	11705	7791	2157	21653	23498
2000	China	4856	14437	14437	450	6800	6047	1140	13987	14437
2000	India	8100	5700	5700	100	4300	500	800	5600	5700
2000	United States	541	1481	2138	239	248	988	166	1402	2138
2000	Argentina	251	395	409	177	142	21	19	182	409
2000	Brazil	102	196	216	3	60	125	18	203	216
2000	Burma	530	640	640	12	390	162	76	628	640
2000	Indonesia	650	1040	1178	0	64	1030	70	1164	1178
2000	Nigeria	1210	1470	1475	0	510	670	290	1470	1475
2000	Senegal	1030	1003	1028	9	420	395	149	964	1028
2000	Sudan	550	370	370	5	210	135	20	365	370
2000	Zaire	491	382	382	0	120	232	30	382	382
2000	World	22644	31120	33225	1387	14174	13886	3021	31081	33225
2002	China	5000	14500	14500	500	6950	5950	1100	14000	14500
2002	India	8100	6700	6700	105	5060	600	935	6595	6700

**TABLE 1** (Continued)

2002	United States	551	1702	2395	293	292	1090	167	1549	2395
2002	Argentina	200	315	335	160	125	21	19	165	335
2002	Brazil	100	195	215	3	60	124	18	202	215
2002	Burma	530	640	640	12	390	162	76	628	640
2002	Indonesia	650	1000	1219	0	62	1075	68	1205	1219
2002	Nigeria	1230	1510	1515	0	528	685	297	1510	1515
2002	Senegal	750	500	523	5	160	252	85	497	523
2002	Sudan	550	370	370	4	211	135	20	366	370
2002	Zaire	500	390	390	0	132	233	25	390	390
2002	World	22507	31837	34155	1518	14901	13971	3056	31928	34155

<sup>1</sup>Data extracted from [http://www.fas.usda.gov/psd/complete\\_files/OIL-2221000.csv](http://www.fas.usda.gov/psd/complete_files/OIL-2221000.csv)



However, China alone has increased exports from 42 MMT to 500 MMT during this same period (Table 1). This increase accounts for nearly one-third of the total world peanut exports. On the other hand, the African continent countries of Nigeria, Senegal, and Sudan have had a decrease in the exporting of peanuts from a combined 447 MMT in 1972 to 9 MMT in 2002.

## 2.2. Peanut Utilization

Peanuts are not a crop that can easily be carried over from one year to the next as noted by a comparison of the production and total consumption values across years (Table 1). Utilization of the peanut crop can be classified into the general areas of crushed, food, feed, seed, and waste. In 1972, the primary utilization in 7 of the 11 listed countries was the crushing of peanuts for oil and utilization of the resultant meal. The United States' food utilization was more than twice that of the next country, Indonesia. In the United States, food utilization was nearly twice that of crushing utilization. In 2002, the number of countries having food as the primary utilization factor had increased to six and the United States and Indonesia were almost equal in food utilization (Table 1).

**2.2.1. Oil** Hammons' (8) review of the origin and early history of the peanut provides extensive insight into writings of the early Spanish and Portuguese explorers and the usage of peanuts as an oil source for many purposes. Spanish recognition of the usefulness of peanut oil is documented by the establishment of an oil mill at the Mediterranean port of Valencia around 1800 (11). Most authorities credit the Portuguese with introducing the peanut into African agriculture from Brazil. West Africa was the primary source of peanut exportation in the nineteenth century. Brooks (12) provides an overview of the development of the peanut industry in Africa and peanut exportation from West Africa to other parts of the world. The first export seems to have been from Gambia to Britain in 1834 involving 213 baskets, but the next year export increased to 47 tons and by the 1840s involved thousands of tons a year. Earliest exports to America were in 1835, and exports to America dominated the Gambian market from 1837 to 1841. The exportation to Britain was for crushing, and the dominant reason for American usage was the pleasing flavor of the roasted peanut.

Development of the European peanut oil industry was stimulated by a worldwide shortage of fats after the Napoleonic wars, an increase in population, a rise in the standard of living, and a new working class. As in Britain, French soap and candle-makers became increasingly dependent on foreign sources of oil supply in the 1830s. Learning of the British peanut imports, French industrialists undertook experimentation of their own on peanut oil. Jaubert, a Gorée trader who had sent a sample of peanut oil to Marseille in 1833, is credited with initiating the industry with a shipment of 722 kg of peanuts from West Africa to Marseilles in 1840, when France reduced the tariff on peanuts (8, 12). Following that shipment, other traders are reported in 1842 to have brought nearly a 1000 tons of peanuts to Marseilles.

Peanut oil production continued to increase, in Europe, throughout the nineteenth century. By 1899, 17 factories at Marseilles were crushing about 200,000 tons. An equal volume was being processed in Britain and other European countries (13). France continued to be a major peanut importer and oil producer, through the mid-1970s with 331 MMT crushed in 1972. However, by 1980 and 2000, the crushed level had dropped to 79 and 8 MMT, respectively (10).

Across the last 30 years, the amount of peanuts crushed for oil worldwide has increased from 7957 to 14,901 MT. (Table 2). Increases in metric tons crushed in China and India and the decreases in the South America countries of Argentina and Brazil account for almost 100% of the changes. The oil produced is virtually all used within the countries of production. It seems appropriate to note that within Japan, the industrial use of peanut oil has increased from 4 to 14 MMT between 1990 and 2002. Exporting of peanut oil has decreased nearly 42% from 1972 to 2002. Of the 252 MMT of oil exported worldwide in 2002, four countries, Argentina, Nigeria, Senegal, and Sudan, account for nearly 70%.

**2.2.1.1. Oil Extraction** Hydraulic pressing, expeller, and/or solvent extraction are the three general methods for extracting oil from the seed. When hydraulic pressing is used, it is followed by hot solvent extraction for nearly total recovery of the oil. Expeller extraction relies on friction and pressure within the expeller, which causes the meal to heat, thus facilitating the oil extraction process. This process removes approximately 50% of the peanut oil. The remaining oil is extracted using hexane, which is later removed through an evaporation–condensation system. Solvent extraction involves petroleum hydrocarbons or other solvents. Solvent extraction is accomplished in closed systems where oil is removed and solvent reclaimed for reuse. The efficiency of extraction with hexane, 95% ethanol, or absolute ethanol on peanut grits has been reported (14). Extracted oil is refined by deacidification with sodium hydroxide to neutralize the free-fatty acids, washing with water at about 82°C to remove the sodium hydroxide, and then bleaching with bleaching clay at about 100°C under reduced pressure. The refined oil is then deodorized by heating under vacuum and blowing superheated steam through the oil. Deacidification and deodorization of peanut oil and other edible oils by dense carbon dioxide extraction has been investigated (15). The purpose of the refining process is to remove nontriacylglycerol components, including free fatty acids, nonhydratable phosphoacylglycerols, sterols, pigments, glucosides, waxes, hydrocarbons, and other compounds that may be detrimental to the flavor or oxidative stability of the refined oil (16).

**2.2.1.2. Alternative Oil Extraction Techniques and Seed Treatment** The complete removal of organic solvents used for extracting seed oils is mandatory if the oil is to be used for human consumption. Supercritical fluid extraction has emerged as an attractive separation technique because it does not introduce any residual organic chemicals. Supercritical CO<sub>2</sub> is the most commonly used supercritical fluid (17). CO<sub>2</sub> is relatively low cost, nonflammable, nontoxic, and

**TABLE 2. Major Countries and World Peanut Oil Production and Utilization (MMT) Across 30 Years.<sup>1</sup>**

Year	Country	Crushed	Oil Production	On Hand	Imports	Total Supply	Domestic Consumption			Total Distribution
							Food	Total	Exports	
1972	China	1018	254	0	0	254	234	234	20	254
1972	India	3511	1060	0	0	1060	1060	1060	0	1060
1972	United States	386	122	15	0	137	72	72	48	137
1972	Argentina	331	78	2	0	80	0	0	80	80
1972	Brazil	401	112	0	0	112	68	68	44	112
1972	Burma	228	73	0	0	73	73	73	0	73
1972	Indonesia	91	29	0	0	29	29	29	0	29
1972	Nigeria	383	122	0	0	122	11	11	111	122
1972	Senegal	385	128	0	0	128	65	65	63	128
1972	Sudan	91	29	0	0	29	29	29	0	29
1972	Zaire	81	26	0	0	26	22	26	0	26
1972	World	7957	2371	17	401	2789	2325	2337	434	2789
1980	China	1667	417	0	0	417	368	368	49	417
1980	India	4059	1177	0	0	1177	1177	1177	0	1177
1980	United States	202	63	20	0	83	44	44	22	83
1980	Argentina	147	42	0	0	42	0	0	36	42
1980	Brazil	196	62	0	0	62	16	16	46	62
1980	Burma	319	102	0	0	102	102	102	0	102
1980	Indonesia	47	16	0	0	16	16	16	0	16
1980	Nigeria	204	65	0	4	69	69	69	0	69
1980	Senegal	258	83	0	0	83	63	63	20	83
1980	Sudan	377	121	0	0	121	105	105	16	121
1980	Zaire	107	34	0	0	34	33	34	0	34
1980	World	8085	2343	60	319	2722	2410	2411	268	2722
1990	China	3250	813	0	5	818	772	772	46	818
1990	India	5999	1740	0	0	1740	1736	1740	0	1740

1990	United States	313	97	10	5	112	90	90	11	112
1990	Argentina	123	40	0	0	40	5	5	35	40
1990	Brazil	50	14	8	15	37	15	15	18	37
1990	Burma	320	99	0	0	99	99	99	0	99
1990	Indonesia	45	14	5	0	19	13	13	0	19
1990	Nigeria	80	37	0	0	37	37	37	0	37
1990	Senegal	480	153	9	0	162	53	58	99	162
1990	Sudan	145	47	0	0	47	44	44	3	47
1990	Zaire	129	41	0	0	41	40	41	0	41
1990	World	11389	3242	54	302	3598	3274	3292	259	3598
2000	China	6800	2115	0	10	2125	2110	2110	15	2125
2000	India	4300	1245	0	0	1245	1235	1245	0	1245
2000	United States	248	81	14	36	131	111	111	6	131
2000	Argentina	142	42	0	0	42	1	1	41	42
2000	Brazil	60	16	2	0	18	17	17	1	18
2000	Burma	390	123	0	0	123	123	123	0	123
2000	Indonesia	64	20	0	0	20	20	20	0	20
2000	Nigeria	510	230	0	0	230	195	195	35	230
2000	Senegal	420	160	6	0	166	58	58	102	166
2000	Sudan	210	67	0	0	67	22	22	45	67
2000	Zaire	120	38	0	0	38	37	38	0	38
2000	World	14149	4301	32	258	4591	4239	4250	312	4591
2002	China	6950	2175	0	10	2185	2170	2170	15	2185
2002	India	5060	1465	0	0	1465	1451	1465	0	1465
2002	United States	292	93	14	20	127	111	111	5	127
2002	Argentina	125	39	0	0	39	1	1	38	39
2002	Brazil	60	16	0	0	16	15	15	1	16
2002	Burma	390	123	0	0	123	123	123	0	123
2002	Indonesia	62	19	0	0	19	19	19	0	19

**TABLE 2** (Continued)

Year	Country	Crushed	Oil Production	On Hand	Imports	Total Supply	Domestic Consumption			Total Distribution
							Food	Total	Exports	
2002	Nigeria	528	238	0	0	238	208	208	30	238
2002	Senegal	160	58	5	10	73	10	10	60	73
2002	Sudan	211	68	0	0	68	24	24	44	68
2002	Zaire	132	41	0	0	41	40	41	0	41
2002	World	14901	4513	30	219	4762	4476	4491	252	4762s

<sup>1</sup>Data extracted from [http://www.fas.usda.gov/psd/complete\\_files/OIL-4234000.csv](http://www.fas.usda.gov/psd/complete_files/OIL-4234000.csv)

easily removed from the oil product by depressurization. However, particle size does have a significant effect on the extraction rate curves (18–20). CO<sub>2</sub> is also U.S. Food and Drug Administration approved and is generally regarded as a safe compound.

Food-grade butane in a supercritical, low-pressure, liquefied gas extraction procedure has also been described for oil extraction from peanuts (21). The extraction process consists of mixing the liquefied butane with the material to form a slurry. The liquefied gas and oil are moved to a solvent recovery system where the oil is removed from the butane. The oil is pumped from the solvent recovery system to a holding tank, and the butane is then transformed into a gas in the solvent recovery system and transported back to the butane storage tank for reuse.

Aqueous enzymatic oil extraction is another ecofriendly extraction procedure. It is based on simultaneous isolation of oil and protein from oilseed by dispersing finely ground seed in water and separating the dispersion by centrifugation into oil, solid, and aqueous phases. The presence of certain enzymes during extraction enhances oil recovery by breaking cell walls and oil bodies (22). For peanuts, a multistep aqueous extraction process has been described with a recovery of about 98% (23). More recently, the relatively new technique of enzyme-assisted aqueous extraction has been applied to peanuts with a reported oil recovery of 86–92% (24).

Microwave treatment, because of its rapid heating of materials, is being explored in a multitude of crops for enzyme inactivation (25–28), for extraction of natural products (29), and oil and fat extraction from seeds and food products (30–32). Microwave treatment of peanut seed prior to press extraction increased oil recovery approximately 10% at an optimum treatment time of 30 seconds (30). However, free fatty acid content initially increased with exposure time as well as peroxide value (30). Research on use of microwave treatment in blanching of peanuts indicated an influence on oil stability depending on treatment conditions (33).

**2.2.1.3. Oil Extraction By-Product** The byproduct of peanut oil production is peanut meal, and depending on the methods used, the oil content remaining in the meal range from about 7% to 1%. Human consumption of peanut meal is negligible except in India and Argentina (Table 3). The primary use of peanut meal is animal feed. When peanut meal is used for human or animal consumption, careful consideration should be given to the quality of the meal. Various oilseeds, edible nuts, grains, and their derived products are subject to mycotoxin contamination (34), and these mycotoxins may have a detrimental effect on both human and animal health (35). Worldwide regulations for mycotoxins have been published (36). Mycotoxins are generally associated with the protein fraction and are not found in refined oil because of the processing procedures. Unrefined or lightly refined oil may contain mycotoxins because of the fine residue particles contained therein. Meal from edible-grade peanuts with low oil content may be processed into flour for human consumption. When poor-quality grades are used, poor extraction efficiencies or lack of hygienic conditions exist, and the residue should be used as a fertilizer.

**TABLE 3. Major Countries and World Peanut Meal Production and Utilization (MMT) Across 30 Years.<sup>1</sup>**

Year	Country	Crushed	On Hand	Production	Imports	Total Supply	Exports	Domestic Consumption			Total Distribution
								Food	Feed: Waste	Total	
1972	China	1018	0	407	0	407	0	0	366	407	407
1972	India	3511	0	1373	0	1373	869	0	504	504	1373
1972	United States	386	1	163	0	164	0	0	161	161	164
1972	Argentina	331	5	136	0	141	85	0	44	44	141
1972	Brazil	401	0	154	0	154	80	0	74	74	154
1972	Burma	228	0	88	0	88	0	0	88	88	88
1972	Indonesia	91	0	35	0	35	0	0	35	35	35
1972	Nigeria	383	6	147	0	153	137	0	16	16	153
1972	Senegal	385	0	148	0	148	135	0	13	13	148
1972	Sudan	91	20	35	0	55	50	0	5	5	55
1972	Zaire	81	0	31	0	31	0	0	31	31	31
1972	World	8098	33	3158	1030	4221	1431	13	2710	2774	4221
1980	China	1667	0	667	0	667	3	0	598	664	667
1980	India	4059	0	1705	0	1705	394	0	1311	1311	1705
1980	United States	202	4	85	0	89	0	0	85	85	89
1980	Argentina	147	7	57	0	64	42	0	8	8	64
1980	Brazil	196	0	72	0	72	46	0	26	26	72
1980	Burma	319	0	121	0	121	0	0	121	121	121
1980	Indonesia	47	0	18	0	18	0	0	18	18	18
1980	Nigeria	204	0	79	0	79	0	0	79	79	79
1980	Senegal	258	0	95	0	95	49	0	46	46	95
1980	Sudan	377	0	145	0	145	75	0	70	70	145
1980	Zaire	107	0	41	0	41	0	0	41	41	41
1980	World	7813	16	3181	488	3685	549	0	3050	3116	3685
1990	China	3250	0	1300	0	1300	160	0	1028	1140	1300
1990	India	5999	0	2520	0	2520	175	5	2340	2345	2520

1990	United States	313	6	136	0	142	35	0	103	103	142
1990	Argentina	123	0	48	0	48	38	3	7	10	48
1990	Brazil	50	0	20	0	20	3	0	17	17	20
1990	Burma	320	0	105	0	105	10	0	95	95	105
1990	France	0	0	0	253	253	3	0	250	250	253
1990	Indonesia	45	16	17	132	165	0	0	145	145	165
1990	Nigeria	80	0	28	0	28	0	0	28	28	28
1990	Senegal	480	34	183	0	217	166	0	22	22	217
1990	Sudan	145	0	56	0	56	52	0	4	4	56
1990	Zaire	129	0	50	0	50	0	0	50	50	50
1990	World	11350	61	4518	717	5296	626	8	4478	4610	5296
2000	China	6800	0	2660	0	2660	15	0	2645	2645	2660
2000	India	4300	0	1810	0	1810	20	10	1780	1790	1810
2000	United States	248	2	104	0	106	5	0	99	99	106
2000	Argentina	142	5	62	0	67	50	3	5	15	67
2000	Brazil	60	0	24	0	24	1	0	23	23	24
2000	Burma	390	0	123	0	123	10	0	113	113	123
2000	Indonesia	64	8	24	7	39	0	0	33	33	39
2000	Nigeria	510	0	163	0	163	0	0	163	163	163
2000	Senegal	420	3	190	0	193	144	0	44	44	193
2000	Sudan	210	0	81	0	81	76	0	5	5	81
2000	Zaire	120	0	46	0	46	0	0	46	46	46
2000	World	14139	22	5254	255	5531	274	35	5204	5239	5531
2002	China	6950	0	2719	0	2719	10	0	2709	2709	2719
2002	India	5060	0	2128	0	2128	50	10	2068	2078	2128
2002	United States	292	2	127	0	129	5	0	122	122	129
2002	Argentina	125	0	55	0	55	47	3	5	8	55
2002	Brazil	60	0	24	0	24	1	0	23	23	24
2002	Burma	390	0	123	0	123	10	0	113	113	123



**TABLE 3** (Continued)

Year	Country	Crushed	On Hand	Production	Imports	Total Supply	Exports	Domestic Consumption			Total Distribution
								Food	Feed: Waste	Total	
2002	Indonesia	62	5	23	0	28	0	0	26	26	28
2002	Nigeria	528	0	169	0	169	0	0	169	169	169
2002	Senegal	160	5	68	0	73	63	0	10	10	73
2002	Sudan	211	0	81	0	81	75	0	6	6	81
2002	Zaire	132	0	51	0	51	0	0	51	51	51
2002	World	14901	17	5502	244	5763	222	37	5495	5532	5763

<sup>1</sup>Data extracted from [http://www.fas.usda.gov/psd/complete\\_files/OIL-0813200.csv](http://www.fas.usda.gov/psd/complete_files/OIL-0813200.csv)

### 3. ENVIRONMENTAL AND GENOTYPE EFFECTS ON THE COMPOSITION PEANUTS

Major factors that influence the oil and other composition components of the peanut include cultivar and maturity (37) as well as the environmental production conditions of light, temperature, water stress, soil constituents, atmospheric constituents, herbicides and insecticides, physical damage, and pest attack (38). In the four major U.S. market-types (runner, virginia, valencia, and spanish), total oil content varies from 44% to 56% (37, 39, 40). Information on the environmental and genotypic effects on oil and fatty acid composition in peanuts is available (40, 41). The effects of production environment on oil composition of varieties grown in Australia (42), India (43, 44), and the United States (45–49) have been reported. In maturity studies, the total oil (as a percentage of dry weight) increased significantly and then decreased slightly (50, 51). The most rapid changes in oil percentage occurred in early maturity stages and corresponded to the time of very rapid increases in seed dry weight (45, 51–54). As the peanut oil content increases across maturity, there is a concurrent change in fatty acid composition (45). Mature seeds contain more stearic and oleic acids and less arachidic, behenic, and lignoceric acids than immature seeds. The oleic/linoleic (O/L) ratio also increases with maturity (41, 45). Development of new high oleic acid peanut cultivars will be discussed in the next section.

Oil content and fatty acid composition have been studied in aboriginal varieties of *Arachis hypogaea* subsp. *hypogaea* and subsp. *fastigiata*. These varieties are important because they contain germplasm that can be used to increase the variability in the genetic base of the cultivated varieties (55, 56). The *A. hypogaea* subsp. *hypogaea* var. *hypogaea* cultivars were higher in oleic acid concentration than the *A. hypogaea* subsp. *fastigiata* var. *fastigiata*, var. *aequatoriana*, and var. *peruviana* cultivars in sources from Peru (57) and Bolivia (58). Similar results were also obtained from Mexican landrace lines of *A. hypogaea* subsp. *hypogaea* var. *hirsuta* (59). In contrast, a survey of 16 wild species of *Arachis* found that the wild species had higher levels of linoleic acid in comparison with the *Arachis hypogaea* genotypes (60).

### 4. MODIFICATION OF OIL CHARACTERISTICS THROUGH BREEDING

Modification of fatty acid composition has been a particular goal of breeding programs because oil quality, fatty acid composition, and protein composition are highly heritable traits. One of the keys to successful progress in a breeding program is the availability of rapid, efficient screening systems. Some of the methods for rapid screening are measurement of the iodine value (IV) by the oil' refractive index (61), estimation of the seed oil content by its specific gravity (62), and estimation of seed fatty acid composition by use of a small tissue fraction and analysis through direct transmethylation (63), which improves on the individual seed analysis method (64). Methods of peanut improvement through breeding programs have been

discussed in detail (65–68). Most peanut genotypes have 36–67% oleic acid (O), 15–46% linoleic acid (L), and O/L ratios between 1.19 and 4.46 (69–72). While surveying peanut genotypes for oil quality, it was found that two closely related experimental lines had 80% oleic acid and 2% linoleic acid (O/L = 40) with an IV of 74 (71). This naturally occurring mutation may have resulted from a mutation of aspartate at position 150 to asparagine in the cDNA that reduced oleoyl-PC desaturase activity (73). Initial oxidative stability studies were done comparing extracted oil from the experimental high-oleic line with that of an isogenic sister line with normal fatty acid composition (74). The results indicated that the high-oleic peanut oil had a greater oxidative stability than the normal-oleic oil. These experimental lines have been used in breeding programs to develop cultivars with high O/L ratios (75–79). Cultivars having these high O/L ratios do not have significant differences in oil content (80) nor do they have significant differences in color, aroma, flavor, or texture (81, 82). It is characteristic of these high oleic acid lines to have a linoleic acid content of 4% or less. High oleic roasted peanut seed have a more stable roasted peanut attribute after 6 weeks storage at 22°C, and their estimated shelf life is approximately two times longer than that of seed from a normal-oleic variety Florunner (83). Comparison of flavor stability in high-oleic and normal oleic roasted peanut seed during storage at low relative humidity (84) or –20°C (85) indicated that the high-oleic sources had better flavor quality and stability. Use of high oleic oil in roasting of peanuts resulted in slight increases in shelf life as measured by oxidative stability index (OSI) and peroxide value (86). The OSI decreased over storage time, but the differential between high-oleic and normal roasting oils was maintained throughout the storage period. The stability of high-oleic peanut, sesame, and soybean blends in comparison with normal-oleic peanut, sesame, and soybean blends has also been investigated (87), as has the effect of the high-oleic trait on roasted peanut flavor heritability (79, 88).

## 5. OIL COLOR

Color is an important quality parameter of edible oil, both in the refining process and in the marketplace. It is frequently monitored in the product line according to some commercial standards to maintain a consistent quality. Each oil has its own characteristic color primarily because of naturally occurring polyphenolic pigments, gossypol, chlorophyll, and carotenoids (89). Therefore, oil color is often specified according to both market and trade rules established by various associations. Peanut oil of the first grade for cooking should not exceed 2 Lovibond red with fixed Lovibond yellow 20 according to Chinese national standard GB5525-85, and for salad use, it should be no more than 1.5 Lovibond red with fixed Lovibond yellow 15 (90). The Lovibond method, American Oil Chemists' Society (AOCS) Method Cc 13e-92 (91), is practiced primarily outside the United States and Canada (90), and AOCS Method Cc 13e-45 or Wesson method is used throughout the Americas (92). Introduction of automated colorimeters made possible the replacement of the manually operated visual color instrument. An international

collaborative study was conducted to establish a broad-scale correlation between an automated colorimeter (Tintometer Model PFX 990 (The Tintometer Ltd)) and the official visual colorimeter (Tinometer Model AF710) (93). The automated colorimeter was concluded to be an appropriate alternative. Recently, digital image analysis has been proposed as an alternative method to the visual Lovibond method (90). The light yellow color of peanut oil is caused by  $\beta$ -carotene and lutein (94). As peanuts mature, a distinct lightening of the oil color can be observed (95). This lightening of oil color has been suggested as a method to assess maturity (96). However, because peanut oil color is affected by factors, such as water stress and rate of curing in addition to maturity (97), this method was replaced by other maturity evaluation methods (98, 99).

## 6. PEANUT OIL EVALUATION AND COMPOSITION

Crude peanut oil has a nutlike flavor, which is removed by refining (14). Flavor quality ballots for oil quality have been described (100) and incorporate separate ballots for grading and flavor intensity. The flavor quality ballot only describes the flavor characteristics and does not include the suspected cause or process of any off-odors (101). Lexicons of roasted peanut flavor terms are available, and the origins of these flavor terms have been discussed (102). Although there is no U.S. standard of identity *per se*, peanut oil must be suitable for human consumption and conform to the identity characteristics defined by the Codex Alimentarius Commission (103). The various chemical and physical characteristics for peanut oil are given in Table 4.

Heat of fusion, or latent heat, is the quantity of heat required to change 1 g of solid to a liquid with no temperature change. This latent heat increases with increasing molecular weight. Heat of combustion is the amount of heat produced by combustion of 1 kg of oil (104). The heat of combustion increases with the chain length of the fatty acids for both monoacylglycerols and triacylglycerols (107).

The Hehner value expresses the percentage of water-insoluble fatty acids plus unsaponifiable matter in an oil or fat (105). This method is of greatest value in testing butterfat purify. Like most vegetable oils, peanut oil has a higher Hehner value than butterfat (108). Lipids with soluble fatty acids will have lower Hehner values than those with a greater proportion of high-molecular-weight fatty acids. The IV, or Wijs iodine number, is the number of grams of iodine absorbed under standard conditions by 100 g of fat. Peanut oil's IV of 82–107 indicates it is more saturated than corn, cottonseed, or linseed oil but is less saturated than coconut, palm, or butter oil (37). Oil from the high oleic peanut varieties has an IV usually between 73 and 77 (41).

Peroxide value is the measure of reactive oxygen content of a fat in terms of milliequivalents per 1000-g fat, following AOCS method Cd 8-53 or AOAC Method 965.33 (109). Elevated peroxide values indicate that lipid oxidation has taken place (110). Free fatty acids can serve as substrates for lipoxxygenase and peroxidase (111), both of which are inactivated during heating (112). Once the cell

TABLE 4. Characteristics of Peanut Oil.

Characteristic	Value	Reference
Acetyl value	8.5–9.5	37
Acid value (maximum)		
Refined	0.6 mg KOH/g oil	103
Cold Pressed	4 mg KOH/g oil	103
Calculated gums (phosphatides x 32)	0.35%	104
Color (Lovibond, maximum)	Yellow 16–25; 2.0 red	37
Color (visual)	Light yellow	37
Flavor and odor		
Refined	Bland	14
Cold Pressed	Shall be characteristic of the natural product Free from foreign and rancid odor or taste	103
Heat of fusion (unhydrogenated)	21.7 cal/g	37
Heating value	40.4 mJ/kg	104
Hehner value	95–96	105
Insoluble Impurities (% maximum)	0.05	103
Iodine no. (Wijs)	86–107	103
Kinematic viscosity (21.1°C)	70.7cSt	104
Melting point	0–3°C	106
Melting point of the fatty acids	22–30°C	106
Moisture and volatiles	0.23%	106
Peroxide value (maximum)		
Refined	10 meq peroxides O <sub>2</sub> /kg oil	103
Cold Pressed	15 meq peroxides O <sub>2</sub> /kg oil	103
Polenske value	0.5	37
Refractive index (n <sub>D</sub> 40°C)	1.46–1.465	103
Reichert-Meissl value	0.5	37
Saponification number	187–196	103
Smoke point (minimum)	~226.4°C	14
Specific gravity (20°C)	0.912–0.920	103
Specific heat (C <sub>p</sub> , liquid oil)	0.4914 + 0.004 T (°C)	107
Surface tension	35.6 mN/m	104
Thiocyanogen value	0.5	37
Titer	26–32°C	37
Unsaponifiable lipids	0.40%	104

structure is disrupted, lipoxygenase reacts with linoleic, linolenic, or arachidonic acid [either as the free acid, triacylglycerols, or methyl or ethyl esters (113)] to form hydroperoxides. Hydroperoxides can undergo further decomposition to form pentanal and hexanal, both of which are detectable by headspace analysis (114). These oxidation products are correlated with reduced flavor scores (100) and cardboard and painty defects (100). Although the peroxide value is used as an indicator of oil oxidation, the Kreis test was found to be a better predictor of oxidation than the peroxide value for peanut oil (115).

The Plenske value and Reichert–Meissel values are indicators of steam-volatile water-soluble (butyric, caproic, and caprylic) or water-insoluble (capric and lauric) fatty acids, respectively (37). These tests were designed for detecting

low-molecular-weight fatty acids in oil and adulteration in butterfat (106). Butterfat has a Reichert–Meissel value of 17–34.5 (110).

The thiocyanogen value (TV) is a measure of the amount of the reagent absorbed by 1 g of fat. GLC methods have largely displaced this method for determining the content of oleic, linoleic, and linolenic acids when IV's are determined (116). Methods for calculating fat composition using the IV and TV have been discussed (110).

For soap making, the melting point of the fatty acids (titer value) is an important parameter (117). The titer value for peanut oil is lower than that for cottonseed oil (30–37°C), cocoa butter, and animal fats and oils (118) but is higher than that for corn (14–20°C) and/or linseed oil (19–21°C) (37).

The unsaponifiable matter is largely sterols and methylsterols (119, 120). Detailed compositional analysis of the unsaponifiable fraction will be discussed under the sterol subheading.

Before the development of gas chromatography and high-pressure liquid chromatography, the presence of peanut oil (as an olive oil adulterant) could be detected because peanut oil contains about 5% arachidic acid. Arachidic acid is insoluble in cold alcohol unlike stearic and palmitic acids (110). Methods for the detection of arachidic acid include the Bellier, Evers, Evers–Bellier, and Renard tests (110, 121). Arachidic acid is predominant in the lecithin and cephalic fractions of peanut oil (122). Detection methods for toxic oils as an adulterant in edible oils such as peanut oil have been reviewed (123, 124).

Advances in instrumentation have brought about proposals of new methods for oil content and quality measurements. Near-infrared transmittance spectroscopy has been used as a nondestructive method for the determination of oil content in peanuts (125). Fourier-transform infrared methodology has been applied as a quality control method in determining peanut oil in high fat products such as peanut butter (126) and monitoring changes in peanut oil and other oils under oxidative conditions (127). Although Fourier-transform–Raman spectroscopy has been applied to the classification of fats and oils including peanut oil (128), differential scanning calorimetry has been used to follow changes in the thermal characteristics of frying oils such as peanut oil (129).

## 6.1. Fatty Acids

Peanut oil is composed of mixed acylglycerol of approximately 80% unsaturated and 20% saturated fatty acids (37). In mature peanuts, the oil is 96% triacylglycerol (130) with the main fatty acids being palmitic, oleic, and linoleic (40). Other fatty acids found in peanut oil are arachidic, 11-eicosenoic, behemic, and lignoceric acids. The long-chain fatty acids are usually found at about or slightly less than 2%. The percent of free fatty acids in peanut oil varies between 0.02% and 0.6% (131). Lipase hydrolysis of triacylglycerols into free fatty acids and glycerol occurs before germination (132) and during adverse storage (97). Consequently, high free fatty acid values indicate poor handling, immaturity, mold growth, or other factors that lead to triacylglycerol hydrolysis (133).

**TABLE 5. Reported Fatty Acid Composition Ranges of Peanut Oil.**

Fatty Acid	Percentage		
	Reference 102	135	141
Palmitic	8.0–14.0	7.4–12.5	5.3–10.4
Stearic	1.0–4.5	2.7–4.9	2.2–4.4
Oleic	35.0–69	41.3–67.4	52.8–82.2
Linoleic	12.0–43.0	13.9–35.4	2.9–27.1
Arachidic	1.0–2.0	1.2–1.9	1.1–1.8
Eicosenoic	0.7–1.7	0.7–1.4	0.7–2.4
Behenic	1.5–4.5	2.1–3.6	2.2–3.9
Lignoceric	0.5–2.5	0.9–1.7	1.0–1.9

With maturation, the percentage of oleic acid increases while linoleic acid percentage decreases slightly (41, 45). Oxidative stability of peanut oil is highly correlated with the ratio of oleic acid to linoleic acid (134); thus, oil stability is correlated with maturity. Cooler production climates lower the O/L ratio, resulting in oil with a shorter shelf life. Other environmental conditions, such as drought (135), and dry-land farming (45) will also lower the O/L ratio, and selecting soils with a more basic pH and increasing iron while avoiding overfertilization will increase the O/L ratio (136). Application of growth regulators has been shown to reduce the O/L ratio (137, 138), decrease the eicosenoic acid content (137), and increase oil yield (139). Herbicides have been shown to have a slight effect on the oleic and linoleic acid content (137, 140). Fatty acid composition of peanut oil can also be widely influenced by cultivar source (141, 142). Varietal variations in fatty acid composition are summarized in Table 5 and by Young (143). It is again important to indicate that in high oleic acid peanut cultivars, the general characteristic is a linoleic acid content of 4% or less (41).

## 6.2. Triacylglycerol Structure

Interest in the triacylglycerol structure of peanut oil arose from observations that peanut oil showed atherogenic effects in rabbits and other animals (144–147). This atherogenicity has been attributed to the triacylglycerol structure of peanut oil (148–150) because treatment of peanut oil with a base, to bring about randomization, reduced the atherogenicity to that of corn oil (151). However, the results of the Kritchevsky studies (148, 149, 151) have been questioned (40) on the basis that they did not include other vegetable oils for comparison and a lack of data for appropriate statistical analysis. More recent studies (152–155) have shown that peanut oil and peanut product-based diets produce a reduction in total and LDL cholesterol.

Various studies have identified anywhere from 18 to 84 different triacylglycerol species in peanut oil (149, 150, 156, 157). Although many different triacylglycerol species have been identified, the data are conclusive concerning a nonrandom distribution of fatty acids in the *sn*-1, -2, -3 positions of the triacylglycerols. As the

composition of the peanut oil changes, so does the spatial arrangement of the triacylglycerols (158). The predominate triacylglycerol species are OOL, OOO, OLL, POL, and POO (O = oleic, L = linoleic, P = palmitic) (157). Oleic acid is present in high concentration at all three positions, and linoleic acid is found primarily in the *sn*-2 position. The shorter chain length saturated fatty acids, palmitic and stearic, are mainly located in the *sn*-1 position and less in the *sn*-3 position. The longer chain length saturated fatty acids, arachidic, behenic, and lignoceric, are located in the *sn*-3 position. Eicosenoic acid is also frequently located in the *sn*-3 position (156, 157, 159). Peanuts are grown under many different environmental conditions, and such environmental differences can also influence the composition of the peanut oil and the triacylglycerol species (160). Because peanut triacylglycerol structure and composition and total oil composition are affected by environmental factors and diverse genetic background (158), their atherogenic potency (148) and oxidative stability (74) may also be affected by these conditions.

### 6.3. Phospholipids

The phospholipid content of peanut oil can vary from 0.6% to 2% depending on the maturity of the peanuts from which the oil is extracted (161). The major phospholipids of peanut oil are phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidylinositol (PI). The composition of the phospholipid fraction is influenced by maturity and by the postharvest stresses to which the peanuts are subjected (162). The concentrations of PA, PE, PC, and PG were higher in immature seed, and PI was lower, when compared with mature seed. The concentration of all phospholipids except PG increased when peanuts were subjected to a curing temperature of 40°C. When the peanuts were frozen before curing, a significant increase was observed in PA and PG, whereas PC and PE decreased in comparison with the controls. Oxidative stability of peanut oil has been postulated for some time to be caused by constituents in addition to the linoleic acid content and tocopherol content (163). More recently, it has been reported that phospholipids act in a synergistic manner with tocopherols in lengthening the onset of the induction period of lipid oxidation (164, 165). The degree of unsaturation of the acyl fatty acid chain has an added effect on the length of the induction period (164). PE and PI appeared to be more effective than PC in increasing oil stability (164). The usually high concentration of PC in raw peanut oil contributes to the efficiency of the degumming process during refining (166). A critical concentration of PC is needed to ensure that a gum is formed for the removal of the phospholipids.

### 6.4. Tocopherols

Tocopherols are considered a moderate antioxidant in the peanut oil. The Codex Alimentaris standard for tocopherols in peanut oil (103) indicates a range of 48–373 mg/kg for alpha-tocopherol, 0–140 mg/kg for beta-tocopherol, 88–389 mg/kg for gamma-tocopherol, and 0–22 mg/kg for delta-tocopherol. Total tocopherol



content ranges from 130 to 1300 mg/kg. Tocotrienols should not be detectable in peanut oil. Tocopherol content in the oil can be affected by variety, production location within the United States, maturity, and temperature of seed storage (37). Storage of peanut seed at 38°C vs. 22°C reduced alpha-tocopherol content by about 25%. A multiyear study on oil composition of peanuts exported from Argentina, China, and the United States found tocopherol content to be the highest in the U.S. source and lowest in the China source (167). Alpha- and gamma-tocopherols were found to be the most abundant forms. Tocopherol form influences the antioxidant capacity. Gamma- and delta-tocopherols were found to be significantly better antioxidants than alpha-tocopherol, in that either of the first two would protect oil approximately twice as long as a similar concentration of the latter (168). In unprocessed expeller-pressed peanut oil, the tocopherol content did not affect antioxidant activity when the oil was stored at 2% relative humidity (RH) vs. 91% RH (169). Total tocopherol content in oil may be reduced during the degumming and the bleaching processes by 20% and 60%, respectively (170). Peanut oil tocopherols are also lost during frying when peanut oil is used as a cooking oil (171). Tocopherols are also known as vitamin E; thus, peanut oil can serve as a good source for this vitamin particularly when the oil is unrefined. The vitamins found in peanuts are given in Table 6.

**TABLE 6. Vitamin Content of Peanuts (Units per 100 g Dry Weight) (37).**

Constituent	Units
Fat soluble	
Vitamin A	26 I.U.
Carotene (provitamin A)	Trace (<1 ug)
Vitamin D	ND
Vitamin E	26.3–59.4 mg/100-g oil
Alpha-tocopherol	11.9–25.3 mg/100-g oil
Beta-tocopherol	10.4–34.2 mg/100-g oil
Delta tocopherol	0.58–2.50 mg/100-g oil
Vitamin K	ND
Water soluble	
B-Complex	
Vitamin B <sub>1</sub> —Thiamine	0.99 mg
Vitamin B <sub>2</sub> —Riboflavin	0.14 mg
Vitamin B <sub>6</sub> —Pyridoxine	0.30 mg
Vitamin B <sub>12</sub> —Cyanocobalamin	ND
Niacin—Nicotinic acid	12.8–16.7 mg
Choline	165–174 mg
Folic acid	0.28 mg
Inositol	180 mg
Biotin	0.034 mg
Pantothenic acid	2.715 mg
Vitamin C	5.8 mg

ND—Nondetectable.

**TABLE 7. Codex Alimentarius Standard Levels of Desmethylsterols in Peanut Oil (102).**

Constituent	% Total Sterols
Cholesterol	ND–3.8
Brassicasterol	ND–0.2
Campesterol	12.0–19.8
Stigmasterol	5.4–13.2
Beta-sitosterol	47.4–69.0
Delta-5-avenasterol	5.0–18.8
Delta-7-stigmasterol	ND–5.1
Delta-7-avenasterol	ND–5.5
Others	ND–1.4
Total sterols (mg/kg)	900–2900

ND—Nondetectable, defined as  $\leq 0.05\%$ .

## 6.5. Sterols

Sterols are a minor constituent of peanut oil, varying from 0.09% to 0.3% (172). Refining can remove nearly 61% of the sterol content. The Codex Alimentaris standards for desmethylsterols in peanut oil (103) are given in Table 7. Detailed analyzes of the unsaponifiable lipid fraction from peanut oil can be found in the literature. Analysis of the unsaponifiable fraction of Nigerian peanut oil indicated the total fraction to be about 0.4%, and when subdivided by TLC, the fractions were sterols 60%, hydrocarbons 27%, the remainder aliphatic alcohols, and other minor components (119, 120). Beta-sitosterol comprised 64% and campesterol 15% of the sterol fraction. The major triterpene alcohols included 24-methylenecycloartanol at 46% and cycloartanol at 33%. A more recent report on the separation of the unsaponifiable components of Madagascar peanut oil (173) indicated that the sterol fraction was composed of 72% beta-sitosterol and about 17% campesterol. The 4-alpha-methylsterol fraction was primarily composed of citrostadienol (20%), obtusifoliol (17%), gramstisterol (15%), and cycloeucalenol (14%). The triterpene alcohol fraction was composed of 14-methyl-cycloeucalenol (42%), cycloartenol (22%), cycloartanol (15%), and lupeol (10%). Use of peanut oil as frying oil also results in the loss of phytosterols (174). The major sterol component, beta-sitosterol, has recently been shown to inhibit cancer growth (175) and may offer protection from colon, prostate, and breast cancer.

## 7. USES

Peanut oil is used mainly for edible purposes in the preparation of shortening, margarines, and mayonnaise, as a cooking and frying oil and as a salad oil. As indicated previously, the primary use of edible peanuts outside North America is the production of peanut oil (Tables 1, 2), and the oil may be hydrogenated into vanaspati, an Indian analogue to margarine (176). Because of the high smoke point (229.4°C),

refined peanut oil is often used in deep-fat frying (14), but hydrolysis of acylglycerols into free fatty acids during frying leads to a decrease in smoke point (107). For both frying and as a salad oil, peanut oil is considered to be superior to soybean oil and develops fewer flavor defects with long-term use (177). Peanut oil is considered to be superior in the manufacture of pourable dressings because of its ability to hold solids in suspension longer (178). However, because peanut oil solidifies at 0–3°C, it does not meet the definition for salad oil, which must remain clear after 5.5 hours of immersion in an ice bath at 0°C (179). A nonedible use of peanut oil as a diesel fuel has been investigated (180–183), but it is more expensive than conventional No. 2 diesel fuel and has the added drawbacks of lower heating value, greater surface tension, greater viscosity, and greater density (104).

### 7.1. Peanut Oil as a Protectant

In developing countries, there is a need for economical and locally available materials that can be used as a protectant, particularly as a seed protectant. In Nigeria, peanut oil is recommended for control of rice weevils (*Sitophilus oryzae* L.) (184) and as a protectant of maize from damage by the maize weevil (*Sitophilus zeamais* Motsch.) (185). Protection can last up to 180 days. Control of *Sitophilus granaries* L. with peanut oil was effective for up to 90 days of storage for wheat (186). The use of peanut oil for the control of *Callosobruchus maculatus* (F) in cowpea grain has been reported (187) and its mode of action investigated (188, 189). Applications of the method have been reported from Gambia (190), Senegal (191), Nigeria, and Colombia, South America (192). In India, peanut oil is used as a protectant against *Callosobruchus chinensis* L. in chickpea (*Cicer arietinum* L.) (193). In Sahel, peanut oil is used for protecting leguminous tree seeds against seed beetles (194). Other protectant applications are its use as a protectant against infestations of *Cryptolestes pusillus* and *Rhyzopertha dominica* in stored grains, such as maize and sorghum (195). Application of peanut oil to apples as a postharvest treatment has been shown to reduce superficial scald (196). Peanut oil has also been evaluated for control of the parasitic tracheal mite [*Acarapis woodi* (Rennie)] in colonies of the honeybee [*Apis mellifera* (L.)] (197).

## 8. DIETARY ASPECTS

Dietary aspects of high fat content products such as peanuts and peanut products and of peanut oil are often in question. One point is the high atherogenic potential of peanut oil, which has been attributed to its triacylglycerol structure (148–150), because treatment of the oil with a base to bring about randomization reduced the atherogenicity to that of corn oil (151). Another study has suggested that the lectin in peanut oil may significantly contribute to its atherogenic properties (198). Continued human epidemiological studies have shown a 30–50% reduction in cardiovascular disease in individuals who ate nuts, including peanuts, four to five times a week (199–201). Another human subjects study found that the use of high oleic

acid peanuts as the fat source in a low-fat–high-monounsaturate diet produced significant positive changes in blood lipids in postmenopausal women, including reduction of total cholesterol from 264 to 238 mg/dl (202). Additional evidence for the benefits of a diet high in monounsaturated and polyunsaturated fats and low in saturated fat on body function is found in a recent study in which the subjects consumed one of five diets: a low-fat diet, one including olive oil, one including peanuts and peanut butter, one including peanut oil, and a typical American diet. Results indicated that the diet including peanuts and peanut butter, the one including peanut oil, and the diet including olive oil (all low in saturated fat and cholesterol, and high in monounsaturated fat) lowered total cholesterol and LDL cholesterol. Further, each of these three diets lowered triacylglycerol levels, but they did not lower the beneficial HDL cholesterol (203, 204). Peanut oil because of its beta-sitosterol may inhibit cancer growth (175) and may offer protection from colon, prostate, and breast cancer. Snacking on peanuts or peanut products has a satiety effect that enables individuals to control hunger without leading to a weight gain (205).

## 9. ALLERGENICITY

The allergenicity of peanuts is well documented (206). Because peanuts are among the most potent allergenic foods, based on the prevalence of peanut allergy and the frequency of reported severe adverse reactions (207–209), peanut oil has been the most thoroughly studied (210). It has been shown that the most peanut-allergic individuals can safely consume refined peanut oil, whereas unrefined oil can provoke reactions in some of the same individuals. However, some other studies report cases of allergic individuals reacting to peanut oil that presumably had been refined (211, 212). This has led to a debate about the safety of refined oils and specifically whether to label each oil individually because of the potential risk of allergenicity. It has been suggested that the discrepancy between these observations was caused by processing differences (210). It was further suggested that there needs to be a standardized and validated methodology for measuring the protein content and immunoreactivity of the residual protein in the peanut oil. Such a standard methodology can then be used to maintain process specifications. Thresholds of reactivity to allergens also need to be established to assess fully the risk from very small amounts. It has been questioned whether high oleic acid peanuts differ in their allergenic properties from normal peanuts. Investigation of this question concluded that a high content of oleic fatty acid has no effect on peanut allergenicity (213).

## REFERENCES

1. H. T. Stalker and C. E. Simpson, in H. E. Pattee and H. T. Stalker, ed., *Advances in Peanut Science*, Am. Peanut Res. & Educ. Soc., Stillwater, Oklahoma, 1995, p. 15.
2. A. Krapovickas (English translation), in P. J. Ucko and I. S. Falk, eds., *The Domestication and Exploitation of Plants and Animals*, Gerald Duckworth Co. Ltd., London, U.K., 1969, pp. 424–441.

3. D. J. Banks, *Am. J. Bot.*, **74**, 724 (Abstr) (1987).
4. D. J. Banks, *Am. J. Bot.*, **75**, 158 (Abstr) (1988).
5. D. J. Banks, T. Pozorski, S. Pozorski, and C. B. Donnan, *Am. Peanut Res. Educ. Soc.*, **25**, 34 (Abstr) (1993).
6. W. Alva, *Nat. Geograph.*, **174**, 510 (1988).
7. C. B. Donnan, *Nat. Geograph.*, **174**, 550 (1988).
8. R. O. Hammons, in *Peanut—Culture and Uses*, Am. Peanut Res. & Educ. Assoc., Stillwater, Oklahoma, 1972, p 17.
9. USDA. FAS, Production Estimates and Crops Assessment Div. (Oct. 2002). *World Agricultural Production*. Available: <http://www.fas.usda.gov>.
10. USDA. (2002). FAS, Production, Supply & Distribution Online, Washington, D.C. Available: [http://www.fas.usda.gov/psd/complete\\_files/default.asp](http://www.fas.usda.gov/psd/complete_files/default.asp).
11. F. R. Johnson, *The Peanut Story*, Johnson Publishing Co., Murfreesboro, North Carolina, 1964, p. 164.
12. G. E. Brooks, *J. African History*, **16**, 29 (1975).
13. Unknown, *Scientific Amer.*, **April 21**, 243 (1900).
14. J. G. Woodroof, *Peanuts: Production, Processing, Products*, Avi Publishing Co., Westport, Connecticut, 1983.
15. G. R. Ziegler and Y-J. Liaw, *J. Amer. Oil Chem. Soc.*, **70**, 947 (1993).
16. G. Hoffman, *The Chemistry and Technology of Edible Oils and Fats and Their High Fat Products*, Academic Press Inc., San Diego, California, 1989.
17. M. Matricardi, R. Hesketh, and S. Farrell, *Technical Note-20*, Supercritical Fluid Technologies, Inc, Newark, Delaware, 2002.
18. E. Stahl, E. Schutz, and H. K. Mangold, *J. Agric. Food Chem.*, **28**, 1153 (1980).
19. J. J. Snyder, J. P. Friedrich, and D. D. Christianson, *J. Amer. Oil Chem. Soc.*, **61**, 1851 (1984).
20. J. W. Goodrum and M. B. Kilgo, *Trans. ASAE*, **30**, 1865 (1987).
21. T. H. Sanders, in *Proc. World Conference on Oilseed Processing and Utilization*, American Oil Chemists' Society, Champaign, Illinois, 2001, p. 141.
22. A. Rosendahl, D. L. Pyle, and K. Niranjana, *Enzyme Microb. Technol.*, **19**, 402 (1996).
23. K. C. Rhee, C. M. Cater, and K. F. Mattil, *J. Food Sci.*, **37**, 90 (1972).
24. A. Sharma, S. K. Khare, and M. N. Gupta, *J. Amer. Oil Chem. Soc.*, **79**, 215 (2002).
25. M. Esaka, K. Suzuki, and K. Kubota, *Agric. Biol. Chem.*, **50**, 2395 (1986).
26. M. Esaka, K. Suzuki, and K. Kubota, *J. Food Sci.*, **52**, 1738 (1987).
27. P. N. Maheswari, D. W. Stanley, and F. R. Van De Voort, *J. Amer. Oil Chem. Soc.*, **57**, 194 (1987).
28. R. Vetrinani, N. Jyothirmayi, P. Haridis Rao, and C. S. Ramadoss, *Lebensm.-Wiss. Technol.*, **25**, 532 (1992).
29. B. Kaufmann and P. Christen, *Phytochem. Anal.*, **13**, 105 (2002).
30. M. Ramesh, P. Haridis Rao, and C. S. Ramadoss, *Lebensm.-Wiss. Technol.*, **28**, 96 (1995).
31. L. E. Garcia-Ayuso, J. Velasco, M. C. Dobarganes, and M. D. Luque-de-Castro, *J. Agric. Food Chem.*, **47**, 2308 (1999).

32. J. R. J. Pare, G. Matni, J. M. R. Belanger, K. Li, C. Rule, B. Thibert, V. Yaylayan, Z. Liu, D. Mathe, and P. Jacquault, *J. Assoc. Offic. Anal. Chem. Int.*, **80**, 928 (1997).
33. T. D. Rausch, M.S. thesis, Dept. Food Sci., North Carolina State University, Raleigh, North Carolina, 2002.
34. L. Stoloff, in J. V. Rodicks, C. W. Hesseltine, and M. A. Mehlman, eds., *Mycotoxins in Human and Animal Health*, Pathotox Publishers, Park Forest South, Illinois, 1977, p. 9.
35. J. E. Smith and R. S. Henderson, eds., *Mycotoxins in Animal Foods*, CRC Press, Boca Raton, Florida, 1991.
36. FAO, Worldwide regulations for mycotoxins 1995: A compendium, FAO Food and Nutrition Paper 64, Rome, Italy, 1997.
37. W. Y. Cobb and B. R. Johnson, in *Peanut—Culture and Uses*, Am. Peanut Res. Educ. Assoc., Stillwater, Oklahoma, 1972, p. 210.
38. J. L. Harwood, *Inform*, **5**, 835 (1994).
39. C. E. Holaday and J. L. Person, *J. Food Sci.*, **39**, 1206 (1974).
40. E. M. Ahmed and C. T. Young, in H. E. Pattee and C. T. Young, ed., *Peanut Science and Technology*, Am. Peanut Res. Educ. Soc., Yoakum, Texas, 1982, p. 660.
41. P. C. Andersen and D. W. Gorbet, *J. Agric. Food Chem.*, **50**, 1298 (2002).
42. B. D. Harch, K. E. Basford, I. H. DeLacy, P. K. Lawrence, and A. Cruickshank, *Genet. Resour. Crop Evol.*, **42**, 243 (1995).
43. R. Jambunathan, R. Sridhar, K. Raghunath, S. L. Dwivedi, and S. N. Nigam, *J. Sci. Food Agric.*, **61**, 23 (1993).
44. S. L. Dwivedi et al., *Peanut Sci.*, **20**, 84 (1984).
45. C. T. Young, M. E. Mason, R. S. Matlock, and G. R. Waller, *J. Amer. Oil Chem. Soc.*, **49**, 314 (1972).
46. R. E. Worthington, R. O. Hammons, and J. R. Allison, *J. Agric. Food Chem.*, **20**, 727 (1972).
47. C. E. Holaday and J. L. Pearson, *J. Food Sci.*, **39**, 1206 (1974).
48. D. F. Brown, S. M. Cater, K. F. Mattil, and J. G. Darroch, *J. Food Sci.*, **40**, 1055 (1975).
49. I. B. Hashim, P. E. Koehler, E. E. Eitenmiller, and C. K. Kvien, *Peanut Sci.*, **20**, 21 (1993).
50. H. E. Pattee, A. E. Purcell, and E. B. Johns, *J. Amer. Oil Chem. Soc.*, **46**, 629 (1969).
51. H. E. Pattee, E. B. Johns, J. A. Singleton, and T. H. Sanders, *Peanut Sci.*, **1**, 57 (1974).
52. T. H. Sanders, J. A. Lansden, R. L. Greene, J. S. Drexler, and E. J. Williams, *Peanut Sci.*, **9**, 20 (1982).
53. T. H. Sanders, A. M. Schubert, and H. E. Pattee, in H. E. Pattee and C. T. Young, eds., *Peanut Science and Technology*, Am. Peanut Res. Educ. Soc., Yoakum, Texas, 1982, p. 624.
54. W. A. Court, R. C. Roy, and J. G. Hendel, *Can. J. Plant Sci.*, **64**, 521 (1984).
55. D. J. Banks, *Crop Sci.*, **16**, 499 (1976).
56. A. J. Norden, O. D. Smith, and D. W. Gorbet, in H. E. Pattee and C. T. Young, eds., *Peanut Science and Technology*, Am. Peanut Res. Educ. Soc., Yoakum, Texas, 1982, p. 101.
57. N. R. Grosso and C. A. Guzman, *J. Agric. Food Chem.*, **43**, 102 (1995).

58. N. R. Grosso, J. A. Zygadlo, A. L. Lamarque, D. M. Maestri, and C. A. Guzman, *J. Sci. Food. Agric.*, **73**, 349 (1997).
59. D. T. Grimm, T. H. Sanders, H. E. Pattee, D. E. Williams, and S. Sanchez-Dominguez, *Peanut Sci.*, **23**, 111 (1996).
60. R. E. Worthington and R. O. Hammons, *J. Amer. Oil Chem. Soc.*, **54**, 105A (1977).
61. A. R. Khan, D. A. Emery, and J. A. Singleton, *Crop Sci.*, **14**, 464 (1974).
62. J. B. Misra, S. K. Yadav, and S. Chauhan, *J. Sci. Food Agric.*, **61**, 231 (1993).
63. W. L. Zeile, D. A. Knauft, and C. B. Kelly, *Peanut Sci.*, **20**, 9 (1993).
64. C. T. Young and G. R. Waller, *J. Agric. Food Chem.*, **20**, 1116 (1972).
65. T. A. Coffelt, in G. Röbbelen, R. K. Downey, and A. Ashri, eds., *Oil Crops of the World: Their Breeding and Utilization*, McGraw-Hill, New York, 1983, p. 319.
66. A. J. Norden, O. D. Smith, and D. W. Gorbet, in H. E. Pattee and C. T. Young, eds., *Peanut Science and Technology*, Am. Peanut Res. Educ. Soc., Yoakum, Texas, 1982, p. 95.
67. H. T. Stalker and J. Moss, *Adv. Agron.*, **41**, 1 (1987).
68. J. C. Wynne and T. A. Coffelt, in H. E. Pattee and C. T. Young, eds., *Peanut Science and Technology*, Am. Peanut Res. Educ. Soc., Yoakum, Texas, 1982, p. 50.
69. H. T. Stalker, C. T. Young, and T. M. Jones, *Oléagineux*, **44**, 419 (1989).
70. R. Jambunathan, A. K. Singh, S. Gurtu, and K. Raghunath, *Oléagineux*, **48**, 415 (1993).
71. A. J. Norden, D. W. Gorbet, D. A. Knauft, and C. T. Young, *Peanut Sci.*, **14**, 7 (1987).
72. K. Treadwell, C. T. Young, and J. C. Wynne, *Oléagineux*, **38**, 381 (1983).
73. A. C. Bruner, S. Jung, A. G. Abbott, and G. L. Powell, *Crop Sci.*, **41**, 522 (2001).
74. S. F. O'Keefe, V. A. Wiley, and D. A. Knauft, *J. Amer. Oil Chem. Soc.*, **70**, 489 (1993).
75. K. M. Moore and D. A. Knauft, *J. Hered.*, **80**, 252 (1989).
76. D. W. Gorbet and D. Knauft, *Crop Sci.*, **37**, 1392 (1997).
77. D. W. Gorbet and D. Knauft, *Crop Sci.*, **40**, 1190 (2000).
78. W. D. Branch, *Crop Sci.*, **40**, 1823 (2000).
79. H. E. Pattee, T. G. Isleib, D. W. Gorbet, K. M. Moore, Y. Lopez, M. R. Baring, and C. E. Simpson, *J. Agric. Food Chem.*, **50**, 7362 (2002).
80. L. C. Mercer, J. C. Wynne, and C. T. Young, *Peanut Sci.*, **17**, 17 (1990).
81. D. A. Knauft, K. M. Moore, and D. W. Gorbet, *Peanut Sci.*, **20**, 4 (1993).
82. H. E. Pattee and D. A. Knauft, *Peanut Sci.*, **22**, 26 (1995).
83. J. C. Braddock, C. A. Sims, and S. F. O'Keefe, *J. Food Sci.*, **60**, 489 (1995).
84. J. B. Mugendi, C. A. Sims, D. W. Gorbet, and S. F. O'Keefe, *J. Amer. Oil Chem. Soc.*, **75**, 21 (1998).
85. H. E. Pattee, T. G. Isleib, K. Moore, D. W. Gorbet, and F. G. Giesbrecht, *J. Agric. Food Chem.*, **50**, 7366 (2002).
86. G. E. Bolton and T. H. Sanders, *J. Amer. Oil Chem. Soc.*, **79**, 129 (2002).
87. G. M. Sumainah, C. A. Sims, R. P. Bates, and S. F. O'Keefe, *J. Food Sci.*, **65**, 901 (2000).
88. H. E. Pattee, T. G. Isleib, D. W. Gorbet, and F. G. Giesbrecht, and Z. Cui, in K. Rajasekaran, T. J. Jacks, and J. W. Finley, eds., *Crop Biotechnology*, ACS Symposium Series 829, American Chemical Society, Washington, D.C., 2002, p. 217.

89. A. E. Bayley, *Cottonseed and Cottonseed Products*, Interscience Publishers, New York, 1948, p. 213.
90. S. Fengxia, Z. Dishun, and Z. Zhanming, *J. Amer. Oil Chem. Soc.*, **78**, 749 (2001).
91. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th ed., AOCS Press, Champaign, Illinois, 1993.
92. A. A. Belbin, *Inform (AOCS)*, **4**, 648 (1993).
93. P. J. Wan, T. W. Hurley, J. D. Guy, and D. L. Berner, *J. Amer. Oil Chem. Soc.*, **74**, 731 (1997).
94. H. E. Pattee and A. E. Purcell, *J. Amer. Oil Chem. Soc.*, **44**, 328 (1967).
95. K. T. Holley and C. T. Young, Proc. Peanut Improvement Working Group, 1963, p. 52.
96. D. A. Emery and C. L. Gupton, *Oléagineux*, **23**, 99 (1968).
97. T. H. Sanders, A. M. Schubert, and H. E. Pattee in H. E. Pattee and C. T. Young, ed., *Peanut Science and Technology*, Am. Peanut Res. Educ. Soc., Yoakum, Texas, 1982, p. 626.
98. T. H. Sanders, E. J. Williams, A. M. Schubert, and H. E. Pattee, *Peanut Sci.*, **7**, 78 (1980).
99. APRES, in E. M. Ahmed, ed., *Quality Methods*, Am. Peanut Res. Educ. Soc., Stillwater, Oklahoma, 1985.
100. K. Warner, in D. B. Minn and T. H. Smouse, ed., *Flavor Chemistry of Fats and Oils*, AOCS Press, Champaign, Illinois, 1985, p. 279.
101. G. V. Civile and C. A. Dus, in A. J. St. Angelo, ed., *Lipid Oxidation in Food*, American Chemical Society, Washington, D.C., 1992, p. 279.
102. T. H. Sanders, H. E. Pattee, J. R. Vercellotti, and K. L. Bett, in H. E. Pattee and H. T. Stalker, eds., *Advances in Peanut Science*, Am. Peanut Res. Educ. Soc., Stillwater, Oklahoma, 1995, p. 528.
103. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, *Codex Alimentarius. Vol. 8 - Fats, Oils and Related Products. 2nd ed. Codex Stan 210-1999*, Rome, Italy, Revised 2001.
104. J. W. Goodrum and S. E. Law, *Trans. ASAE*, **25**, 897 (1982).
105. M. B. Jacobs, *The Chemical Analysis of Foods and Food Products*, 3rd ed., Robert E. Krieger Publishing Co., Huntington, New York, 1973.
106. C. E. McMichael and A. E. Bailey, in M. J. Jacobs, ed., *The Chemistry and Technology of Food and Food Products*, Interscience Publishers, New York, 1951, p. 1134.
107. M. W. Formo, in D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 1, 4th ed., Wiley, New York, 1979, p. 172.
108. A. G. Woodman, *Food Analysis*, McGraw-Hill, New York, 1931.
109. AOAC, *Official Methods of Analysis of the Association of Official Analytical Chemists*, AOAC, Arlington, Virginia, 1960.
110. G. A. Jacobsen, *Inform (AOCS)*, **4**, 811 (1993).
111. J. A. Singleton and H. E. Pattee, in G. Charalambous, ed., *Analysis of Foods and Beverages-Headspace Techniques*, Academic Press, New York, 1978, p. 359.
112. A. J. St. Angelo, J. C. Kuck, and R. L. Ory, *J. Agric. Food Chem.*, **27**, 229 (1979).
113. A. J. St. Angelo and R. L. Ory, *Phytopathology*, **73**, 315 (1982).
114. J. A. Singleton and H. E. Pattee, *J. Amer. Oil Chem. Soc.*, **57**, 405 (1980).



115. S. Narasimhan, K. G. Raghuvver, C. Arumughan, K. K. Bhat, and D. P. Sen, *Food Sci. Technol.*, **23**, 273 (1986).
116. N. O. V. Sonntag, in D. Swern, *Bailey's Industrial Oil and Fat Products*, Vol. 2, 4th ed., Wiley, New York, 1979, p. 407.
117. F. A. Norris, in D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 1, 4th ed., Wiley, New York, 1979, p. 470.
118. W. Heimann, *Hanbuch der Lebensmittelchemis, Band IV: Fette und Lipide (Lipids)*, Springer-Verlag, Berlin, 1969.
119. T. Itoh, T. Tamura, and T. Matsumoto, *J. Amer. Oil Chem. Soc.*, **50**, 122 (1974).
120. T. Itoh, T. Tamura, and T. Matsumoto, *J. Amer. Oil Chem. Soc.*, **50**, 300 (1974).
121. L. W. Aurand, A. E. Woods, and M. R. Wells, *Food Composition and Analysis*, Van Nostrand Reinhold, New York, 1987.
122. F. Osman, A. E. Ashour, and A. M. Gad, *Fette, Seifen, Anstrichmit.*, **71**, 264 (1969).
123. H. E. Cox and D. Pearson, *The Chemical Analysis of Foods*, Chemical Publishing Co., New York, 1962.
124. Nasirullah and K. V. Nagaraja, *Oléagineux*, **42**, 35 (1987).
125. J. B. Misra, R. S. Mathur, and D. M. Bhatt, *J. Sci. Food Agric.*, **80**, 237 (2000).
126. F. R. van de Voort, J. Sedman, and A. A. Ismail, *Food Chem.*, **48**, 213 (1993).
127. M. D. Gullen and N. Cabo, *J. Sci. Food Agric.*, **80**, 2028 (2000).
128. V. Baeten, P. Hourant, M. T. Morales, and R. Aparicio, *J. Agric. Food Chem.*, **46**, 2638 (1998).
129. H. Gloria and J. M. Aguilera, *J. Agric. Food Chem.*, **46**, 1363 (1998).
130. T. H. Sanders, *J. Amer. Oil Chem. Soc.*, **57**, 8 (1980).
131. J. D. Guthrie, C. L. Hoffpuir, M. F. Stansbury, and W. A. Reeves, *USDA Agricultural Circular*, 61, USDA, Washington, D.C., March 1949.
132. T. H. Sanders and H. E. Pattee, *Lipids*, **10**, 50 (1975).
133. J. I. Davidson, T. B. Whitaker, and J. W. Dickens, in H. E. Pattee and H. T. Stalker, eds., *Advances in Peanut Science*, Am. Peanut Res. Educ. Soc., Stillwater, Oklahoma, 1995, p. 574.
134. S. P. Fore, N. J. Morris, C. H. Mack, A. H. Freeman, and W. G. Bickford, *J. Amer. Oil Chem. Soc.*, **30**, 298 (1953).
135. I. B. Hashim, P. R. Koehler, R. R. Eitenmiller, and C. K. Kvien, *Peanut Sci.*, **20**, 21 (1993).
136. S. L. Dwivedi, S. N. Nigam, R. Jambunathan, K. L. Sahrawat, G. V. S. Nagabhusanam, and K. Raghunath, *Peanut Sci.*, **20**, 84 (1993).
137. C. P. Malik, P. Singh, and U. Parmar, *Phytochemistry*, **28**, 2651 (1986).
138. I. B. Hashim, P. R. Koehler, and C. K. Kvien, *Peanut Sci.*, **20**, 106 (1993).
139. P. Singh, U. Parmar, C. P. Malik, M. K. Grewal, and D. S. Bhatia, *Peanut Sci.*, **18**, 3 (1991).
140. E. W. Hauser, G. A. Buchanan, W. J. Ethredge, M. D. Jellum, and S. R. Cecil, *Peanut Sci.*, **3**, 56 (1976).
141. R. E. Worthington, R. O. Hammons, and J. R. Allison, *J. Agric. Food Chem.*, **20**, 727 (1972).

142. P. C. Andersen, K. Hill, D. W. Gorbet, and B. V. Brodbeck, *J. Food Comp. Anal.*, **11**, 100 (1998).
143. C. T. Young, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., Wiley, New York, 1996, p. 377.
144. G. A. Gresham and A. N. Howard, *Br. J. Exp. Pathol.*, **41**, 395 (1960).
145. R. F. Scott, E. W. Morrison, R. J. Thomas, and S. C. Nam, *Exp. Mol. Pathol.*, **3**, 421 (1964).
146. H. Imai, K. T. Lee, S. Pastori, E. Ponzilio, R. Florantin, and W. A. Thomas, *Exp. Mol. Pathol.*, **5**, 273 (1966).
147. D. Kritchevsky, S. A. Tepper, H. K. Kim, J. A. Story, D. Vesselinovitch, and R. W. Wissler, *Exp. Mol. Pathol.*, **24**, 375 (1966).
148. D. Kritchevsky, S. A. Tepper, D. Vesselinovitch, and R. W. Wissler, *Atherosclerosis*, **17**, 225 (1973).
149. J. J. Myher, L. Marai, A. Kuksis, and D. Kritchevsky, *Lipids*, **12**, 775 (1977).
150. F. Manganaro, J. J. Myher, A. Kuksis, and D. Kritchevsky, *Lipids*, **16**, 508 (1981).
151. D. Kritchevsky, *Am. J. Clin. Nutr.*, **23**, 1105 (1970).
152. L. Cordain, *Lipids*, **33**, 229 (1998).
153. S. Satchithanandam, T. J. Flynn, R. J. Calvert, and D. Kritchevsky, *Lipids*, **34**, 1305 (1999).
154. P. M. Kris-Etherton, G. Zhao, A. E. Binkoske, S. M. Coval, and T. D. Etherton, *Nutr. Rev.*, **59**, 103 (2001).
155. R. L. Hargrove, T. D. Etherton, T. A. Pearson, E. H. Harrison, and P. M. Kris-Etherton, *J. Nutr.*, **131**, 1758 (2001).
156. G. Sempore and J. Bezard, *J. Amer. Oil Chem. Soc.*, **59**, 124 (1982).
157. J. A. Singleton and H. E. Pattee, *J. Amer. Oil Chem. Soc.*, **64**, 534 (1987).
158. J. C. Hokes and R. E. Worthington, *J. Amer. Oil Chem. Soc.*, **56**, 953 (1979).
159. T. H. Sanders, *Lipids*, **14**, 630 (1979).
160. T. H. Sanders, *J. Amer. Oil Chem. Soc.*, **59**, 346 (1982).
161. T. H. Sanders, *J. Amer. Oil Chem. Soc.*, **57**, 8 (1980).
162. J. A. Singleton and L. F. Stikleather, *J. Amer. Oil Chem. Soc.*, **72**, 485 (1995).
163. S. P. Fore, N. J. Norris, C. H. Mack, A. F. Freeman, and W. G. Bickford, *J. Amer. Oil Chem. Soc.*, **30**, 298 (1953).
164. D. H. Hildebrand, J. Terao, and M. Kito, *J. Amer. Oil Chem. Soc.*, **61**, 552 (1984).
165. T. Ohshima, Y. Fujita, and C. Koizumi, *J. Amer. Oil Chem. Soc.*, **70**, 269 (1993).
166. J. A. Robertson, W. H. Morrison, and D. Burdick, *J. Amer. Oil Chem. Soc.*, **50**, 443 (1973).
167. T. H. Sanders, J. R. Vercellotti, K. L. Crippen, R. T. Hinsch, G. K. Rasmussen, and J. H. Edwards, *J. Amer. Oil Chem. Soc.*, **69**, 1032 (1992).
168. R. M. Parkhurst, W. A. Skinner, and P. A. Sturm, *J. Amer. Oil Chem. Soc.*, **45**, 641 (1968).
169. A. G. Gopala Krishna and J. V. Prabhakar, *J. Amer. Oil Chem. Soc.*, **71**, 1245 (1994).
170. E. M. Prior, V. S. Vadke, and F. W. Sosulski, *J. Amer. Oil Chem. Soc.*, **68**, 401 (1991).
171. K. I. Holownia, M. C. Erickson, M. S. Chinnan, and R. R. Eitnmiller, *Food Res. Int.*, **34**, 77 (2001).

172. A. Seher, *Fett. Wissenschaft Technologie*, **89**(1), 27 (1987).
173. E. M. Gaydo, J. P. Bianchini, and J. V. Ratovohery, *J. Agric. Food Chem.*, **31**, 833 (1993).
174. L. L. Oehrl, A. P. Hansen, C. A. Rohrer, G. P. Fenner, and L. C. Boyd, *J. Amer. Oil Chem. Soc.*, **78**, 1073 (2001).
175. A. B. Awad, K. C. Chan, A. C. Downie, and C. S. Funk, *Nutr. Cancer*, **36**, 238 (2000).
176. D. K. Salunke and B. B. Desai, *Postharvest Technology of Oilseeds*, CRC Press, Boca Raton, Florida, 1986, p. 35.
177. J. C. Cowen, H. Moser, G. R. List, and C. D. Evans, *J. Amer. Oil Chem. Soc.*, **48**, 835 (1971).
178. L. Branscome and C. T. Young, *Peanut Oil*, Georgia Agricultural Commodity Commission for Peanuts, Tifton, Georgia, 1972.
179. R. G. Krishnamurthy, in D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 1, 4th ed., Wiley, New York, 1982, p. 315.
180. J. W. Goodrum, *Trans. ASAE*, **27**, 1257 (1985).
181. M. A. Mazed, J. D. Summers, and D. G. Batchelder, *Trans. ASAE*, **27**, 1371 (1985).
182. M. A. Mazed, J. D. Summers, and D. G. Batchelder, *Trans. ASAE*, **27**, 1375 (1985).
183. J. W. Goodrum, V. C. Patel, and R. W. McClendon, *Trans. ASAE*, **39**, 817 (1996).
184. M. F. Ivbijaro, C. Ligan, and A. Youdewei, *Agric. Ecosyst. Environ.*, **14**, 237 (1985).
185. M. F. Ivbijaro, *Proc. Ecol.*, **6**, 267 (1984).
186. E. Tembo and R. F. A. Murfitt, *J. Stored Prod. Res.*, **31**, 77 (1995).
187. S. R. Singh, R. A. Luse, K. Leuschner, and D. Nangju, *J. Stored Prod. Res.*, **14**, 77 (1978).
188. K. N. Don-Pedro, *Pestic. Sci.*, **26**, 107 (1989).
189. K. N. Don-Pedro, *Pestic. Sci.*, **30**, 295 (1990).
190. S. D. Cockfield, *Trop. Pest Manage.*, **38**, 268 (1992).
191. G. Pierrard, *Trop. Pest Manage.*, **32**, 197 (1986).
192. Anonymous, *League Int. Food Educ. Newsl.*, May, 3-4 (1979).
193. S. K. Singal, Z. Singh, *Trop. Pest Manage.*, **36**, 314 (1990).
194. C. Cardet, T. Kandji, A. Delobel, and P. Danthu, *Agrofor. Syst.*, **40**, 29 (1998).
195. D. Obeng-Ofori, *Entomol. Exp. Appl.*, **77**, 133 (1995).
196. K. J. Scott, C. M. C. Yuen, and G. H. Kim, *Postharvest Biol. Technol.*, **6**, 219 (1995).
197. N. W. Calderone and H. Shimanuki, *J. Econ. Entomol.*, **88**, 805 (1995).
198. D. Kritchevsky, S. A. Tepper, and D. M. Klurfeld, *Lipids*, **33**, 821 (1998).
199. G. E. Fraser, J. Sabate, W. L. Beeson, and T. M. Strahan, *Arch. Inter. Med.*, **152**, 1416 (1992).
200. J. Sabate and G. E. Fraser, *Prim. Cardiol.*, **19**, 65 (1993).
201. F. B. Hu, M. J. Stampfer, J. E. Manson, E. B. Rimm, G. A. Colditz, B. A. Rosner, F. E. Speizer, C. H. Hennekens, and W. C. Willett, *Brit. Med. J.*, **317**, 1341 (1998).
202. D. J. O'Byrne, D. A. Knauft, and R. B. Shireman, *Lipids*, **32**, 687 (1997).
203. P. M. Kris-Etherton, T. A. Pearson, Y. Wan, R. Hargrove, K. Moriarty, V. Fishnell, and T. D. Etherton, *Am. J. Clin. Nutr.*, **70**, 1009 (1999).
204. P. M. Kris-Etherton, G. Zhao, A. E. Binkoski, S. M. Coval, and T. D. Etherton, *Nutr. Rev.*, **59**, 103 (2001).

205. S. V. Kirkmeyer and R. D. Mattes, *Int. J. Obes. Relat. Metab. Disord.*, **24**, 1167 (2000).
206. W. Burks, H. A. Sampson, and G. A. Bannon, *Allergy*, **53**, 725 (1998).
207. C. Loza and J. Brostoff, *Clini. Exper. Allergy*, **25**, 493 (1995).
208. S. M. Tariq, M. Stevens, S. Matthews, S. Ridout, R. Twiselton, and D. W. Hide, *Brit. Med. J.*, **313**, 514 (1996).
209. J. O. Hourihane, S. A. Kilburn, J. A. Nordlee, S. L. Taylor, and J. O. Warner, *Brit. Med. J.*, **314**, 1084 (1997).
210. R. W. R. Crevel, M. A. T. Kerkhoff, and M. M. G. Koning, *Food Chem. Toxicol.*, **38**, 385 (2000).
211. A. Olszewski, L. Pons, F. Moutété, I. Aimone-Gastin, G. Kanny, D. A. Moneret-Vautrin, and J. L. Guéant, *Clini. Exper. Allergy*, **28**, 850 (1998).
212. D. A. Moneret-Vautrin, F. Rance, G. Kanny, A. Olszewski, J. L. Guéant, G. Dutau, and L. Guérin, *Clini. Exper. Allergy*, **28**, 1113 (1998).
213. S-Y. Chung, S. Maleki, E. T. Champagne, K. L. Buhr, and D. W. Gorbet, *J. Agric. Food Chem.*, **50**, 878 (2002).

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## *Rice Bran Oil*

Frank T. Orthoefer

### 1. INTRODUCTION

Rice oil, also called rice bran oil, has been used extensively in Asian countries such as Japan, Korea, China, Taiwan, Thailand, and Pakistan (1, 2). It is the preferred oil in Japan for its subtle flavor and odor. Interest in rice oil in the United States was initiated after WWII, primarily to provide an additional revenue stream to the rice miller. More recently, interest in rice oil escalated with its identification as a “healthy oil” that reduces serum cholesterol (3, 4).

Three facilities were constructed in the United States to produce rice oil (5). The first facility began operation in the late 1950s, and a second facility was started in the 1960s. Both were shut down in the early 1980s because of economics. A third production facility began operation in the early 1990s and continues producing both bulk and packaged oils for the domestic and export markets. Attempts at further development of rice oil production have not been successful because of high capital requirement to construct an oil extraction plant and refining facility and limited availability of stabilized rice bran (6).

Rice oil is a minor constituent of rough rice when compared with the carbohydrate and protein content. Two major classes of lipids are present: those internal within the endosperm and those associated with the bran. The internal lipids contribute to the nutritional, functional, and sensory qualities of rice (7).

Rice bran is the main source of rice oil. The majority of available bran continues to be used for animal feeds without being extracted for the oil. The food industry

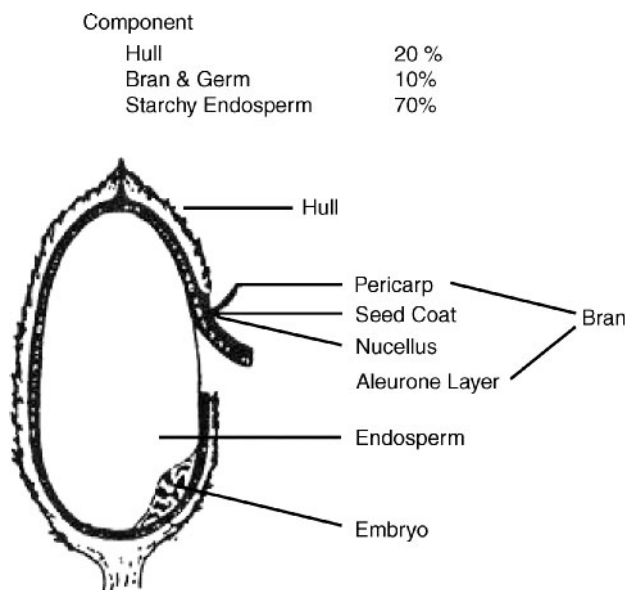
uses minor quantities of stabilized rice bran as a source of dietary fiber, protein, and desirable oil.

This chapter reviews the source and composition of rice bran oil, its nutritional characteristics, production, and refining of the oil and its applications.

## 2. COMPOSITION OF RICE AND RICE BRAN LIPIDS

The structure of the rice kernel is given in Figure 1. Lipids are present as spherosomes or lipid droplets less than 1.5 mm in diameter in the aleurone layer, less than 1.0 mm in the subaleurone layer, and less than 0.7 mm in the embryo of the rice grain (7, 9). Most of the lipids in the endosperm are associated with protein bodies and the starch granules as bound lipids (10). The lipids are broadly classified as nonstarch and starch lipids (Table 1). The majority of the lipids are the nonstarch lipids. Starch lipids consist primarily of lysophospholipids, triacylglycerols, and free fatty acids (13). Major phospholipid species are lysophosphatidylethanolamine and lysophosphatidylcholine. The major fatty acids are palmitic and linoleic acids along with oleic acid. Minor amounts of monoacylglycerols, diacylglycerols, and sterols are also found. Glycolipids found are diglycosyl monoacylglycerols and monoglycosyl monoacylglycerols. The component sugars are galactose and glucose.

The nonstarch lipids in the aleurone, subaleurone, and germ layers were 86–91% neutral lipids, 2–5% glycolipids, and 7–9% phospholipids, although these are variable because of different milling degrees (11). The fatty acid composition of



**Figure 1.** Relative proportion of major rice caryopsis components (8).

**TABLE 1. Lipid Composition of Rice and its Fractions (7, 9, 11, 12).<sup>a</sup>**

Property	Nonstarch Lipids in Rice Fractions						Nonwaxy Starch Lipid in	
	Hull	Brown	Milled	Bran	Germ	Polish	Brown Rice	Milled Rice
Lipid content	<b>0.4</b>	<b>2.7</b>	<b>0.8</b>	<b>18.3</b>	<b>30.2</b>	<b>10.8</b>	<b>0.6</b>	<b>.05</b>
Saponification no.	145	181	190	184	189			
Iodine no.	69	94	100	99	101			
Unsaponifiable matter	26	6	6	6	34			
Fatty acid composition Wt % of total								
Palmitic	18	23	33	23	24	23	46	45
Oleic	42	35	21	37	36	35	12	11
Linoleic	28	38	40	36	37	38	38	40
Others	12	4	6	4	3	4	4	4
Neutral lipids, % of total lipids	64	86	82	89	91	87	28	26
Triglyceride	—	71	58	76	79	72	4	2
Free fatty acids	—	7	15	4	4	5	20	21
Glycolipids, % of total lipids	25	5	8	4	2	5	19	16
Phospholipids, % total lipids	11	9	10	7	7	8	53	58
Phosphotidylcholine	—	4	9	3	3	3	4	4
Phosphatidylethanolamine	—	4	4	3	3	3	5	5
Lysophosphatidylcholine	—	<1	2	<1	<1	<1	21	23
Lysophosphatidylethanolamine	—	—	1	—	—	—	22	25

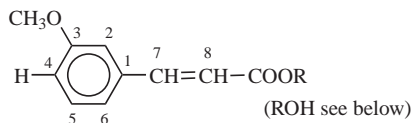
<sup>a</sup>Based on 6% bran-germ, 4% polish, and 90% milled rice from brown rice.

nonstarch lipids showed 22–25% palmitic, 37–41% oleic acid, and 37–41% linoleic acid (Table 2). The brown rice non-starch lipids was 14–18% in germ, 39–41% in bran, 15–21% in polish, and 25–33% in milled rice. The composition was 83–87% triacylglycerol together with 7–9% free fatty acids, diacylglycerols, sterols together with sterol esters, hydrocarbons, and wax. Oil extracted from rice bran contained 20.1% total lipid, 89.2% neutral lipids, 6.8% glycolipid, and 4.0% phospholipid (14). A component of rice bran oil that has promise as a nutraceutical compound is  $\gamma$ -oryzanol (15).  $\gamma$ -Oryzanol was first isolated from soapstock from rice oil

**TABLE 2. Major Lipid Classes of Crude Bran Oil Extracted from Raw Rice Bran and Their Fatty Acid Composition (14).**

Lipid class <sup>a</sup>	wt%	Fatty Acid Composition (%)								saturated	unsaturated
		14:0	16:0	18:0	18:1	18:2	18:3	20:0			
TL	20.1	.40	22.21	2.21	38.85	34.58	1.14	0.61	25.43	74.57	
NL	89.2	0.43	23.41	1.88	37.24	35.29	1.07	0.68	26.40	73.60	
GL	6.8	0.09	27.34	0.28	36.45	35.76	0.18		27.61	72.39	
PL	4.0	0.11	22.13	0.16	38.11	39.32	0.17		22.40	77.60	

<sup>a</sup>TL = total lipids; NL = neutral lipids (nonpolar lipid and free fatty acids); GL = glycolipids; PL = phospholipids.

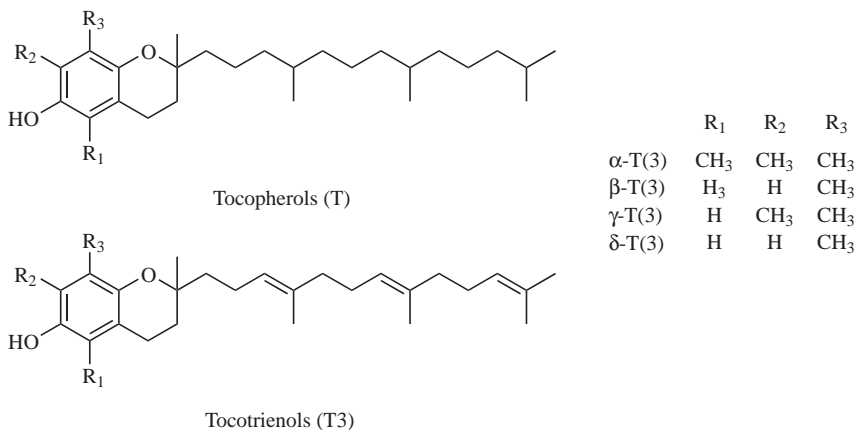


ROH = campesterol  
 = O sitosterol  
 = cycloartenol  
 = 24 methylene-cycloartenol  
 = cyclobranol

**Figure 2.** Major ferulates in oryzanol (9).

refining (16). Although originally thought to be a single compound, it is now known to be a mixture of steryl and other triterpenyl esters of ferulic acids (cycloartenyl ferulate, 24 methylenecycloartenyl ferulate, and  $\beta$  sitosterol ferulate and campesteryl ferulate) (Figure 2). It is present at 1.5–2.9% of rice bran oil with a m.p. of 138.5°C. The oryzanol content is dependent on rice grain variety with long grain rice at 6.42 mg/g and medium grain rice at 5.17 mg/g (17).

Tocopherols and tocotrienols (tocols) are present in rice oil (Figure 3). Crude rice bran oil was found to contain, per 100 g of oil, 19–46 mg of  $\alpha$ -tocopherol, 1–3 mg of  $\beta$ -tocopherol, 1–10 mg of  $\gamma$ -tocopherol, and 0.4–0.9 mg of  $\delta$ -tocopherol, 14–33 mg of  $\alpha$ -tocotrienol, and 9–69 mg of  $\gamma$ -tocotrienol (18, 19) (Table 3). The mean tocol content was 93 mg/100 g for crude oil and 50 mg/100 g for refined oil (19). Close to 370 mg/100 g has been reported (20). Rice bran stabilization and storage (21) and method of extraction (22) affects the concentration of tocols in the oil.  $\gamma$ -Tocotrienol is more stable and persists to a greater extent during storage than other tocols (21). Other factors influencing tocol content are milling and variety (17, 23). Long-grain varieties have higher levels of tocotrienols than medium grain rice (17).



**Figure 3.** Structure of tocopherol and tocotrienol (9).



**TABLE 3. Tocopherol and Tocotrienol Concentrations (mg/100g) in Raw Rice Bran and Commercially Available Refined Oil (14).**

Source	$\alpha$ -T	$\beta$ -T	$\gamma$ -T	$\delta$ -T	$\alpha$ -T3	$\gamma$ -T3	$\delta$ -T3
Rice bran	6.3	0.9	3.20	0.20	3.8	12.0	0.7
Brown rice <sup>a</sup>	0.63	0.09	0.32	0.02	0.38	1.2	0.07
Crude oil <sup>a</sup>	31.50	4.50	16.00	1.00	19.0	60.0	3.5
Refined oil	8.2		12.80	1.3	2.1	42.9	3.5

<sup>a</sup>Calculated values.

Waxes are present as long-chain fatty acid esters with fatty alcohols, methanol, and ethanol. Fatty acid analysis showed that behenic (C:22), lignoceric (C:24), and palmitic acids (C:16) are the major fatty acid for longer alkyl esters and oleic and palmitic for the shorter alkyl esters (Table 4) (24). The major alcohols found are for longer alkyl esters. These are as follows:

**TABLE 4. Fatty Acids of Sterol and Alkyl Esters, Alcohols of Longer Alkyl Esters, and Alkanes and Alkenes of Rice Bran Waxy Lipids (24).**

Fatty Acids Composition of:						
Carbon and Double Bond No.	Longer Alkyl			Alcohols of Longer Alkyl		
	Sterol Esters	Esters	Shorter Alkyl Esters	Alkane	Alkenes	
14:0	0.6	1.8	2.2	—	—	—
16:0	11.1	23.8	35.5	—	—	—
18:0	1.0	3.8	0.8	—	—	—
18:1	33.1	2.9	60.2	—	—	—
18:2	51.1	0.3	1.5	—	—	—
18:3	2.0	—	—	—	—	—
20:0	0.7	3.6	—	0.1	—	—
22:0	—	32.6	—	2.0	—	—
23:0	—	31.2	—	—	1.3	—
24:0	—	—	—	11.2	0.2	—
25:0	—	—	—	—	2.0	—
26:0	—	—	—	6.3	0.8	—
27:0	—	—	—	—	9.5	7.9
28:0	—	—	—	12.5	3.6	1.3
29:0	—	—	—	—	46.5	38.8
30:0	—	—	—	19.1	3.0	0.9
31:0	—	—	—	—	23.7	20.8
32:0	—	—	—	10.5 (5.1)	1.4	0.5
33:0	—	—	—	—	6.5	18.8
34:0	—	—	—	6.6 (18.3)	0.7	1.4
35:0	—	—	—	—	0.8	8.4
36:0	—	—	—	3.0 (5.4)	—	0.1
37:0	—	—	—	—	—	1.6

Major alcohols:

Tetratriacontanol	C34:0
Triaccontanol	C30:0
Dotriacontanol	C32:0
Octacosanol	C28:0
Tetracosanol	C24:0

Straight-chain alkanes, alkenes, and branched-chain alkenes (squalene) are detected in the hydrocarbon fraction. The squalene content is 120 mg/100 g.

Hard and soft waxes are recovered from crude rice bran oil with m.p. of 79.5°C and 74°C (25). The hard wax consists of 64.5% fatty alcohols, 33.5% fatty acids, and 2% hydrocarbons. Soft wax includes 51.8% fatty alcohols, 46.2% fatty acids, and 2% hydrocarbons.

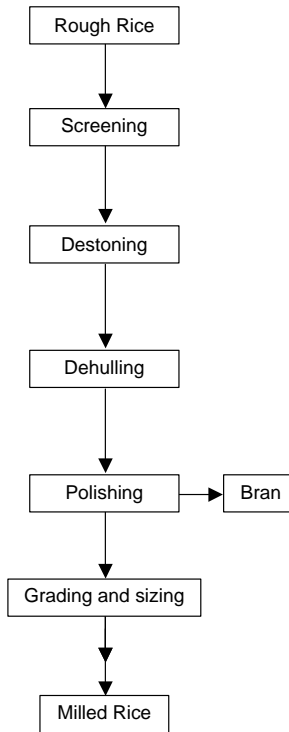
### 3. MILLING OF RICE

Today's modern rice mills efficiently separate hulls from paddy rice followed by bran removal (Figure 4) (6). Milling consists of rubber roll dehullers, paddy separators, abrasive milling (whitening), and possibly friction mills. The bran and polish consist mainly of the outer layers of rice caryopsis. These include the pericarp, seed coat, nucellus, aleurone layer, germ, and part of the subaleurone layer of the starchy endosperm. Rice bran makes up 5–8% of rough rice, and the polish may account for an additional 2–3% (5). Commercial rice bran is a fine, floury material made up of the outer layers of the brown rice plus pulverized germ, some hull fragments, and some endosperm (white rice fragments) (8). The particle size distribution of the bran is shown in Table 5 (26). The particle size of the bran varies significantly with type of milling and milling condition. The composition of the bran also varies as a function of milling degree (Table 6) (27). Generally, a low degree of milling is practiced.

Rice bran is rich in lipids, proteins, minerals, vitamins, phytin, trypsin inhibitor, lipase, and lectin (hemeagglutinins) (5). Compared with other cereal brans, rice bran with germ is a little higher in fat content but comparable in protein, fiber, and ash (Table 7). The high phosphorous content is among the highest of the cereal grains. Rice bran is also high in silica probably because of the presence of rice hull fragments. Bran is high in B vitamins and tocopherol, but it contains only a little Vitamin A and C (28).

Rice bran and germ are used in animal feeds as a low-cost source of protein and oil (6). "Rice mill feed" is a combined product produced by huller mills, where dehulling and milling is a single processing step (5). Raw rice bran, when dehulling is a separate processing step, has about four times the oil content (17–20%) of rice mill feed (6). Parboiled rice bran produced by cooking of rough rice prior to milling has a greater oil content, usually above 20%, than raw rice bran. The higher oil content may be caused by less endosperm contamination, better extractability of the oil

## Steps in Rice Milling



**Figure 4.** Steps in rice milling.

**TABLE 5.** Particle-Size Distribution (%) of Raw and Heat-Stabilized Brans (26).

Mesh	Particle Size (mm)	Raw Bran	Moist Heat-Stabilized Bran
18	>1000	0	0
18–30	1000–595	2.4	18.6
30–50	595–297	30.0	32.7
50–80	297–177	12.2	18.5
80–100	177–149	8.5	10.8
<100	<149	46.7	19.4

**TABLE 6.** Variation in Rice Bran Composition as a Function of the Degree of Milling (27).

	Degree of Milling (%)	Protein	Bran Composition (%)			NFE <sup>a</sup>
			Fat	Fiber	Ash	
1st Cone	0–3	17–0	17.7	10.5	9.8	45.0
2nd Cone	3–6	17.6	17.1	10.3	9.4	45.2
3rd Cone	6–9	17.0	16.5	5.7	8.4	52.5
4th Cone	9–10	16.7	14.2	5.7	7.5	55.9

<sup>a</sup>Nitrogen-free extract.

**TABLE 7 Composition (% at 14% Moisture) of Rice Bran and Polish and Other Cereal Brans (26).**

Constituent	Rice								
	Bran	Polish	Wheat Bran	Corn Bran	Barley Bran	Rye Bran	Oat Bran Shorts	Sorghum Bran	Millet Bran
Crude protein (% N × 6.25)	12.0–5.6	11.8–13.0	14.5–15.	7.8–11.5	11.5	14.6	8.8–16.2	7.7–15.0	11.5
Crude fat (%)	15.0–19.7	10.1–12.4	2.9–4.3	4.4–8.1	2.8	2.6	3.0–6.8	4.6–4.7	8.0
Crude fiber (%)	7.0–11.4	2.3–3.2	6.8–10.4	2.6–9.4	9.6	6.6	20.5	7.4–9.1	—
Available carbohydrates (%)	31.1–52.3	51.1–55.0	50.7–59.2	58.9–62.6	58.4	58.0	61.4	54.3–64.1	56.0
Crude ash (%)	6.6–9.9	5.2–7.3	4.0–6.5	1.9–3.4	3.6	4.2	6.3	2.1–3.0	10.5
Calcium (mg/g)	0.3–1.2	0.5–0.7	1.2–1.3	0.3–0.4	2.8	0.9–1.2	0.9	—	0.8
Magnesium (mg/g)	5–13	6–7	5.6	2.5	—	—	3.0	—	4.0
Phosphorus (mg/g)	11–25	10–22	9–13	1–6	5–8	7.2–10.5	8.1	—	—
Phytin phosphorus (mg/g)	9–22	12–17	10	—	3.1	6.9	—	—	—
Silica (mg/g)	6–11	2–3	2	—	—	—	—	—	—
Zinc (µg/g)	43–258	17–60	105	—	21	56	—	—	—
Thiamine (B <sub>1</sub> ) (µg/g)	12–24	3–19	5.4–7.0	4.2	—	2.5	4.1	—	10.6
Riboflavin (B <sub>2</sub> ) (µg/g)	1.8–4.3	1.7–2.4	2.4–8.0	1.5	—	0.2	3.3	—	—
Niacin (µg/g)	267–499	224–389	181–550	—	—	22.6	1.5	—	—

by solvents, and outward movement of the oil from aleurone and germ cells to the bran layer (28).

The final physical and chemical nature of bran depends on the following:

1. Rice variety
2. Treatment of the grain before milling
3. Type of milling system
4. Degree of milling
5. Fractionation that occurs during milling (29).

The preferred method for milling of rice that gives hulls, bran, and milled rice is referred to as "multistage" or "multiple break" where shellers (dehullers), polishers, and whiteners are used. The hull is first removed in shellers, and the dehulled brown rice undergoes subsequent whitening operations. The amount of contaminants in the bran affects the total lipid content. Contaminants are broken rice and layers from the endosperm. Addition of calcium carbonate, usually at 0.25% of rough rice as a milling aid during whitening, further reduces the oil content. Other milling aids such as diatomaceous earth and ground limestone have also been used.

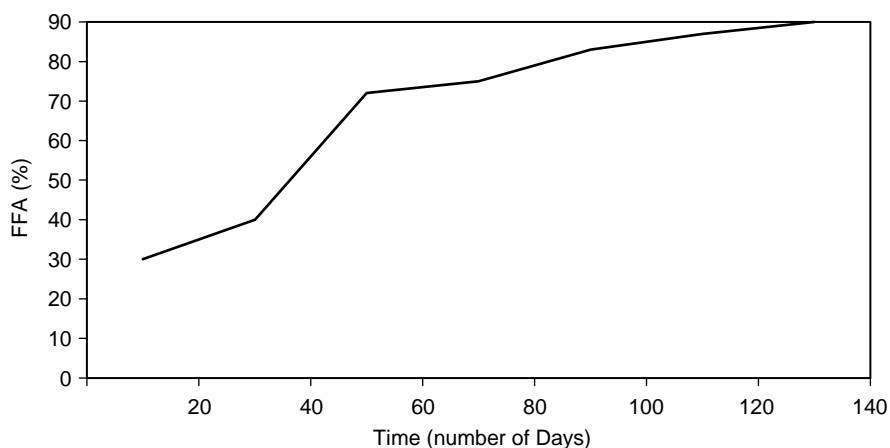
In developing countries, most rice is milled in a one-stage (huller) mill that removes hull, bran, and germ as a single mixture. It is estimated that less than 25% of rough rice is fractionated into hull and bran fractions (29).

#### 4. ENZYMES IN RICE BRAN

Rice bran contains active enzymes (30). Germ and the outer layers of the caryopsis have higher enzyme activities. Some enzymes that are present include  $\alpha$ -amylase,  $\beta$ -amylase, ascorbic acid oxidase, catalase, cytochrome oxidase, dehydrogenase, deoxyribonuclease, esterase, flavin oxidase,  $\alpha$  and  $\beta$ -glycosidase, invertase, lecithinase, lipase, lipoxygenase, pectinase, peroxidase, phosphatase, phytase, proteinase, and succinate dehydrogenase.

Particularly lipase, but also lipoxygenase and peroxidase, are probably most important commercially because they affect the keeping quality and shelf life of rice bran.

Lipase promotes the hydrolysis of the oil in the bran into glycerol and free fatty acids (FFA) (5). The lipase has been studied extensively. In the intact grain, the lipases are localized in the testa-cross layer of the rice grains while the oil is in the aleurone and subaleurone layers and in the germ (26, 26a). The germ, where 60% of the lipase occurs, is similarly compartmentalized. During milling, the enzyme and substrate are brought together. The rate of FFA formation is highly dependent on environmental conditions. Formation of 5–7% free fatty acids per day has been reported (29). Up to 70% FFA has been reported for a single month of bran storage. Production of FFA in a clean U.S.-produced bran is shown in Figure 5 (31). Rice



**Figure 5.** Free fatty acid (FFA) increase in raw bran during a 135-day storage period (8).

bran oil contains 2–4% FFA at the time of milling. Less than 5% FFA is desirable for producing rice bran oil because high FFA results in high refining losses. The composition of crude rice bran oil produced by hexane extraction of stabilized bran is shown in Table 8.

Lipase has a molecular weight of about 40,000 Da and an isoelectric point (pI) of 8.56 (32). It is activated by calcium and inhibited by heavy metals. The optimum pH is 7.5–8.0, and the optimum temperature is 37°C. It is inactivated by heating at 60°C for 15 minutes. Rice bran lipase preferentially hydrolyzes fatty acids from the

**TABLE 8. Crude Rice Bran Oil Composition (8).**

Lipid Type	Percent
<b>Saponifiable lipids</b>	90–96
Neutral lipids	88–89
Triacylglycerols	83–86
Diacylglycerols	3–4
Monoacylglycerols	6–7
Free fatty acids	2–4
Waxes	6–7
Glycolipids	6–7
Phospholipids	4–5
<b>Unsaponifiable lipids</b>	4–2
Phytosterols	43
Sterol esters	10
Triterpene alcohols	28
Hydrocarbons	18
Tocopherols	1

1 and 3 positions in the triacylglycerol molecules. Two subunits are suggested for lipase, and these are held together by disulfide bonds.

A second rice bran lipase has a pI of 9.1 and an optimum temperature of 27°C (33). It has a high specificity for triacylglycerols having short-chain fatty acids.

The enzyme, lipoxygenase, is associated with the oxidation of the polyunsaturated fatty acids (PUFA) having a cis, cis-pentadiene structure. The carbonyl products from the degradation, particularly hexanal, have been implicated in the stale flavor of rice. Lipoxygenase activity is highest in the germ fraction. Three forms of lipoxygenase have been isolated differing in pH optimum and specificity (34).

## 5. STABILIZATION OF RICE BRAN

The instability of rice bran has long been associated with lipase activity (35). As long as the kernel is intact, lipase is physically isolated from the lipids (29). Even dehulling disturbs the surface structure allowing lipase and oil to mix. Oil in intact bran contains 2–4% free fatty acids (2). Once bran is milled from the kernel, a rapid increase in the FFA occurs. In high humidity storage, the rate of hydrolysis is 5–10% per day and about 70% in a month as shown earlier. The objectives of rice bran stabilization are as follows:

- Arrest lipase and lipoxygenase activity.
- Improve oil extraction efficiency.
- Reduce fines in crude oil.
- Sterilize the bran.
- Reduce color development.

The lipoxygenase and peroxidase enzymes also have a negative impact on the oxidative state of the bran (Table 9). Further degradation of the oil occurs as reflected in an increase in peroxide and thiobarbituric acid value and a decrease in iodine value. Both lipoxygenase and peroxidase enzymes are inactivated with lipase inactivation.

**TABLE 9. Changes in the Composition of Bran Lipids During Storage of Milyang 23 Rice Bran at 30°C and 80% RH (28).**

Oil Property	Storage Period (weeks)					
	0	1	2	3	4	5
Free fatty acids (% as oleic acid)	3.6	33.0	40.3	45.8	61.8	68.2
Peroxide value (meq/kg)	32.8	73.2	96.0	109.3	90.6	91.0
Iodine value (%)	96.8	90.2	85.4	83.2	79.0	74.7
TBA <sup>a</sup> (mg of malonaldehyde equivalents/Kg)	0.5	0.8	1.1	0.7	0.7	0.6

<sup>a</sup>Thiobarbituric acid.

Lipase activity results in hydrolytic rancidity. There is little or no change in flavor of the bran with an increase in FFA (5). Lipoyxygenase activity, however, increases with the presence of FFA resulting in oxidative rancidity (36). It is oxidative deterioration that is responsible for the flavor and odor of rancid rice bran.

Peroxidase is used as a convenient index of lipase activity. The inactivation temperature for lipases and associated enzymes is dependent on the moisture content. At 4% moisture, inactivation temperature for lipoyxygenase is 40°C, lipase is 55°C, and peroxidase is 70°C (28).

Methods for stabilization of rice bran have been reviewed (37). These include dry heating, wet heating, and extrusion. The most practical method has been the use of extrusion or expansion methods.

In retained heating methods (dry heat), a simple hot air drying reduces the moisture content to 3–4%. The bran must be kept dry in moisture-proof containers, or the rehydrated bran will regain its lipase activity. If the bran is heated in the presence of moisture, the lipase is permanently denatured.

The types of retained-moisture heating methods include extrusion cookers and sealed rotating drums. Extrusion cooking results in both lipase denaturation and bran sterilization. When pressure is released, part of the superheated moisture evaporates with little or no drying being required. Expanders or expellers are also used to permit addition of moisture (wet heating) through steam and the formulation of collets or pellets from the bran. The collets aid handling and oil extraction.

Extrusion (dry heat) cookers have been ideal for stabilization because excess moisture is not added, eliminating the need for drying. The heating of the bran occurs through conversion of mechanical energy of the screw drive to heat the bran. Temperatures used for stabilization vary from 100° to 140°C. The bran is kept hot for 3–5 minutes after extrusion to ensure lipase inactivation. The hot bran is then cooled using ambient air.

Extrusion cooking of the bran was pioneered by the Western Regional Research Laboratory (28, 29, 29a). Dry extrusion was found more suitable for stabilizing bran to be used as a food ingredient (38). Stabilization within 1 hour after milling is considered ideal for bran quality.

Wet heating is more effective for bran stabilization for oil extraction than is dry heating. Lipase is inactivated in 3 minutes at 100°C (37). The equipment that can be used include steam cookers, blanchers, autoclaves, and screw extruders with injected steam and water (30). Extrusion with steam injection and up to 10% added water reduces the temperature required for lipase inactivation. Temperatures are reduced to 100–120°C. Product may be held at 100°C for 1.5–3.0 minutes before drying to a stable moisture content. Bran expands as it exits the extruder, and water flashes to steam (8). Porous pellets assist in solvent percolation during oil extraction. Fines are agglomerated as well.

Addition of water/steam to bran during wet extrusion requires drying after stabilization. Hot air is simply passed through a bed of pellets. Although this increases the cost of stabilization, lipase inactivation is permanent with less nutritional damage to the bran. The recovered oil is lighter in color with lower refining losses.



The stabilized bran may be stored for extended periods, although extraction should be completed within 1 month for best quality oil (39).

Parboiling of rice is also an example of wet heat stabilization. The lipase in rough rice is completely inactivated by either autoclaving for 3–20 minutes or by parboiling.

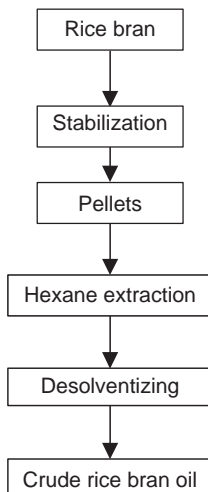
Other stabilization methods that have been investigated are as follows:

1. Refrigeration to reduce the rate of hydrolysis (8)
2. Lowering pH to reduce lipase activity (4)
3. Chemical additions such as sodium metabisulfite (39a)

## 6. RICE BRAN TO RICE BRAN OIL

Rice bran is the source of rice bran oil (30). Various commercial efforts to extract the oil have been made over the past 50 years. Initially, use of the oil in traditional foods was targeted. More recent efforts have emphasized the nutritional benefits of rice bran oil.

Rice bran oil with a low free fatty acid content can be extracted with hexane from extrusion stabilized bran. The process flow is shown in Figure 6. Nonstabilized bran, although having a high free fatty acid, can also be used for production of oil. With nonstabilized bran, the extraction is similar to that of extracting a fine powder. Preprocessing of the bran through an extruder, expander, or expeller may be used to form either a flake or pellet that results in improved solvent flow through an extraction bed (40). Flaked bran with only 7–12% passing a 25 mesh screen gave a percolation through a 60-cm bed of 563–620 L/m<sup>2</sup>/min. The oil extraction rate



**Figure 6.** Process for rice oil production (8).

was rapid, with 96% of the oil being removed in 5 minutes and only 0.7% residual oil remaining after 1 hour of extraction.

Earlier methods to recover the oil used hydraulic pressing (28). In a Japanese system for pressing, the raw bran is cleaned by sifting and air classification to remove whole and broken grains and hulls, and, in some instances, to recover rice germ. The bran is then steam cooked, dried, prepressed, and finally expeller pressed.

Hexane extraction may be batch, battery, or continuous type (12). All three systems were recently operating in Japan. Continuous systems operate in Brazil, Burma, Egypt, India, Mexico, Taiwan, Thailand, and the United States. The bran in the most efficient systems is stabilized, pelletized, and, if required, dried. After the pretreated bran is placed in the extractor, hexane is pumped in and allowed to percolate through the bran to extract the oil. Countercurrent extraction is used. The miscella (solvent plus oil) is passed through filters to remove the bran fines before evaporation for solvent and crude oil recovery. The production of fines from expander stabilized bran depends on stabilization condition (38). Flake size is larger if expanded at 120°C, but the flakes are fragile and easily broken. Flakes with high moisture content were more resistant to breakage. Final bran moisture was about 6%.

Pelletizing of the bran improves percolation and minimizes fines in the miscella. Pellets are 6–8 mm in diameter. Moistening during palletizing reduces the fines problem. Parboiled bran does not produce the hard pellets found for raw bran possibly because of protein denaturation during parboiling (33). Binding of the fines in the pellet is assisted by starch gelatinization during heating of the bran. Parboiled bran also presents problems with sticking to dryer surfaces resulting in self-ignition in the dryer. Prior mixing with raw bran alleviates the problem.

The X-M process combines solvent extraction and milling of the rice (41). Brown rice is pretreated with warm rice oil (0.5%) for 2–3 hours to soften the bran. The rice is then milled in the presence of a rice oil miscella. The solvent slurry is then removed from the rice and the rice oil is recovered. Advantages are that stabilization is not required and the resultant oil had a minimum FFA level. This process is no longer used.

Extraction of rice bran oil by supercritical fluid has been investigated (5). Minor reductions in oil yield may occur. The oil yield with supercritical CO<sub>2</sub> is 17.98%, with CO<sub>2</sub>-ethanol 18.23%, and with hexane 20.21%.

## 7. REFINING OF THE OIL

The color of crude rice bran oil is dark greenish brown to light yellow depending on the condition of the bran, extraction method, and composition of the bran. The pigments include carotene, chlorophyll, and Maillard browning products (12, 28). Oil from parboiled rice bran is generally darker in color than oil from raw rice bran.

The composition of crude rice bran oil has a major effect on refining. The crude oil typically contains up to 0.5% bran fines and 0.5–5% wax. Agitated storage tanks are required. Heated tanks and lines also are necessary to prevent crystallization of

waxes. Refining losses may be in excess of ten times the FFA when the crude oil has a relatively low FFA (<10%). Lower refining losses of approximately two times the FFA have been reported (2, 6, 40).

Refining of crude rice oil involves dewaxing, degumming, neutralization of free fatty acids, bleaching to improve color, and steam deodorization. Refined rice bran oil is a light yellow color (Lovibond 3.0 R 30Y) with a mild background odor and flavor reminiscent of rice. Similar to peanut oil, the flavor and odor are complementary to the flavor of many fried foods, such as fish, chicken, and chips.

## 8. DEWAXING

Waxes can increase refining losses (8). The wax content of crude oil depends on the variety of rice, milling technique, method of oil extraction, and extraction temperature (2). Extraction temperature affects both the type of wax present and its quantity (42). For example, extraction at 50°C yields two to three times more wax than extraction at 20°C.

Initial dewaxing may simply be gravity settling followed by decanting (43). The oil is gradually cooled to allow for wax crystallization followed by filtration or centrifugation to recover the wax sludge. The foots recovered may be added back to the defatted bran, sold as an animal feed oil, or further processed for oil recovery and wax purification. Wax recovery involves acetone washing and fractionation with isopropanol.

The characteristics of the wax are as follows:

Iodine value	11.1–17.6
FFA (%)	2.1–7.3
Phosphorous (%)	0.01–0.15
M.P (°C)	75.3–79.9

Attempts have been made to recover the wax using cold and hot extraction (2). Wax yields of 1.29–1.82% of the crude oil are obtained. Continuous dewaxing of rice bran oil by chilling the oil or miscella to less than 20°C followed by filtration through plate and frame filters is practiced. Kinsey and Hummell (44) reported on the use of sodium silicate as an aid for dewaxing. The characteristics and physical properties of a purified rice bran wax are similar to carnauba wax (45).

Additional dewaxing may be used during degumming and alkali refining (8). Dewaxing of refined, bleached oil by cooling to approximately 5°C followed by filtration is necessary for production of a high-grade, chill-proof oil.

## 9. DEGUMMING AND DEACIDIFICATION

The phospholipids in rice oil are similar in composition to other oil sources. These may be recovered as rice lecithin (5). Production of food-grade lecithin requires

prior removal of bran fines and waxes. Regular water degumming may be used. Temperatures above 80°C are required to prevent crystallization and removal of waxes with the gums. If food-grade lecithin is not being produced, filtration of bran fines is not required. Pretreatment with phosphoric or organic acid is necessary to remove nonhydratable phospholipids. Food-grade surfactants may be added to improve wax removal (46). Degumming at less than 50°C actually assists in wax removal. Wet gums may be added to defatted bran as a method for disposal (8).

Both alkali and physical refining have been used for FFA removal (5). With alkali refining, batch or continuous methods may be used. Oil may be pretreated with phosphoric or organic acid for phospholipid hydration. The oil is then treated with 16–30 baume (Be') caustic with 20–40% excess. The soaps settle and may be recovered as “soapstock” or “foots” (47).

Continuous refining consists of in-line mixers, heaters, and centrifuges (8). The combined oils plus alkali are rapidly heated to 55–70°C to assist in breaking the emulsion of hydrated soap in oil. In instances where neutralization is combined with dewaxing, separation is performed at 28–32°C. Water washing or post-neutralization treatment with silicates to remove final traces of soaps and phospholipids is the same as for conventional oils. Miscella refining, or refining while still in solvent, may also be used (47). Higher refining yields and good-quality neutralized oil with less color are advantages of miscella refining. Losses were near the calculated amount (48) based on titrated values. Rice oil miscella is often variable.

Excessive losses may occur in refining of rice oil. A 5% FFA crude oil has losses ranging from 12% to 40% by the cup method. The cause of high refining losses is unknown. It is assumed the losses are caused by the presence of partial esters, oxidized components, and waxes, as well as high FFA acidity (8). Steam refining is practiced by various refineries in Japan and the United States (2).

In calculating the amount of caustic required for caustic neutralization, the oil is titrated to a phenolphthalein end point. This titration endpoint includes not only the FFA, but also the oryzanol compounds. With the higher caustic addition, the oryzanol is transferred to the soapstock away from the oil. The nutritional benefit of these compounds is lost. An alternative indicator for titration uses alkali blue (8). This indicator reflects the acidity contributed only by the free fatty acids.

## 10. BLEACHING, HYDROGENATION AND DEODORERIZATION

Standard methods are used for bleaching, hydrogenation, and deodorization of rice bran oil. Bleaching uses activated carbon or bleaching earth (47). Activated carbon is seldom used because of high cost and handling difficulties. Bleach clay dosage depends on the characteristics of the rice oil as well as that of the bleaching earth. Dosages range from 2% to 10%. Newer silica bleaching earths are more effective in reaching satisfactory oil colors.

Deodorization or steam stripping is used to remove objectionable odors resulting from peroxides, aldehydes, and ketones as well as characteristic rice oil odors and flavors (12). The oil is heated to 220–250°C under 3–5-mm Hg vacuum. Semicontinuous deodorizer units are the most common types used. Other designs have been evaluated (43). After deodorization, the oil is cooled to 60°C and filtered. Storage of deodorized rice bran oil is the same as for other oils.

Physical refining, also called steam refining, combines deacidification with deodorization. Physical refining is more efficient for high FFA oils giving better yields of neutralized oil than alkali refining (2).

## 11. WINTERIZATION

In addition to wax removal, rice bran oil contains sufficient saturated and high melting glycerides to require winterization to gain a cold test of 5 hours (8, 43). Without winterization, dewaxed rice oil is frequently cloudy or turbid even at room temperature or slightly lower.

Winterization consists of cooling the oil under defined rates and to specific temperatures followed by filtration. With rice oil, winterization consists of cooling 30–35°C oil slowly at a uniform rate to 15°C over a 12-hour period with slow agitation, then further cooling to 4–5°C without agitation followed by holding over a 24–48-hour period, allowing higher melting components to crystallize. The type of crystals formed depends on the cooling rate and the temperature differentials. Large, stable crystals are desired for filterability. Filter aids may be added to assist separation of the crystals from the viscous oil. Cold tests of the winterized oil of 5–7 hours are near maximum.

Miscella winterization more effectively separates the high melting solids from rice oil. Hexane, acetone, and isopropyl acetate are among the solvents used. The miscella is slowly cooled to 15°C over 12 hours with agitation, then to 4–5°C without agitation, and held for 24–48 hours before filtering.

## 12. CO-PRODUCTS FROM PROCESSING

As with all oils, coproducts of refining represent a significant revenue stream. Waxes may be concentrated and refined to compete with other organic waxes. The hard, high melting waxes are preferred for most applications.

Soapstock contains fatty acid soaps and, for oil that is caustically refined, oryzanol (5–10%). The soaps may be acidulated for feed use and the oryzanol isolated (16). Diethyl ether, alumina chromatography, and crystallization are used for purification of the oryzanol.

The deodorizer distillate, about 1% of deodorizer feed, contains tocopherols, tocotrienols, and sterols (Table 10). The tocopherols are shown in Table 11 and the sterols in Table 12. Its value is similar to other oil distillates.

**TABLE 10. Rice Oil Deodorizer Distillate Composition (20).**

Component	Percent (range)
Free fatty acids	25–40
Tocopherols	1.5–3.0
Tocotrienols	4.0–6.0
Sterols	15–25
Squalene	15–25
Monoacylglycerols, diacylglycerols, etc	15–25

**TABLE 11. Approximate Tocol Composition of Rice Oil Distillate (20).**

	Tocol (percent)	
	Tocopherol	Tocotrienols
Alpha	67	21
Beta	3	tr.
Gamma	30	77
Delta	tr.	2

**TABLE 12. Sterol Composition of Rice Oil Deodorizer Distillate (20).**

Sterol	Percent
Beta-sitosterol	38
Stigmasterol	18
Campesterol	13
Delta-7-stigmasterol	10
Delta-7-avenasterol	6
Delta-5-avenasterol	5
Others	10

### 13. COMPOSITION OF REFINED RICE BRAN OIL

A typical specification for finished rice bran oil is shown in Table 13. These are similar to that for other oils. Rice bran oil has a characteristic nutty, earthy flavor not unlike peanut oil.

The fatty acid composition of rice bran oil is most similar to peanut or ground nut oil (Table 14) (8). Palmitic, oleic, and linoleic acids make up more than 90% of the fatty acids present. The major molecular species of triacylglycerols are palmitic-linolenic-oleic, oleic-linoleic-palmitic, palmitic-linoleic-linoleic, linolenic-linoleic-palmitic, and trioleic. As with peanut oil, rice bran oil is most suited for general frying and cooking applications.

**TABLE 13. Product Specification of Refined, Bleached, and Deodorized Rice Bran Oil (8).**

Characteristic	Value
Iodine value (Wijs method, g/100 g sample)	99–108
Peroxide value (meq/kg)	1.0 max
Moisture (%)	0.05 max
Color (5.25-in Lovibond red)	5.0 max
Free fatty acid (% as oleic)	0.05 max
Flavor/odor	7 min
Chlorophyll (ppb)	75 max
Saponification value	180–190
Unsaponifiable matter	3–5
Smoke point	213°C
Refractive index	1,470–1,473
Specific gravity	0.916
AOM <sup>a</sup> (hr)	17.5

<sup>a</sup>Active oxygen method.

**TABLE 14. Chemical Composition of Rice Bran Oil (8).**

Physicochemical Parameters	Value
Acid value	1.2
Iodine value	100.0
Saponification value	211.8
Unsaponifiable matter	4.2
<b>Fatty acid composition</b>	<b>Percent</b>
C14:0	0.6
C16:0	21.5
C18:0	2.9
C18:1	38.4
C18:2	34.4
C18:3	2.2
C20:0	—
C22:0	—

## 14. RICE BRAN OIL NUTRITION

The initial interest in rice bran oil resulted from work with the stabilized rice bran. Rice bran was shown to be equivalent in serum cholesterol reduction to oat bran in hamster trials (Table 15) (1). Two clinical studies showed rice bran reduced serum low-density lipoprotein (LDL) cholesterol in humans (49,50). Defatted bran was less effective in lowering cholesterol than full fat bran (1). The cholesterol-lowering activity was concentrated in the unsaponifiable fraction of rice bran oil (Table 16) (51). Oryzanol was found to contribute to the hypocholesterolemic activity of rice

**TABLE 15. Effect of Rice and Oat Brans on Serum Cholesterol in Hamsters (1).**

Bran in Diet	Serum Cholesterol (mg/dL)
Cellulose (10%)	395
Rice bran (47.8%)	270
Defatted rice bran (24.7%)	347
Parboiled rice bran (31.8%)	297
Defatted parboiled rice bran (19.6%)	377
Oat bran (53.7%)	289

**TABLE 16. Hypocholesterolemic Activity of Unsaponifiable Matter of Rice Bran Oil in Rats (51).**

Diet	Serum Cholesterol <sup>a</sup> (mg/dL)		
	Total	HDL	LDL + VLDL
Control (peanut oil) (10%)	374	43	331
Rice bran oil (10%)	228	48	240
Control + 0.2% unsaponifiables	387	48	339
Control + 0.4% unsaponifiables	243	48	195

<sup>a</sup>HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein.

oil in rats (52) and primates (53). A clinical study with 3.1 g/day of rice bran oil unsaponifiables over a 12-month period resulted in a 14.1% reduction in total cholesterol and a 20.5% reduction on LDL-cholesterol (Table 17) (54). HDL-cholesterol rose, and triacylglycerols decreased significantly. Tocotrienols, also present in rice bran oil, have been reported to reduce serum cholesterol (55).

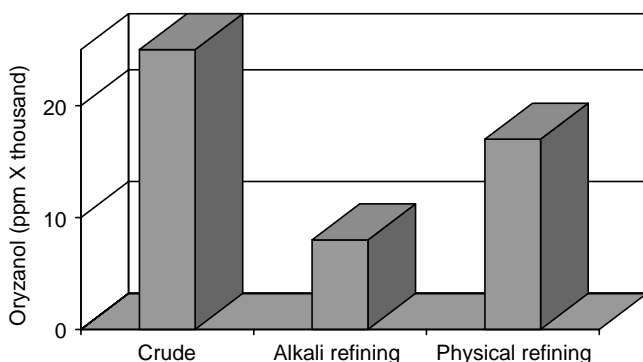
The refining method used in rice oil production affects the oryzanol content of finished oil (3). With alkali refining, most of the oryzanol is removed (Figure 7), whereas with steam or physical refining, most of the oryzanol (66%) remains in

**TABLE 17. Effect of Daily Addition of Rice Bran Unsaponifiables (RBN) on Serum Lipids (mmol/L) in Hypercholesterolemic Subjects (54).**

Serum Lipids <sup>a</sup>	Start	12 months	<i>p</i>
RBN			
Cholesterol	6.18 ± 0.33	5.31 ± 0.20	<0.05
LDL cholesterol	4.28 ± 0.37	3.40 ± 0.18	<0.05
HDL cholesterol	0.17 ± 0.02	0.24 ± 0.02	<0.025
Triacylglycerol/HDL	2.16 ± 0.35	1.21 ± 0.21	<0.05
RBN placebo			
Cholesterol	5.70 ± 0.21	6.06 ± 0.32	ns
LDL cholesterol	3.95 ± 0.18	4.05 ± 0.31	ns
HDL cholesterol	0.21 ± 0.06	0.22 ± 0.01	ns
Triacylglycerol/HDL	1.54 ± 0.31	1.55 ± 0.20	ns

<sup>a</sup>LDL = low-density lipoprotein, HDL = high-density lipoprotein, ns = not significant.





**Figure 7.** Effect of the refining process on the oryzanol content of rice bran oil (8).

the oil (56). Physically refined rice oil gave a serum lipid response similar to that of crude rice bran oil. Various refining methods to preserve the oryzanol in the oil have been attempted. Sodium carbonate instead of sodium hydroxide has been partially successful in which two-thirds of the original oryzanol in the crude oil is preserved in the refined oil (57). Adding back unsaponifiables to the oil has been patented (58). Clinical trials have not been performed with high oryzanol rice bran oil. An unsaponifiable concentrate was prepared by extracting the soapstock, with hexane giving a deacidified concentrate with 30% unsaponifiable content.

## 15. RICE BRAN OIL UTILIZATION

Rice bran oil is used in foods, feed, and industrial applications. Only high-quality oil is targeted to foods. The use of rice bran oil in Japan, where it is the largest volume domestically produced vegetable oil, is as a frying oil where its flavor is preferred over alternative oils. The oxidative stability of rice bran oil is equivalent to peanut oil and cottonseed oils in deep frying applications (Table 18) (8, 59).

**TABLE 18.** Frying Evaluation of Rice Oil (15-day results) (8).

Oil type <sup>a</sup>	Days to Maximum Level <sup>b</sup>				
	FFA	FOS	LY	LR	TPM
Rice (without additives)	3.91	3.74	6	28.0	31.9
Rice (with additives)	5.62	3.46	7	49.6	34.6
Peanut (with additives)	6.87	3.92	8	21.2	37.5
Cottonseed (with additives)	7.22	4.07	7	28.8	37.2

<sup>a</sup>Specifications. 40 lb (18.2 kg) gas fryers, frying temperature 350°F (177°C); hourly rotation: breaded chicken, fish, onion rings, French fries: 5-ppm dimethyl polysiloxane antifoam, 200-ppm tertiary butyl hydroquinone.

<sup>b</sup>FFA = free fatty acids, FOS = food oil sensor; LY = Lovibond yellow; LR = Lovibond red; TPM = total polar material.

**TABLE 19. Frying Results Using Blends of Rice and Soybean Oils (8).**

Oil Type	Total Polar Material (%)	
	10 days	13 days
Rice	21.12	32.78
Peanut	21.07	35.53
Rice/soybean 50:50	24.11	35.80
Rice/soybean 25:75	23.25	40.42

**TABLE 20. Days at 145°F (62.8°C) Before Rancid Odor is Detected (8).**

Oil Type	Days to Detect Rancid Odor
Rice (without additives)	20
Rice (with additives)	25
Peanut (without additives)	14
Cottonseed (with additives)	31

Blends of rice bran oil with soybean oil reduces the increase in total polar material (TPM) depending on the amount of rice bran oil in the blend (Table 19). Potato chips fried in rice bran oil show flavor and odor stability at elevated temperatures between that of peanut and cottonseed oils (Table 20).

Winterized rice bran oil is an acceptable oil for salad dressing and mayonnaise. The hard fraction of rice bran oil may be used to replace the plastic fats in margarines and shortening. Hydrogenated rice bran oil is adaptable to specialty shortenings and margarines.

The nonfood uses of rice bran oil are feed formulations, soaps, and glycerin. Waxes may be used as a carnauba wax replacement in confectionery, cosmetics, and polishing compounds products.

Use of rice bran oil grows as a specialty ingredient in the cosmetic/personal care market. The demand is for natural, value-added healthy ingredients (60).

## 16. RICE OIL PRODUCTION (POTENTIAL)

World rice production is greater than 500 million metric tons. Rice oil production is estimated at 722.2 thousand metric tons (Table 21). India, China, and Japan are the leading producers. More than half of rice is processed in small rice mills. This leaves approximately 20–25 million metric tons of bran available for oil production. The rice bran oil potential is, then, 3–4 million metric tons.

In the United States, most bran is also produced in small rice mills scattered in rice production areas with insufficient bran production to justify oil extraction.

**TABLE 21. Production of Rice Bran Oil (61).\***

Country	Thousand Metric Tons
Bangladesh	1.5
Brazil	1.5
Cambodia	4.6
China	90.0
India	472.7
Indonesia	0.15
Japan	65.0
Korea	11.7
Republic of Korea	9.2
Laos	2.6
Burma	17.6
Nepal	7.6
Pakistan	3.7
Sri Lanka	5.5
Thailand	7.8
Vietnam	7.6
<b>Total</b>	<b>722.2</b>

\* Does not include U. S. production, which is 15.9–18 thousand metric tons.

Production estimates are for less than 80 thousand metric tons. Only 15.9 to 18 thousand metric tons are produced currently in the United States at a single oil extraction facility.

## 17. SUMMARY

Rice bran is an underused coproduct of rice milling. The value is partially captured through extraction and refining of the rice bran oil. The capital costs have limited the ability of the U.S. rice milling industry to capture this value. However, rice bran oil has performance properties competitive to other widely used oils. An additional advantage of rice bran oil is certainly its nutritional benefits, which include a balance of fatty acids meeting AHA recommendations. Rice oil contains a mixture of antioxidants and promotes cholesterol reduction beyond that of more unsaturated oils. Its taste and performance is complementary to salad, cooking, and frying applications.

## REFERENCES

1. T. Kahlon et al., *Cereal Chem.*, **69**(5), 485–489 (1992).
2. F. T. Orthoefer, presented at 85th American Oil Chemists Soc. Annual Meeting, Atlanta, GA, May 9, 1994.
3. R. Cheruvanky, in W. R. Bidlack et al., eds. *Phytochemicals as Bioactive Agents*, Technomic Press, Lancaster Press, 2001.

4. R. Cheruvanky, in L. Johnson and G. Williamson, eds. *Phytochemical Functional Foods*, Woodhead Publishing Limited, Cambridge, U.K., 2003.
5. F. T. Orthoefer, in Y. H. Hui, ed. *Bailey's Industrial Oil and Fat Products*, 5 ed., vol. 2, J. W. Wiley and Sons, Inc. New York, 1996.
6. F. T. Orthoefer and J. Eastman, in E. Champagne, ed. *Rice Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, MN, 2003.
7. B. O. Juliano, in P. J. Barnes, ed. *Lipids in Cereal Technology*, Academic Press, London, 1983.
8. F. T. Orthoefer, in R. F. Wilson, ed. *Proceedings of the World Conference on Oilseed Processing and Utilization*, AOCS Press, Champaign, Illinois, 2001.
9. J. S. Godber and B. O. Juliano, in E. Champagne, ed. *Rice Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, MN, 2003.
10. W. R. Morrison, in Y. Pomeranz, ed. *Cereal Lipids*, Vol. 2, American Association of Cereal Chemists, St. Paul, MN, 1978.
11. N. H. Choudhury and B. O. Juliano, *Phytochemistry*, **19**, 1385–1389 (1980).
12. B. O. Juliano, in P. J. Barnes, ed. *Lipids in Cereal Technology*, Academic Press. New York, 1983.
13. W. R. Morrison, *J. Cereal Sci.*, **8**, 1–15 (1988).
14. T. S. Shin and J. S. Godber, *J. Agric. Food Chem.*, **44**, 567–573 (1996).
15. C. Rukmini and T. C. Raghuram, *J. Am. Coll. Nutr.*, **10**, 593–601 (1991).
16. G. S. Seetharamaiah and J. V. Prabhakar, *J. Food Sci. Tech.*, **23**, 270–273 (1986).
17. B. J. Lloyd, T. Siebenmorgen, and K. W. Beers, *Cereal Chem.*, **77**, 551–555 (2000).
18. K. Tanabe, M. Yamaoka, and A. Kato, *Jpn. Oil Chem. Soc.*, **30**, 116–118 (1981).
19. K. Tanabe et al., *Yuk agaku*, **31**, 205–208 (1982).
20. L. Gingras, presented at Amer. Oil Chem. Soc. Ann. Meeting, May 2002.
21. T. S. Shin, J. S. Godber, D. E. Martin, and J. H. Wells, *J. Food Sci.*, **62**, 704–728 (1997).
22. W. Hu, J. H. Wells, T. S. Shin, and J. S. Gober, *J. Am. Oil Chem. Soc.*, **73**, 1653–1656 (1996).
23. D. E. Martin, M. S. Thesis, Louisiana State University, Baton Rouge, p. 143.
24. Silto, T. Susuki, and Y. Fujino, *Nippon Nogei Kagaku Kaishi*, **55**, 247–253 (1981).
25. S. H. Yoon and J. S. Rhee, *J. Am. Oil Chem. Soc.*, **59**, 561–563 (1982).
26. B. S. Luh, S. Barber, and C. B. deBarger, in B. S. Luh, ed. *Rice Utilization*, Vol. II, Avi Book, Van Nostrand Reinhold, New York, 1991.
- 26a. B. S. Shastry and R. M. R. Rau, *Chereal Chem.*, **53**(2), 190–200 (1976).
27. S. Danforth and F. T. Orthoefer, presented at 64th Tri-state Oil Mill Assoc. Mtg., Biloxi, Mississippi, 1989.
28. B. O. Juliano, in B. O. Juliano, ed. *Rice: Chemistry and Technology*, Amer. Assoc. of Cereal Chem. St. Paul, Minnesota, 1985.
29. R. M. Saunders, *Food Rev. Int.*, **1**, 465–495 (1986).
- 29a. J. M. Harper, in C. Mercier, P. Linko and J. M. Harper, eds. *Extrusion Cooking*. American Assoc. of Cereal Chemists, St. Paul, Minnesota, 1989.
30. S. Barber and C. Benedito de Barber, in B. S. Luh, ed. *Rice: Production and Utilization*, Avi, Westport, Connecticut, 1980.

31. F. T. Orthoefer and R. J. Nicolosi, presented at 84th American Oil Chemist Society Annual Meeting, Anaheim, California, Apr. 29, 1993.
32. M. Funatsu et al., *Agr. Biol. Chem.*, **35**, 734–774 (1971).
33. Y. Aizono, et al., *Agr. Biol. Chem.*, **40**, 317–324 (1976).
34. S. Ida, Y. Masaka, and Y. Moriton, *Agr. Biol. Chem.*, **47**, 637–641 (1983).
35. C. A. Brown, *J. Am. Chem. Soc.*, **25**, 248–954 (1903).
36. T. Galliard, J. Allen and R. Hamilton, eds. in *Rancidity in Cereal Products*, Elsevier Applied Science, New York, 1989.
37. R. N. Sayre et. al., *Cereal Foods World*, **27**, 317–322 (1982).
38. J. M. Randall et al., *J. Food Sci.*, **50**, 361–364, 368 (1985).
39. D. R. McCaskill, S. D. Danforth, and F. T. Orthoefer, presented at 23rd Rice Technical Working Group, Biloxi, Mississippi, 1990.
- 39a. R. N. Sayre, D. K. Nagyar, and R. M. Saunders, *J. Amer. Oil Chem. Soc.*, **62**, 1040–1043 (1985).
40. J. V. Prabhakar and K. V. L. Venkatesh, *J. Am. Oil Chem. Soc.*, **63**, 644–646 (1986).
41. J. W. Hunnel and J. F. Nowlin, in D. F. Houston, ed. *Rice: Chemistry and Technology*, 1st ed., Amer. Assoc. of Cereal Chemists, St. Paul, Minnesota, 1972.
42. C. Kao and B. S. Luh, in B. S. Luh, ed. *Rice Utilization*, Van Nostrand Reinhold, New York, 1991.
43. S. C. Chang, R. M. Saunders, and B. S. Luh, in B. S. Luh, ed. *Rice Production and Utilization*, Avi Publishing, Westport, Connecticut, 1980.
44. D. N. Kinsey and J. W. Hunnell, U.S. Patent 3,481,960, Dec. 2, 1969.
45. R. Lasztity, United Nations Industrial Development Organization, ID/W. G. 89/10, March 9, 1971.
46. A. Sah, B. K.D Agrawal, and L. S. Shukla, *J. Amer. Oil Chem. Soc.*, **60**(2), 466 (1983).
47. A. C. Bhattacharyya, S. Majumdar, and D. K. Bhattacharyya, *J. Amer. Oil Chem. Soc.*, **63**, 1189–1191 (1986).
48. P. B. V. Reddi, K. S. Murti, and R. O. Feugi, *J. Amer. Oil Chem. Soc.*, **25**, 206–211 (1948).
49. M. Hegsted, M. Windhauser, and S. Lester, *Faseb*, **4**, 368A (1990).
50. A. Gerhardt and N. B. Gallo, *Food Chem. News*, **31**, (1989).
51. R. Sharma and C. Rukmini, *Indian J. Med Rev.*, **85**, 278–281 (1987).
52. R. Sharma and C. Rukmini, *Lipids*, **21**, 715–720 (1986).
53. R. J. Nicolosi et al., in J. Wadsworth and W. Marshall, eds. *Rice Science and Technology*, Marcel Dekker, New York, 1992.
54. T. R. Watkins et al., *Environ. Nutr. Interact.*, **3**, 115–122 (1990).
55. A. Qureshi et al., *Amer. J. Clin. Nutrition*, **53**, 10215–10265 (1991).
56. A. G. Krishna et al., *J. Amer. Oil Chem. Soc.*, **78**(2), 127–131 (2001).
57. L. Gingras, *Inform II*, Nov, 2000.
58. H. Hitasumatsu and Y. Takeshita, U.S. Patent 5, 290, 579 (1994).
59. L. Gingras, D. Hutchinson, and D. McCaskill, presented at *Amer. Oil Chem. Soc. Annu. Meeting*, May 2003.
60. D. DeGuzman, *Chemical Marketing Reporter*, **263**, 12 (2003).
61. Food and Agriculture Organization of the United Nations (FAOSTAT 1998).

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## *Safflower Oil*

Joseph Smith

### 1. HISTORY AND BOTANICAL DESCRIPTION

Safflower, *Carthamus tinctorius* L., has a long history of cultivation. Some would class it as the world's most ancient crop (1); others feel that olives, dates, and sesame predate safflower (2). Safflower was produced in Egypt more than 4000 years ago (3), but the most likely area of its origin is in the Euphrates basin (4–6). From there it apparently was introduced into Egypt and Ethiopia. Muslim traders carried safflower seeds across the northern coast of Africa and into present day Spain, while Arabs introduced it into many parts of east Africa. By the sixteenth century, safflower was found in several parts of Europe. Turks carried safflower into all parts of the Middle East, from where it spread to Iran, Afghanistan, and India. From Afghanistan it spread into China more than 2000 years ago (7). It spread to Japan in the third century A.D. (8). Spanish and Portuguese conquerors brought safflower to the New World, and later emigrants from Portugal and Russia did the same (9). For much of its history, safflower was used primarily as a source of dye, a food coloring, a cosmetic, or for medicinal purposes (7, 10, 11). Dried safflower florets are commonly used as an adulterant or substitute for colorful saffron, *Crocus sativus* L., a much more costly spice (12–14). Production of safflower oil was carried out in the reign of Ptolemy II (10), and Pliny pointed out that it could be used as a substitute for castor oil for nonedible purposes (15). While it had become known as an edible oil during pre-Christian times in Mesopotamia (16), it was only in more recent times that it began to be used in India as an

edible oil, and of course, it was not until the middle of this century that it began to enter world commerce, first as an industrial oil and then as an edible product (17).

Because safflower was introduced to many lands, it is known by a number of different names, some of which are azafrancillo, bastard saffron, benihana, cartamo, cnikos, false saffron, ghurtom, hung hua, kafsha, kahil, kajireh, kardi, khardam, kusumba, onickus, safflor, thistle saffron and ssuff (1, 3).

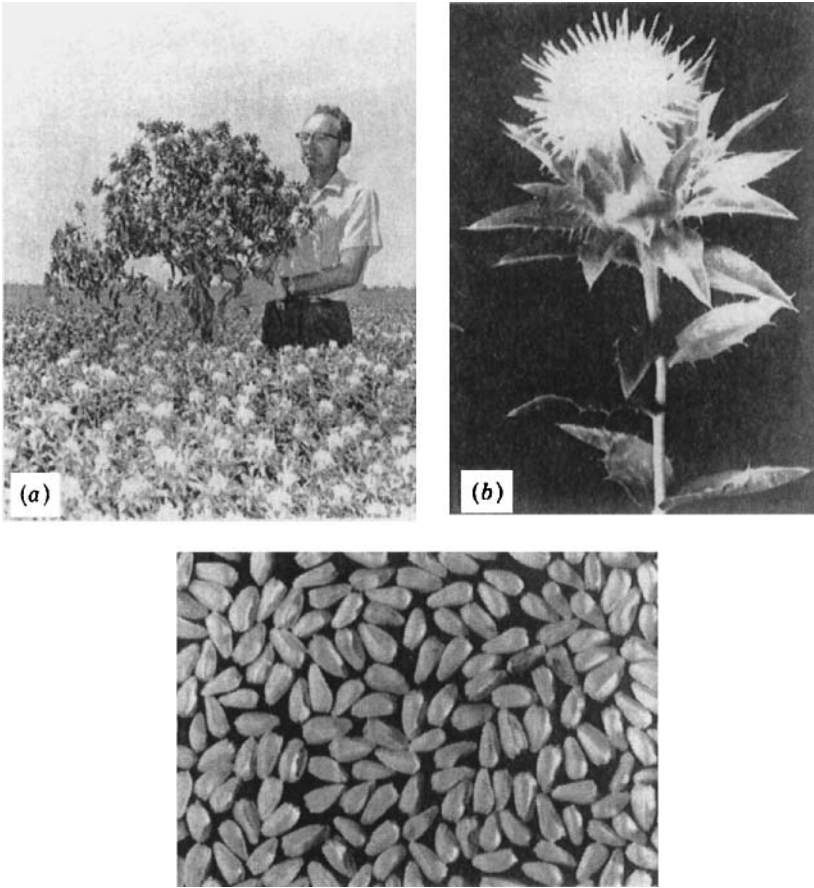
The safflower plant is a member of the Compositae family. Other members of this family are the artichoke, chrysanthemum, niger, and sunflower. There are at least 25 species of the *Carthamus* genus that grow in the wild (18), but only *C. tinctorius*, which we call safflower, has been domesticated; some quantities of *C. oxyacantha* have been gathered and used as oil or food sources in India and Pakistan (19).

The safflower plant as we know it resembles the Scottish thistle but has yellow, orange, or red florets rather than the purple bloom of the thistle. However, the commercial species of safflower, *C. tinctorius*, does not become a weed. The plant grows to a height of 30–150 cm, develops many branches (unless affected by natural or artificial environmental conditions), and develops a thickened taproot that can extend down to 4 m.

Each branch terminates in an inflorescence which is a dense capitulum of florets (individual tubular corollas), commonly called a flower. Each floret flower protrudes from a conical head surrounded by layers of bracts. The leaves, which develop along the stalk and branches, and the outer layers of bracts usually are spiny, although the types of safflower grown for the production of dye or food coloring are spineless, or nearly so. The seeds of the safflower plant develop within the head in a concentric pattern and are oblate with a flattened top, usually white, and about the size of a barley kernel (Figure 1) (20).

Safflower is a plant of desert origins, as evidenced by its deep taproot, waxy leaves, and relatively thick hull. It responds well to moisture and nitrogen. Its seed has the ability to germinate almost immediately if exposed to moisture at the proper temperature, unlike a sunflower seed, which must go through a period of dormancy before germination. The deep root and the many fine laterals that extend from it have the ability to seek out water and nutrients deep in the soil. These properties, while they allow safflower to survive in periods of moisture shortage also limit the areas of the world where safflower can be cropped successfully. Safflower is normally planted after soil temperatures exceed 4.5°C and does not begin growing fast until temperatures exceed 15°C. In the interim period, it goes into a rosette stage after emergence. During this time, it establishes its deep root system. As temperatures increase, the stem of the plant begins to elongate and can grow as much as 2.5 cm per day, until maximum height is attained. Branches and buds form until the plant flowers, after 70 days or more (depending on temperature at planting time). Flowering can last from 10 days to 3 weeks, and the crop usually is ready for harvest 45 days after time of full flower.

Flowering normally takes place during the warmest part of the growing season. If a protracted period of rainfall occurs at the same time, or until harvest time, unharvested safflower seeds still in the head will germinate and begin to form

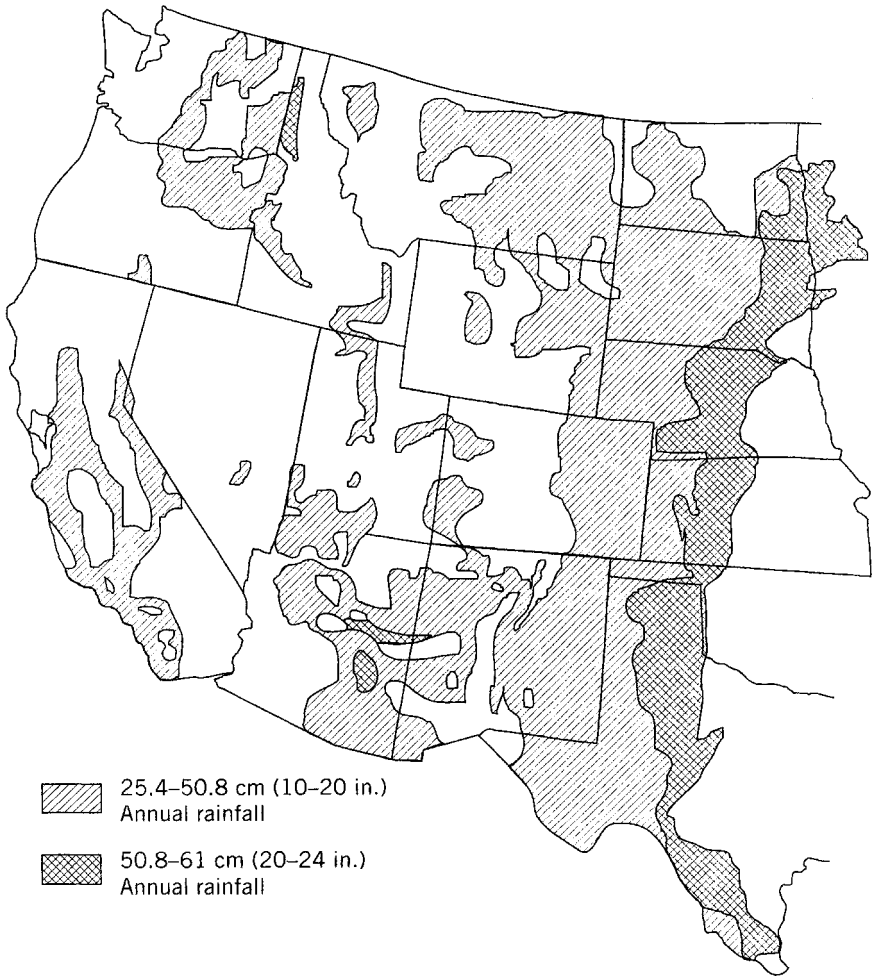


**Figure 1.** a, Dr. Carl E. Claassen, father of the modern-day safflower, among fully branched safflower. b, Safflower blossom. c, Safflower seed.

sprouts, which quickly can reduce oil content, increase color and FFA of the contained oil, and eventually result in total loss of the crop. Therefore, to grow safflower successfully, it must be planted in regions that have a minimum 120 frost-free growing days, 300–500 mm, of annual rainfall or irrigation, and that do not experience rainfall during the period when safflower is in flower or thereafter.

Most of the farming areas of the world receive some summer rains. If rain occurs, safflower has a chance of surviving, but this greatly increases the chance of the plant being attacked by various leaf and head molds, which can limit yield severely. So safflower production is limited to areas such as California's central valley and southern Arizona; isolated areas of Mexico and Australia; and the drier parts of China, India, and the Middle East. Areas where safflower can be grown, but with greater risk, are U.S. Northern and Great Plains, southern Idaho, and northern Utah; much of northern Mexico; far northern Argentina; and the drier parts of India and China (Figure 2).





**Figure 2.** Areas in the United States that can support safflower production.

Safflower does not require any specialized equipment to be farmed successfully. In developed parts of the world, 15–70 kg of seed are planted per hectare, with the lower ranges being planted either because moisture is a limiting factor or because the crop is to be managed in cultivated rows. In areas with only 300–400 mm of annual rainfall, 15 kg of seed are planted with a grain drill, much as a crop of wheat would be picked. In an area where plentiful irrigation water is available, a 20 kg of seed may be planted per hectare. Seed may be planted in three or four drilled or precision-planted rows on an elevated bed; the groups of rows are spaced 50–60 mm apart. In areas where moisture is plentiful, 35–70 kg of seed may be planted per hectare; the higher rate is used to ensure that weeds do not gain a competitive edge. Normal dates of planting in the United States range from December to March in Arizona, January to March in California's San Joaquin Valley, February to early

May in California's Sacramento Valley and last half of April to the first half of May in the rest of the country.

Good practice is to incorporate a trifluran herbicide into medium to heavy loam soil before planting; the seed is placed at a 35–100-mm depth, depending on the moisture level. A well-incorporated herbicide is necessary where weeds can be a problem, because safflower is not a good weed competitor in its early stages of growth. Almost 120 units of nitrogen are necessary to attain maximum production. For the crop to generate maximum yield, it must receive enough rainfall or have enough moisture in the soil either through preirrigation or subsequent row irrigation in order to maintain a bright green color and to prevent drying of its lower leaves until it is past flowering. As the plant approaches maturity, the flowers dry, and the entire crop attains a golden brown color.

Harvesting is accomplished with a standard grain combine generally set to run internally at a slightly lower speed than for grain, which prevents cracking of the seed. The combine should be set to cut only as deep as is necessary to capture all heads. Harvesting should not begin until the seed has dried in the head to a level of 8% moisture content or lower. Most safflower is harvested at a 4–5% moisture level.

In parts of India and China, much of the production is done by hand, and generally red-flowered, lower oil content, spineless varieties are used. Young people pass through the fields at time of flowering and pluck the florets from the seed heads, putting them into purses strung around their necks; the seeds are subsequently harvested when the crop has matured and dried, to recover oil. The florets are carefully dried out of the sun and then used for food coloring or (in China and Sri Lanka) for the production of either red or yellow dye. In India, some green safflower plants are used as a vegetable (21). In Australia, India, and Pakistan the plant is occasionally used as a grazing crop or fodder for cattle (22–24). After harvesting, the remaining stubble consists of hollow stalks, dried leaves, empty heads, and some empty hulls or immature seeds. Sheep and cattle were allowed to graze on this stubble in the United States during the 1950s; today such grazing is confined to occasional employment by sheep ranchers. Most fields in Mexico are grazed by cattle after harvest.

Until the twentieth century, safflower tended to be a local crop. No effort was expended to find species that had better oil content, since most of the interest centered around the crop as a medicinal or dye stuff source.

In 1925, the U.S. Department of Agriculture obtained samples of safflower seed from the then U.S.S.R. and India, and over the next 10 years various agricultural experiment stations and some farmer cooperatives conducted trials. In 1935, the USDA produced a circular summarizing the trial results; it concluded that safflower had possibilities as an oil seed crop in the northern Great Plains and far west (25). A Montana farmer conducted trials with safflower beginning in 1928 and contacted paint companies, researchers, and others who might have an interest in safflower oil (26). Several favorable reports ensued (27–29). In 1937, a comprehensive report based on seed obtained from Montana and elsewhere praised safflower oil's good properties (30). Others in Europe had earlier written favorable evaluations of safflower as a drying oil (31) and as a source of high protein meal (32). In 1947,

a comprehensive report on safflower production and drying oil capability was published (33).

Cargill, Inc. contracted for and processed about 1000 t of safflower seed in the U.S. northern Great Plains during 1947 and 1948; the company concluded that the crop was not sound at that time (34). Two men and one company provided the real impetus for getting safflower established as a crop in the United States: Claassen, Knowles, and the Pacific Vegetable Oil Corp. (PVO).

Claassen was employed in 1941 as a research agronomist by the University of Nebraska to assist the newly created Chemurgy Project in evaluating crops that could become significant contributors to the state of Nebraska (35, 36). After testing many new crops, he settled on safflower and began a breeding program (9).

Claassen found that most safflower introductions were in the 22–29% oil content range but found introductions from Sudan and Egypt that ranged from 33 to 37% oil content (37). Claassen began to do selection and breeding work, and by 1949, he had released several lines and described cultural methods for obtaining relatively consistent yields (37–41). The most important line released, N-852, had an oil content of 32–34% and good yielding ability. Several safflower processing companies were formed in Colorado and Nebraska to commercialize the new releases, but they quickly failed (34).

Claassen's work came to the attention of Knowles at the University of California at Davis. Claassen had sent portions of his new lines to a number of western cooperators for testing, and results that Knowles obtained were quite exciting (42). Claassen was encouraged by Hoagland, who was by then living in California, to come out for a visit to see the potential that safflower had in that state. Claassen visited California in 1949 and traveled to various oil processing companies. Claassen was convinced to resign from the university and join Hoagland in starting up a safflower planting seed and promotion concern called Western Oilseeds Co. (9).

Initially, Oil Seed Products Co. of Fresno, California, displayed the most interest in Claassen and Hoagland's work, but it soon became apparent that PVO could offer much more help because of its strong background in the production and sales of industrial oils. The primary interest in safflower oil at the time was coming from paint companies, whereas most oil millers in California were suppliers to the food industry. The N-852 variety, although it had good yields, was susceptible to phytophthora root rot under irrigation. Thus, the first tries at growing safflower in California in 1950 resulted in severe losses, because of the stress of irrigation. This turned away many growers and millers in California's cottonseed production areas, which helped PVO, because its mill was farther north. Claassen was soon joined by Hoffman, his former assistant at Nebraska; they formed an alliance with PVO. After Hoagland departed after a dispute, Pacific Oilseeds, Inc. (POI) was formed, jointly owned by PVO, Claassen, and Hoffman (34).

The combination of PVO and POI formed a near monopoly, dominating the safflower business until 1962. Approximately 95% of the safflower oil sold during that period went to the paint, varnish, and coatings market in which PVO had the strongest hand. POI's tie with PVO meant that only growers who contracted their safflower crop with PVO got the best seed as new varieties began to be released. This

system was the real key to PVO's dominance, which was probably the first time that a nonperishable crop was grown under contract. Growers were given contracts that guaranteed a floor price for the entire production from a given acreage, provided the best planting seed available for cash or credit, and offered field service and advice free of charge. In addition to the floor price the grower received a bonus based on PVO's profits in marketing the crop.

In ensuing years, PVO began to offer long-term contracts under which the grower was paid 50% of PVO's safflower profit as measured by the firm's auditors and the grower committed his or her entire production of safflower to PVO for 3–7 years. POI was also offered bonuses, reflecting 50% of the value of any increase in oil or protein levels in new seeds it developed. In turn, PVO went to great lengths to keep Knowles and various farm advisers and university officials interested in safflower. Banks, truckers, and warehouses were fully informed about PVO's future plans and how the safflower business was doing. In addition to making growers, researchers, and POI feel that they were part of a partnership aimed at improving safflower's lot, PVO kept oil and meal prices as low as possible to encourage market development. Buyers were also offered long-term requirements contracts that allowed them to purchase oil more than 1 year ahead of delivery date and, in fact, committed both parties to an assurance that the customer would always be able to obtain his or her full requirements. To some consumers, PVO also offered technical assistance agreements wherein a chemist or engineer was sent to the customer's plant to help solve processing problems and find new, profitable markets for safflower products. As markets grew, PVO expanded with them, enlarging processing capability in California, building new oil mills in Montana and Nebraska, and expanding production to Spain, Mexico, and Australia (34).

Coinciding with this development was the publication of *Calories Don't Count* (43), which became an instant best-seller in 1961. The book advocated a diet that featured daily capsulized doses of safflower oil. Medical research was beginning to demonstrate the close relationship between diet and heart disease. Eminent researchers began to show the relationship of cholesterol to heart disease and, more important, to show that polyunsaturates such as safflower oil would lower blood serum cholesterol for many people (44–46).

A further development enlarging the market for safflower oil was the increasing importance of the Japanese vegetable oil market. Japan became a consumer of U.S. safflower seed because of a fluke in the duty and quota structures set up after World War II. A duty of 20% was established to protect Japanese soybean producers from cheap imports. To be consistent, this duty was applied to a list of other oilseeds in addition to soybeans. Imports were also controlled under a very tight quota system to protect Japanese foreign exchange. But the framers of the duty structure were unaware of safflower, which was thus omitted from the list. Japanese oil mills became large clients for duty-free safflower seed, until the duties were gradually reduced as Japan became a major importer of soybeans and rapeseed (3). In the United States, safflower oil was being employed primarily in the coatings industry, but in Japan it was employed primarily as an edible oil. Safflower became quite popular in tempura oil blends.

During the early years of increasing exports of safflower seed to Japan, PVO did the lion's share of the business and was able to tie up a majority of the Japanese importers through a series of long-term requirements contracts wherein PVO agreed to supply the Japanese mills' increasing needs each year for an agreement to buy exclusively from PVO. The increasing Japanese demand and the growing popularity of safflower oil in the United States fueled PVO's expansion in production first to the western Great Plains and then to Mexico, Spain, and Australia. PVO began to lose its monopoly position in 1957 with the public release of the Gila variety of safflower seed (47, 48). This seed had a good yield and oil content and was also resistant to phytophthora root rot. As the seed became available, practically every cottonseed milling company in California and Arizona was able to become a producer and supplier of edible safflower oil.

Up to that point, safflower seed production had been on a continued upward spiral in the United States, which carried through to 1963. Safflower oil had been price competitive with soybean oil, particularly in the western United States and Japan, since soybean oil produced in the Midwest was at a freight disadvantage. The introduction into California of new varieties of wheat developed by the Borlaug program in Mexico allowed California farmers to achieve increasingly better wheat yields. In the 1950s, safflower was easily able to compete with wheat or barley as a rotation crop for California's rice or cotton farmers, but once wheat yields increased and safflower yields remained constant, safflower seed prices (and consequently oil prices) were forced to rise to compete for the farmer's favor.

Rising prices for safflower and increasingly better water-based paints formulated from petroleum-based polymers rather than vegetable oils quickly cut industrial consumption of safflower oil. PVO attempted to stem this tide by introducing products that combined safflower oil with water emulsion technologies, but it was too late (49-51).

The polyunsaturated bubble almost burst when the U.S. Food and Drug Administration began attacking refiners' claims about the ability of these products to reduce incidence of heart disease and lower cholesterol, but subsequent supportive statements by the American Medical Association and the American Heart Association softened the effect of the attack.

Increasingly competitive supplies of the former U.S.S.R. and U.S. sunflower seeds and oil helped erode the international market for U.S. safflower producers. Safflower oil has the highest level of polyunsaturation of the commercial oils, and the market has recently stabilized in the United States, northern Europe, and Japan. In Japan in particular, safflower oil has achieved an increasingly larger share of the gift pack market, wherein fancy tins of safflower oil are exchanged during the summer and Christmas gift-giving seasons.

Industrial use of safflower oil has declined to 2-3% of the total market. Conjugated safflower oil (52) competes with dehydrated castor or tung oils and very high quality alkyds. Table 1 illustrates the rise and fall of safflower supply and disappearance in the United States in comparison with major and minor crops (53).

**TABLE 1. Edible Fats and Oils: U.S. Supply and Disappearance, 10<sup>6</sup> lb.**

Item	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002 <sup>a</sup>	2003 <sup>b</sup>
<b>Stocks October<sup>a</sup></b>													
Coconut	277	188	251	164	163	84	150	393	152	136	260	227	148
Corn	138	196	150	118	241	116	129	102	135	267	117	104	114
Cottonseed	137	78	81	106	82	94	66	79	76	49	93	39	40
Lard	24	27	26	34	24	23	20	40	21	18	14	10	5
Palm	53	44	33	35	15	31	46	35	48	48	61	70	42
Palm kernel	53	49	88	73	55	22	51	64	73	49	155	128	50
Peanut <sup>c</sup>	25	51	50	25	40	65	86	41	40	32	31	32	50
Safflower	28	28	18	31	21	44	27	38	48	36	21	17	19
Soybean	1,786	2,239	1,555	1,103	1,137	2,015	1,520	1,382	1,520	1,993	2,767	2,359	1,486
Sunflower	47	100	56	65	82	147	93	60	121	157	136	23	25
Canola	41	71	67	137	54	77	65	112	169	206	110	52	55
Tallow, edible	41	33	41	36	52	34	48	46	43	40	49	24	35
<b>Imports</b>													
Coconut	841	1,163	999	1,100	874	1,188	1,438	791	926	1,115	1,093	860	970
Corn	5	7	7	10	11	14	28	42	18	27	61	65	65
Cottonseed	18	38	26	0	0	0	0	48	8	0	0	22	0
Lard	2	3	3	2	2	1	2	2	2	3	6	10	10
Olive	216	253	262	260	227	304	333	355	397	455	455	485	540
Palm	220	267	368	218	236	322	282	284	345	399	490	425	440
Palm kernel	342	302	304	280	262	392	359	401	393	351	330	470	475
Peanut <sup>c</sup>	1	0	11	4	5	14	10	73	12	79	39	70	70
Canola	815	861	902	938	1,086	1,075	1,088	1,060	1,139	1,193	1,108	929	1,215
Safflower	22	15	16	26	35	30	51	51	33	34	40	43	45
Soybean	1	10	68	17	95	53	60	83	83	73	46	50	85
Sunflower	9	0	7	1	2	22	8	5	4	8	36	60	5
Tallow, edible	6	10	15	18	8	5	2	3	10	32	7	11	10
<b>Production</b>													
Corn	1,821	1,878	1,906	2,227	2,139	2,231	2,335	2,374	2,501	2,403	2,461	2,453	2,650
Cottonseed	1,280	1,126	1,119	1,312	1,229	1,216	1,224	832	939	847	876	725	865

**TABLE 1** (Continued)

Item	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002 <sup>a</sup>	2003 <sup>b</sup>
Lard	1,016	1,011	1,015	1,052	1,013	979	1,065	1,106	1,069	1,050	1,080	1,075	1,100
Peanut <sup>c</sup>	356	286	212	314	321	221	176	145	229	179	230	286	219
Canola	32	49	406	299	355	342	451	548	617	641	585	541	629
Safflower	69	87	111	115	127	103	115	111	91	88	76	89	91
Soybean	14,345	13,778	13,951	15,613	15,240	15,752	18,143	18,078	17,825	18,420	18,898	18,435	17,020
Sunflower	911	730	580	1,165	860	840	959	1,177	1,046	873	673	320	595
Tallow, edible	1,515	1,414	1,535	1,550	1,559	1,407	1,517	1,677	1,792	1,764	1,932	2,075	2,000
<b>Exports</b>													
Coconut	22	0	19	18	12	12	6	11	14	8	7	8	10
Corn	566	712	717	865	977	988	1,118	989	970	951	1,172	890	900
Cottonseed	269	184	248	329	221	232	208	111	141	131	150	110	115
Lard	131	129	119	140	94	103	122	140	189	93	90	105	100
Olive	20	15	11	21	24	21	19	15	12	9	10	12	12
Palm kernel	2	9	4	2	2	2	2	2	2	2	2	2	2
Palm	7	7	7	13	20	9	11	11	11	11	10	11	10
Peanut <sup>c</sup>	151	52	61	97	108	21	13	10	18	14	8	42	19
Canola	15	16	76	153	147	295	349	272	284	187	255	166	157
Safflower	73	65	75	93	122	83	83	92	51	35	37	37	40
Soybean	1,644	1,461	1,531	2,683	992	2,033	3,079	2,372	1,375	1,401	2,519	2,250	850
Sunflower	471	586	450	978	628	709	815	800	630	545	453	110	200
Tallow, edible <sup>d</sup>	333	306	316	277	241	181	236	322	224	338	475	485	490
<b>Domestic disappearance</b>													
Coconut	910	1,084	1,067	1,083	941	1,111	1,189	1,021	927	983	1,119	930	958
Corn	1,202	1,220	1,228	1,250	1,298	1,244	1,271	1,394	1,417	1,630	1,363	1,618	1,804
Cottonseed	1,088	975	873	1,007	996	1,012	1,004	772	833	672	780	636	750
Lard	885	886	890	924	922	880	925	987	886	964	1,000	985	990
Olive	216	253	262	260	227	304	333	355	397	455	455	473	528
Palm	223	271	359	225	201	298	282	260	335	375	471	425	427
Palm kernel	344	254	315	295	293	362	344	390	414	243	355	511	458
Peanut	179	236	187	206	193	194	217	208	233	244	260	296	275

Canola	801	898	1,162	1,165	1,271	1,134	1,143	1,287	1,435	1,744	1,496	1,301	1,687
Safflower	15	47	40	57	17	67	73	59	86	102	89	93	95
Soybean	12,248	13,012	12,939	12,913	13,465	14,267	15,262	15,652	16,059	16,318	16,833	17,108	16,522
Sunflower	396	188	129	171	168	207	186	320	385	357	370	268	385
Tallow, edible	1,197	1,109	1,239	1,275	1,345	1,218	1,286	1,360	1,581	1,449	1,488	1,590	1,515

<sup>a</sup> Preliminary and estimated.

<sup>b</sup> ERS and WAOB forecast.

<sup>c</sup> August-July year beginning 1982.

<sup>d</sup> Disappearance, as defined by the USDA-ERS, means beginning food stocks, production, and imports minus exports, shipments to U.S. territories, and ending stocks.

*Source:* Bureau of the Census.



**TABLE 2. Typical Fatty Acid Composition of Linoleic and Oleic Safflower Oils (%).**

Fatty Acid	Normal	Oleic
Palmitic	5.25	4.5
Stearic	1.50	1.5
Oleic	15.00	77.00
Linoleic	77.00	15.00
Others	1.25	2.00

In 1957, scientists in Australia and California independently reported a mutation that came to be known as oleic safflower (54–56). This mutation occurred naturally and produces a plant and seed that look exactly like linoleic safflower, except for an oil whose fatty acid distribution is a mirror image of linoleic safflower oil (Table 2). The initial oleic safflower variety released by Knowles, UC-1 (57), was lower in oil content and had a poorer yield than conventional varieties available at the time. This meant that oleic safflower oil was initially sold at a premium. But agronomic research has since produced varieties that equal or even exceed normal safflower in yield and that are comparable in oil content.

Oleic safflower oil interested buyers in Japan and the United States when it was first commercially released—in Japan as an ingredient for a new mayonnaise and in the United States as a replacement for peanut oil in most of Frito-Lay's western plants. These markets evaporated, however, when producers were forced to raise prices because of increasing competition with wheat for western farmland. The development of markets for oleic safflower oil has been a constant series of steps forward and then back. The oil has enjoyed good success as an ingredient in artificial baby milks (because of its excellent stability), in production of premium chips and snacks (again because of its stability and good frying characteristics), in the production of cocoa butter substitutes, and as an oil for blending with olive oil because of its similar fatty acid structure.

In recent years as more research has focused on the role of monounsaturates versus polyunsaturates and their effects on cholesterol reduction, oleic safflower oil has begun to receive more attention. In the United States, Saffola Grocery Products has introduced a grown-without-pesticides salad oil in which linoleic safflower oil has been replaced by the oleic type. In Japan, several bottlers have begun to feature oleic safflower oil in their gift-pack campaign both as an individually identified product and also in blends with the linoleic type.

The emergence of countries other than the United States as exporters of safflower products became increasingly important. Since 1986, Mexico has been able to take an increasing percentage of its total supply to world markets, generally at lower prices than U.S. oils. Earlier, Mexico was limited by government controls over its exports and poorer varieties of planting seed. These restraints have been eliminated.

Argentina is able to produce safflower at prices similar to sunflower oil prices and also has access to good U.S. planting seed varieties. However, Argentina is able to sell at low prices only by planting safflower in the far north, immediately after soybeans are harvested. If timing is perfect, a good yield can result. If soybean harvest is delayed, safflower is also delayed; thus the harvest may be pushed into the rainy season, causing sprouting and potential loss of the crop.

Recently, Australia has transferred most of its safflower production from Queensland and New South Wales to Victoria and the south, where rainfall is more reliable. Australia has not been able to produce a sustained, reliable production for world markets. Severe quarantine laws have not allowed more modern varieties of safflower planting seed to be imported; thus most Australian seed is quite low in oil content.

China has begun to produce some safflower oil. Chinese varieties have traditionally been under 30% oil content, since the bulk of the research there was aimed at improved seed and floret yields. Safflower produced in the far west and northern parts of China is high in linoleic levels, and Japan has continually imported small quantities of Chinese seed because of this. With China's emergence into the world's economic and political venues, its safflower could become more important as it produces or imports better varieties of seed and improves its internal transportation system.

Canada produces limited amounts of safflower, which is aimed primarily at bird feed rather than oil production. Even more so than in the U.S. northern Great Plains, Canada's producers face a tough battle trying to squeeze in a long enough growing season.

The world's largest acreage of safflower is found in India, and Indian scientists have undoubtedly published more details about safflower production than scientists in any other country. India is trying to encourage more safflower production, but sunflower offers more hope there because of its resistance to rain. India basically consumes all of the safflower it produces, and this is unlikely to change.

Spain used to be an important producer of safflower, but sunflower hybrids have shown much more promise, because of their tolerance of summer rains. *Pseudomonas* almost wiped out safflower production in Spain, and current EEC policy (which offers subsidies to sunflower producers but ignores safflower) does not leave safflower much chance (58).

The FAO's estimate of world safflower production for the period 1950–1992 is shown in Table 3. U.S. safflower acreage fell 13% in 2001 to 188,000 acres. Below average yields also contributed to a cut in safflowerseed production to 242 million pounds, making it the smallest crop since 1983. As a result, crush and exports of safflowerseed in 2001/02 fell to 190 million and 43 million pounds, respectively. For safflowerseed oil, a recovery in U.S. shipments to Japan boosted 2001/02 exports to 40 million pounds (53). Safflower's future will continue to be limited by its relatively high cost of production, unless hybrids are developed that can be produced cheaply.

TABLE 3. World Safflower Production by Crop Year (Year of Harvest/Milling) (59, 60).<sup>a,b</sup>

Year	Argentina		Australia		Ethiopia		India		Mexico		Spain		United States		World Total	
	(ha)	(t)	(ha)	(t)	(ha)	(t)	(ha)	(t)	(ha)	(t)	(ha)	(t)	(ha)	(t)	(ha)	(t)
1950	-	-	-	-	44	19	370	50	-	-	-	-	30	16	458	91
1951	-	-	-	-	45	20	390	53	-	-	-	-	11	9	460	88
1952	-	-	-	-	45	21	400	56	-	-	-	-	23	24	482	107
1953	-	-	-	-	45	21	406	57	-	-	-	-	26	25	491	109
1954	-	-	-	-	45	22	410	57	-	-	-	-	10	13	479	91
1955	-	-	-	-	45	22	415	60	-	-	-	-	23	35	497	122
1956	-	-	1	-	46	23	420	55	-	-	-	-	36	66	517	150
1957	-	-	1	-	46	23	425	60	-	-	-	-	37	66	523	155
1958	-	-	3	2	48	24	430	60	-	-	-	-	65	67	560	159
1959	-	-	5	2	48	24	433	63	-	-	-	-	99	113	599	209
1960	-	-	2	1	50	25	435	61	26	32	-	-	131	152	658	278
1961	-	-	4	2	51	26	440	66	33	41	-	-	166	160	709	305
1962	-	-	2	2	52	26	445	67	37	47	-	-	217	334	768	486
1963	-	-	8	5	54	27	450	68	36	47	-	-	223	358	761	514
1964	-	-	19	13	55	27	455	62	36	47	-	-	147	255	710	413
1965	-	-	24	10	56	28	460	72	59	80	-	-	146	262	746	459
1966	-	-	38	25	57	29	462	69	165	236	55	30	182	304	955	701
1967	-	-	42	16	59	30	478	72	100	149	70	56	181	311	925	643
1968	-	-	19	10	60	32	513	78	86	102	55	39	83	169	812	441
1969	-	-	11	4	61	34	578	94	145	209	11	5	113	212	939	566
1970	-	-	28	9	62	36	580	142	175	288	14	8	102	180	968	673
1971	2	1	34	15	64	39	588	154	265	511	22	13	115	227	1126	891
1972	5	3	11	4	63	24	598	131	199	271	16	8	110	208	1039	676
1973	7	5	12	7	64	24	423	82	198	298	34	20	95	156	874	615
1974	8	8	36	31	64	30	614	191	192	272	34	17	77	150	1054	713
1975	4	3	40	18	64	25	648	212	363	532	34	16	84	175	1269	1001
1976	8	6	13	6	64	30	674	238	185	240	36	20	37	69	1057	632
1977	3	2	39	26	64	30	683	220	404	518	29	13	113	171	1350	989
1978	2	2	75	58	64	30	707	188	429	616	15	15	145	186	1443	1108
1979	2	1	54	30	64	30	703	209	528	635	17	14	152	205	1516	1129
1980	1	1	18	8	65	31	733	279	416	480	20	20	84	111	1340	936
1981	1	1	33	20	65	31	720	335	391	372	12	4	65	101	1289	870
1982	1	1	12	20	66	32	749	421	189	221	20	13	72	117	1114	832
1983	2	2	55	31	66	32	782	396	349	277	19	13	35	92	1320	853
1984	3	2	44	32	66	32	831	501	227	209	20	14	89	124	1290	894
1985	3	2	44	28	66	32	870	497	190	180	19	16	88	110	1288	876
1986	14	10	30	19	67	33	911	348	204	161	15	13	148	147	1385	737
1987	15	11	38	25	67	33	892	353	200	219	12	11	107	155	1331	811
1988	15	11	46	41	68	34	1052	462	200	247	18	13	95	146	1498	960
1989	50	33	33	21	68	34	816	445	150	142	7	6	91	159	1219	850
1990	50	35	19	10	69	34	842	487	157	159	1	1	108	139	1248	873
1991	50	35	37	24	69	35	821	327	94	88	-	-	84	115	1160	620
1992	50	35	34	32	69	35	831	350	80	82	-	-	136	148	1199	690

<sup>a</sup>In units of 1000.<sup>b</sup>Some data from PVO, Agricom International, and Oilseeds International, Ltd.

## 2. PHYSICAL AND CHEMICAL PROPERTIES

### 2.1. Safflower Seed

Safflower seed (technically an achene) (61) consists of a tough fibrous hull that protects a kernel comprised of two cotyledons and an embryo. Applewhite (62) reported that hulls make up 18–59% of the seed weight (62), Weiss (63) characterized normal hulled seeds as 38–49%, and Li et al. (64) noted percentages of 25–87.5%. This diversity also shows up in seed weight per 1000 seeds (14–105 g), oil content (11.48–47.45%), and fatty acid distribution (linoleic acid, 11.13–85.6%; oleic acid, 6.74–81.84%, stearic acid, 0.01–4.88%, and palmitic acid, 2.1–29.03%) (57).

Safflower seeds are normally cream to white, but since 1960, breeding has resulted in great variation in color, ranging from normal hull to thin hull (which tend to show part of the underlying colored layers) to types with gray, purple, or brown-striped hulls. Most of this research has been aimed at creating a thinner hull to increase oil content (Table 4). Although reduction of the hull fraction

**TABLE 4. Analyses of U.S. Safflower Seed (65).<sup>a</sup>**

Type	Oil	Protein	Fiber
Analyses of whole seed			
Gila	38.1	16.7	22.3
U-5	38.5	17.2	21.1
US-10	36.8	19.4	22.3
Frio	40.1	15.4	20.8
Thick-hull hybrid	37.8	17.3	21.5
Brown striped	47.7	20.3	11.7
Pigmentless brown striped	42.8	22.5	13.6
Thin hull	47.2	21.1	11.2
Analyses of hull			
Gila	3.2	4.3	57.1
U-5	2.2	5.0	58.4
US-10	1.4	3.6	60.0
Frio	2.7	4.1	60.4
Thick-hull hybrid	2.2	4.1	63.9
Brown striped	5.7	8.4	46.9
Pigmentless brown striped	5.6	8.6	46.2
Thin hull	5.1	10.0	45.3
Analyses of kernel			
Gila	60.9	24.9	1.5
U-5	61.8	25.4	1.5
US-10	59.0	29.4	1.5
Frio	64.0	23.0	1.0
Thick-hull hybrid	58.1	24.7	2.8
Brown-striped	52.7	24.8	0.9
Pigmentless brown striped	55.9	27.4	2.7
Thin-hull	62.6	25.5	0.9

<sup>a</sup>All analyses are percentages on a moisture-free basis.

increases valuable oil and protein percentages, too much reduction can produce other problems. Brown-striped seeds have a distinctly musty odor (66). Thin hull types can create harvesting, storage, handling, and processing problems (67, 68), although the more gentle combine harvesters in use for the last decade can probably deal with the harvesting worry. Brown-striped seeds also were shown to contain colorless precursors in the hull and kernel that could create dark extracted oil (68). Ways to remove the precursors and color bodies have been published (68, 69). Three phosphatides have been identified that are responsible for color formation from oil extracted from the kernel of safflower seed: phosphatidyl ethanolamine, phosphatidyl myoinositol, and phosphatidyl choline (70–72).

Attempts have been made to produce commercial hybrids of safflower seed by exploiting heterosis to increase seed or oil content yields (73–76). In addition, many Indian scientists have published on the hybrid theory of safflower. POI marketed a near hybrid for a short while, which exhibited oil contents in excess of 50%, but it was not popular with growers and proved difficult to manage in the oil mill. Cargill marketed hybrids in India for several years. They were initially popular with growers because of 25% higher yields than standard varieties, but high production costs and 25% lower oil content caused the program to be phased out. Other hybrid work (76) was based on white-flowered genetic male-sterile germplasm (77–79), which in turn resulted from colchicine treatment of an introduction from Afghanistan.

Oleic types of safflower are produced primarily in the United States and to a minor degree in Mexico. The commonly available types exhibit oleic fatty acid levels in the 76–81% range. Linoleic level decreases proportionally as oleic level increases. Safflower seeds in the Northern Hemisphere tend to be higher in linoleic acid at

**TABLE 5. Characteristics of Commercial Safflower Production.**

Country or Region	Oil Content	Moisture (%)	Protein (% in Solvent Extracted Meal)	Linoleic (% in Total Fatty Acids)
United States				
California	39.5–44	4–5	25	75–78
Arizona	39–41.5	4–5	25	72–78
Northern Great Plains <sup>a</sup>	25–41	5–9	24	76–81
Utah and Idaho	38–42	5–7	25	76–78
Canada	32–35	5–9	24	76–81
Mexico				
San Jose and Quiriego	30–38	5–12	24	60–70
Normal types	35–37	5–12	24	72–77
U.S. types	35–39	6–12	23–24	72–77
Argentina	35–36	6–12	23–24	70–76
India	32	7–8	21–24	72–78
China	28–32	7–8	25–28	76–82
Australia	35–38	5–9	24	70–76

<sup>a</sup>Wide range caused by loss of oil content in years of early frost. High, basis-S-541 variety; normal range for local varieties is 35–38%.

**TABLE 6. Mineral and Trace Element Composition of Some Indian Oilseeds (80).<sup>a</sup>**

Factor	Sesame	Mustard	Groundnut	Safflower
Number of varieties	6	8	19	6
Ash (g %)	6.6 ± 0.02	4.7 ± 0.21	3.3 ± 0.09	2.1 ± 0.15
Phosphorus (mg/100 g)	872 ± 35	767 ± 41	500 ± 8	367 ± 10
Calcium (mg/100 g)	1232 ± 28	318 ± 25	77 ± 6	214 ± 28
Magnesium (mg/100 g)	521 ± 42	273 ± 18	239 ± 4	241 ± 18
Iron (mg/100 g)	9.3 ± 0.43	7.9 ± 0.34	2.5 ± 0.23	4.6 ± 0.13
Zinc (µg/g)	122 ± 21	48 ± 3.7	30 ± 1.6	52 ± 3.3
Manganese (µg/g)	13.2 ± 0.26	25.6 ± 4.09	11.0 ± 0.76	11.0 ± 0.78
Copper (µg/g)	22.9 ± 1.70	8.3 ± 0.44	9.0 ± 0.53	15.8 ± 1.54
Molybdenum (µg/g)	2.02 ± 0.068	0.89 ± 0.105	1.66 ± 0.173	0.54 ± 0.08
Chromium (µg)	0.87 ± 0.052	0.63 ± 0.058	0.48 ± 6.031	0.45 ± 0.060

<sup>a</sup>All values are mean ± SEM for dry weight of the sample.

more northern latitudes because cooler temperatures are usually experienced at and after the time of flowering. Similarly, oleic levels increase in safflower exposed to higher temperatures. Oil contents of commercially available oleic types tend to be slightly lower than linoleic types, but field yields are equal or superior to linoleic types. Tables 5 to 8 list some of the characteristics of safflower seeds.

## 2.2. Safflower Oil

Normal safflower oil is pale yellow to golden and has a slightly nutty flavor. Table 9 summarizes the important chemical and physical characteristics of U.S. safflower oil. Safflower oil exhibits the highest level of linoleic fatty acid of any commercially available oil. This high level, combined with an absence of linolenic fatty acid, is what has made safflower oil attractive to consumers, initially as a

**TABLE 7. Identification and Distribution of Sugars in Safflower Hull and Kernel (81).**

Safflower Component	Sugars Present	Percent Distribution of Sugars	Percent Sugars	Percent Sugars on Defatted Basis (calculated)
Kernel	Uronic sugar glycosides	14.3	0.43	—
	Raffinose	35.8	1.08	—
	Sucrose	46.9	1.42	—
	Galactinol	3.0	0.09	—
	Total	—	3.02	7.74
Hull	Uronic sugar glycosides	38.9	0.37	—
	Raffinose	6.8	0.06	—
	Sucrose	22.6	0.21	—
	Galactinol	2.7	0.025	—
	D-Glucose	14.6	0.14	—
	D-Fructose	14.2	0.13	—
	Total	—	0.94	0.79



**TABLE 9. Physical and Chemical Characteristics of U.S. Safflower Oil (82).**

Characteristic	Usual Range		
	of California Oil	Minimum <sup>a</sup>	Maximum <sup>a</sup>
<b>Physical</b>			
Color (Gardner)	8—10	—	11 <sup>b</sup>
Color after heat bleaching, 315.5°C (600°F)	2—3+ <sup>b</sup>	—	4 <sup>b</sup>
Color, refined, bleached, deodorized <sup>c</sup>	0.5—1.0 red <sup>d</sup>	—	15 yellow and 1.5 red <sup>d</sup>
Specific gravity, 25/25°C	0.919—0.924	—	—
Refractive index, $n_D$ 25°C	1.473—1.476	—	—
Titer, °C	15—17	—	—
Flash point, °C (°F)	148.8+(300+)	121.1(250)	—
<b>Chemical</b>			
Free fatty acids, % as oleic	0.15—0.6	—	2
	0.03—0.05 <sup>d</sup>	—	0.05 <sup>d</sup>
Saponification value	186—194	—	—
Iodine value (Wijs)	141—147	140	155
Unsaponifiable, %	0.3—0.6	—	1.5
Peroxide value (at time of shipment)	0—1.0 <sup>d</sup>	—	1.0 <sup>d</sup>
Moisture and volatile, % <sup>e</sup>	0.03—0.1	—	0.8
Insoluble impurities, % <sup>f</sup>	0.01—0.1	—	0.3
Moisture and impurities, %	0.05—0.1 <sup>b</sup>	—	0.1 <sup>b</sup>
Principal fatty acids, % TFA			
Palmitic	4—6	—	—
Stearic	1—2	—	—
Oleic	16—12	—	—
Linoleic	75—79	72	—
Linolenic	Nil	—	—

<sup>a</sup>Per NIOP trading rules.<sup>b</sup>Nonbreak grade, NIOP.<sup>c</sup>AOCS method Cc 13b-45.<sup>d</sup>Edible grade, NIOP.<sup>e</sup>AOCS method, Ca 2d-25.<sup>f</sup>AOCS method Ca 3-46.

quick-drying oil that could produce films that would not yellow with age and, more recently, as an edible oil with the highest available level of polyunsaturation. As an edible oil, the high level of unsaturation also creates problems. Home consumers using safflower oil for frying must be careful to clean pans quickly after use or a tough varnish film results, which is difficult to remove. Fresh safflower salad-grade oil has excellent flavor and odor characteristics, and because it lacks linolenic fatty acid, it does not display the fishy or beany odors sometimes associated with poorly refined soybean oil. Unfortunately, it does have a relatively short shelf life (typically 9–12 h AOM), which means the oil should be kept cool after the bottle is opened to maintain freshness.

Oleic safflower oil displays most of the same characteristics as the linoleic type, except for its fatty acid structure (see Table 2). It has been noted that a blend of linoleic and oleic edible oils would improve the dietary value of commercial safflower oil (83). Blends of this type began to be marketed in Japan in 1990 and appear to be achieving good acceptance by the public.



A great variation in fatty acid, oil, and protein levels occurs in the world collection of safflower seeds. Knowles's pioneering work in understanding and subsequently finding ways to modify these differences inspired many researchers to publish extensively on this subject (34, 84). Recently, most research on safflower oil modification has been performed in the United States by private planting seed companies and by the Sidney Experiment Station of Montana State University; little has been published.

A few studies have reported on the location of the fatty acids on the triglyceride in varying ways. One study used argentation TLC with lipase hydrolysis on a sample of safflower oil from Kenya that contained 10% total saturated fatty acids. It was found that 2 mol% was configured with two saturated acyl chains (S) and one unsaturated acyl chain (U), 26 mol % had a SU<sub>2</sub> configuration, and 72 mol % had a U<sub>3</sub> configuration. It was also reported that 3 mol % had two double bonds attached, 3 mol % had three double bonds, 23 mol % had four double bonds, 19 mol % had five double bonds, and 47 mol % had six or seven double bonds (85). Another study measured the position of linoleic acid on the triglyceride in a study on hydrogenation (86). It was found that 84.6 mol % of linoleic acid was located in the 2-position in a safflower oil containing 76.4% linoleic acid.

### 2.3. Safflower Meal

The by-product of the extraction of safflower oil is a grayish tan to brown cake or meal that exhibits flakes or shreds of whitish safflower hulls. Table 10 presents typical analysis for safflower meal. Most meal produced in the United States is of a solvent-extracted type. The amino acid and mineral contents of meal are shown in Table 11.

Australian data from 1959 indicated up to 17,324 kg/ha of green matter (2762 kg/ha of dry matter) could be gained by grazing safflower as a green crop

**TABLE 10. Typical Analyses for U.S. Safflower Meal (87).**

Characteristic	A <sup>a</sup>	B <sup>b</sup>	C <sup>c</sup>	D <sup>d</sup>
Crude protein, %	21.03	20.00	42.0	25.4
Crude fat, %	6.6	0.5	1.3	1.5
Moisture, %	9.0	10.0	9.2	8.0
Crude fiber, %	32.2	37.0	15.1	32.5
Ash	3.7	5.0	7.8	5.9
Calcium, %	0.23	0.24	0.4	0.37
Total phosphorus, %	0.61	0.24	0.4	0.8
NFE, %	—	—	—	40.0
TDN, %	—	—	—	57.0

<sup>a</sup>Expeller pressing of safflower seed without decortication.

<sup>b</sup>The low end fraction of meal that resulted from prepress-solvent extraction of safflower seed followed by two fraction tail-end decortication.

<sup>c</sup>The high end fraction of meal that resulted from prepress-solvent extraction of safflower seed followed by two-fraction tail-end decortication.

<sup>d</sup>Prepress-solvent extraction of safflower seed without decortication. Typical California, 1992.

**TABLE 11. Amino Acids and Minerals in Safflower Meal (87, 88).<sup>a</sup>**

Factor	A	B	C
Methionine	0.4	0.33	0.69
Cystine	0.5	0.35	0.7
Lysine	0.7	0.7	1.3
Tryptophane	0.3	0.26	0.6
Threonine	0.47	0.5	1.35
Isoleucine	0.28	0.27	1.7
Histidine	0.48	0.5	1.0
Valine	1.0	1.0	2.3
Leucine	1.1	1.2	2.5
Arginine	1.2	1.9	3.7
Phenylalanine	1.0	1.0	1.85
Glycine	1.1	1.1	2.4
Calcium	0.28	0.37	0.44
Phosphorus	0.78	0.80	1.41
Potassium	0.79	0.79	1.33
Magnesium	0.36	0.37	1.33

<sup>a</sup>See Table 10 for explanation of A, B, and C. Numbers are percents.

with 11–12% protein (22). Indian researchers have presented several papers that showed promise concerning the production of fodder and ratoon seed in northern India (82–92). In 1993, a U.S. farmer was able to harvest approximately 8000 kg/ha of green hay, which measured 18% protein from a safflower crop that failed to mature.

### 3. PROCESSING

#### 3.1. Extraction

Much of the safflower processed in India in the past was crushed by a mortar-and-pestle-like device called a *ghani*. Seed was cleaned by hand and then introduced into a *chakki*. This machine, which consisted of two horizontal stone wheels, one of which was turned by a blindfolded bullock, partially dehulled the cleaned seed passing between the stones. Hand winnowing and sieving next removed the hulls from the seed kernels. The meats were pressed into balls after the addition of about 6% water. About 15 kg of the “balled” kernels were introduced into the *ghani*, an inverted conical mortar into which a heavy pole was placed. The pole was held to the side of the mortar by heavy weights and dragged around the perimeter by a team of oxen. A small amount of heated oil was added, and crushing then proceeded for 45 min, after which the oil was allowed to drain out through a small hole. A *ghani* could process about 100–120 kg of seed per day.

More recently, *ghanis* capable of processing 150–175 kg per day were sometimes motor driven. Animal-powered *ghanis* could obtain 11–16% residual oil in the extracted meats, while motor driven models could extract 10–12%. Today,

some cast-iron *ghanis*, expellers, and solvent-extraction plants are used, in addition to the older stone devices (93–95). The oil extracted by a *ghani* is clarified by settling and decanting or by water washing. The oil is placed in tins for local sale.

Most safflower was first processed in the United States by continuous screw press expellers. Some processors attempted decortication, but the nature of safflower seed acts against successful decortication. To prevent the oil from scorching, water-cooled shafts were recommended. Oil so treated could easily be heat bleached to a level below 4 Gardner color. Expellers such as the Anderson Super Duo Duplex could process about 15 t of safflower seed per day, leaving 7–8% residual oil in the remaining cake. However, the principal problem encountered in processing safflower seed through expellers was the propensity of expeller-processed safflower meal to burn in storage (96). The combination of a reactive polyunsaturated residual and the fibrous texture of safflower meal created many fires in the 1950s and early 1960s. Once safflower processing shifted to prepress–solvent extraction, which brought residual oil contents down below 1.5%, most storage problems were eliminated.

These same expellers, if employed in a prepressing mode wherein 15–17% residual oil remains in the cake that is sent to the solvent extraction unit, can process 45–50 t of seed per day. Prepressing of safflower produced under California conditions (or the equivalent) requires no cooking, flaking, or cracking of the seed before extraction and results in oil capable of being heat bleached to 1–3 Gardner color.

In the early 1960s, PVO produced an air gun device that decorticated safflower seed satisfactorily, but the idea was abandoned because it required too much energy and was extremely noisy (34). A PVO researcher developed a method for decorticating safflower meal after extraction, which employed the principle that the fine particles produced in grinding safflower cake are high in protein and the coarser particles are more fibrous (97). This method, using vertical hammer mills to grind the cake and a combination of air classification and screening was employed by several California mills in the 1960s and 1970s to produce safflower meal of 42% protein, in addition to an 18–20% protein middle fraction and a 6% protein hull fraction. More recently, most mills have returned to only producing “as is” meal of approximately 25% protein content, because the high amount of energy consumed by the tail-end process cost more than the additional return gained from the high protein fraction.

The high cost of energy encouraged some mills in the 1980s and 1990s to replace or supplement prepress expellers with caged expander-extruders, which are capable of removing approximately 66% of the available oil through the caged portion of the extruder and to produce collets that are ideal for efficient solvent fraction. Extruders require much less horsepower per ton of seed processed than expellers and cost less to maintain (98–100).

Horizontal basket or moving bed solvent extractors are preferred over vertical tower extractors in processing safflower cake. The fibrous nature of safflower provides a natural channel through which the solvent can move, and the bed acts as a natural filter medium. Tower extractors generally have problems extracting

safflower seed because the hulls tend to float, sometimes carry over in the top of the extractor, and cause excessive wear in a tower's rotary seal.

### 3.2. Refining, Bleaching, and Deodorizing

Safflower oil that is extracted from seed in good condition is easy to refine because it is low in FFA and contains few gums or impurities. Conventional caustic refining systems work well. This most important factor in handling safflower oil, destined for edible use, is to limit exposure to air throughout the extraction, refining, bleaching, deodorizing, and packaging cycle. Nitrogen blanketing should be employed if deodorized oil is to be stored for more than a few hours. Generally speaking, safflower oil processed by expeller processing will contain just enough free fatty acids and impurities to require refining before deodorization; in most cases, safflower oil prepressed from California, Arizona, or northern Mexico seed can be introduced directly to deodorizers. California prepress oil normally will meet a varnish maker's nonbreak grade without further processing.

Safflower seed that is produced in areas with late summer rains or cool weather cycles that interfere with maturation can produce dark-colored or greenish oils that are often higher in FFA as well. If the seed has sprouted before or during harvest or has been attacked by *Alternaria*, *Pseudomonas*, or other head-rot diseases, the resulting oil can be quite difficult to refine and extremely difficult to bleach.

While safflower oil may, on occasion, display minute traces of a fine, lacy wax (101), most U.S. refiners neither winterize nor dewax safflower oil, feeling that a brilliant oil can be delivered without it. Japanese refiners generally insist on refining and bleaching safflower oil to under 1.0 red color, followed by winterization to avoid problems with minute amounts of wax that may appear in the oil in the winter months in the north.

### 3.3. Production of Margarine and Mayonnaise

If proper steps are not taken, physical crystal changes (polymorphism) can take place in the production of safflower margarines, resulting in a product with a sandy texture (102). The  $\beta$ -crystalline form that results consists of large crystals instead of the smooth, uniform mixture desired in a margarine; safflower's uniform triglyceride structure encourages production of  $\beta$ -crystals. This problem can be solved by incorporation of a small amount of more saturated oil into the margarine mix. PVO solved the problem in its Saffola margarine by adding 5% cottonseed oil, which also improved the product's "mouth" feel (34). A 1966 patent described a blending of liquid safflower with selectively hydrogenated safflower and peanut oils (103).

Soft safflower margarines, wherein a highly hydrogenated safflower lattice was employed to encapsulate a larger portion of liquid safflower oil, have been successfully produced by several companies (34). The methods employed to produce these types of margarine structures have been reviewed (104, 105). It has been shown that  $\text{Cr}(\text{CO})_3$  catalysts can be used to selectively hydrogenate safflower oil and retain a 90–95% cis configuration (106–108). Several studies have reported on safflower oil's

good taste, appearance, odor, and texture in mayonnaise and frozen salad dressings, where it exhibits excellent qualities in repeated freeze–thaw cycles (34, 102, 109, 110).

### 3.4. Industrial Processing

Although this Chapter is concerned with oils that are used in edible products, it is well to remember that safflower oil's recognition in modern times occurred because of interest in its excellent properties as a semidrying oil. Safflower oil's light color, ability to heat bleach to near water whiteness, low level of free fatty acids and impurities, and lack of linolenic acid make it an ideal vehicle for white house paints and varnishes and for the production of alkyd resins. It is easy to polymerize via kettle bodying without the need for vacuum equipment; capable of producing excellent blown, limed, or maleated oils; and acts as a good source for conjugation or methyl esters (34).

## 4. ECONOMICS AND MARKETING

As mentioned, safflower is a crop that has been grown for thousands of years, primarily for local use. As people traveled they carried safflower seeds with them, generally for personal use. It is only in recent times that safflower has entered world commerce; still much of what is produced remains in the country where it is grown.

The price of wheat has been the dominant factor affecting the price that U.S. farmers must receive for safflower seed to put safflower into their cropping plans. In its early years of U.S. production, safflower oil competed directly with soybean oil for market share and soybean futures on the Chicago Board of Trade, offered as a reasonable medium for hedging safflower seed and oil prices. But, more recently, safflower prices have borne little relationship to the market for soybean oil, and safflower oil has become a product that is impossible to hedge.

In 1997, U.S. farm wholesale prices were the following: safflower oil, tanks, \$0.59/lb; soybean oil, tanks, \$0.24/lb. In 2002, prices for were safflower oil, tanks, \$0.79/lb, soybean oil, tanks, \$0.19/lb (53).

### 4.1. Safflower Seed

In the United States, most safflower seed is grown by farmers who have agreed to a contract of sale with an oil mill or grain dealer before planting the crop. Because there is no daily market for safflower seed posted in the newspaper and there are no quotations available from the commodity futures markets, most banks or other financing agencies encourage farmers to contract their crop in advance. There is no other way for the bank to protect any funds that have been loaned with the crop as collateral.

The usual safflower production contracts state that the farmer will deliver the entire yield from a given number of acres or hectares. The buyer assumes the

risk of yield. Besides stating the number of hectares to be planted and their location, contracts usually specify the type of planting seed, the name of a landlord (if any), what compensation he or she is to receive, and of course, the price and point of delivery.

The National Institute of Oilseed Products (NIOP) publishes an annual rule book that covers specifications and standards of trade for many vegetable oils, including safflower and oleic safflower. Rules 7.1 g and h (formerly 110 g and h) and 7.1 i (formerly 110 i) are the NIOP rules for safflower seed and oleic seed, respectively. When combined with the state of California's official standards for safflower seed, little room exists for argument as to the meaning of a contract between buyer and seller.

Safflower seed is usually sold domestically on a dockage-free basis with no limit on the amount of dockage a shipment of seed might contain. Dockage is defined as any foreign material plus parts of the safflower plant other than seed, empty or partly filled seeds and broken parts of the seed small enough to pass through a screen opening of 1.78 mm. Moisture content is required to be <8%, unless the buyer is willing to accept a higher percentage in exchange for a penalty. Most oil mills will accept limited quantities of seed up to 12% moisture content, if their schedule permits such seed to be processed immediately. Moisture content levels higher than 12% cause problems in expeller operation. Some mills also operate grain dryers, which allow them to accept higher moisture content seed.

Normally, buyer's require safflower seed that contains more than 5% dockage or green foreign matter or that is higher than 8% moisture to be cleaned before accepting it. This is to prevent heating in storage. Because most buyers in California purchase safflower seed from the farmer free on board a truck at the edge of the field, making the buyer responsible for the cost of freight to the elevator or oil mill site, the farmer is normally also charged for the cost of freight involved on dockage in excess of 5%. In other states, in Mexico, and most other parts of the world, the farmer is responsible for the delivery of the seed to the buyer's location.

In most parts of the world, except India, safflower seed is handled in bulk. In California this is accomplished in large aluminum-sided, bottom-dumping, open-top truck trailers of approximately 10–12 t capacity each, two of which are hauled in tandem to a field by a truck tractor unit. The trailers are left by the field to be filled by the farmer, and the tractor unit returns and hauls the full trailers directly to the oil mill or export terminal (in some cases up to 250 km away) or to a closer grain elevator for intermediate storage. In other parts of the United States, safflower is delivered in many types of grain trucking equipment and much of it is delivered to small country elevators where it is stored, cleaned if necessary, and subsequently loaded onto trucks or railroad hopper cars (which can hold between 50 and 70 t of safflower seed) for delivery to a buyer.

Correct methods for sampling of safflower seed are specified in the NIOP rules. Three probes in each truck trailer with a grain probe is the preferred method. Sampling takes place on delivery of the seed to the first place of rest and is conducted if possible by a third party (a representative of the California State Department of Agriculture or an employee of the receiving elevator company) to minimize

disputes. Moisture content and refractive index (if oleic safflower is delivered) of the parcel is checked immediately and a carefully split portion of the sample is then forwarded to the nearest state of California or other independent laboratory for determination of dockage or, should the sampled seed be defective, other factors.

In other states besides California and Arizona, the purchase contract specifies a price that is based on a dockage-free sample, a certain level of oil content, and the moisture content at the time of delivery. This is necessary because of the effect that cold temperatures, rain, snow, disease, or drought can have on the oil content of an individual crop. In California and Arizona, little variation in oil content occurs from year to year in a particular variety of safflower. In the northern Great Plains states, the oil content of a seed that might be 38–40% in a normal year can be as low as 20–25% under adverse conditions. In the mountain states, these variations are usually less extreme. Safflower seed in the Great Plains is normally purchased on a 38% oil content basis (sometimes 40% is used as a basis) with reciprocal allowance of 2% for each 1% variation in oil content (fractions in proportion) applied to the agreed on price.

Because the United States does not use the metric system, prices in California and Arizona are normally quoted in dollars per short ton of (2000 lb, 907.185 kg) and in the Great Plains and mountain states most transactions are fixed in cents per pound (453.59 g). In the rest of the world, metric tons or quintals prevail, except in some parts of India and China. Prices paid to farmers in various parts of the United States vary because of quality and distance from final markets.

The price offered for safflower seed in California is shaped by several market elements. The principal factor is the amount of land in the central valley that will be committed to rice, cotton, and tomatoes, the three primary income-producing crops in the area. Safflower, sugar beets, grain, and corn compete for the remaining cultivated land; the competition between wheat and safflower is the most intense. Experienced farmers favor safflower over wheat if the contracting price for safflower multiplied by an average yield of 2.5 tons/ha equals or exceeds the perceived price for wheat multiplied by a yield of 5.0–6.0 tons/ha. Safflower buyers usually begin negotiating with farmers in October, since farmers must make the decision to withhold planting wheat at that time. Wheat is normally planted in late November through January, and safflower is planted during February through April. During the 1980s the acreage of safflower planted in California would decline sharply when prices fell below \$275/t. In the 1990s, this value was about \$330/t, because of inflation and the increasing prices for other crops.

The second factor that affects the price of safflower is the condition of the market for safflower oil. For example, there may be a surplus of oil from the previous crop, Mexico may be forecasting a large harvest (which occurs 3–4 months before the U.S. harvest), or Japanese buyers may be experiencing a slowdown in their domestic market.

Because safflower oil is a specialty that serves a market that responds little, if at all, to price changes, these two factors tend to slow down dealer's desire to buy the seed, and a rationing process takes place. Dealers either delay their opening gambits to contract for safflower seed from the next crop or offer low prices that do not

compete with other alternatives. Side issues that affect supply (drought, disease, or floods; longshore or transportation strikes; etc.) and price (changes in government support for competing crops or in import or export regulations, etc.) also affect these decisions. Safflower prices are not affected by prices for other commodity types of oils such as sunflower, soya, and canola, except in periods of wild upward price movements. If prices for other oils climb above \$880/t safflower oil prices move up accordingly or the safflower oil disappears into export markets as a replacement.

Of course, the demand for safflower can be changed by longer-term fundamental changes. In Japan, safflower oil is identified as an eminently healthy oil that is given as a gift. Should medical research find that polyunsaturates, and particularly safflower oil, cause medical problems that outweigh its benefits, demand for the oil would crumble. On the other hand, if long-term medical studies show that mono-unsaturates, including oleic safflower oil, are preferred over the types of oils, even over linoleic safflower oil, there might be a shift in the ratio of linoleic to oleic safflower oil consumption. This appears to be happening in Japan.

Safflower seeds produced in California are located close to the ultimate domestic safflower oil markets as well as near export terminals for ocean shipping to Japan or Europe. Safflower seeds produced in the Great Plains, however, are generally priced \$50/t below California prices for several reasons. First, Great Plains safflower seed is generally 3–5% lower in oil content than western seed, and in years of bad weather it can be much lower. Second, while the oil produced therefrom is generally 2–4% higher in linoleic fatty acid than California seed, it is normally 0.25% higher in FFA and 1–2 Gardner color units higher, with generally a greenish tinge, all of which necessitates higher refining and bleaching costs. Great Plains prepress oil normally cannot be deodorized without prior refining. Finally, Great Plains seed must face a long railroad trip to markets in California, or if delivered locally for processing, the oil and meal produced from it face long trips to consumer markets. Safflower growers in the mountain states face similar discounting problems. Mountain-grown seed usually is closer to California seed in quality but has no local milling or customer base so all seed must be delivered over a long distance.

These factors do not apply to the markets for safflower seed sold for bird feed. Birdseed buyers' specifications emphasize seed color (pure white seed is preferred) test weight (a weight in excess of 0.4739 kg/L is desired), and purity (less than 1–2% foreign material is preferred). Oil content is not a factor. Seeds that have heavy white hulls and, accordingly, low oil content are preferred for birdseed use. Consequently, birdseed buyers, whose customers are located predominantly in the eastern half of the United States or overseas, prefer to contract in the Great Plains and mountain states, where they compete with \$50/ton lower seed prices and enjoy a \$40–50/ton freight advantage to eastern markets.

It is hard to judge the exact size of the market for birdseed safflower, but as feeding of wild birds increases in the United States, most dealers believe it has exceeded 20,000 t annually. China generally enjoys the reputation of supplying the best birdseed quality, since much of Chinese seed is below 30% in oil content and normally has white hulls. Weather and transportation factors sometimes increase difficulties



for marketers of Chinese-origin seed. Indian seed was a factor in world birdseed markets until 1989, when the Indian government banned the exportation of seed to improve local supplies of oil. This undoubtedly contributed to the increase in the U.S. birdseed market.

Exportation of safflower seed to Japan was the largest factor in the expansion of U.S. safflower seed production. When high duties on the oilseeds entering Japan began to be relaxed, U.S. safflower seed exports declined. Once more than 10 oil mills were engaged in processing safflower seed in Japan, now only 2 mills continue to crush safflower seed there. The remainder of Japan's needs for safflower oil are covered by imports of safflower oil. Safflower seed exports are governed by the NIOP Rule 7.1 g (former Rule 110 g Export). Many factors are involved in the Domestic Rule (Rule 7.1 h), but export terms require measurement of oil content and payment of a premium or discount much as most seed is purchased in the Great Plains states. The export safflower seed rule establishes a price basis point of 34% oil content with a premium/discount of 2% for each 1% of oil content variation with fractions in proportion. The 34% level is used, even though most seed exported today is in the range of 41–43% oil content, because this was the level of oil content available when safflower first started being exported. Export rules also allow only a maximum of 3% dockage for an export shipment to be considered correct, although provisions are made for allowing shipments containing up to 6% maximum in exchange for a penalty of an additional 0.2% for shipments measuring between 3 and 6% dockage.

Because one oceangoing vessel normally carries 3,000–5,000 t, and up to 15,000 t, the sampling methods are different from those used for truckloads. The NIOP rules call for oil, moisture, and dockage analyses to be performed separately on samples representing each 1,000 t, or fraction thereof, loaded on a vessel. In the case of oil content analyses, identical samples of each 1,000-t lot are presented to five different independent laboratories, each laboratory reports its analyses for the entire load on a weighted average basis, the results of the laboratory with the highest and lowest oil contents are discarded and the results of the remaining three are averaged and used for payment purposes. In this manner a fair analysis is made, because safflower oil content is difficult to measure accurately.

Almost all safflower seed exported to Japan from the United States has come from California and in some years, Arizona. This is because the Japanese prefer the quality of West Coast production, preferring not to pay a high ocean freight cost on a seed that is lower in oil content; generally produces higher color and refining costs; and may contain the fungus *Sclerotinia sclerotina*, which although found regularly in the Great Plains has not yet been observed in California. Japan imports some safflower seed from Australia and a small quantity from China, to obtain the +80% linoleic safflower oil that Chinese seed can guarantee. Japan also imported safflower seed from Mexico at one time. The United States has exported safflower seed to Europe, but recently the high price of safflower seed and low value of safflower meal in Europe have made the processing of safflower seed impractical, and imports have been confined to safflower or oleic safflower oils.

## 4.2. Safflower Oil

In today's marketplace safflower occupies a unique position. It is the oil with the highest level of linoleic acid available commercially. It continues to enjoy a favorable reputation in the mind of consumers, which is a legacy of the polyunsaturated boom of the 1960s. Most safflower oil produced today reaches consumers as a refined, deodorized, and bleached salad oil; as a principal ingredient in margarine; and in several forms of mayonnaise and salad dressings. A small percentage of the total oil produced, primarily prepress oil, is bottled and sold to consumers without any further refining, bleaching, or treatment of any kind other than filtration. In the United States and Europe, a small segment of the market wants an oil that has not been exposed to chemicals (in this case hexane). Bottled prepress oil generally has a short shelf life, perhaps of less than 2 weeks, and once opened the oil needs to be refrigerated so it does not develop strong odors.

Two sellers dominate the U.S. grocery market for all safflower-edible products, although a number of other companies produce small quantities for health food venues. Bottled safflower salad oil generally retails at more than \$1 per bottle higher than canola, corn, sunflower, or soybean oil. Customers for safflower oil make up a small but dedicated segment of the market. Safflower salad oil brands have never achieved over a 7% market share, and without heavy advertising, this level drops in half.

In Japan, the premium price is almost an advantage, and the companies marketing safflower oil enjoy better margins for the product than other oils produce. They exert strict quality controls, market the oil in beautiful and expensive gift packs, and engage in heavy advertising to maintain market share. In Japan's gift-giving seasons, safflower oil has achieved a premier status among all oils. Some say it has captured up to 85% of this market.

In Mexico, safflower oil occupied a preferred status for many years in grocery stores catering to the affluent. When first produced in Mexico, a sizable portion of the safflower oil produced was used as an adulterant in sesame oil. Over time, safflower became the premier oil in the marketplace and *puro cartamo* would command a substantial premium. Safflower oil itself soon began to be adulterated with sunflower and other oils, and eventually consumers became aware of this and switched loyalty to branded oils that were cheaper. Little safflower oil is found in Mexico because it is generally exported to the United States or Europe, and lower priced sunflower, canola, or soybean oil is imported in its place.

A similar situation has taken place in Australia, where the bulk of safflower grown is no longer processed for the local market but is exported as seed or oil. A small amount of Australia's safflower total is devoted to producing so-called organic safflower oil. Because Australia still has virgin farmland, it is possible to produce a crop of safflower using no herbicides, insecticides, or fertilizer. Some organic safflower oil is also produced in the United States. In the last 3 years the most successful program has been operated by Saffola Grocery Products Co., which markets so-called Grown Without Pesticides safflower oil. Saffola has chosen to use this method, because it wishes to establish its own definition for the "purity" of its

product, in contrast to organic oils, which are usually defined by government edict, subject to periodic change. The Grown Without Pesticides (GWP) regime requires that the safflower is planted on land that has had no chemicals applied to it for at least 6 months and that shows no residue levels. The farmer is allowed to apply fertilizer but no planting seed fungicides (allowed in organic farming) or other chemicals. A thorough auditing scheme that includes inspection of the crop throughout the growth cycle and inspection of harvesters, trucks, and storage facilities for cleanliness and lack of chemical sprays is employed. This is more rigorous than the standards employed by the organic industry, which works primarily on the honor system.

The GWP program for oleic safflower oil has been successful when there is heavy advertising. Saffola has not been able to expand beyond the regional market because of the cost of advertising. The Japanese gift-pack market, which also uses heavy advertising, includes some oleic safflower oil. One manufacturer is selling a blend of linoleic and oleic safflower oils to combine the good attributes of both oils in a single package; its largest competitor markets the oils separately to give the customer a choice.

In India much oil is still sold by small mills that simply filter oil from the press and supply the product in small tins or even in the consumer's own vessel. Safflower production is by and large a neighborhood affair in India. While the government is encouraging more production of all types of oilseeds, sunflower, which has much wider adaptation than safflower, enjoyed spectacular increase in production in the 1990s.

The European market consists of three areas. First, safflower oil is an ingredient in sunflower-based margarines, helping to maintain a guaranteed level of polyunsaturation. This market area may be in decline, because some manufacturers lowered their polyunsaturated guarantee levels in early 1994, opting perhaps to feature low saturation or higher monounsaturated attributes in the future. Safflower oil also finds a small but dedicated audience because of its high level of unsaturation. A portion of this market prefers to use either unrefined prepress oil or a form that has been gently deodorized. The third market area is for safflower, and particularly oleic safflower, oil that is used for blending with other oils. When safflower oil became more expensive than other oils, this market area virtually vanished. Much more rigorous and sophisticated control measures by government authorities have also restricted attempts at blending.

Like safflower seed most safflower oil also is traded under rules established by the National Institute of Oilseed Products, in this case Rule 6.11 and 6.12. Rules for both domestic and export shipments are in force, with the primary difference being that the export rules require more analyses to be performed before payment. Of course, some U.S. buyers, many of whom have never heard of the NIOP, establish their own specifications for the safflower oil they purchase, but by and large their standards meet or exceed the NIOP grades.

It is outside the scope of this article to examine the medical literature that fueled the polyunsaturated boom of the 1960s (34) and that has continued to provide impetus to the U.S., European, and Japanese safflower oil markets. Whether

**TABLE 12. Historic U.S. Oleic Safflower Plantings and Production.**

Crop Year	Plantings (ha)	Production (t)
1967	405	953
1968	5,221	11,031
1969	8,580	23,014
1970	5,868	12,973
1971	13,462	32,922
1972	8,843	20,321
1973	15,480	24,222
1974	11,615	22,801
1975	21,004	43,316
1976	9,632	23,678
1977	9,594	24,540
1978	14,569	21,037
1979	19,010	36,940
1980	13,345	31,351
1981	9,340	24,721
1982	4,856	12,701
1983	4,917	12,610
1984	11,550	31,026
1985	10,958	27,994
1986	11,635	31,425
1987	4,290	11,340
1988	8,094	20,684
1989	10,805	29,393
1990	11,343	29,908
1991	10,891	22,803
1992	33,634	48,680
1993	36,430	73,564

monounsaturations or simply lack of saturation will become the wave of the future is unknown, but from an historic viewpoint it is interesting to observe how oleic safflower production slowly increased since the crop was introduced in 1967 (Table 12).

In the United States in 2003,  $221 \times 10^3$  acres of safflower were planted and  $212 \times 10^3$  acres were harvested. Forecasts for 2004 are for  $142 \times 10^3$  acres to be planted and  $133 \times 10^3$  acres to be harvested (111).

### 4.3. Safflower Meal

Safflower meal, the by-product of the production of safflower oil, contains all of the hull. The high fiber content of the hull limits its value. In California, safflower meal is employed primarily as an ingredient in dairy feeds; it is also used in beef cattle feed and to a limited extent in poultry mixes. In the 1960s and 1970s, when safflower meal was being decorticated by the tail end process, the resultant high (38–42%) protein fraction found good employment in chicken and turkey rations. PVO produced three meal fractions of 42, 20, and 6% protein. Although PVO and

others spent considerable time searching for alternative uses for safflower hulls, the best bet in that period was to export the 6% fraction to Japan, where safflower hulls were used as low cost filler in many types of compound feeds. Japan was also a regular consumer of 20% protein (sometimes purchased basis 20% protein-fat combined analysis), but in today's market, safflower meal from the United States is not competitive in the Japanese market.

Safflower hulls find their best market when incorporated in safflower meal, and none has been produced separately for many years in the United States, because most mills produced only two fractions when decorticating, 20 and 42% portions. Today, the energy consumed in separating safflower meal fractions exceeds the premium that can be gained from the high protein fraction, so most mills confine themselves to offering as is meal of 25% protein.

Numerous studies have shown safflower oil to be a good feed product for beef cattle (112–116), dairy cattle (117–119), poultry (120–123), and lamb (124–127), and is generally available at price levels that are similar to the lowest prices for alfalfa hay, grain screenings, almond hulls, and other low protein feeds.

Promising experiments have been done to produce protein flour or protein isolates from safflower meal. The USDA compared safflower protein isolate with isolate from soy and found the safflower product to be quite useful. The study also outlined the cost of investment and production for the process envisioned (128–130). Other researchers have written extensively on this subject (131–134). A factory would need considerably more than the total U.S. supply of safflower meal to produce an economically viable protein isolate. Unless a scientific breakthrough can materially reduce the hull portion of a safflower seed while retaining satisfactory yields, meal will continue to sell for a modest price and to be considered a second-rank product. NIOP Rules 8.1.1–8.1.3 established the factors guiding the trade in safflower meal.

## 5. QUALITY ASSESSMENT

Although most of the standard tests for measuring physical and chemical characteristics of a product work well for safflower seed and its products, some unique problems have arisen over the years.

### 5.1. Safflower Seed

When safflower was first introduced into the United States, the Fred Stein Co. was the first to produce a chart, the Steinlite moisture meter, calibrated specifically for safflower seed, allowing moisture to be rapidly and correctly determined at the elevator. Most moisture meters available today work well on safflower seed.

During its growth cycle, a safflower head will respond to the amount of moisture available. If there is plenty of moisture, many of the individual seeds that have begun to form in the head will fill completely. If moisture is restricted or if a sudden trauma such as disease or removal of water occurs, some of the seeds that have

begun to fill will stop filling and others will not even begin. This results in a mixture in each safflower head of some seeds that are plump and completely filled and others that appear to be the same but that, on inspection, are empty or only partially filled. For the laboratories performing dockage tests on the thousands of samples representing each truckload delivered, it can be a daunting task to find which seeds are empty or partially filled. Originally, the dockage analysis method adopted by the state of California employed a series of hand screens, followed by winnowing through a Bates aspirator and hand picking of the resultant sample to find empty seeds that escaped the aspirator. This method was too slow, and when used to measure samples containing high amounts of empty hulls, as is often encountered in Great Plains safflower, up to 30 passes through an aspirator were required to find all empties.

During the 1950s and early 1960s, PVO and the California State Department of Agriculture performed hundreds of experiments together aimed at producing a simpler and more reproducible test for dockage. As a result, modifications to the Carter Dockage Tester were developed that allow consistent measurement of dockage. This method was adopted by the California Department of Agriculture and by the NIOP, incorporating the procedure as part of Rules 7.1 g D and E (135).

Determining the oil content of safflower seed in the laboratory by solvent extraction is also more difficult than for other oilseeds because of the vast difference in texture of the hull compared to the kernel within. The hull must be cracked or all of the oil will not be extracted. But in cracking the seed, the kernel tends to mash as well and small amounts of oil can be lost in the process, a small amount is important when the sample contains only 5 g of seed. Since many people expressed dissatisfaction in safflower oil content analyses, PVO's control laboratory worked for a long time to develop a better method than the standard AOCS procedure (136). This method of analysis is now part of the NIOP rules for safflower (137).

The NIOP also conducted extensive tests to develop methods for better sampling of safflower seed. Field run safflower seed is fairly difficult to sample. Although pure safflower seed is relatively smooth flowing, the seed delivered by a farmer can contain portions of stalks and stems; parts of the head that held seed; and leaves, and other foreign material. Safflower seed, which has traveled over bumpy roads for 50–200 km inside a truck, for 3000 km in a railroad hopper car, or for 10000 km in the hold of a heavy grain carrier on its way to Japan, tends to stratify, and unless the sampling device reaches all levels of the product, the sample is not representative. Japanese buyers, who were receiving 5,000–15,000 tons of safflower at a time, found that the oil content and dockage analyses performed at time of shipment did not reflect what the oil mills obtained as a final outturn in the milling of the same seeds. The NIOP adopted standard sampling and dividing procedures aimed at reducing variation in results, and these procedures now are incorporated in their rules (138).

It is particularly important to remember that the sample used in analyzing safflower oil contents must be first cleaned of all dockage (including empty hulls), unlike the common method of measuring sunflower oil contents, which is

performed on seed containing admixture. This puts a premium on good sampling, good cleaning, good division of the sample, and consistent performance of the analysis itself.

Safflower seed oil content can also be determined by the use of nuclear magnetic resonance (NMR), and today most plant breeders employ NMR techniques to measure their new lines. NMR techniques can be performed on only one half of a seed, so the other half can be planted if the results of the analysis are promising. In its earlier versions, processors tended to feel that NMR analysis produced oil content results that were slightly higher than found by standard solvent extraction analysis or than what was actually obtained at the oil mill. This has been disproven in the case of safflower seed, and the industry has adopted NMR analyses in large part to speed up paperwork. Because of the relatively small amount of safflower seed being measured for oil content annually, no one has taken the time to prove that present-day NMR procedures should be used to substitute for the standard AOCS procedure.

The USDA published what may have been the first practical procedure for quickly determining if a truckload of seed is a linoleic or oleic variety (138). It involves squeezing a few seeds in a small hand-powered press to obtain a few drops of oil. A drop of oil is placed on the glass prism cell of a hand-held refractometer. The refractive index has a straight-line relationship with the iodine value or fatty acid distribution of the oil, hence it is easy to determine if the seed in question meets an oleic standard or not, so long as a temperature correction is applied. Recently, it has become simpler to compare the unknown sample to a known oil standard, eliminating the need to apply a temperature correction. Temperature corrections are difficult to measure accurately in the field under the time pressure of harvest.

## 5.2. Safflower Oil

Measurement of safflower oil's various chemical and physical characteristics is quite straightforward and only minor changes have occurred over 50 years in the rules governing the safflower trade. In 1990, the requirement for certification that safflower oil demonstrate a negative halphen test was dropped. The emergence of better and better GLC technology eliminated the need for a color test of cottonseed oil adulteration.

The U.S. Department of Agriculture Utilization Laboratories at Philadelphia, Peoria, and particularly Albany, California, contributed a major body of work that measured various factors that affect the quality of safflower and its reaction to various processes. Oxidation reactions of safflower oil and methods for following heat-generated changes in composition during deep-fat frying were studied in depth (139–143). USDA scientists at Albany (144) and Peoria (145) analyzed the headspace volatiles of safflower methyl esters and safflower oil, respectively, subjected to accelerated oxidation and found them to be the most reactive of all oils tested. One study showed vinyl-*n*-amylkelone to be the compound responsible for the generation of metallic off-flavors in oxidized safflower oil (146). USDA researchers

demonstrated the effects of oxyfatty acids, malonaldehyde and diclorocarbenes, respectively, on oil flavor and storage reactions (147–149).

It has been demonstrated that tocopherols in linoleic safflower oil were more stable than tocopherols in oleic safflower oil (150). The USDA did room odor studies that showed that oleic safflower did well compared with all other oils used in the study (151). A broad study was conducted of the effects of various substances on the oxidation of safflower oil in deep frying (152); of high temperature reactions in the presence of amino acids (153); and of the effect of amino acids on emulsions (154), dried emulsions (155, 156), and chemical and organoleptic properties (155, 156).

### 5.3. Safflower Meal

As mentioned, safflower meal tends to stratify in storage so the principal problem in quality control is making sure a truly representative sample is obtained. The USDA Regional Utilization Laboratory at Albany, California, produced a body of work concerning safflower meal that allows a better understanding of its attributes and deficiencies. A survey of the world collections for seeds high in lysine was undertaken (157), and this work has been continued for both lysine and methionine at the Eastern Experiment Station of Montana State University (158). The work included studies of safflower steroids (159–161). Another study demonstrated how to remove deleterious glucosides from safflower meal and then demonstrated possibilities for removal of these and other negative factors to make safflower meal a more useful product (162). Others have isolated three conjugated serotonin factors (163) and their related phenolic factors (164).

## 6. STORAGE AND TRANSPORTATION

### 6.1. Safflower Seed

The most important element in the storage of safflower seed is anticipation of problems. If safflower seed buyers maintain contact with the suppliers and inspect the fields, most problems can be solved before they escalate. Safflower seed that is below 8% moisture; is free of green weed, seeds, or trash; and has been brought to room temperature gradually is quite stable and can be stored indefinitely with no problem. Arranging for outside cleaning and/or drying before delivery to the oil mill and possible rejection, if the grower is unable to cope with weeds in the field, is much better than handling such problems on an emergency basis at time of delivery.

Because oleic and linoleic safflower seeds are virtually identical in appearance, extreme care is necessary to prevent inadvertent mixing. If a positive paper trail can be established for identifying fields of linoleic and oleic safflower from time of planting until delivery to the oil mill or storage point, much more confidence is possible when the samples are taken and the seed is checked for refractive index to



verify positively the type delivered. Each load can be properly directed to its appropriate discharge point. If safflower seed is put into storage free of included green weeds or other plant material, with moisture level that has been brought to equilibrium at 8% or under, it can be stored indefinitely.

At the time of the year that safflower is harvested, air temperatures are often in excess of 38°C. If safflower seed is being harvested from a particularly weedy field, the farmer must be careful to monitor the temperature of the seed in the truck or trailer into which the harvest is loaded. If a truck is forced to wait overnight in such conditions, the seed can begin heating to a dangerous level. Similarly, if seed is brought to a warehouse on a hot day, it is prudent for the warehouse to pull air through the seed pile or silo until the temperature of the entire mass reaches equilibrium. Monitoring the temperature within the seed mass by means of thermocouples is mandatory. If temperatures start to rise, air circulation can be started again until equilibrium is restored. If a hot spot cannot be controlled, it is prudent to reach that area as soon as possible by turning part of the seed column (in a tank or silo) or by digging into the side of the pile (if stored in a flat warehouse). This is not a time to move slowly; tear off the side of the building if that is what it takes to reach a hot spot. Safflower seed seems to be quite hygroscopic, absorbing moisture from wet material adjacent to it, which in turn causes germination to start, and hence heating. Safflower seed that has heated will char to dark brown to black mass and will develop strong odors that can permeate the storage unit if not separated quickly. This is not to portray safflower seed as a problem seed. Most seed is received in fine condition and stores without problems.

If a problem load is received, it is usually best to process it through a simple grain cleaner to remove the problem foreign material or, if that is not possible, to pile the seed in a thin layer on a concrete slab for a few days until it stabilizes. Safflower seed can be dried in forced air grain dryers. It is wise, however, to avoid letting temperatures in the dryer exceed 82°C to prevent safflower oil from scorching. If necessary, two passes through a dryer are to be preferred to one pass at high temperatures.

Safflower seed is normally not attacked by grain weevils, but these pests are often attracted to the foreign material contained in seed stored longer than 5 months. Again, careful monitoring is necessary if seed is destined for export. Treatment with approved fumigants in a timely fashion will avoid having the shipment graded "weevily," which in turn makes the safflower seed sample grade and subject to rejection by the buyer.

Safflower seed is quite stable under carriage in ocean vessels. Although natural separations within the vessel's interior are preferred, safflower seed can be successfully separated from safflower bulk cargoes in the hold by a temporary separation built from layers of plywood, plastic, and burlap.

## 6.2. Safflower Oil

Crude safflower oil can be stored and conveyed in normal black-iron vessels or pipelines without problems. The most important factor is to avoid exposure to air.

Safflower salad oil, because of its reactivity, should be stored and shipped in stainless equipment. The USDA in Peoria found that safflower was more light stable than soybean or cottonseed oils but still recommended packaging in brown bottles (165). It was found that  $\alpha$ -tocopherol does little to inhibit photooxidation, and it has been recommended that brown bottling be used to inhibit photooxidation and to reduce formation of free radicals, which generate objectional off-flavor (166). The USDA presented an early study of antioxidants (167), and the super qualities of 2', 4', 5'-trihydroxybutyrophenone (TBHQ) have been demonstrated (168). The limited effects of tempeh oil have been studied (169), and some researchers have recommended chromans, particularly Trolox C, as producing superior results in stabilizing safflower oil (170). TBHQ was the preferred antioxidant when these products were fashionable. In today's world of environmental concerns, the only antioxidant used is the addition of citric acid during deodorization. If safflower salad oil must be stored, nitrogen sparging and blanketing is recommended.

### 6.3. Safflower Meal

As noted, expeller safflower meal is dangerous to store under any conditions. Solvent-extracted safflower meal is much more stable and can be stored safely so long as moisture levels are kept low in storage. Safflower meal can hold 10% moisture under California weather conditions, but it is preferable to maintain it at a 5–7% moisture content if storage is for an extended period. Long-term storage in small diameter tanks should be avoided, because safflower meal tends to bridge under such conditions and become difficult to remove.

## 7. UNIQUE USES

### 7.1. Bird Feed

Approximately 60 million people in the United States provide feed for wild birds (171). Safflower seed is employed in many wild bird feeding mixes because of its high oil content; it is also used to feed caged birds, particularly parakeets and parrots. Safflower seed is used in the feeding of tame ducks in China and Taiwan and for tame pigeons in Europe. Safflower fields are considered excellent venues in the United States by hunters of white wing dove and pheasants, and safflower is often planted at duck clubs to attract ducks and pheasants.

### 7.2. Ornamental

Safflower seeds and flowers were used in Egypt to make ornaments, wreaths, and jewelry (172). Horticultural use is limited because of safflower's prickly foliage, but it is employed in some places for this reason as a protection for other plants against children and dogs. The spineless varieties are often used by Spanish and Portuguese farmers as garden flowers and as sources of food coloring. More recently, the red or

orange flowers of spineless safflower have been harvested and then either dried or sold as a fresh display by florists. If dried carefully, safflower plants and flowers retain their color and can be used for years in a static display.

### 7.3. Food Coloring

Safflower has been employed as a source of color and flavor in cooking in every country where it has been grown with the exception of the countries that have started large-scale commercial production in the last 40 years. Except for recent immigrants, safflower has not been extensively so used in the United States. References on the use of safflower florets in cooking are available (1,3). In the areas of Spain and Portugal where saffron is grown, fields of safflower exist that the local farmers do not like to acknowledge. Almost surely it was being used as an adulterant to the much more expensive saffron.

### 7.4. Dye

Another ancient use for safflower is to make dyes. The principal dye, carthamin, is a bright red colorant that is extracted from red-flowered plants after the yellow dye has been leached with water. Carthamin ( $C_{43}H_{42}O_{22}$ ) imparts a scarlet red color to silk and cotton (173–176). Fine examples of its durability from ancient times can be found in museums in Egypt, China, and Japan (3). Safflower yellow pigment ( $C_{16}H_{20}O_{11}$ ) must be removed to allow the red dye to be extracted; in earlier times the yellow was discarded. A factory has been established in Xinjiang, China, to manufacture large quantities of both types of dye (173).

### 7.5. Medicinal

Safflower seed, pollen, florets, and oil have been used for medicinal purposes almost since cultivation began. In the first century A.D., Pliny wrote that safflower oil, called *oleum cnicium*, was used as a milder substitute for castor oil, and Pedanius Dioscorides, in *De Materia Medica* (the leading Western pharmacological text for 16 centuries), described the use of safflower to color and flavor various potions and unguents and to act as mild laxatives and flavoring agents (157).

Several Arab texts dating to 1000–1500 mention safflower as an antidote for poisons and as an agent to induce sweating as a fever cure (172). Known as *Kusumbha* oil, safflower was regarded as a purgative in ancient Africa and India. Charred safflower oil is used in India as a treatment for sores and rheumatism, and safflower seeds are employed as diuretics and tonics (177). China has a long history of use of all parts of the safflower plant in medicinals combined with many herbal products. A tea is manufactured in Beijing (178), and a polyamino acid nutrient is manufactured from safflower protein isolates in inner Mongolia. Many discussions on ancient and modern Chinese medicine are available (7, 173).

USP safflower oil has been used as a carrier for penicillin. Data on use of safflower oil as a vehicle for injection of androgens (179) have been published, and it

has been demonstrated that safflower oil could be used as a dipersant for solutions of intravenous feedings of dextrose (109).

## 7.6. Cosmetic

Safflower florets were used to color ceremonial ointments in Egyptian tombs (10). The *Journal of American College of Toxicology* published a report on the safety of safflower oil in 1981. The article concluded that safflower oil was safe as a cosmetic ingredient in the current practices of use (180). Oleic safflower is considered equally safe, and safflower oil was found to be nonallergenic (181).

Powdered safflower florets have been employed since ancient times as a rouge in Egypt, China, and Japan, where it was known as *beni* (7, 177). The *Misehnz* of ancient Hebrew literature speaks of safflower's use as a rouge (182). It was mixed with French chalk and used as a rouge in old England (9). Soot from charred safflower plants was used until recently as the source of *kohl*, a cosmetic used to darken the eyelids of Egyptian women (183). In India, safflower oil is used as an ingredient in soap and the preparation of *Macassar* hair oil (180).

**TABLE 13. Supply of Potential Biodiesel Feedstocks.**

Oil Type	Total Oil Production <sup>a</sup>	
	Pounds	Gallons <sup>b</sup>
	Millions	
Crops		
Total	20,030	2,601.3
Soybean	14,935	1,939.6
Corn	2,076	269.6
Cottonseed	1,220	158.4
Sunflowerseed	868	112.7
Canola	353	45.8
Peanut	282	36.6
Flaxseed/linseed	175	22.7
Safflower	118	15.3
Rapeseed	3	0.4
Animal fat		
Total	8,772	1,139.2
Lard	1,026	133.2
Edible tallow	1,490	193.5
Inedible tallow	3,623	470.5
Yellow grease	2,633	341.9
Total supply	28,802	3,740.5

<sup>a</sup>Pounds of oil production are a 3-year average (1993–1995) from *Oil Crops Yearbook*, October 1997, USDA, ERS with the following exceptions: rapeseed was calculated by multiplying oil per acre times the 1993–95 average number of acres harvested. Number of harvested acres comes from USDA, NASS, January 1996. Inedible tallow and yellow grease supply comes from U.S. Department of Commerce, Bureau of the Census, *Fats and Oils, Production, Consumption and Stocks, Annual Summaries 1993–1995*.

<sup>b</sup>Pounds are converted to gallons of oil using a 7.7 pounds-to-gallon conversion rate.

## 7.7. Biodiesel Fuel

Safflower has been examined as a possible biodiesel feedstock. Table 13 is an example of the amount of oil produced annually in the United States by type of feedstock. Amounts are reported by weight (i.e., millions of pounds), which are converted to a liquid volume basis to provide a gallon estimate for each feedstock. Soybean oil is the largest potential feedstock source for biodiesel. Corn oil, cottonseed and sunflowerseed are also relatively large contributors to U.S. vegetable oil supplies. The production of oil from the other feedstocks is minor, ranging from 353 million pounds for canola to around 3 million pounds for industrial rapeseed. The total production of oil from crops is about 20 billion pounds per year. Animal fats and yellow grease add about another 8.8 billion pounds, resulting in about 29 billion pounds of total oil.

On a liquid fuel basis, these feedstocks would equal about 3.7 billion gallons of diesel fuel, about 13% of the 28 billion gallons of diesel fuel consumed in the United States for transportation in 1996. If biodiesel was blended with petroleum diesel fuel, e.g., 20% biodiesel and 80% petroleum (B20), the total supply of this blended fuel would be about 18.7 billion gallons, or 67% of U.S. annual diesel consumption. This example uses the total average supply of all crop oils, animal fats, and yellow grease as the available feedstock supply (182).

## REFERENCES

1. D. K. Salunkhe, J. K. Charan, R. N. Adjule, and S. S. Kadam, *World Oilseeds*, Van Nostrand Reinhold, Co., Inc., New York, 1992, p. 326.
2. N. W. Simmons, ed., *Evolution of Crop Plants*, Longman, New York, 1976, pp. 219, 230, 231–233.
3. E. A. Weiss, *Castor, Sesame and Safflower*, Barnes & Noble, New York, 1971, pp. 530–554.
4. A. Ashri, *Divergence and Evolution in the Safflower Genus, Carthamus, Final Research Report USDA PL 480*, Project No. **A-10-CR-18**, Hebrew University, Rehovot, Israel, 1973.
5. A. Ashri and P. F. Knowles, *Agron. J.*, **52**(1), 11–17 (1960).
6. A. Ashri and P. F. Knowles, *Abst. Am. Soc. Agron.*, 50 (1977).
7. D. Li, ed., *Proceedings of the Third International Safflower Conference*, Chinese Academy of Sciences, Beijing, 1993, pp. 35–46.
8. Ref. 3, p. 534.
9. C. E. Claassen, ed., *Proceedings of the First International Safflower Conference*, University of California, Davis, 1987, pp. 28–35.
10. L. Keimer, *Die Gartendflanzen im Alten Agypten*, Hamburg, Germany, 1924.
11. G. Schweinfurth, *Bot. Jahr.*, **68**, 1–6 (1887).
12. J. S. Ingram, *Trop. Sci.*, **11**(3), 177–184 (1969).
13. C. L. Madan, B. M. Kapur, and U. S. Gupta, *Econ. Bot.*, **20**(4), 377–385 (1966).
14. D. Basker and M. Negbi, *Econ. Bot.*, **37**(2), 228–236 (1983).

15. Ref. 3, p. 531.
16. Ref. 3, p. 529.
17. J. R. Smith in D. R. Erickson, ed., *Edible Fats and Oils Processing*, American Oil Chemists Society, Champaign, Illinois, 1990, p. 324.
18. G. Yuan and co-workers, *Safflower Genetic Resources and Their Utilization*, Science and Technology Publishing House, Beijing, 1989.
19. Ref. 3, pp. 586–590.
20. Ref. 3, p. 742.
21. W. R. Aykroyd, *Health Bulletin*, No. 23, 4th ed., Ministry of Health, New Delhi, India, 1951.
22. P. J. C. Brauns, *Experiences in 1957 and 1959 of the Grazing Value of Safflower*, Biloela Regional Experiment Station, Australia, 1960.
23. S. K. Ranjhan, *Ind. Vet. J.*, **36**(6), 267–276 (1959).
24. Ref. 3, p. 677.
25. F. Rabak, *USDA Circ.*, **366** (1935).
26. A. Rehbein Sr., *Mont. Farmer-Stockman*, **35**(5), 6 (1948).
27. L. L. Carrick and H. K. Nielsen, *Amer. Paint J.*, **22**(22), 7–9, 18–16; **22**(45), 13–21, 44–46; **22**(47), 12–14, 20, 43–48; **22**(48), 20–29; **22**(49) 52–60 (1938).
28. F. Schofield, *Natl. Paint, Varn. Lacquer Assoc. Sci. Sec. Cir.*, **519** 522 (1936).
29. J. S. Remington, *Paint Manufac.*, **6**, 50–52 (1936).
30. J. Van Loon, *Verfkronief*, **10**(4), 280, 282 (1937).
31. H. H. Mann and N. V. Kankar, *J. Soc. Chem. Ind.*, **38**, 36–38 (1919).
32. F. Honcamp et al., *Tierernahr*, **1**, 3–29 (1929).
33. A. T. Pugsley and G. Winter, *Aust. Munitions Supply Labs Rep.*, **171**, (1947).
34. J. R. Smith, *Safflower*, AOCS Publications, Champaign, Illinois, in press.
35. J. Muir, *Farm Q.*, **88**, 168–172 (1960).
36. R. E. Woodward, *Industrial Survey of Safflower*, University of Nebraska, Lincoln, 1949.
37. C. E. Claassen and T. A. Kiesselbach, *Experiments with Safflower in Western Nebraska*, Bulletin **376**, Nebraska Agriculture Experiment Station, Lincoln, Nebraska, 1945.
38. C. E. Claassen, *Chem. Dig.*, **7**(3), 11–17 (1948).
39. C. E. Claassen, *Nebraska Sta. Circ.*, **87** (1949).
40. C. E. Claassen, M. L. Shuster, and W. W. Ray, *Plant Dis. Rep.*, **33**(2), 73–94 (1949).
41. C. E. Claassen and A. Hoffman, *Crops Soils*, **1**(8), 5–7 (1949).
42. P. F. Knowles, *Safflower Production in California*, Davis, University of California, Davis, Agricultural Experiment Station, 1949, pp. 1–7.
43. H. Taller, *Calories Don't Count*, Simon & Schuster, New York, 1967.
44. L. W. Kinsell et al., *J. Clin. Endocrinol. Metab.*, **12**, 909 (1952).
45. E. H. Ahrens et al., *Lancet.*, **1**, 6976 (1957).
46. B. B. Stewart et al., *Lancet.*, **2**, 521 (1956).
47. D. D. Rubis and D. S. Black, *Safflower Recommendations for Arizona*, Report **170**, Agriculture Experiment Station, University of Arizona, Tucson, Oct. 1958.
48. D. D. Rubis and D. S. Black, *Gila—A New Safflower Variety*, Bulletin **301**, Agriculture Experiment Station, University of Arizona, Tucson, Nov. 1958.

49. L. O. Cummings and I. A. Kneeland, U.S. Patent 3,266,977, Aug. 16, 1966.
50. L. O. Cummings, U.S. Patent 3,669,900, June 13, 1972.
51. L. O. Cummings, J. W. Sjoquist, and J. A. Kneeland, U.S. Patent 3,332,899, July 25, 1967.
52. W. E. Rathjen, L. O. Cummings, and J. A. Kneeland, U.S. Patent 3,278,567, Oct. 11, 1966.
53. *Oil Crop Situation and Outlook Yearbook*, Economic Research Service, USDA, Oct. 2002, updated, accessed Aug. 2004.
54. B. Horowitz and G. Winter, *Nature*, **179**, 582 (1957).
55. P. F. Knowles and A. Mitwaki, *Econ. Bot.*, **17**, 139 (1963).
56. P. F. Knowles and A. B. Hill, *Crop Sci.*, **4**, 406 (1964).
57. P. F. Knowles, A. B. Hill, and J. E. Ruckman, *Calif. Agr.*, **19**(12), 15 (1965).
58. J. Dominguez Gimenez, *Sesame Safflower Newslett.*, **3**, 63 (1987).
59. FAO, *World Crop and Livestock Statistics, 1948–1955*, Process Statistic Series 1, FAO, Rome, 1987, pp. 332–349.
60. FAO, *Yearbook Production*, Statistical Series 104, Vols. 40–46, FAO, Rome, 1987–1993, pp. 116, 119, 126, 165.
61. Ref. 3, p. 570.
62. T. H. Applewhite, *J. Amer. Oil Chem. Soc.*, **43**(6), 406–408 (1966).
63. E. A. Weiss, *Oilseed Crops*, Longman, London, 1983.
64. D. Li, M. Zhou, and V. R. Rao, eds., *Characterizations and Evaluations of Safflower Germplasm*, Geological Publishing House, Beijing, China, 1993, pp. 96–233.
65. J. Guggolz et al., *J. Amer. Oil Chem. Soc.*, **45**(10), 689–693 (1968).
66. A. L. Urie and D. E. Zimmer, *Crop Sci.*, **10**, 371–372 (1970).
67. W. W. Elbert and P. F. Knowles, *Am. J. Bot.*, **55**(4), 421–430 (1968).
68. A. L. Urie, *Abst. Amer. Soc. Agron.*, 65 (1976).
69. H. J. Burkhardt and G. Fuller, *J. Amer. Oil Chem. Soc.*, **47**, 219–221 (1970).
70. H. J. Burkhardt, *J. Amer. Oil Chem. Soc.*, **45**(1), 96–99 (1968).
71. H. J. Burkhardt, *J. Amer. Oil Chem. Soc.*, **47**(2), 69–72 (1970).
72. H. J. Burkhardt, *J. Amer. Oil Chem. Soc.*, **48**(11) 697–699 (1971).
73. A. B. Hill, *Oilseeds and New Crops Workshop*, University of California, Davis, Feb. 27, 1986.
74. D. L. Smith and C. E. Claassen, *Abst. Am. Soc. Agron.*, **90** (1963).
75. D. D. Rubis, ed., *Proceedings of the Third Safflower Research Conference*, Davis, California, 1969, pp. 27–32.
76. A. R. Deshmukh, R. M. Patil, and N. Nimbkar, eds., *Abstracts Second International Safflower Conference*, Hyderabad, India, 1989, pp. 32–33.
77. T. C. Heaton, P. F. Knowles, and B. D. Webster, *Abst. Am. Soc. Agron.*, 163 (1979).
78. T. C. Heaton and P. F. Knowles, *Crop Sci.*, **20**(4), 554 (1980).
79. T. C. Heaton and P. F. Knowles, *Crop Sci.*, **47**(7), 520–522 (1982).
80. Y. C. Doesthale, *J. Amer. Oil Chem. Soc.*, **58**(11), 988–990 (1981).
81. R. M. Saunders, *J. Amer. Oil Chem. Soc.*, **47**(7), 254–255 (1970).
82. NIOP, *Trading Rules*, 1993–1994 NIOP, Washington, D.C., pp. 77–79.

83. Ref. 1, p. 340.
84. G. Röbbelen, R. K. Downey, and A. Ashri, *Oil Crops of the World*, McGraw-Hill, New York, 1989, pp. v–viii.
85. F. D. Gunstone and M. I. Quresh *J. Amer. Oil Chem. Soc.*, **42**(11), 961–965 (1965).
86. T. N. B. Kaimal and G. Lakshminarayana, *J. Amer. Oil Chem. Soc.*, **56**(5), 578–580 (1979).
87. R. B. Allen, *Feedstuffs*, **62**(31), 24–30 (1990).
88. D. Bath et al., *Feedstuffs*, **62**(31), 31–27 (1990).
89. N. Mehrotra and G. P. Shukla, *Haryana Agr. Univ. J. Res.*, **3**, 195–196 (1973).
90. N. Mehrotra, S. K. Arora, and P. P. Jain in *Symposium on the Strategy and Experimental Approaches for Genetic Improvement of Forages for Increased Animal Productivity*, Indian Society of Genetics, Haryana Agricultural University, Hissar, 1975.
91. I. J. Singh and N. Mehrotra, in Ref. 9, pp. 225–227.
92. N. Mehrotra and B. D. Chaudhary, in Ref. 9, pp. 155–159.
93. Ref. 3, pp. 761–765.
94. P. F. Knowles, *Econ. Bot.*, **21**(2), 156–162 (1967).
95. Ref. 1, p. 334.
96. Ref. 3, p. 328.
97. G. A. Kopas and J. A. Kneeland, U.S. Patent 3,271,160, Sept. 6, 1966.
98. M. A. Williams, *Oil Mill Gazet.*, **95**(3) (1989).
99. M. A. Williams and J. F. Crawford, in press.
100. M. A. Williams, U.S. Patent 4,901,635, Feb. 20, 1990.
101. G. M. Neumunz, *J. Amer. Oil Chem. Soc.*, **55**(5), 396A–398A (1978).
102. J. E. Blum, *J. Amer. Oil Chem. Soc.*, **43**(6), 416–417 (1966).
103. W. H. Schmidt, J. P. McNaught, and K. S. Baker, U.S. Patent 3,240,608, Mar. 15, 1966.
104. T. H. Applewhite, ed., *Bailey's Industrial Oil and Fat Products*, vol. 3, Wiley, New York, 1985, p. 60–86.
105. S. Zalewski and F. A. Kummerow, *J. Amer. Oil Chem. Soc.*, **45**(2), 87–92 (1968).
106. G. Ben-et, A. Doley, M. Schimmel, and R. Stern, *J. Amer. Oil Chem. Soc.*, **49**(4), 205–207 (1972).
107. E. N. Frankel, *J. Amer. Oil Chem. Soc.*, **47**(1), 11–14 (1970).
108. E. N. Frankel, F. L. Thomas, and J. C. Cowan, *J. Amer. Oil Chem. Soc.*, **47**(12), 497–500 (1970).
109. R. S. Singleton, R. R. Benerito, and J. L. White, *J. Amer. Oil Chem. Soc.*, **37**(2), 88–92 (1960).
110. H. L. Hanson and L. R. Fletcher, *Food Technol.*, **15**, 256–261 (1961).
111. *Acreage*, National Agricultural Statistics Service, USDA, Washington, D.C., released June 30, 2004.
112. M. L. Baker and co-workers, *Feeding Safflower Meal.*, Bulletin **402**, Nebraska Agriculture Experiment Station, Jan. 1951.
113. M. L. Baker, G. N. Baker, and J. K. Matsushima, *Safflower Meal as a Supplement for Cattle*, Bulletin **447**, Nebraska Agriculture Experiment Station, Aug. 1959.



114. G. N. Baker, T. Kosku, and M. L. Baker, *Uncorticated Safflower Meal as a Protein Supplement for Wintering Calves*, Bulletin **458**, Nebraska Agriculture Experiment Station, Sept. 1960.
115. N. W. Hilston, C. B. Boubicik and L. Paules, *Comparative Value of Soybean Meal, Safflower Meal and Urea for Fattening Steers*, Wyoming Agriculture Experiment Station, Aug. 1951.
116. N. W. Hilston, C. P. Stroble, and L. Paules, *Comparative Value of Soybean Meal, Safflower Meal and Urea for Fattening Steers*, Wyoming Agriculture Experiment Station, June 1952.
117. Anon., *Idaho Agr. Sci.*, **38**(3), 2 (1953).
118. R. H. Ross, G. W. Cleveland, and D. L. Fourt, *Am. Dairy Sci. Soc., West. Div. Proc.*, **34**, 72–73 (1953).
119. R. H. Ross, G. W. Cleveland, and D. L. Fourt, *J. Dairy Sci.*, **37**(6), 671–672 (1954).
120. D. W. Pittman and C. I. Draper, *Safflower, Its Possibilities and Culture in Utah*, Agriculture Experiment Station, Logan, Utah, 1955.
121. F. M. Kratzer and D. Williams, *Poultry Sci.*, **26**(6), 623–625 (1947).
122. F. M. Kratzer and D. Williams, *Poultry Sci.*, **30**(3), 417–421 (1951).
123. H. R. Halloran, *Feedstuffs*, **33**(45), 70–71 (1961).
124. E. K. Faulkener and L. Paules, *Saffmeal for Fattening Lambs*, Wyoming Agriculture Experiment Station, Aug. 1951.
125. E. K. Faulkener and L. Paules, *Saffmeal for Fattening Lambs—2nd Year's Trials*, Wyoming Agriculture Experiment Station, Mar. 1952.
126. H. Goss and K. K. Otagaki, *Calif. Agr.*, **8**(5), 15 (1954).
127. R. M. Dixon et al., in D. J. Farrell, ed., *Recent Advances in Animal Nutrition in Australia*, 1989, p. 15–24.
128. A. A. Betschart, *J. Amer. Oil Chem. Soc.*, **56**(3), 454–457 (1979).
129. A. A. Betschart, and R. M. Saunders, *J. Food Sci.*, **43**, 964 (1978).
130. A. A. Betschart, R. Y. Fogn, and M. M. Hanamoto, *J. Food Sci.*, **44**(4), 1022–1026, 1035 (1979).
131. O. Paredes Lopez and C. Ordorica Falomir, *J. Sci. Food Agr.*, **37**(11), 1097–1103 (1986).
132. O. Paredes Lopez and C. Ordorica Falomir, *J. Sci. Food Agr.*, **37**(11), 1104–1108 (1986).
133. O. Paredes Lopez, C. Ordorica Falomir, and T. A. Carabez, *Leb.-Wissensch. Technol.*, **21**(6), 328–333 (1988).
134. D. N. Kulkarni, K. D. Kulkarni, and D. R. Vencatasamy, in the Third International Safflower Conference, Beijing, China, 1993.
135. Ref. 82, pp. 99–101.
136. E. Jacobsen in *First Research Conference on Utilization of Safflower*, USDA ARS 74–74, Albany, California, 1967, pp. 28–31.
137. Ref. 82, pp. 97–99.
138. M. H. Neustadt, *Rapid Testing of Oilseeds for Oil Quality and Iodine Number of Oil*, USDA Technical Bulletin **1171**, Washington, D.C., Oct. 1977.
139. E. N. Frankel, C. D. Evans, and J. C. Cowan, *J. Amer. Oil Chem. Soc.*, **37**(9), 418–424 (1960).
140. C. D. Evans et al., *J. Amer. Oil Chem. Soc.*, **37**(12), 452 (1960).

141. E. N. Frankel et al., *J. Amer. Oil Chem. Soc.*, **37**(12), 418 (1960).
142. E. N. Frankel et al., *J. Amer. Oil Chem. Soc.*, **38**(3), 130–134 (1961).
143. E. N. Frankel et al., *J. Amer. Oil Chem. Soc.*, **38**(9), 134–137 (1961).
144. R. J. Horvat et al., *J. Amer. Oil Chem. Soc.*, **42**(12), 1112–1115 (1965).
145. J. M. Snyder, E. N. Frankel, and E. Selke, *J. Amer. Oil Chem. Soc.*, **62**(12), 1675–1679 (1985).
146. D. A. Forss, E. H. Ramshaw, and W. Stark, *J. Amer. Oil Chem. Soc.*, **39**(6), 308 (1962).
147. D. P. Schwartz and A. H. Rady, *J. Amer. Oil Chem. Soc.*, **67**(10), 635–641 (1960).
148. P. Fitton, E. H. Pryde, and J. C. Cowan, *J. Amer. Oil Chem. Soc.*, **42**(1), 14–19 (1965).
149. H. G. Kenney, D. Kamanowski, and A. N. Wrigley, *J. Amer. Oil Chem. Soc.*, **42**(1), 19–22 (1965).
150. G. Yuki, and Y. Ishikawa, *J. Amer. Oil Chem. Soc.*, **53**(11), 673–676 (1976).
151. C. D. Evans et al., *J. Amer. Oil Chem. Soc.*, **49**(10), 578–582 (1972).
152. R. J. Sims, J. A. Fiorti, and M. J. Kanuk, *J. Amer. Oil Chem. Soc.*, **49**(5), 298–301 (1972).
153. R. J. Sims and J. A. Fiorti, *J. Amer. Oil Chem. Soc.*, **52**(5), 144–147 (1975).
154. T. Riisom, R. J. Sims, and J. A. Fiorti, *J. Amer. Oil Chem. Soc.*, **57**(10), 354–358 (1980).
155. J. A. Fiorti, H. S. Stahl, J. Cseri, and R. J. Sims, *J. Amer. Oil Chem. Soc.*, **52**(10), 395–399 (1975).
156. J. A. Fiorti, M. J. Kanuk, and R. J. Sims, *J. Amer. Oil Chem. Soc.*, **51**(6), 219 (1974).
157. R. Palter, G. O. Kohler, and P. F. Knowles, *J. Agr. Food Chem.*, **17**(6), 1298–1300 (1969).
158. Ref. 9, pp. 214–217.
159. R. Palter, W. F. Haddon, and R. E. Luddin, *Phytochemistry*, **11**(7), 2327–2328 (1972).
160. R. Palter, R. E. Luddin, and G. Fuller, *Phytochemistry*, **11**(2), 819–822 (1972).
161. R. Palter, R. E. Luddin, and W. F. Haddon, *Phytochemistry*, **11**(9), 2871–2874 (1972).
162. C. K. Lyon et al., *J. Amer. Oil Chem. Soc.*, **56**(5), 560–564 (1979).
163. S. Sakamura, Y. Terayama, and S. Kawakatsu, *Agr. Biol. Chem.*, **42**(9), 1805–1806 (1978).
164. S. Sakamura et al., *Agr. Biol. Chem.*, **44**(12), 2951–2954 (1980).
165. H. A. Moser, C. D. Evans, J. C. Cowan, and W. F. Kwolek, *J. Amer. Oil Chem. Soc.*, **42**(1), 30–33 (1965).
166. J. A. F. Faria and M. K. Mukai, *J. Amer. Oil Chem. Soc.*, **60**(1), 77–81 (1983).
167. R. E. Beal, H. A. Moser, and O. L. Brekke, *J. Amer. Oil Chem. Soc.*, **35**(2), 97–99 (1958).
168. J. W. Thompson and E. R. Sherwin, *J. Amer. Oil Chem. Soc.*, **43**(12), 683–696 (1966).
169. P. György, K. Murata, and Y. Sugimoto, *J. Amer. Oil Chem. Soc.*, **51**(8), 377–379 (1974).
170. W. M. Cort, A. J. W. Scott, and M. Araujoetal, *J. Amer. Oil Chem. Soc.*, **56**(6), 174–178 (1975).
171. D. Lilleboe, *Sunflower*, **20**(1), 12–14, 1994.
172. Ref. 3, p. 530.
173. D. Li, ed., *Abstracts on Safflower*, Chinese Academy of Sciences, Beijing, 1993.
174. S. Buelaverie et al., eds., *The Merck Index*, 11th ed., Merck & Co., Inc., Rahway, New Jersey, 1989, p. 285.

175. F. Mayer, *The Chemistry of Natural Colouring Matters*, Reinhold Publishing, New York, 1943.
176. R. S. Lewis, Sr., ed., *The Condensed Chemical Dictionary*, 11th ed., Van Nostrand Reinhold, New York, 1993, p. 227.
177. Ref. 3, pp. 533–534.
178. D. Li and Y. Han, in Ref. 7, pp. 837–843.
179. S. L. Shapiro, L. Freeman and S. Kobrin, *Arch. Int. de Pharmacodyn.*, **3**(1), 30–35 (1957).
180. L. E. Cathers, ed., *J. Am. Coll. Toxicol.*, **4**(5), 171–197 (1985).
181. D. H. Goodman, *J. Allergy.*, **35**, 38–42 (1964).
182. I. Low, *Die Flora der Juden*, Vol. 1, Vienna, 1926.
183. G. Brunton, *Qau and Badri*, Vol. 1, London, p. 63.
184. *U.S. Biodiesel Development: New Markets*, Economic Research Service, USDA, 1997.

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## *Sesame Oil*

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### **1. INTRODUCTION**

Sesame (*Sesamum indicum* L.) is believed to be one of the most ancient crops cultivated by humans (1). It was first recorded as a crop in Babylon and Assyria over 4000 years ago. The seeds of the crop are used both as condiment and oil source. The Babylonians made wine and cakes with sesame seeds, whereas sesame oil was used for cooking, medicinal, and cosmetic purposes. Ancient Indians used sesame oil as lighting oil, and sesame seeds were commonly used in the religious rites of Hindus. The Chinese believed that sesame seeds could promote health and longevity.

Sesame seed has higher oil content (around 50%) than most of the known oil-seeds although its production is far less than the major oilseeds such as soybean or rapeseed due to labor-intensive harvesting of the seeds. Sesame oil is generally regarded as a high-priced and high-quality oil. It is one of the most stable edible oil despite its high degree of unsaturation. The presence of lignan type of natural antioxidants accounts for both the superior stability of sesame oil and the beneficial physiological effects of sesame.

In Asia, sesame oil is obtained by pressing the roasted oilseeds and consumed as a naturally flavored oil without refining. In the western world, sesame oil is extracted by a multiple-step mechanical expeller and either the virgin oil or the

refined oil is used for salad dressing. After pressing out oil, the remaining sesame meal contains high-quality protein suitable for human consumption as well as animal feed. It is also a good source of water-soluble antioxidants.

In this chapter, the properties and processing of sesame oil will be presented, and the antioxidative components and their effects on oil stability and health will be summarized.

## 2. BOTANY OF SESAME

Sesame (*Sesamun indicum* L., synonymous with *Sesamun orientale* L.), also known as benniseed (Africa), benne (Southern United States), gingelly (India), gengen (Brazil), sim-sim, semsem (Hebrew), and tila (Sanskrit), is the world's oldest oil crop. It belongs to the *Tubiflorae* order, *Pedaliaceae* family, which comprises of 16 genera and some 60 species (2). There are 37 species under the *Sesamum* genus (3). Among the 37 species, only *Sesamum indicum* is widely cultivated. The wild species such as *S. angustifolium*, *S. calycium*, *S. baumii*, *S. auriculatum*, *S. brasiliense*, *S. malabaricum*, *S. prostratum*, *S. indicatum*, *S. radiatum*, *S. occidentale*, and *S. radiatum* are cultivated in Africa, India, or Sri Lanka in small areas. The wild species, although low in oil contents, may contribute to favorable agronomic characters (such as resistance to disease, pests, and drought) when used in plant breeding.

As most of the wild species of sesame were found in Africa, it is generally believed that sesame originated in Africa. India may also be the origin of some species (*S. capense*, *S. prostratum*, and *S. schenckii*) of sesame (2, 4). The sesame species in the Middle East are similar to Africa; they are believed to be spread from Africa via Egypt (2). Sesame seeds were brought to India and Burma from Africa and the Middle East (4). Cross-fertilization of the species from Africa and India results in a large variety of sesame species. India, therefore, became the secondary center of diversity. Both China and Japan are the major consumers of sesame seeds; their sesame seeds were introduced from the Middle East as early as in 500 to 700 B.C. Sesame was brought to the United States by slaves from Africa in the late seventeenth century. The sesame seeds are still known as benne in the southern parts of United States, a term similar to the African name of sesame (benniseed).

Sesame grows in tropical and subtropical areas about 40°N latitude to 40°S latitude (5). *Sesame indicum* L. is the commonly cultivated species of sesame. It has 26 somatic chromosomes ( $2n = 26$ ). Sesame is an annual, erect herb that may grow between 50 cm and 250 cm in height, depending on the variety and growing conditions. The stems (Figure 1) may have branches and are obtusely quadrangular, longitudinally furrowed, and densely hairy. The extent of hairiness on the stem can be classified as smooth, slightly, and very hairy; it is related to the variety of sesame. The degree and type of branching of the stem are also important varietal characters (6).

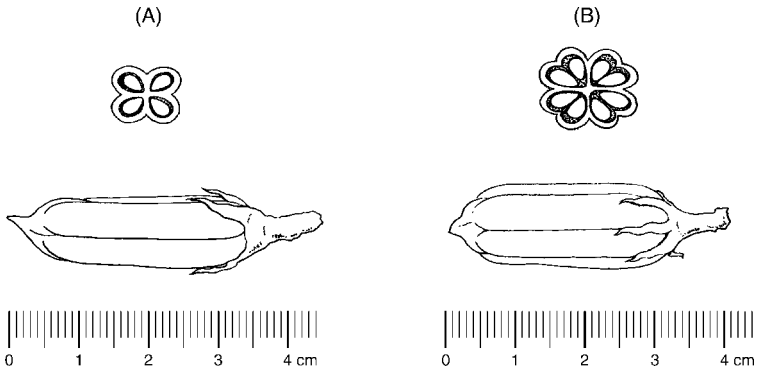


**Figure 1.** *The plant of sesame.*

Sesame leaves are hairy on both sides and are highly variable in shape and size not only among different varieties but also on the same plant. The lower leaves are opposite, ovate, sometimes palmately lobed or palmately compound, dull green in color, 3–17.5 cm long and 1–7 cm wide, and coarsely serrate, and the petiole is 5 cm in length. The upper leaves are alternate or subopposite, lanceolate, and entire or with a few coarse teeth, and the petiole is 1–2 cm long. The arrangement of leaves influences the number of flowers born in the axils and thus the seed yield per plant.

Sesame has large, white, bell-shaped flowers. The flowers are zygomorphic, in axils of upper leaves, born singly or 2~3 together, short-pedicelled, and geniculate. The calyx is small and five parted, and the segments are ovate-lanceolate and 0.5–0.6 cm long. The corolla is tubular-campanulate, 3–4 cm long, widened upward, two-lipped, five-lobed with middle lower lobe longest, pubescent outside, white, pink, or purplish in color with yellow or purple blotches, spots, and stripes on inner surface. The stamens are four in number, didynamous, and inserted on the base of the corolla; the anthers are sagittate. The ovary is superior and two-celled (7).

The fruit of sesame is a capsule (2–5 cm long and 0.5–2 cm in diameter), and it is erect, oblong, brown or purple in color, rectangular in section, deeply grooved with a short, triangular beak (Figure 2). The capsules may have four, six or eight rows of seeds in each capsule (Figure 2). Most of the sesame capsules have four rows of seeds, with a total of 70 seeds per capsule. The capsules with a wider

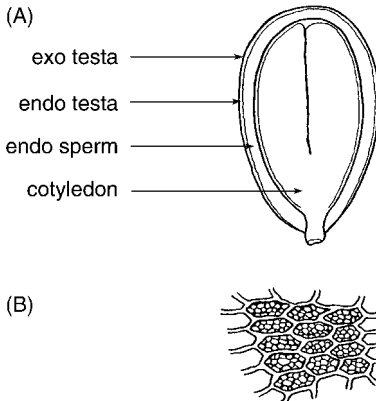


**Figure 2.** Sesame fruits with four (A) or eight (B) rows of seeds in each capsule.

diameter will usually have higher rows of seeds and the total number of seeds per capsule can be as high as 100~200. When the fruit is ripened, it dehisces by splitting along the septa from top to bottom (so called “open sesame”).

Sesame seeds are small (3~4 mm long and 1.5~2 mm wide), flat, ovate (slightly thinner at the hilum than at the opposite end), smooth, or reticulate. The color varies from white, yellow, gray, red, brown, to black. The weight of 1000 seeds is around 2.5 to 3.5 g. Sesame seeds consists of testa (exo and endo), endosperm, and cotyledon (Figure 3). The oil drops are located in the cotyledon. It is generally believed that the light-colored seeds with thin coats are higher in quality and oil content than the dark-colored seeds.

Although sesame seeds are higher in oil contents than most other oilseeds and sesame oil has good flavor and oxidation stability, sesame seeds have never been a major oil source. The low yield (400~500 kg/ha) of sesame seeds and the labor-intensive harvesting procedure are the limiting factors. When sesame capsules



**Figure 3.** Structure of the sesame seed (A) and the oil drops in cotyledon (B).

are mature, they are fragile and will burst open easily, scattering the seeds on the ground and thus difficult to collect. Harvesting of sesame seeds is usually performed by cutting the plant stalks and stacking them vertically under the sun with the cut-ends downward in the threshing yard. Each dried stalk is then shaken or beaten over a cloth to catch the seeds that fly out from the dried capsules. The plant breeders have been trying to develop sesame varieties that do not dehisce when the capsules are mature and thus can be adapted to mechanical harvest (8–10). In the middle of the twentieth century, horticulturists developed sesame with “papershell capsules,” which is indehiscence allowing mechanical harvesting and is easier to thresh than the normal type (11). Until today, however, more than 99% of the sesame produced in world is still harvested manually. Numerous efforts have been made to move sesame from a labor-intensive harvest crop to a mechanically harvest crop for the past 60 years. Considerable progress was made between 1940 and 1965, but there was still a limited amount of manual labor necessary in the harvest. The first completely mechanized cultivars were developed in the early 1980s, and there has been continuing progress. Progress in mechanizing sesame has been slow because of the need to combine many characters in order to compromise between machine-harvesting and plant characteristics such as seed yield and quality, disease resistance, insect resistance, hail resistance, and drought resistance. Sesame can become a major oilseed only with lower price achieved by increasing yields and reducing production costs (12).

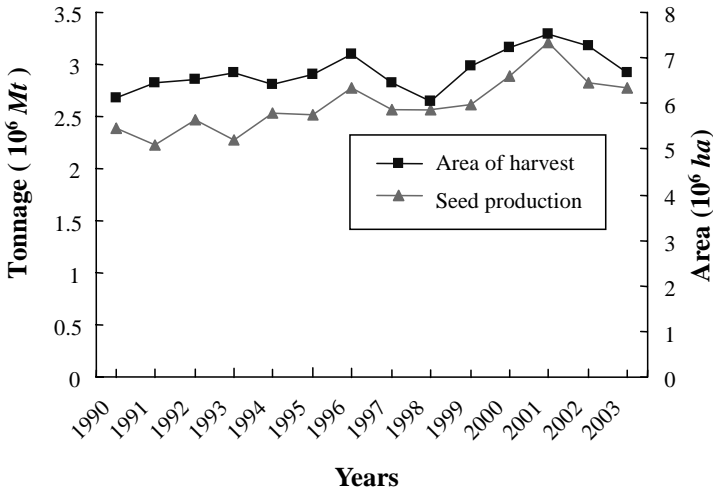
### 3. WORLD PRODUCTION

#### 3.1. Sesame Seed

Sesame ranks eighth in the world production of edible oil seeds. The total annual production of sesame seeds is around 3 million metric tons (MT) worldwide from 2000 to 2002. This number has increased 33% since 1990. Figure 4 shows the total tonnage together with the total area of world sesame production from 1990 to 2003. It is evident that there is a steady increase of both the seed production and the area of harvest. The highest sesame seed production reached 3.2 million MT in 2001, with a total harvesting area of 7.5 million hectares (ha) worldwide. The average yield of sesame seed is around 400 kg/ha worldwide (Table 1). Among the five continents, Asia has the highest area of harvest (4.6 million ha), which produces 2 million MT of sesame seed annually. Europe has the lowest quantity of seed production (only 0.057% of the world total) but the highest yield (4968.5 kg/ha) of sesame seed. This yield is ten times that of Asia where more than 70% of world's sesame seeds are produced. Africa, the origin of sesame seed, is the second largest sesame-producing continent. It has, however, the lowest yield (only 328 kg/ha) of sesame seed.

China, India, Sudan, Myanmar, and Uganda are the world's major sesame seed producing countries. In 2003, China produced 825 thousand MT of sesame seed and was the world's largest sesame-producing country followed by India (620,000 MT),





(Data source: FAOSTAT database)

**Figure 4.** World production of sesame seed (1990–2003). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

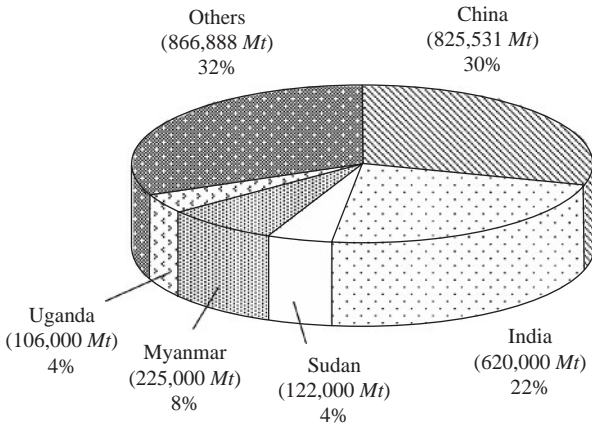
Myanmar (225,000 MT), Sudan (122,000 MT), and Uganda (106,000 MT). These five countries together supply nearly 70% of the world’s total sesame seed (Figure 5). Figure 6 shows the fluctuation in annual seed production by these countries from 1990 to 2003. As the crop yield is very dependent on moisture, the seed production can vary up or down in any given year due to rainfall. According to FAO statistics (13), the yield of sesame seed in China grew rapidly from around 700 kg/ha in 1990 to 1099 kg/ha in 2003, whereas India remained around 300 kg/ha for the past 15 years. Sudan is the lowest among the five major sesame producing countries in per hectare yield (150~220 kg/ha) followed by Myanmar (170~380 kg/ha). Uganda has a relatively high yield (500 kg/ha) of sesame seed, but the area of harvest is the lowest among the five countries.

**TABLE 1. Production of Sesame Seed in the Five Continents in 2003.<sup>1</sup>**

Continent	Seed Production (1000 Mt)		Area of Harvest (1000 ha)		Yield (kg/ha)
Africa	603.827	(21.835%) <sup>2</sup>	1840.382	(27.547%)	328.099
Asia	2014.492	(72.846%)	4602.432	(68.889%)	437.702
Europe	1.575	(0.057%)	0.317	(0.005%)	4968.454
North and Central America	65.870	(2.382%)	127.254	(1.905%)	517.626
South America	79.655	(2.880%)	110.485	(1.654%)	720.958
<b>World Total</b>	<b>2765.419</b>	<b>(100%)</b>	<b>6680.870</b>	<b>(100%)</b>	<b>413.931</b>

<sup>1</sup>Based on FAOSTAT database (2003).

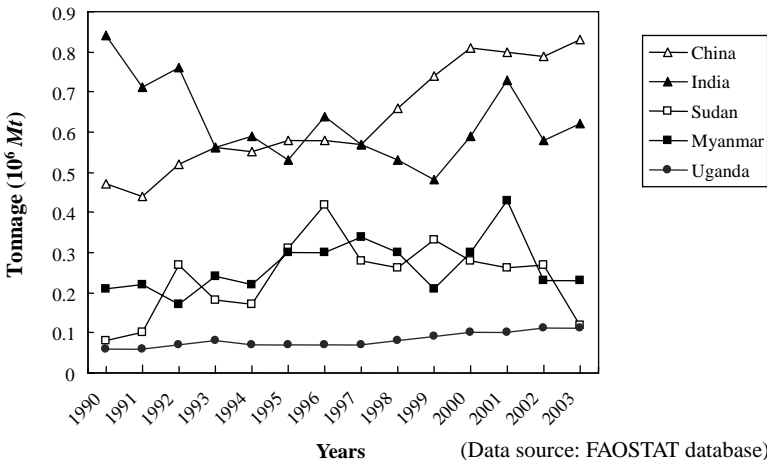
<sup>2</sup>Data in parenthesis are the percentage of total.



(Data source: FAOSTAT database)

**Figure 5.** Major sesame seed-producing countries and their percentage shares of the world production in 2003.

In 2000, the world trade of sesame seed was 620,000 MT, which was 21.5% of the total production. Japan imported 165,000 MT (26% of the world imports) and was the largest importer of sesame seed. South Korea was the second largest importer (70,000 MT) followed by United States (49,000 MT), Taiwan (35,000 MT) and Egypt (34,000 MT). Although China and India are the top two sesame seed producers, most of the seeds are consumed locally. Only 12~15% of the sesame seeds produced in India were exported in the past ten years. China was the world number one sesame seeds exporting country, which exported



(Data source: FAOSTAT database)

**Figure 6.** Major sesame seed-producing countries (1990–2003). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

17~25% of its sesame production before 1996. Because of the fast economic growth in China, domestic demand of sesame seed increased tremendously after 1996. Although China became the world's biggest sesame seed producer since 1997 (Figure 6), the export of sesame seed from China dropped from 119,000 MT (in 1996) to 41,000 MT (in 1997). Starting from 1996, Sudan became the world's top sesame exporting country followed by India and China.

### 3.2. Sesame Oil

Each year, the world consumes close to 120 million MT of edible fats and oils (14). Soybean oil is the leading oil that accounts for 30% of the world production of edible fats and oils. In 2003, it is closely followed by palm oil, whereas rapeseed oil ranked third has only one-third of the production tonnage of soybean oil. Sesame oil, with an annual production of 760,000 MT in 2003, is the twelfth largest vegetable oil produced in the world, higher in quantity than olive oil and safflower oil (13). The production of sesame oil increased 20% in the recent 10 years, it was 632,000 MT in 1992. China has almost doubled the production of sesame oil (from 142,000 to 210,000 MT), whereas India has decreased the production by 44% (from 236,000 to 131,000 MT) in the above period. Both China and India are the largest producers of sesame oil, together they account for nearly half of the total world production of sesame oil. Besides China and India, Myanmar, Sudan, and Japan are the top five sesame oil producers.

## 4. CHEMICAL COMPOSITION

Sesame seed contains high levels of fat and protein. The chemical composition of sesame seed varies with the variety, origin, color, and size of the seed. The fat content of sesame seed is around 50% whereas the protein content is around 25%. Table 2 lists the proximate composition of sesame seeds from different sources. Sesame seed contains about 5% of ash, whereas the fiber and carbohydrate contents show large variation. Crude fiber from one variety of Nigerian black sesame was reported to have 19.6% of crude fiber (15), whereas one variety of Taiwanese

**TABLE 2. Proximate Composition of Sesame Seed (%).**

Sesame	Crude Fat	Crude Protein	Carbohydrate	Crude Fiber	Ash	Moisture	Reference
Black sesame	35.8	17.2	9.19	19.6	4.01	4.73	15
White sesame	34.6	20.8	9.19	14.2	10.1	4.14	15
Brown sesame	41.3	20.2	10.3	18.6	5.19	4.12	15
Yellow sesame	53.8	22.0	6.85	13.0	6.09	4.28	15
Black sesame	48.4–56.7	22.8–30.3	3.4–10.8	2.8–7.2	4.4–5.5	4.6–6.4	16
White sesame	50.1–51.7	22.6–24.1	7.9–13.2	5.3–7.5	4.2–4.5	4.4–4.7	16
Brown sesame	46.3–53.1	21.8–27.6	4.7–13.6	3.7–7.3	3.9–5.4	5.0–8.2	16
Nigerian sesame whole seed	51.5	20.0	12.5	6.0	5.0	5.0	17
Dehulled seed	55.0	24.3	10.4	2.0	3.0	5.3	17

black seed contained only 2.81% of crude fiber (16). The carbohydrate content ranged from 3% to 14% (15–17).

Sesame seed has about 17% seed weight as hull, which is high in oxalic acid (2~3%), calcium, and crude fiber. Oxalic acid could complex with calcium and reduce its bioavailability; indigestible fiber would reduce the digestibility of protein. Sesame seed hull is therefore recommended to be removed if sesame meal is used for human food (18). When sesame seed is properly dehulled, the oxalic acid content can be decreased to less than 0.25% of the seed weight (19). After dehulling, the fat and protein contents are raised, whereas the fiber, ash, and carbohydrate contents are lowered (Table 2).

#### 4.1. Content of Oil

Sesame seed is a rich source of edible oil. It contains more oil than the major oil-seeds, such as soybean, rapeseed-canola, sunflower seed, and cotton seed. The oil content of sesame seed varies with the variety of sesame; it may range from 28% to 59% (20–22). The wild seeds contain less oil (around 30%) than the cultivated seeds because the oil content is an important criterion for seed selection in agriculture practice. In general, the cultivated seed has around 50% oil, whereas the color of the seed coat exhibits slight influence on the oil content. Black seeds appear to contain slightly less oil than the white and brown seeds in the Japanese strains (Table 3). The oil content was found to be influenced also by the growing condition, daily mean temperature, and the cumulative degrees of daily temperatures during reproductive stage, which showed negative correlation with the oil content (23).

**TABLE 3. Oil Content of Sesame Seed.**

Sesame Species	Color of Seed Coat	Oil Content (% Seed)	Reference
<i>Sesamum indicum</i> L.			
Sudan strains <sup>a</sup>	Black	50.7	20
Sudan strains	Brown	52.3	20
Sudan strains	White	47.4–55.5	20
Japanese strains <sup>b</sup>	Black	43.4–51.1	21
Japanese strains	Brown	50.5–56.5	21
Japanese strains	White	51.8–58.8	21
Turkish strains <sup>c</sup>	Black	43.3–48.2	22
Turkish strains	Brown	42.8–46.9	22
Turkish strains	White	43.1–46.3	22
<i>Sesamum alatum</i> T. <sup>d</sup>	Brown	28.1–29.8	20
<i>Sesamum radiatum</i> S. and T. <sup>d</sup>	Black	30.3–33.4	20
<i>Sesamum angustifolium</i> E. <sup>d</sup>	Black	29.2–29.7	20

<sup>a</sup> The cultivated species of sesame grown in Sudan.

<sup>b</sup> Forty-two species of sesame grown in Japan.

<sup>c</sup> The cultivated species of sesame grown in Turkey.

<sup>d</sup> The wild species of sesame grown in Sudan.

Table 4 lists the chemical and physical properties of sesame oil (24).

**TABLE 4. Chemical and Physical Characteristics of Sesame Oil (24).**

Properties	Range
Relative density (20°C/water at 20°C)	0.915–0.924
Refractive index (ND 40°C)	1.465–1.469
Saponification value (mg KOH/g oil)	186–195
Iodine value	104–120
Unsaponifiable matter (g/kg)	≤20

## 4.2. Fatty Acid Composition

Sesame oil belongs to the oleic-linoleic acid group. It has less than 20% saturated fatty acid, mainly palmitic (7.9~12%) and stearic (4.8~6.1%) acids. Oleic acid and linoleic acid constitute more than 80% of the total fatty acids in sesame oil. Unlike other vegetable oils in this group, the percentages of oleic acid (35.9–42.3%) and linoleic acid (41.5–47.9%) in the total fatty acids of sesame oil are close (Table 5).

Table 5 lists the first FAO/WHO Codex Alimentarius Standard of the sesame oil fatty acid composition as reported by O'Connor and Herb (25) and the most recent Codex Standard (24). Besides the four major fatty acids, there are low

**TABLE 5. Fatty Acid Composition of Sesame Oil (% Total Fatty Acids).**

Fatty Acid	Codex(24)	O'Connor(25)	Kamal-Eldin and Appelqvist(20) Cultivated <sup>a</sup>	Wild <sup>b</sup>
Myristic (C14:0)	ND <sup>c</sup> -0.1	<0.5		
Palmitic (C16:0)	7.9–12.0	7.0–12	9.0–9.6	8.2–12.7
Palmitoleic (C16:1)	0.1–0.2	<0.5	0.1–0.2	0.2–0.3
Heptadecanoic (C17:0)	ND-0.2			
Heptadecenoic (C17:1)	ND-0.2			
Stearic (C18:0)	4.8–6.1	3.5–6.0	5.6–6.4	5.6–9.1
Oleic (C18:1)	35.9–42.3	35–50	41.9–45.2	34.3–48.1
Linoleic (C18:2)	41.5–47.9	35–50	38.0–41.6	33.2–48.4
Linolenic (C18:3)	0.3–0.4	<1.0	0.5–0.6	0.6–0.9
Arachidic (C20:0)	0.3–0.6	<1.0	0.3	0.2–0.8
Eicosenoic (C20:1)	ND-0.3	<0.5	0.1	0.1
Behenic (C22:0)	ND-0.3	<1.0	0.1	0.1
Lignoceric (C24:0)	ND-0.3		trace	trace

<sup>a</sup>*Sesamum indicum* L.

<sup>b</sup>*Sesamum alatum* T, *Sesamum radiatum* S. and T., *Sesamum angustifolium* E.

<sup>c</sup>ND: Not detected.

percentages (less than 1%) of other fatty acids—myristic (ND-0.1), palmitoleic (0.1–0.2), heptadecanoic (ND-0.2), heptadecenoic (ND-0.2), linolenic (0.3–0.4), arachidic (0.3–0.6), eicosenoic (ND-0.3), behenic (ND-0.3), and lignoceric acid (ND-0.3). Fatty acid composition varies with the species of sesame seed (20, 22). Species with high oleic acid and linoleic acid contents are often selected for plantation (22). Sesame oils from the wild seeds, therefore, are higher in saturated fatty acids than oils from the cultivated sesame seeds (Table 5).

Fatty acid compositions of different lipid classes in sesame oil also show variation. The major sesame seed lipid is triacylglycerol, which represents nearly 90% of the total lipid (20). It has a lower percentage of saturated fatty acids and a higher percentage of unsaturated fatty acids than the other lipid classes, namely, diacylglycerol, free fatty acid, polar lipid, and steryl ester. Slightly higher percentages of long-chain fatty acids (20:0, 20:1, 22:0, and 24:0) were found in lipid classes other than triacylglycerol (20, 26).

### 4.3. Sterols

Sesame oil is relatively high in unsaponifiable matter (~2%) compared with other vegetable oils. The unsaponifiable matter includes sterols, triterpenes and triterpene alcohols, tocopherols, and sesame lignans. Sterols are present in vegetable oils in free form or as sterol esters, sterol glucosides, or esterified steryl glucosides, but free sterols and sterol esters are often the dominant forms. Among the three classes of sterols, desmethylated sterol is the major one (85~89% of total sterols) followed by monomethylated (9~11%) and dimethylated (2~4%) sterols in sesame oil (27). According to the Codex Standard, sesame oil may contain as high as 1.9% of total sterols; it is one of the richest oil source of phytosterols (24). Table 6 lists the levels of desmethylsterols composition in sesame oil.  $\beta$ -sitosterol is the most abundant sterol in sesame oil. There are also campesterol, stigmasterol,  $\Delta^5$ -avenasterol,  $\Delta^7$ -ave-

**TABLE 6. Levels of Desmethylsterols in Sesame Oil.<sup>a</sup>**

Desmethyl Sterol	Codex (24)	Kamal-Eldin and Appelqvist (27)	
		Cultivated Sesame <sup>b</sup>	Wild Sesame <sup>c</sup>
Cholesterol	0.1–0.5	0.1–0.2	0.2–0.3
Brassicasterol	0.1–0.2	—	—
Campesterol	10.1–20.0	12.5–16.9	10.3–20.5
Stigmasterol	3.4–12.0	6.0–8.7	4.4–14.2
$\beta$ -sitosterol	57.7–61.9	57.5–62.0	33.9–60.2
$\Delta^5$ -avenasterol	6.2–7.8	8.1–11.5	12.4–23.5
$\Delta^7$ -stigmasterol	0.5–7.6	0.4–3.1	0.1–3.0
$\Delta^7$ -avenasterol	1.2–5.6	0.3–1.3	0.9–3.7
Others	0.7–9.2	3.6–6.1	4.6–7.3
Total sterols (mg/kg)	4500–19000	4335–6764	3420–10005

<sup>a</sup> Expressed as a percentage of total sterols.

<sup>b</sup> The cultivated species of sesame grown in Sudan.

<sup>c</sup> The wild species of sesame grown in Sudan.

nasterol, and  $\Delta^7$ -stigmasterol present in descending abundance. Only a trace amount (<0.5%) of cholesterol was found in sesame oil. Oils from the wild species of sesame contain higher levels of sterols, especially  $\Delta^5$ - and  $\Delta^7$ -avenasterols. These two sterols having the  $\Delta^{24,28}$  ethylidene side chain showed antipolymerization effects that could protect vegetable oils from high-temperature oxidation (28).

Phytosterols and cholesterol have similar structures; phytosterols are therefore competitors of cholesterol absorption. Consumption of phytosterol may lower blood cholesterol and thus protect from cardiovascular diseases (29). Phytosterol, especially,  $\beta$ -sitosterol, inhibits the growth of human colon cancer cell (30), prostate cancer cell (31), and breast cancer cell (32).

#### 4.4. Tocopherols

Sesame oil is well known for its oxidative stability; one of the reasons for this extra-stability is attributed to its tocopherol content. The total tocopherol content of sesame oil ranges from 330-mg/kg to 1010-mg/kg oil according to the Codex Standard. Sesame oil from black sesame seeds contains less tocopherols than oils from brown or white sesame seeds (Table 7). The wild species of sesame, *Sesamum angustifolium* E. and *Sesamum radiatum* S. and T., have higher levels of total tocopherol (760 mg/kg and 810 mg/kg, respectively) in the oil than the cultivated species (486–680 mg/kg) although they have a black seed coat. Regardless of the species and the color of seed coat,  $\gamma$ -tocopherol is the predominant tocopherol in sesame oil, whereas  $\delta$ -tocopherol accounted for less than 5% of the total tocopherols.  $\alpha$ -Tocopherol is present in sesame oil in trace amount only. Among the different tocopherol isomers,  $\gamma$ -tocopherol is a more potent antioxidant in oils (33), but it has lower Vitamin E value in biological systems than  $\alpha$ -tocopherol (34).

TABLE 7. Levels of Tocopherols in Sesame Oil.

Sesame Species	Color of Seed Coat	Tocopherol (mg/kg Oil)				Reference
		$\alpha$	$\gamma$	$\delta$	Total	
<i>Sesamum indicum</i> L.						
Japanese strains <sup>a</sup>	Black	5.2	468.5	12.2	485.9	26
	Brown	6.2	517.9	13.6	537.7	26
	White	3.8	497.8	20.5	522.1	26
Sudan strains <sup>b</sup>	Black	ND <sup>d</sup>	527.0	12.6	540	27
	Brown	4.8	663.7	11.6	680	27
	White	3.1	603.9	13.0	620	27
<i>Sesamum alatum</i> T. <sup>c</sup>	Brown	2.9	310.1	7.0	320	27
<i>Sesamum radiatum</i> S. and T. <sup>c</sup>	Black	6.5	800.3	3.2	810	27
<i>Sesamum angustifolium</i> E. <sup>c</sup>	Black	ND	754.7	5.3	760	27
Codex standard	—	ND–3.3	521–983	4–21	330–1010	24

<sup>a</sup>The cultivated species of sesame grown in Japan.

<sup>b</sup>The cultivated species of sesame grown in Sudan.

<sup>c</sup>The wild species of sesame grown in Sudan.

<sup>d</sup>ND: Not detected.

## 4.5. Protein

The protein content of sesame seed is approximately 25% with a range of 17~31% depending on the source of the seed. Sesame protein is low in lysine (3.1% protein), but it is rich in sulfur-containing amino acids methionine and cystine (6.1%), which are often the limiting amino acids in legumes. Comparing with the standard values recommended by FAO and WHO for children, sesame protein is borderline deficient in other essential amino acids such as valine, threonine, and isoleucine. Sesame seed protein, however, contains an adequate amount of tryptophan, which is limiting in many oilseed proteins. Because of its characteristic amino acid composition, sesame seed protein is regarded as an excellent protein source for supplementing many vegetable proteins such as soybean and peanut to increase their nutritional value.

The protein efficiency ratio (PER) of sesame seed protein is 1.86 (35). The PER value can be raised to 2.9 when sesame seed protein is supplemented with lysine (36). El-Adawy (37) added sesame products including sesame meal, sesame protein isolate, and protein concentrate to red wheat flour to produce flour blends. It was found that water absorption, development time, and dough weakening were increased as the protein level increased in all blends; however, dough stability decreased. Sesame products could be added to wheat flour up to 16% protein without any detrimental effect on bread sensory properties. The addition of sesame products to red wheat flour increased the contents of protein, minerals, and total essential amino acids; the *in vitro* protein digestibility also increased significantly.

Inyans and Nwadiimkpa (17) investigated the protein functionality of dehulled sesame seed flour. They reported that the emulsification capacity was higher at alkaline condition and ranged from 25-ml oil/g at pH 4 to 66-ml oil/g at pH 10. The highest foaming capacity (315%) was observed at pH 2. Protein solubility ranged from 7.9% at pH 2 to 14.2% at pH 10. The viscosity of the flour dispersion ranged from 2.5 cps at 1% concentration to 7.0 cps at 10% concentration. The sesame flour could impart desirable characteristics when incorporated into products such as ice cream, frozen dessert, sausage, baked food, and confectionary.

When sesame seeds were boiled or allowed to sprout, in order to reduce bitter taste, there was a slight increase in protein content of sprouted seeds and the foaming capacity of flour from boiled seeds was increased (38). The emulsion stability was improved after sprouting or boiling, whereas the emulsion capacity was lowered after boiling. The bitter taste was not detected in flour from boiled seed but still persisted in that from sprouted seed.

## 5. SESAME LIGNANS AND LIGNAN GLYCOSIDES

### 5.1. Lignans

Sesame oil contains high levels of unsaturated fatty acids (more than 80% of total fatty acids); however, it is highly resistant to oxidative deterioration as compared with other edible vegetable oils (39, 40). The superior oxidative stability is not



only attributed to the presence of tocopherols, but it is mainly associated with the unique group of compounds-lignans (41). Lignans are compounds formed by oxidative coupling of  $\rho$ -hydroxyphenylpropane. They are widely distributed in all parts of plants. Oilseeds such as sesame and flaxseed are well known to contain abundant lignans (42). Two types of lignan compounds existed in sesame seeds, the oil soluble lignans and the water soluble lignan glycosides. In raw sesame seed, sesamin and sesamol are the two major lignans. Sesamin has been found in other plants, whereas sesamol is characteristic of sesame and has not been found in plants other than *Sesamum*. Fukuda et al. (43) determined the lignan contents of 14 varieties of commercial sesame seeds grown in Japan and noticed that sesamin content was always higher than sesamol content and that the average ratio of sesamol to sesamin in the black varieties (0.6~1.0) was greater than the white varieties (0.2~0.5). Other types of lignans such as sesamol, P1, sesamolol, and sesamolol were only present in minor quantity as shown in Table 8 (43). The structures of the sesame lignans are illustrated in Figure 7.

Tashiro et al. (21) further investigated the oil and lignan (sesamin and sesamol) contents in 42 strains of *Sesamum indicum* L. originated from different parts of the world. The strains included white-, brown-, black-, and yellow-colored seed types. The results of this study indicated that the sesamin content in the oil ranging from 0.07% to 0.61% with an average of 0.36%, whereas the sesamol content was lower (ranging from 0.02% to 0.48% with an average of 0.27%). There was a significant positive correlation between the oil content of the seed and the sesamin content of the oil, whereas no correlation existed between the oil and the sesamol

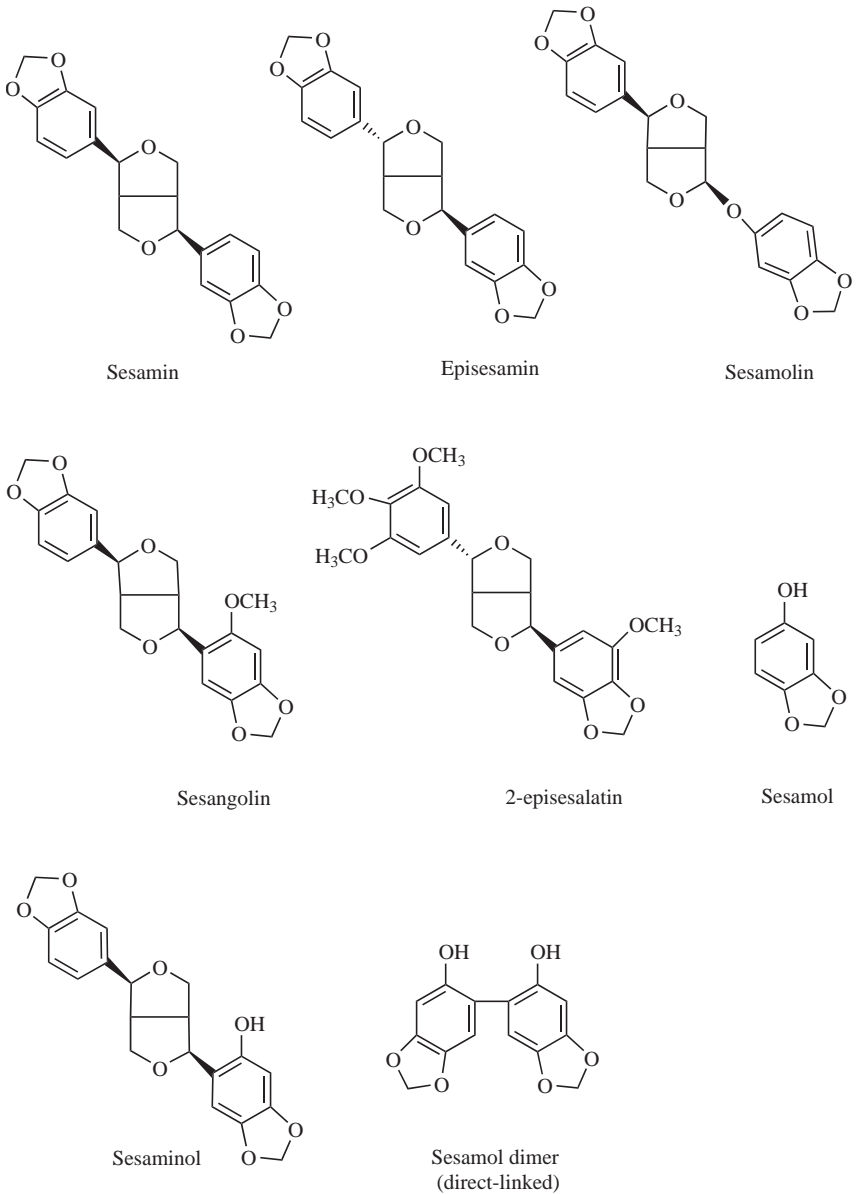
**TABLE 8. Lignan Contents in Different Strains of Sesame Seeds.<sup>a,b</sup>**

Strain no.	Color of Seed Coat	Sesamolol/ Sesamin						
		Sesamin	Sesamolol	Sesamin	Sesamol	P1	Sesamolol	Sesamolol
48	White	821.3	441.2	0.537	2.0	1.6	1.0	1.4
611	White	410.6	441.2	0.537	2.5	1.3	1.0	1.0
630	White	522.7	123.5	0.236	2.5	2.3	0.9	0.3
638	White	885.2	476.5	0.538	ND <sup>c</sup>	2.9	1.1	1.0
643	White	464.0	229.4	0.494	5.0	2.0	1.1	1.0
785	Yellow	453.3	247.0	0.545	Trace	2.0	0.9	0.3
673	Violet	464.0	317.6	0.684	2.5	1.8	1.5	1.1
675	Brown	528.0	264.6	0.501	Trace	3.8	0.6	0.7
126	Brown	682.7	458.8	0.672	4.0	2.9	1.2	1.0
201	Black	502.5	441.2	0.878	3.6	2.5	1.2	1.1
601	Black	314.3	235.3	0.749	10.8	1.6	1.9	1.1
631	Black	362.7	229.4	0.632	2.5	1.5	0.8	0.5
792	Black	154.7	152.9	0.988	4.9	1.5	0.9	0.9
801	Black	293.3	294.0	1.002	6.5	1.6	1.1	1.2
Mean		490.6	300.4		3.4	2.1	1.1	0.9
SD		198.6	113.6		2.9	0.7	0.3	0.3

<sup>a</sup>Data adapted from (43).

<sup>b</sup>Unit: mg/100-g oil.

<sup>c</sup>ND: Not detected.



**Figure 7.** Structures of lignans.

contents. It was also noticed that the black seed types contained significantly less oil and a high ratio of sesamolin to sesamin. In the wild species of sesame seeds, Fukuda et al. (43) found that an Indian variety had an extreme low sesamolin content (only 14% of its sesamin content), whereas one variety from Borneo contained

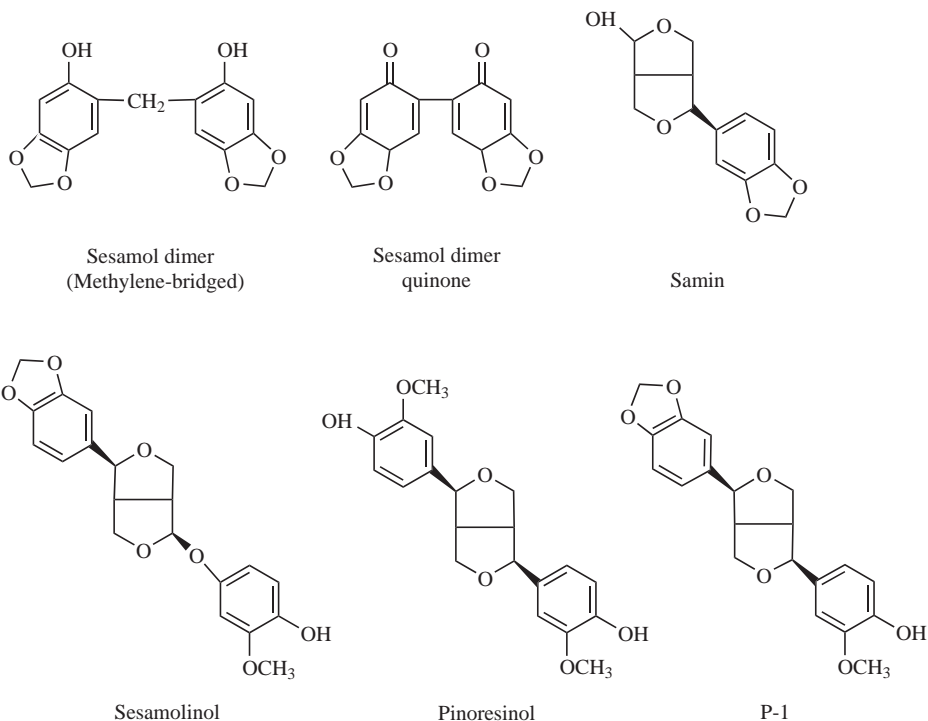


Figure 7. (Continued)

several times more sesamin (1152.3 mg/100 g oil) and sesamol (1360.7 mg/100 g oil) than in other species. Kamal-Eldin and Appelqvist (27) determined the contents of sesamin and sesamol in three wild species of *Sesamum*. They reported that *S. radiatum* was extremely high in sesamin (2.40% in oil) but contained only a minor amount of sesamol (0.02%), whereas *S. alatum* contained minor amounts of sesamin and sesamol (both were 0.01%); *S. angustifolium* possessed reasonable amounts of both sesamin (0.32%) and sesamol (0.16%).

Other types of lignans were found in wild species of *Sesamum*. Sesangolin was present in *S. angolense* (44) and was the major lignan in *S. angustifolium*, which contained 3.15% sesangolin in its oil (27). 2-Episesalatin occurred in *S. alatum* (45) and was its most abundant lignan present at 1.37% in its oil (27). The structures of sesangolin and 2-episesalatin are shown in Figure 7. The contents of different lignans present in sesame oil are listed in Table 9.

## 5.2. Lignan Glycosides

Lignan glycosides are the glycosylated forms of lignans; they are water soluble. Although most lignans are found in the oil-soluble part of sesame seed, lignan glycosides are present in sesame meal. Sesaminol, sesamolinol, and pinoresinol

TABLE 9. Levels of Lignans in Sesame Oil.

Sesame Species	Color of Seed Coat	Lignan Contents (% Oil)					Reference
		Sesamin	Sesamol	Sesamol	Sesangolin	2-Epsesalatin	
<i>Sesamum indicum</i> L.							
Eleven strains	Black	0.24 (0.07–0.40)	0.27 (0.13–0.40)	—	—	—	21
Twelve strains	Brown	0.36 (0.11–0.61)	0.30 (0.13–0.42)	—	—	—	21
Fifteen strains	White	0.44 (0.12–0.61)	0.25 (0.02–0.48)	—	—	—	21
Japanese strains	Black	0.45	0.54	ND <sup>a</sup>	—	—	26
Japanese strains	Brown	0.46	0.66	ND	—	—	26
Japanese strains	White	0.66	0.42	ND	—	—	26
Sudan strains	Black	0.45	0.54	—	ND	ND	27
Sudan strains	Brown	0.46	0.66	—	ND	ND	27
Sudan strains	White	0.23–0.72	0.39–0.41	—	ND	ND	27
<i>Sesamum alatum</i> T. <sup>b</sup>	Brown	0.01	0.01	—	ND	1.37	27
<i>Sesamum radiatum</i>							
S. and T. <sup>b</sup>	Black	2.4	0.02	—	ND	ND	27
<i>Sesamum angustifolium</i> E. <sup>b</sup>	Black	0.32	0.16	—	3.15	ND	27

<sup>a</sup>ND: Not detected.<sup>b</sup>The wild species of sesame grown in Sudan.

glucosides (Figure 8) are the major lignan glycosides in sesame. Acetone extract of sesame seed contained sesamolol and sesaminol (46, 47), and it was revealed that they were released after treating defatted sesame seed flour with  $\beta$ -glucosidase (48). Later, three pinoresinol diglucosides (KP1, KP2, and KP4) and one pinoresinol triglucoside (KP3) were isolated from the ethanol extract of sesame seed (49, 50). Kuriyama et al. (51) analyzed the lignan glycosides composition of white sesame seed with high-performance liquid chromatography (HPLC) and found eight lignan glycosides. There were two pinoresinol glucosides with two or three glucose units, three sesaminol glucosides with one to three glucose units, two sesamolol glucosides with one or two glucose units, and one P-1 glucoside with two glucose units. The total contents of lignan glycosides in white sesame seed were around 100–170-mg/100-g seed, with sesaminol triglucoside the most predominant one. In black sesame seed, the lignan glycosides content varied greatly with the species of the sesame (from 6.4 to 361.3-mg/100-g seed), whereas sesaminol triglucoside was still the major lignan glycoside (52). This effect of sesame variety on the lignan glycoside contents was also noticed by Ryu et al. (53). They reported that a significant difference existed between the black and white sesame seeds in their sesaminol contents, which were analyzed after hydrolysis of the sesaminol glucosides. White sesame seeds contained an average of 84.5-mg sesaminol in 100-g seed (ranging from 32.5 to 98.5 mg/100 g), and black sesame seeds contained 113.2 mg/100 g of sesaminol in average with a range of 41.5 to 134.5-mg/100-g seed. Table 10 lists the contents of sesaminol glucosides in various sesame seeds.

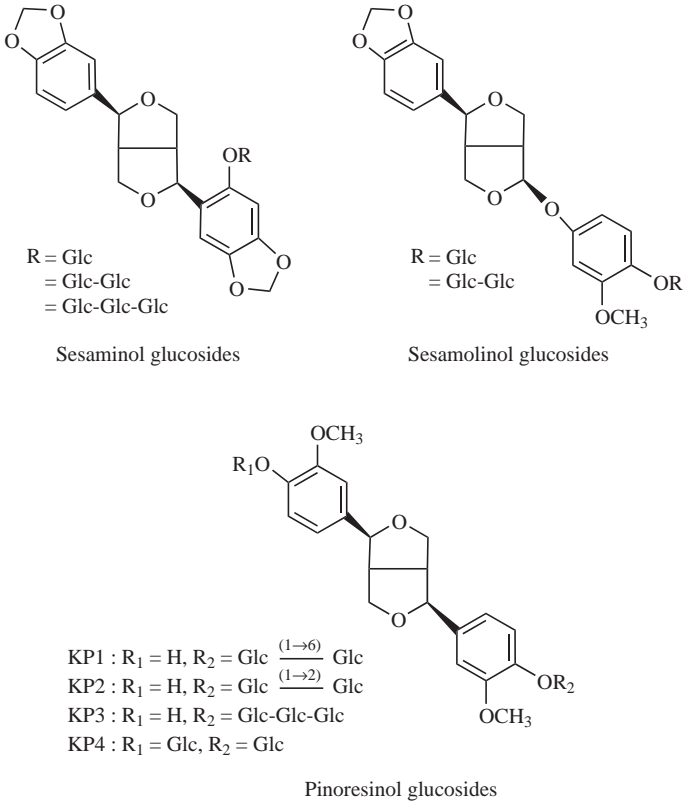


Figure 8. Structures of lignan glucosides.

TABLE 10. Contents of Sesaminol Glucosides in Different Sesame Seeds.<sup>a</sup>

Color of Seed Coat	Sesaminol Glucosides (mg/100g Seed)		
	Mean <sup>b</sup>	Range	CV (%) <sup>c</sup>
Black (n = 10) <sup>d</sup>	113.2**	41.5–134.5	23.5
Brown (n = 5)	78.5*	39.4–91.4	8.9
White (n = 10)	84.5*	32.5–98.5	11.8

<sup>a</sup>Data adapted from (53).

<sup>b</sup>Mean values bearing different superscripts are different significantly at 1% level.

<sup>c</sup>CV: coefficient of variance.

<sup>d</sup>n: represents the number of samples analyzed.

## 6. PROCESSING

Sesame oil has a long history of human consumption. The processing of sesame seed to yield sesame oil varies from region to region. The major differences are (1) whether the seed coat is removed and (2) whether the seed is roasted. Figure 9 shows the flow diagrams of the processing of three major types of sesame oils produced worldwide, namely (1) refined sesame oil, which is produced from unroasted sesame seed either with seed coat or without seed coat; (2) roasted sesame oil, which is produced from roasted sesame seed; and (3) small mill sesame oil, which is produced from roasted dehulled sesame seed.

Refined sesame oil is the salad oil grade of sesame oil. It is the most common type of sesame oil consumed worldwide except in the Orient. Sesame seeds are cleaned and cooked before oil extraction with expeller. Crude sesame oil is refined by alkali-refining, bleaching, and deodorization to obtain the refined sesame oil (Figure 9). Sesame cake from oil extraction with expeller may still contain 18~22% of residual oil (54). It is often extracted with solvent or pressed again to obtain more oil. The desolventized sesame cake can then be processed into food grade sesame flour if the dehulled sesame seed is used. If the seed coats are not removed, the sesame cake can only be used as feedstuff because it contains undesirable constituents. The dehulling process will be discussed later.

Roasted sesame oil has a strong characteristic flavor of roasted sesame seed. It is the most popular sesame oil consumed in China, Japan, and Korea. It is also believed to be beneficial to health (40). As shown in Figure 9, sesame seeds are roasted at 140~200°C prior to oil extraction. The conditions of the roasting process

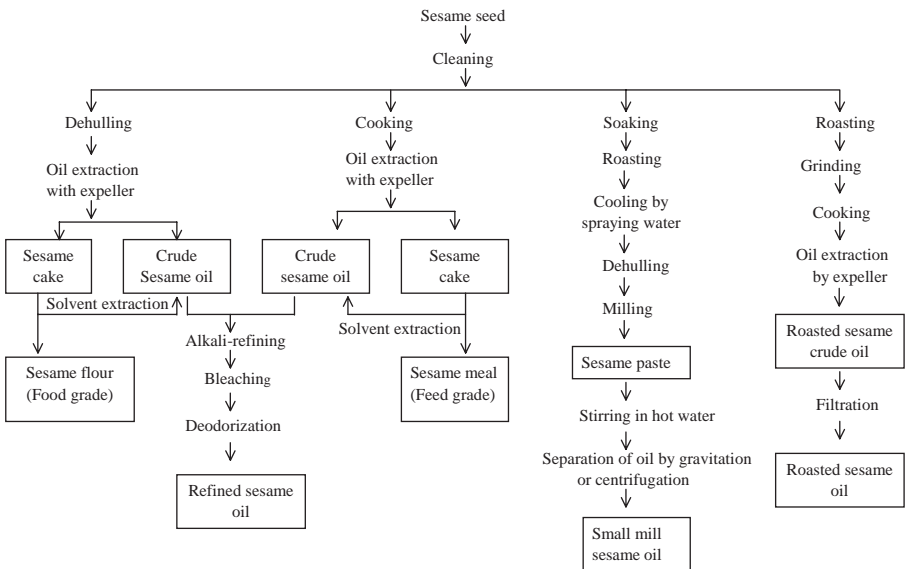


Figure 9. Flow diagram showing the processing of different sesame oils.

is of prime importance to the quality of the roasted sesame oil. The effect of roasting on sesame seed and oil will be discussed later. After roasting, sesame seeds are ground, cooked, and pressed to obtain the crude roasted sesame oil. The crude oil is simply filtered without further purification to produce roasted sesame oil. The color of roasted sesame oil ranges from light yellow to dark brown depending on the roasting conditions.

Small mill sesame oil, also known as Shiang-you, is a unique sesame oil product of Northern China. It has a light roasted sesame flavor and is light brownish in color. Shiang-you is often used as seasoning oil for cold dishes; it is seldom used for cooking purpose. Roasted sesame oil, however, is mainly used as cooking oil. The processing scheme of small mill sesame oil is shown in Figure 9. Sesame seeds are cleaned and soaked in water for about an hour in order for the sesame seed to reach a water content of 35%, which can facilitate protein denaturation, assure even heating, and avoid burning during the subsequent roasting process. Roasting process is recommended to conduct at 200°C for 30 min. The roasted sesame seeds are cooled to 140~150°C by spraying water. Before milling the roasted sesame seeds with stone mill, the seed coats are removed by blowing air or sieving through screen. The milling process is important for the separation of oil from sesame paste. Successful milling will result in sesame paste with fine particle size, which will give rise to a higher oil yield (55). After milling, hot water is added to the sesame paste and stirred slowly (around 30 rpm). The addition of hot water (temperature above 90°C) is usually conducted three to four times with decreasing amount of added water. Sesame oil will slowly rise to the top by gravitational force when the addition of water is completed and the paste is allowed to stand for 1 hour (56). The processing of Shiang-you is labor-intensive, and the oil yield (around 40%) is low. Many efforts were made to increase the yield of Shiang-you. Yen and Tsai (57) tried to include soybean oil in hot water to separate oil from sesame paste. They reported that the highest yield of Shiang-you was obtained with the combination ratio of sesame paste-soybean oil-boiling water (10:9:7, w/w).

## 6.1. Dehulling

Generally, sesame seeds are processed without removal of seed coat. Seed coat contains undesirable oxalic acid and indigestible fiber that may lower the nutritional value of the meal. The presence of seed coat will also impart a dark color and bitter taste to the meal. In India, where sesame meal is an important food, dehulling is an indispensable step of sesame oil processing. Sesame meal prepared with dehulled sesame seed is non-bitter, light-colored, low in fiber, and rich in protein. Dehulling is performed either manually at village level or mechanically in conventional oil mills in India (58, 59). Manual dehulling involved soaking sesame seeds in water and removal of hulls from the swell and burst seeds by light pounding or rubbing on stone or wooden block. It is tedious, labor intensive, and inefficient; therefore, it limits the production of sesame oil and its meal.

Mechanical dehulling can be processed either by soaking sesame seeds in water followed by removal of seed coat mechanically (58) or by alkali treatment (60–62).

In alkali treatment or lye peeling method, sesame seeds are treated with hot lye for a short time. Either hot 0.6% NaOH for 1 minute (60) or 6% NaOH at 60°C for 10 seconds (61) have been used to decorticate sesame seed. There was no appreciable loss in protein and oil contents after alkali treatment. Nag et al. (63) reported that dehulling not only increased oil content but also produced oil of better color quality compared with the whole seed. Sesame oil extracted from dehulled sesame seeds, however, was oxidatively less stable (measured by the Rancimat test) than that extracted from whole seeds (64). The presence of natural antioxidants such as  $\gamma$ -tocopherol, sesamin, and sesamol in the seed coat may contribute to the oxidative stability of whole sesame seed oil. In addition, Chang et al. (65) recently reported that the sesame seed coat also contained phenolic compounds and tetranortriterpenoids, which had good antioxidative activity. Dehulled sesame seeds are not suitable for roasting process either. Abou-Gharbia et al. (66) demonstrated that sesame oil prepared from coated seeds had better oxidative stability than from dehulled seeds either roasted at 200°C for 20 min or without roasting as evaluated by peroxide value, conjugated diene formation, and TBA value.

## 6.2. Roasting

In Oriental countries such as China, Japan, and Korea, sesame seeds are often roasted prior to oil extraction. Roasting is important for the development of desirable color and flavor for sesame oil, and it will enhance the oxidative stability of sesame oil (67). The conditions of roasting may influence the sensory quality and composition of the roasted sesame oil. When sesame seeds were roasted between 180°C and 260°C for 30 min, Yen (68) reported that the red color unit of the roasted sesame oil increased with temperature up to 220°C and then decreased while the flavor score showed an optimum at 200°C. There was almost no change in fatty acid composition until the roasting temperature was above 220°C (68, 69). The antioxidant, sesamol, content also increased with roasting temperature up to 220°C and then decreased with higher roasting temperature. The roasting temperature of 200°C was therefore recommended (68, 70).

Yoshida and Takagi (69) compared the quality of sesame oils prepared at roasting temperatures between 160°C and 250°C. They found that the typical dark-brown color was apparent after 15 min, and the roasted sesame seeds had a burnt smell when the roasting temperature was above 220°C. Roasted sesame oil obtained from seeds roasted at temperature above 220°C had burnt and bitter tastes; the peroxide, anisidine, carbonyl, and TBARS values were also higher indicating poor oil quality. They suggested that a high-quality roasted sesame oil would be obtained by roasting for 25 min at 160°C and 180°C, 15 min at 200°C, and 5 min at 220°C.

**6.2.1. Effect of Roasting on Antioxidative Activity** Roasted sesame oil was reported to be much more antioxidative than unroasted purified sesame oil (71). Yen and Shyu (67) found that roasted sesame oils prepared from sesame seeds with different roasting temperatures (between 180°C and 210°C) exhibited



differences in their oxidative stability. The oxidative stability appeared to increase with roasting temperature; sesame oil prepared from 200°C roasted seed was found to exhibit the best stability.

Koizumi et al. (72) also noticed the relationship between the roasting conditions of sesame seeds and the development of antioxidative activity. The antioxidative activity of oil obtained from sesame seed roasted at 200°C for as short as 5 min was higher than at 180°C for 30 min. This observation indicated that the development of antioxidant activity in roasted sesame oil depends primarily on temperature. In an attempt to investigate the contributing antioxidants in roasted sesame oil, Fukuda et al. (73) reported that either sesamol alone or  $\gamma$ -tocopherol alone at the concentrations present in roasted sesame oil showed weak antioxidant activity. Even the combination of both was not enough to explain the strong antioxidative activity of roasted sesame oil. As roasting of sesame seed caused significant browning (74), the browning products from roasted sesame seed were isolated and found to show weak antioxidative activity (73). The combination of  $\gamma$ -tocopherol, sesame lignans (sesamol and sesamin), and the browning products was shown to be responsible for the superior oxidative stability of roasted sesame oil (73).

**6.2.2. Effect of Roasting on Different Classes of Lipids** Roasting of sesame seed not only affects the antioxidative activity and the lignans of sesame oil, the lipid composition will also be affected. The lipids in sesame seeds consist of neutral lipids, phospholipids, and glycolipids. The major lipid fraction is neutral lipids, which constitute about 91~96% of the total lipids. Phospholipids and glycolipids represent around 3% and 0.3~6% of the total lipids, respectively (69, 70, 75-78).

Roasting will cause a significant reduction in the phospholipids content in sesame seed because of browning reaction (75). Phospholipids fraction in sesame seeds decreased with roasting temperature and time (69, 70, 75-77). There was no appreciable change in phospholipids content when sesame seeds were roasted at 160-180°C for 10 min (69); the reduction in phospholipids content was 69~73% at 220°C for 25 min (70, 77) and 96% at 250°C for 25 min (69). Even with microwave roasting, phospholipids in sesame seed decreased appreciably; more than half of the original phospholipids were lost after microwaving at 2450 MHz for 15 min (76), and less than 14% were left after 30 min (78). The highest rate of phospholipid loss was observed in the phosphatidyl ethanolamine (PE) fraction followed by the phosphatidyl choline (PC) and phosphatidyl inositol (PI) fractions. This trend became more pronounced with longer roasting time and higher roasting temperature. After roasting at 220°C for 25 min, PE was completely destroyed while there were still 22% of PC and 42% of PI left in the roasted sesame seeds (77). The amino group of PE or PC was suggested to be involved in browning reaction and donation of hydrogen or electron to tocopherol or sesamol (79).

Glycolipids content of sesame seed, on the other hand, increased with roasting temperature and time (75, 78). When sesame seeds were roasted in an electric oven from 120°C to 250°C for 30 min, the glycolipids content was found to increase from 6.9-mg/1000-g seeds (0.5% of total lipids) to 262.9-mg/1000-g seeds (17.2%

of total lipids) as reported by Yoshida (75). Glycolipid components of microwaved sesame seeds increased slowly in the first 25 min of heating and rapidly thereafter; a ninefold increase in glycolipids content after 30 min of microwaving at 2450 MHz was observed (78). The color of sesame seeds become brownish after heating, indicating that browning reaction has taken place. The browning substances are generally very polar, and the increase in browning substances may be attributed to the increase of glycolipids (78).

The dominant component of sesame lipids, neutral lipids, did not change in its content when sesame seeds were roasted at temperature below 200°C for 30 min. As the roasting temperature increased to 220°C and 250°C, a significant decrease in the neutral lipids content was noticed (75). This decrease became more severe when the roasting time was increased (69, 70, 77). Yoshida et al. (70) examined the effect of roasting on the molecular species of triacylglycerols. They reported that roasting for 10 min at 220°C caused a significant decrease not only in molecular species containing more than four double bonds, but also in the amount of diene and triene species present in triacylylycerols. They also confirmed that no significant changes in molecular species or fatty acid distribution of triacylglycerols would occur within 25 min of roasting at 180°C.

### 6.3. Extraction of Oil

The tradition way of extracting sesame oil from unroasted sesame seeds in India is done by ghani, which is basically a large pestle and mortar. The ghani is driven by bullocks (79). Sesame seeds are cleaned and dehulled before used in the ghani. In many parts of India, water or brown sugar is added to sesame seed in the ghani to facilitate oil extraction (80). Sesame oil is removed from the ghani after milling and allowed to settle, skimmed, and sometimes strained through a cloth before sale. The bullock-driven ghani is replaced by power-driven mills in most of the Indian villages in order to improve the efficiency of oil production (58, 80).

The modern commercial methods of oil extraction from oilseeds include (1) batch hydraulic pressing: Oil seeds are expressed by hydraulic pressure to yield oil; (2) continuous mechanical pressing: Oil seeds are squeezed through a tapering outlet and oil is expressed by the increasing pressure; and (3) solvent extraction: Oil seeds are extracted with solvent followed by removal of solvent to yield oil. These methods are also employed in the extraction of sesame seeds with some modification.

For unroasted sesame seeds, the commercial extraction of oil is carried out using a continuous screw-press or hydraulic press. Sesame seeds are small; they are usually cooked prior to oil extraction. Sesame oil is generally extracted in three stages (60, 79). The first stage is cold press; the cold-pressed oil obtained after filtration is ready to use and has very good quality. It is light in color and agreeable in taste and odor. The second stage pressing is conducted with sesame residue under high pressure; it yields a highly colored oil that needs refining before used for edible purpose. The residue left after the second stage pressing is extracted for the third time under similar conditions as the second stage. Sesame oil obtained

from the third stage pressing has very low quality and is used for nonedible purposes.

Alternatively, unroasted sesame seeds are pressed once followed by solvent extraction to recover the oil from residue. The oxidative stability of sesame oil was found to be dependent on the extraction method and seed pretreatment (64). Extraction of the sesame seeds after effective seed crushing with polar solvent, heptane-isopropanol (3:1, v/v), would yield a more stable oil from whole sesame seeds because more antioxidative substances and phospholipids could be extracted. Phospholipids may act as synergists to antioxidants (81).

The extraction of sesame oil from roasted sesame seed is generally performed with pressing. Solvent extraction is not used because the desirable roasted flavor may be removed during evaporation of solvent. In commercial production, continuous screw-press or hydraulic press is employed (42). The hydraulic press can be vertical or horizontal. The continuous screw may be operated twice in order to increase the oil yield (82). Proper cooking (100°C, 7 min) and addition of water (12.5%) after roasting can also raise the oil yield (83).

#### 6.4. Refining

Sesame oil from roasted sesame seed has the characteristic flavor and color of the roasted sesame oil; the filtered crude oil is used without further refining. Sesame oil from cold-pressed unroasted sesame seed is also used directly after filtration as a flavored oil. Crude sesame oil from unroasted sesame seeds after screw-press or hydraulic press or solvent extraction, which varies in color from yellow to dark amber, may need further refining. Refined sesame oil is usually pale yellow in color.

Crude sesame oil does not require extensive purification and refining. The suspended meal particles in crude oil can be removed by settling, screening, and filtering. The filtered crude oil can be used directly or be further refined to remove impurities such as phospholipids, resins, free fatty acids, and coloring substances. The refining steps include removal of free fatty acids, gums, and some water-miscible substances by alkaline treatment, removal of pigments by bleaching, and removal of odorous substances by deodorization. Degumming is not necessary because sesame oil contains a limited amount (<3%) of phospholipids (69).

Alkali-refining of sesame oil can use sodium carbonate as the neutralizing agent in order to reduce the refining loss, because sodium carbonate does not attack the neutral triacylglycerols. The free fatty acids are first neutralized by sodium carbonate, and then a weak sodium hydroxide (NaOH) wash is given to improve color. Liberation of carbon dioxide, which makes the separation of soapstock difficult, has limited the practice of using sodium carbonate in alkali-refining. Mukhopadhyay et al. (84) reported an easy way of refining sesame oil with alkali-enriched dry sodium metasilicate (SMS). This method precluded emulsion formation, and thus the separation of soapstock could be easily achieved. It is superior to the sodium carbonate process because no liberation of carbon dioxide is involved. The reduction in free fatty acids by this dry refining process was dependent on the

alkalinity of SMS-NaOH mixture. Color of the oil was not markedly improved by this process. The process appears to be useful for the refining of crude oils with low free fatty acids and medium color. Therefore, it is specially useful for the alkali-refining of sesame oil.

After alkali-refining, the neutralized sesame oil is bleached with a relatively lower quantity of bleaching earth as compared with that required for most other vegetable oils. Bleaching conditions and the bleaching agent employed may influence the bleaching efficiency. Increase in bleaching temperature was found to increase bleaching efficiency until a maximum was reached and then decreased (85). Agitation speed also affect the result of bleaching; 40 rpm (86) or 50~100 rpm (85) was found to be the optimal condition. The higher the ratio of adsorbent/edible oil, the higher is the bleaching ability of adsorbent (87). Recently, activated rice hull ash was investigated as the bleaching agent of sesame oil (88, 89). Rice hull ashed at 500°C for 30 min followed by activation with 6N H<sub>2</sub>SO<sub>4</sub> at 30°C for 60 min was found to possess the maximum bleaching efficiency (88). Using this acid-activated rice hull ash as bleaching agent, sesame oil could be successfully bleached at 120°C with an agitation speed of 80 rpm employing 25 mg of rice hull ash per gram of sesame oil (89).

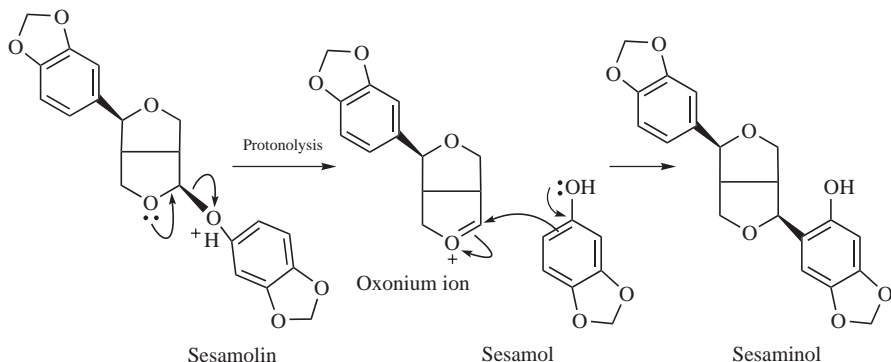
Bleaching removes most pigments, and the bleached oil is light in color. In order to produce a bland oil suitable for salad dressing, the bleached sesame oil is further deodorized. Deodorization is conducted in vacuum with steam at 200~250°C as most other vegetable oils.

## 6.5. Changes of Lignans During Processing

The two major lignans, sesamin and sesamol, present in sesame seed are reported to be responsible for many unique chemical and physiological properties of sesame seed oil (39). Sesamin and sesamol, however, do not have antioxidative activity in themselves because of a lack of phenolic groups (90). The high oxidative stability of sesame oil came mainly from the transformation products of these sesame lignans. Sesamol, which possesses antioxidative activity and is usually present in trace amount in the oil of raw sesame seed, may be released from sesamol during the roasting process of sesame seed prior to pressing for oil (46). Sesamol could also be formed from the hydrolysis of sesamol after heating at frying temperature for 1–2 hours (47). During sesame oil refining, the antioxidative sesaminol was formed in high concentration from sesamol under the acidic anhydrous condition of bleaching (acid clay are used for bleaching) as reported by Fukuda et al. (41).

Sesamol is first decomposed to sesamol by protonolysis to form an oxonium ion, and then the carbon-carbon bond is formed; thus, it is hypothesized that sesaminol is formed from sesamol by intermolecular group transformation (91). The conversion of sesamol to sesamol and sesaminol is illustrated in Figure 10. Both sesamol and sesaminol are strong antioxidants; they contribute to the superior oxidative stability of refined or roasted sesame oil.

Sesamol is unstable to heat and is completely destroyed when the roasted sesame oil is heated at the deep frying temperature of 180°C for 4 hours. Sesaminol,



**Figure 10.** Conversion of sesamol to sesamol and sesaminol.

however, is more heat stable. The retention of sesaminol in the roasted sesame oil after heating at 180°C for 6 hours was 40.5% (52).

The other sesame lignan, sesamin, will undergo epimerization upon heating with acid (92). A marked change in the sesamin content of sesame oil was observed after bleaching and deodorization. The decrease in sesamin was accompanied by the formation of epi-sesamin. The deodorization process of oil refining will also destroy the thermally liable lignan sesamol. Sesamol produced from sesamol during the bleaching step was lost in the next deodorization step (93). Only trace amounts (<20 ppm) of sesamol were found in the commercially deodorized sesame oil (41).

The changes in the contents of sesame lignans during industrial refining of unroasted sesame oil, which included alkaline treatment, warm water washing, bleaching with acid clay, and deodorization, are listed in Table 11. These data clearly revealed that the significant changes in the sesame lignans contents occurred at the bleaching step. There were epimerization of sesamin (41%), disappearance of sesamol, and formation of sesamol, sesaminol, epi-sesaminol, and a minor amount of sesamol dimer. It was also evident that the contents of

**TABLE 11.** Contents of Sesame Lignans and Tocopherol in Unroasted Sesame Oil During Industrial Refining Process (mg/100-g Oil).<sup>a</sup>

Refining Stage	Sesamin	Epi-Sesamin	Sesamol	Sesamol	Sesaminol	$\gamma$ -Tocopherol
				(Sesamol Dimer)	(Epi-Sesaminol)	
Crude sesame oil	813.3	0	510.0	4.3 (0)	0 (0)	33.5
Alkaline-refining	730.6	0	458.0	2.5 (0)	0 (0)	23.4
Warm water washing	677.8	0	424.8	0.7 (0)	0 (0)	22.6
Bleaching	375.5	277.6	0	46.3 (trace)	33.9 (48.0)	21.8
Deodorizing	258.3	192.6	0	1.7 (trace)	28.4 (34.4)	18.4

<sup>a</sup>Data adapted from (41).

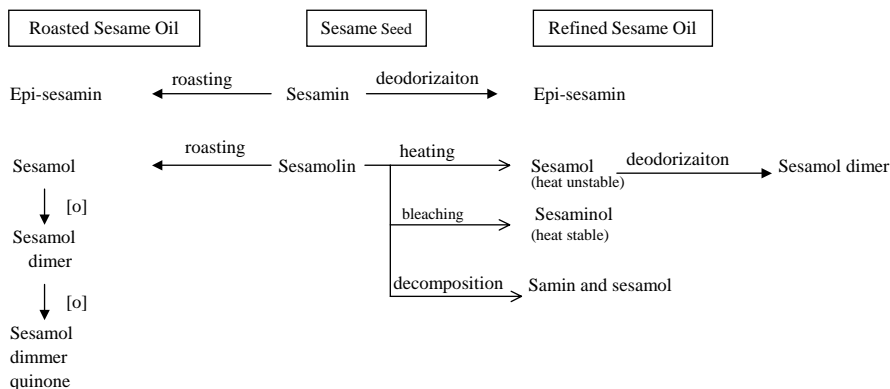
**TABLE 12. Effect of Processing Method on the Retention of Sesamin and Sesamolins in Sesame Oil (mg/100-g Oil).<sup>a</sup>**

Processing Method	Sesamin				Sesamolins			
	Coated Seed		Dehulled Seed		Coated Seed		Dehulled Seed	
	Fresh	Stored <sup>b</sup>	Fresh	Stored <sup>b</sup>	Fresh	Stored <sup>b</sup>	Fresh	Stored <sup>b</sup>
Raw seed	649 ± 20 <sup>d,x</sup>	584 ± 15 <sup>d,y</sup>	610 ± 21 <sup>d,x</sup>	461 ± 16 <sup>d,y</sup>	183 ± 7 <sup>d,x</sup>	123 ± 6 <sup>d,y</sup>	168 ± 5 <sup>d,x</sup>	117 ± 5 <sup>d,y</sup>
Roasting	576 ± 14 <sup>e,x</sup>	436 ± 10 <sup>g,y</sup>	489 ± 15 <sup>f,x</sup>	315 ± 14 <sup>f,y</sup>	146 ± 5 <sup>e,x</sup>	73 ± 2 <sup>f,y</sup>	119 ± 3 <sup>f,x</sup>	55 ± 1 <sup>g,y</sup>
Steaming	601 ± 18 <sup>e,x</sup>	514 ± 14 <sup>e,y</sup>	531 ± 16 <sup>e,f,x</sup>	325 ± 13 <sup>f,y</sup>	129 ± 5 <sup>f,x</sup>	88 ± 4 <sup>e,y</sup>	108 ± 4 <sup>g,x</sup>	52 ± 1 <sup>g,y</sup>
Roasting plus steaming	583 ± 15 <sup>e,x</sup>	506 ± 13 <sup>e,f,y</sup>	555 ± 18 <sup>e,x</sup>	411 ± 15 <sup>e,y</sup>	146 ± 6 <sup>e,x</sup>	115 ± 7 <sup>d,y</sup>	139 ± 4 <sup>e,x</sup>	106 ± 3 <sup>e,y</sup>
Microwaving	590 ± 17 <sup>e,x</sup>	475 ± 12 <sup>f,y</sup>	520 ± 12 <sup>e,f,x</sup>	422 ± 16 <sup>e,y</sup>	123 ± 3 <sup>f,x</sup>	71 ± 3 <sup>f,y</sup>	129 ± 2 <sup>e,f,x</sup>	75 ± 2 <sup>f,y</sup>

<sup>a</sup>Data adapted from (94).

<sup>b</sup>The extracted oil was stored at 65°C for 35 days.

<sup>c</sup>Results are mean values of three determinations ± SD. Values in each column with different superscripts (d-g) are significantly ( $p < 0.05$ ) different from one another. Values of fresh and stored oil with different superscripts (x and y) are significantly ( $p < 0.05$ ) different from each other.



**Figure 11.** Changes of sesame lignan during processing.

sesaminol and its epimer did not decrease by deodorization as much as sesamol. In refined unroasted sesame oil, sesaminol, epi-sesaminol, and  $\gamma$ -tocopherol are thus the antioxidative substances responsible for its excellent oxidative stability (41).

Shahidi et al. (94) investigated the effect of different processing methods, including roasting (200°C for 20 min), steaming (100°C for 20 min), roasting (200°C for 15 min) plus steaming (100°C for 7 min), and microwaving (2450 MHz for 15 min) on the endogenous antioxidants in the resultant sesame oil and upon storage. Sesamin content in oil was well retained (nearly 90%) in oil from coated seed immediately after processing, but the decrease was more pronounced (nearly 50%) in oil from dehulled seed especially after the oil was stored (65°C for 35 days). The roasting process resulted in the highest loss of sesamin. The corresponding changes in sesamolin contents were more drastic than sesamin (Table 12).

The changes of sesame lignans during processing is summarized in Figure 11.

Recently, Asakura et al. (95) have prepared the ortho methylene-bridged and direct-link oligomers from sesamol. The structures are shown in Figure 7. The methylene-bridged oligomers showed much stronger antioxidant activities on the autoxidation of lard than the sesamol monomer because of a greater average number of hydroxyl groups per sesamol unit. The direct-linked oligomers prepared in acidic conditions were better antioxidants for lard than the sesamol monomer, whereas oligomers prepared under neutral and alkaline conditions did not improve the antioxidant effect of sesamol.

## 7. NUTRITIONAL CHARACTERISTICS

### 7.1. Effect on Polyunsaturated Fatty Acid Metabolism

Linoleic acid and  $\alpha$ -linolenic acid are essential fatty acids and are the important fatty acids involved in the metabolic pathway of prostaglandin synthesis.

Converting linoleic acid to  $\gamma$ -linolenic acid and dihomo- $\gamma$ -linolenic acid (DGLA) is catalyzed by  $\Delta^6$ -desaturase, whereas  $\Delta^5$ -desaturase catalyzes the transformation of DGLA to arachidonic acid. Shimizu et al. (96) reported that sesame oil could cause an accumulation of DGLA acid in the cell. Sesamin was discovered to be the active component in sesame oil; it can inhibit the activity of  $\Delta^5$ -desaturase (97). When rats were fed sesamin, there was an accumulation of DGLA in liver phospholipids and the ratio of DGLA to arachidonic acid increased. Arachidonic acid is the precursor of eicosanoids such as 2 series' prostaglandin and 4 series' leukotriene. Consequently, sesamin tended to reduce the production of eicosanoids from arachidonic acid (98), and the plasma concentration of PGE<sub>2</sub> was decreased (99). Fujiyama-Fujiwara et al. (100) also reported that sesame lignans (sesamin and episesamin) inhibited  $\Delta^5$  desaturation from DGLA (n-6) to arachidonic acid (n-6), but not from 20:4(n-3) to eicosapentaenoic acid (EPA, n-3) in cultured rat hepatocytes, and Umeda-Sawada et al. (101) confirmed this finding in vivo and also found that dietary sesame lignans decreased arachidonic acid content and increased n-6/n-3 ratio. Umeda-Sawada et al. (102) further examined the effect of dietary sesame lignans on hepatic metabolism and n-6/n-3 ratio of essential fatty acids in rats; they concluded that sesame lignans could inhibit extreme changes of n-6/n-3 ratio and function to bring it close to the appropriate n-6/n-3 ratio. Epidemiological and clinical studies have shown that the plasma n-6/n-3 ratio is associated with the prevalence of thrombosis (99, 103). Therefore, sesame lignans would be beneficial to the prevention of thrombosis.

## 7.2. Hypocholesterolemic Effect of Sesame Lignans

Sesame oil was reported to lower the absorption of fatty acid and cholesterol in lymph by 50% when rats were fed diet containing 24% sesame oil as compared with control diet containing no sesame oil (104). As the lymphatic system is the major route for the transport of absorbed fatty acids and cholesterol, serum and liver cholesterol levels were significantly reduced, especially LDL-cholesterol (105). Crude lignan fraction separated from sesame oil was found to have a weak but significant hypocholesterolemic activity (98). The cholesterol-lowering activity depended on the dietary level of the lignans. With purified sesame lignan (sesamin), the hypocholesterolemic effect was clearly demonstrated (106). As shown in Table 13, sesamin (0.5%) significantly reduced the serum cholesterol in rats fed a cholesterol-enriched diet (Exp. I) or a commercial chow diet (Exp. II). Sesamin lowered intestinal absorption of cholesterol by precipitating cholesterol from the bile acid micelles, and thus the serum cholesterol level is reduced. Table 13 also shows that liver cholesterol concentration was also significantly lowered when rats were fed a sesamin-containing diet because of the reduction in the activity of liver microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), the key enzyme in the cholesterol synthesis pathway of liver. Sesamin thus possess a unique function in that it can simultaneously inhibit cholesterol synthesis and absorption. It is, therefore, a potential hypocholesterolemic agent of natural origin.



**TABLE 13. Effect of Sesamin on the Concentrations of Serum Cholesterol, Liver Cholesterol, and the Liver Enzyme Activity.**<sup>1,2</sup>

Diet	Serum Cholesterol (mg/dl)	Liver Cholesterol (mg/g liver)	Liver HMG-CoA Reductase (pmol/min/mg protein)
Exp. I			
Purified diet	108 ± 4 <sup>a</sup>	2.54 ± 0.13 <sup>a</sup>	203 ± 12 <sup>a</sup>
Diet + sesamin (0.5%)	110 ± 5 <sup>a</sup>	1.95 ± 0.06 <sup>b</sup>	151 ± 11 <sup>b</sup>
Diet + cholesterol (0.5%)	136 ± 8 <sup>b</sup>	20.8 ± 2.2 <sup>c</sup>	51.6 ± 2.0 <sup>c</sup>
Diet + cholesterol (0.5%) and sesamin (0.5%)	102 ± 5 <sup>a</sup>	9.13 ± 1.02 <sup>d</sup>	29.0 ± 2.4 <sup>d</sup>
Exp. II			
Commercial chow	69.1 ± 5.2 <sup>a</sup>	2.86 ± 0.19 <sup>a</sup>	269 ± 27 <sup>a</sup>
Chow + sesamin(0.5%)	55.5 ± 3.0 <sup>b</sup>	1.82 ± 0.04 <sup>b</sup>	172 ± 13 <sup>b</sup>

<sup>1</sup>Data adapted from (106).<sup>2</sup>Values are means ± SEM (n = 6 ~ 8). Values with different letters are significantly different in each experiment (*p* < 0.05). Male Wistar rats were fed experimental diets for 4 weeks.

The hypocholesterolemic effect of sesamin could be enhanced by  $\alpha$ -tocopherol (107). Data shown in Table 14 clearly indicated that rats fed sesamin together with tocopherol (1%), the serum cholesterol-lowering effect of sesamin, could be demonstrated at a much lower level (0.05%). This synergistic effect was found to be related to both the levels of sesamin and cholesterol in the diet. The combination of  $\alpha$ -tocopherol with sesamin has a practical value for the treatment of hypercholesterolemia. The cholesterol-lowering effect of sesamin has also been demonstrated in humans with dietary supplementation of sesamin at 64.8-mg/day level (108).

With regard to the mechanism underlying the hypocholesterolemic effect of dietary sesamin, Hirose et al. (109) demonstrated in rats that it increased fecal

**TABLE 14. Combined Effects of Sesamin and  $\alpha$ -Tocopherol on Serum Cholesterol Levels of Rats.**<sup>1,2</sup>

Group	Serum Cholesterol (mg/dl)
Cholesterol diet	490 ± 94 <sup>a</sup>
Diet + 1.0% tocopherol	460 ± 70 <sup>a</sup>
Diet + 0.05% sesamin	437 ± 76 <sup>a</sup>
Diet + 0.05% sesamin + 1.0% tocopherol	244 ± 23 <sup>b,c</sup>
Diet + 0.2% sesamin	371 ± 28 <sup>a,c</sup>
Diet + 0.2% sesamin + 0.2% tocopherol	243 ± 5 <sup>c</sup>
Diet + 0.2% sesamin + 1.0% tocopherol	149 ± 9 <sup>c</sup>

<sup>1</sup>Data adapted from (107).<sup>2</sup>Values are means ± SE (n = 6 ~ 9). Values with different letters are significantly different in each experiment (*p* < 0.05). Male Wistar rats were fed experimental diets for 4 weeks.

cholesterol excretion and reduced the hepatic activity of HMG-CoA reductase. In addition, Ashakumary et al. (110) examined the effect of sesame lignan (a 1:1 mixture of sesamin and episesamin) on hepatic fatty acid oxidation in rats. They concluded that sesame lignan greatly increased the activity and gene expression of hepatic fatty acid oxidation enzymes and thus increased the rate of fatty acid  $\beta$ -oxidation through the activation of peroxisome proliferator activated receptor (PPAR) $\alpha$ . Sesame lignan was also demonstrated to decrease the hepatic fatty acid synthesis in rats by decreasing the activity and gene expression of many hepatic enzymes involved in fatty acid synthesis (111) because sesame lignan contains both sesamin and episesamin. The effect of each component was examined by Kushiro et al. (112). They found that episesamin caused a larger magnitude of increase in the activity and gene expression of enzymes in fatty acid oxidation than sesamin. Sesamin and episesamin showed no difference, however, in lowering the activity and gene expression of hepatic lipogenic enzymes.

### 7.3. Effect on Vitamin E

Sesame seed has long been regarded as a health food for longevity. Namiki et al. (113–115) examined the effect of sesame seed in aging by using a senescence-accelerated mouse, and they have found that the advancement of senescence was suppressed by long-term feeding of sesame seed. Vitamin E is recognized as a food component that may exert an anti-aging effect (116). Sesame seed, however, contains mainly  $\gamma$ -tocopherol whose Vitamin E activity is only 6–16% that of  $\alpha$ -tocopherol (117, 118), although it exhibits a stronger antioxidative activity in vitro than  $\alpha$ -tocopherol (119, 120). The effects of sesame seed and sesame lignans on Vitamin E activity were, therefore, studied extensively to elucidate if sesame is a good source of Vitamin E.

Yamashita et al. (121) first reported that sesame seed and its lignans could raise the bioactivity of  $\gamma$ -tocopherol to almost the same level as  $\alpha$ -tocopherol in rats. Later, they reported that sesame seed lignans could also act synergistically with  $\alpha$ -tocopherol to enhance its Vitamin E activity in rats fed a low  $\alpha$ -tocopherol diet (122). Kamal-Eldin et al. (123) showed that feeding rats with sesamin, a lignan from sesame oil, increased  $\gamma$ -tocopherol and  $\gamma$ -/ $\alpha$ -tocopherol ratio in the plasma, liver, and lung. Sesamin appears to enhance the bioavailability of  $\gamma$ -tocopherol in rat plasma and tissues, and this effect persists in the presence of  $\alpha$ -tocopherol. Dietary sesame seed can also elevate the tocotrienol concentration in the adipose tissue and skin of rats fed tocotrienol-rich diet (124). The effect of sesame lignans on the levels of tocopherols was also demonstrated in humans. In a study with 40 healthy Swedish women (mean age 26), serum  $\gamma$ -tocopherol concentrations were raised significantly after consuming a diet that contained 22.5 g/day of sesame oil (125). Coonery et al. (126) gave muffins containing equivalent amounts of  $\gamma$ -tocopherol from sesame seeds, walnuts, or soy oil to nine volunteers; they observed that consumption of as little as 5 mg of  $\gamma$ -tocopherol per day over a 3-day period from sesame seeds but not from walnuts nor soy oil significantly elevated serum  $\gamma$ -tocopherol levels in the volunteers.

#### 7.4. Effect on Blood Pressure

Sesamin, the most abundant lignan present in sesame seed and sesame oil, was demonstrated to suppress the development of hypertension in rats induced by deoxycorticosterone acetate (DOCA) and salt (127). Dietary sesamin was also reported to effectively prevent the elevation of blood pressure and cardiac hypertrophy in two-kidney, one-clip (2k, 1c) renal hypertensive rats (128). In the stroke-prone spontaneously hypertensive rats (SHRSP), sesamin feeding was much more effective as an anti-hypertensive regimen in salt-loaded SHRSP (with 1% salt in drinking water) than in unloaded SHRSP (129).

#### 7.5. Antioxidative Effect in Biological System

In the development of atherosclerosis, oxidative modification of low-density lipoprotein (LDL) is the critical step and is therefore a target for interventions aimed at slowing down the progression of atherogenesis (130). Antioxidants such as Vitamin E, probucol, and N,N'-diphenylphenylenediamine (DPPD) were suggested to prevent the oxidation of LDL (131–133). Sesame oil is highly resistant to oxidative deterioration because of the presence of endogenous antioxidants such as sesaminol, sesamol, pinorelinol, and P1. Sesaminol exerted a strong inhibitory effect on the 2,2'-azobis (2,4-dimethylvaleronitrile) (AMVN)-induced peroxidation of LDL by acting as a chain breaker in the lipid peroxidation cascade *in vitro* (134). In inhibiting either Cu<sup>2+</sup>-induced or 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced lipid peroxidation in LDL, sesaminol was found to be more effective than  $\alpha$ -tocopherol and probucol. Sesaminol was also the strongest antioxidant among the sesame lignans (sesamol, pinorelinol, and P1) for protecting LDL from oxidative modification (135). The reason for the strong antioxidative effect of sesaminol is possibly because of its highly lipophilic nature that makes it act within the LDL particle to exert a sparing effect on tocopherol (122, 123).

The *in vivo* antioxidative activity of sesame lignan was examined in an animal model (136). When SD rats were fed a diet containing 1% sesamol, the lipid peroxidation activity (measured as 2-thiobarbituric acid reactive substances, TBARS) in the liver and kidney was significantly lowered. The amount of 8-hydroxy-2'-deoxyguanosine, a DNA base-modified product generated by reactive oxygen species and a good marker for oxidative damage (137), was also significantly lower in the sesamol-fed rats. Sesamol is one of the major sesame lignans present in the oil fraction of sesame; however, it does not possess any appreciable *in vitro* antioxidant activity (138). The significant *in vivo* antioxidative activity of sesamol came from its metabolites, sesamol and sesamolol, when sesamol was supplemented in rats diet (136). Feeding rats with a diet containing 40% of dietary energy as either sesame, soybean, olive, or canola oils for 7 weeks, sesame oil was shown to be the most effective one in lowering lipid peroxidation (139). Sesame seeds rich in sesame lignans, sesamin and sesamol, could lower the activities of enzymes involved in fatty acid synthesis, and thus the serum triacylglycerol levels were lower in rats fed diets high in sesame lignans (140).

Sesame seeds contain two types of lignans, the oil-soluble lignans such as sesamin and sesamol and the water-soluble lignan glycosides including pinoresinol glucosides (141) and sesaminol glucosides (142). Both of the glucosides were lower in peroxy radical scavenging activity than their corresponding aglycones because of the lack of phenolic group. Using hypercholesterolemic rabbit as the animal model, Kang et al. (143) were able to demonstrate that dietary defatted sesame flour (containing 1% sesaminol glucoside) could decrease the peroxidation in liver and serum. Sesaminol, the principal metabolite of sesaminol glucoside and the active antioxidant, was found in abundant quantities in the serum and liver of rabbit (143). In an insulin-resistance animal model, rats were fed with high fructose diet in order to develop insulin-resistance, which was accompanied by a high oxidative stress status (144). When the insulin-resistant rats were given 1.0 g/kgBW of crude lignan glycosides, liver TBARS were significantly lowered and the insulin sensitivity was improved, indicating an alleviation of oxidative stress (145).

## 7.6. Effect on Cancer

Antioxidants are well recognized to play an important role in the defense against oxidative stress, which may cause damage to membrane, nucleic acid, and protein resulting in circulatory ailments, senility, mutation, and cancer (146). As sesame lignans possess antioxidative ability, their effect on the model systems for in vivo peroxidation, such as the peroxidation of ghost membranes of rabbit erythrocyte and the peroxidation of rat liver microsome, were investigated (147). Sesame lignans were found to suppress lipid peroxidation equal to or stronger than tocopherol in these systems. One of the sesame lignan, sesaminol, was observed to be as strongly suppressive as tocopherol in mutagenicity of *E. Coli* WP2s induced by peroxidation of membrane lipid of erythrocytes (147).

As mentioned earlier that sesame lignans, especially sesamin and epi-sesamin, could influence the metabolism of polyunsaturated fatty acid and the production of prostaglandins. As prostaglandin is one of the most influential factors for mammary carcinogenesis, Hirose et al. (99) studied the effect of sesamin on dimethylbenzanthracene (DMBA)-induced mammary cancer. Their results showed that sesamin at a dietary level of 0.2% considerably reduced the cumulative number and mean number of mammary cancer; the effectiveness of sesamin was similar to  $\alpha$ -tocopherol.

The anti-tumor promotion activity of topically and orally administered sesame components was tested in ICR mice using a two-stage skin tumorigenesis model (148). Skin tumor was initiated with 7,12-dimethylbenz [a]-anthracene (DMBA) and promoted with 12-*o*-tetra-decanoylphorbol-13-acetate (TPA). The sesame components applied topically after TPA treatment were able to delay the formation of papilloma remarkably. It was suggested that sesame components had radical scavenging ability toward the reactive oxygen species or peroxidized molecules generated by TPA. Therefore, the inhibition of tumorigenesis by sesame components was the result of metabolic inactivation. When sesame components were administered orally, the formation of skin papilloma was also inhibited effectively,

indicating that the sesame components could be absorbed and remained active even after passing through digestive organs (149).

Sesamin, however, did not significantly reduce the number of N-nitrosobis-(2-oxopropyl)-amine(DOP)-induced pancreatic cancer in hamsters (150). It was noticed that 2% sesamol in the diet exerts forestomach carcinogenic activity in rats and mice (151). Fortunately, human beings do not have a forestomach and daily ingestion of sesamol is much lower than 2%.

### 7.7. Effect on Liver Function

Sesamin fed to rats at a level above 0.5% caused a temporary liver enlargement because of an increase in liver phospholipids; no specific histological changes were observed, and the activities of serum GOT and GPT remained unchanged (99, 106). It was suggested that sesamin could act as a stimulus to the liver function, particularly in the endoplasmic reticula. When mice were exposed to a high concentration of carbon tetrachloride or continuously inhaled ethanol to cause liver damage, sesamin was able to improve the liver function (152). Furthermore, rats previously given sesamin were found to reduce their plasma ethanol levels more rapidly than the control rats. This effect of sesamin on alcohol metabolism was studied in human trials. Male adults given sesamin (100 mg/day for 7 days) were found to have a significantly faster rate of ethanol reduction in their blood (153).

The effect of dietary sesamin and sesaminol on the ethanol-induced modulation of immune indices related to food allergy has also been studied. Although chronic ethanol drinking would increase the plasma IgA, IgM, and IgG concentrations, 0.2% sesamin in the diet could suppress this increase of IgA and IgM, whereas sesaminol was not effective. In addition, the increase in relative liver weight because of ethanol consumption was alleviated by dietary supplementation of sesamin but not by sesaminol (154).

### REFERENCE

1. D. Bedigian and J. R. Harlan, *Econ Bot.*, **40**, 137 (1986).
2. N. M. Nayar, in N. W. Simmonds, ed., *Sesame: Evolution of Crop Plants*, Longman, London and New York, 1976, pp. 231–233.
3. T. Kobayashi, in A. Ashri, ed., *Sesame: Status and Improvement Proceedings of Expert Consultation, The Type Classification of Cultivated Sesame Based on Genetic Characters*, FAO Plant Production and Protection Paper 29 Rome, 1981, pp. 86–89.
4. N. M. Nayar and K. L. Mehra. *Econ. Bot.*, **24**, 20 (1970).
5. A. Ashri, in G. Roebblen, R. K. Downey, and A. Ashri, eds., *Oil Crops of the World*, McGraw Hill, New York, 1989, p. 375.
6. J. J. Ochse, J. Soule, M. J. Dijkman, and C. Wehlburg, *Tropical and Subtropical Agriculture Vol. II*, Macmillan, New York, 1961, pp. 1089–1093.

7. J. W. Purseglove, *Tropical Crops: Dicotyledons*, Longman, Harlow, Essex, 1982, pp. 430–435.
8. D. G. Langham, *J. Hered.*, **36**, 245 (1945).
9. D. G. Langham, *J. Hered.*, **37**, 149 (1946).
10. D. G. Langham, *J. Hered.*, **38**, 347 (1957).
11. T. W. Culp, *J. Hered.*, **51**, 146 (1960).
12. D. R. Langham and T. Wiemers, in J. Janick and A. Whipkey, eds., *Trends in New Crops and New Used*, ASHS Press, Alexandria, VA, 2002, p. 157.
13. Food and Agriculture Organization of the United Nations (FAO). (2003). Available: <http://faostat.fao.org/faostat/collections?subset=agriculture>.
14. D. H. Siraj, *Palm Oil Link*, **12**, 1–5 (2002).
15. D. A. Dashak and C. N. Fali, *Food Chem.*, **47**, 253 (1993).
16. G.-C. Yen, S.-L. Shyu, and J.-S. Lin, *J. Agric. Forest. (Taiwan)*, **35**, 177 (1986).
17. U. E. Inyang and C. U. Nwadiimkpa, *J. Amer. Oil Chem. Soc.*, **69**, 819 (1992).
18. J. E. Kinsella and R. R. Mohite, in A. M. Altsechull and H. L. Wilcke, eds., *New Protein Foods*, Academic Press, New York, 1985, 435–456.
19. L. A. Johnson, T. M. Suleiman, and E. W. Lusas, *J. Amer. Oil Chem. Soc.*, **56**, 463 (1979).
20. A. Kamal-Eldin and L.-Å. Appelqvist, *J. Amer. Oil Chem. Soc.*, **71**, 135 (1994).
21. T. Tashiro, Y. Fukuda, T. Osawa, and M. Namiki, *J. Amer. Oil Chem. Soc.*, **67**, 508 (1990).
22. H. Baydar, I. Turgut, and K. Turgut, *Tr. J. Agric. Forest.*, **23**, 431 (1999).
23. H. Baydar and I. Turgut, *Tr. J. Agric. Forest.*, **18**, 387 (1994).
24. Codex Alimentarius, *Named Vegetable Oils 8*, Codex Standard 210, (2001).
25. R. T. O'Connor and S. F. Herb, *J. Amer. Oil Chem. Soc.*, **47**, 186A (1970).
26. H. Yoshida, J. Shigezaki, S. Takagi, and G. Kajimoto, *J. Sci. Food Agric.*, **68**, 407 (1995).
27. A. Kamal-Eldin and L.-Å. Appelqvist, *J. Amer. Oil Chem. Soc.*, **71**, 149 (1994).
28. R. J. Sims, J. A. Fioriti, and M. J. Kanuk, *J. Amer. Oil Chem. Soc.*, **49**, 298 (1972).
29. T. A. Miettinen, T. Puska, H. Glyying, H. Vanhanen, and E. Vartianen, *N. Engl. J. Med.*, **333**, 1308 (1995).
30. A. B. Awad, Y.-C. Chen, C. S. Fink, and T. Hennessey, *Anticancer Res.*, **16**, 2797 (1996).
31. R. L. Von Holtz, C. S. Fink, and A. B. Awad, *Nutr. Cancer*, **32**, 8 (1998).
32. A. Downie, C. S. Fink, and A. B. Awad, *FASEB*, **13**, A433 (1999).
33. C. H. Lea and R. J. Ward, *J. Sci. Food Agric.*, **10**, 537 (1959).
34. G. W. Burton and M. G. Traber, *Ann. Rev. Nutr.*, **10**, 375 (1990).
35. M. S. Narasinga Rao in H. V. Srivastava, S. Bhaskaran, B. Vatsya, and K. K. G. Menon, eds., *Oilseeds Production: Constraints and Opportunities*, Oxford and IBH, New Delhi, India, 1985, pp. 625–634.
36. M.C.S. Sastry, N. Subramanian, and H.A.B. Parpia, *J. Amer. Oil Chem. Soc.*, **51**, 115 (1974).
37. T. A. El-Adawy, *Food Chem.*, **59**, 7 (1997).
38. G. I. O. Badifu and E. M. Akpagher, *Plant Foods for Human Nutrition*, **49**, 119 (1996).

39. P. Budowski and K. S. Markely, *Chem. Rev.*, **48**, 125 (1951).
40. M. Namiki, *Food Rev. Int.*, **11**, 281 (1995).
41. Y. Fukuda, M. Nagata, T. Osawa, and M. Namiki, *J. Amer. Oil Chem. Soc.*, **63**, 1027 (1986).
42. P. K. J. P. D. Wanasundara, F. Shahidi, and V. K. S. Shukla, *Food Rev. Int.*, **13**, 225 (1997).
43. Y. Fukuda, T. Osawa, S. Kawakishi, and M. Namiki, *Nippon Shokuhin Kogyo Gakkaishi*, **35**, 483 (1988).
44. W. A. Jones and M. Beroza, *J. Org. Chem.*, **27**, 3232 (1962).
45. A. Kamal-Eldin and G. Yousif, *Phytochemistry*, **31**, 2911 (1992).
46. T. Osawa, M. Nagata, M. Namiki, and Y. Fukuda, *Agric. Biol. Chem.*, **49**, 3351 (1985).
47. M. Nagata, T. Osawa, M. Namiki, and Y. Fukuda, *Agric. Biol. Chem.*, **51**, 1285 (1987).
48. Y. Fukuda, T. Osawa, M. Namiki, and T. Ozaki, *Agric. Biol. Chem.*, **49**, 301 (1985).
49. H. Katsuzaki, M. Kawasumi, S. Kawakishi, and T. Osawa, *Biosci. Biotech. Biochem.*, **56**, 2087 (1992).
50. H. Katsuzaki, S. Kawakishi, and T. Osawa, *Phytochemistry*, **35**, 773 (1994).
51. K.-I. Kuriyama, K.-Y. Tsuchiya, and T. Murui, *Nippon Nōgeikagaku Kaishi*, **67**, 1693 (1993).
52. T. Osawa, H. Kumon, M. Namiki, S. Kawakishi, and Y. Fukuda, in M. W. Pariza, H. U. Aeschbacher, J. S. Felton, and S. Sato, eds., *Mutagens and Carcinogens in the Diet*, Wiley-Liss, New York, 1990, p. 233.
53. S. N. Ryu, C.-T. Ho, and T. Osawa, *J. Food Lipids*, **5**, 17 (1998).
54. G.-C. Yen, S.-L. Shyu and T.-C. Lin, *Food Sci. (Chinese)*, **13**, 198 (1986).
55. G.-C. Yen, and S.-L. Shyu, *J. Chinese Agric. Chem. Soc.*, **26**, 50 (1988).
56. A. J. Shi, *Food Res. Develop. (China)*, **22**, 43 (2001).
57. G.-C. Yen, and Y.-E. Tsai, *Food Sci.*, **17**, 87 (1990).
58. Oil Technological Research Institute, *Profiles in Oil Technology*, OTRI, Anantpur, India, 1977, p. 157.
59. B. D. Green, in E. W. Lusas, D. R. Erickson, and W. Nip, eds., *Food Uses of Whole Oil and Protein Seeds*, Amer. Oil Chem. Soc., Champaign, Illinois, 1989, p. 218.
60. B. S. Ramachandra, M. C. S. Sastry, and L. S. Subbarao, *J. Food Sci. Technol. (Mysore)*, **7**, 127 (1970).
61. Y. G. Moharram, A. M. Moustafa, and H.O.A. Osman, *Lebensm. Wiss. Technol.*, **14**, 137 (1981).
62. G. M. Yehai, O. A. Hussein, and I. A. Y. Abou-El-Khier, *Food Nut. Bull.*, **121**, 1 (1985).
63. D. K. Nag, D. Pandit, M. Sen, D. K. Bhattacharya, M. M. Chakraborty, and S. Mukherjee, *Sci. Culture*, **65**, 163 (1999).
64. A. Kamal-Eldin and L.-Å. Appelqvist, *J. Amer. Oil Chem. Soc.*, **72**, 967 (1995).
65. L.-W. Chang, W.-J. Yen, S. C. Huang, and P.-D. Duh, *Food Chem.*, **78**, 347 (2002).
66. H. A. Abou-Gharbia, F. Shahidi, A. A. Y. Shehata, and M. M. Youssef, *J. Amer. Oil Chem. Soc.*, **74**, 215 (1997).
67. G.-C. Yen and S.-L. Shyu, *Food Chem.*, **31**, 215 (1989).
68. G.-C. Yen, *J. Sci. Food Agric.*, **50**, 563 (1990).

69. H. Yoshida and S. Takagi, *J. Sci. Food Agric.*, **75**, 19 (1997).
70. H. Yoshida, Y. Hirakawa, and S. Takagi, *J. Sci. Food Agric.*, **80**, 1495 (2000).
71. Y. Fukuda, M. Nagata, T. Osawa, and M. Namiki, *Agric. Biol. Chem.*, **50**, 857 (1986).
72. Y. Koizumi, Y. Fukuda, and M. Namiki, *Nippon Shokuhin Kagaku Kogaku Kaishi*, **43**, 689 (1996).
73. Y. Fukuda, Y. Koizumi, R. Ito, and M. Namiki, *Nippon Shokuhin Kagaku Kogaku Kaishi*, **43**, 1272 (1996).
74. G.-C. Yen and S.-L. Shyu, *Food Sci.*, **19**, 25 (1992).
75. H. Yoshida, *J. Sci. Food Agric.*, **65**, 331 (1994).
76. H. A. Abou-Gharbia, A. A. Y. Shehata, and F. Shahidi, *Food Res. Int.*, **33**, 331 (2000).
77. H. Yoshida, S. Abe, Y. Hirakawa, and S. Takagi, *J. Sci. Food Agric.*, **81**, 620 (2001).
78. H. Yoshida and G. Kajimoto, *J. Food Sci.*, **59**, 613 (1994).
79. D. K. Salunkhe, J. K. Chavan, R. N. Adsule, and S. S. Kadam, *World Oilseeds: Chemistry, Technology and Utilization*, Van Nostrand Reinhold, New York, pp. 371–402 (1992).
80. H. G. Muralidhara, in *A Panorama of the World of Oils*, National Education Society, Shimoga, India, 1981, pp. 56–57.
81. P. Brandt, E. Hollstein, and C. Franzke, *Lebensm. Ind.*, **20**, 31 (1973).
82. G.-P. Dong, *China Oil Fat*, **18**, 55 (1993).
83. S. Y. Tsai and Y. H. Chu, *Report of FIRDI (Taiwan)*, **21** (1991).
84. S. B. Mukhopadhyay, P. K. Gupta, and A. K. Basu, *J. Amer. Oil Chem. Soc.*, **69**, 294 (1992).
85. L. L. Richardson, *J. Amer. Oil Chem. Soc.*, **55**, 777 (1978).
86. H. B. W. Patterson, *J. Amer. Oil Chem. Soc.*, **53**, 339 (1976).
87. K. Y. Liew, A. H. Yee, and M. R. Nordin, *J. Amer. Oil Chem. Soc.*, **70**, 539 (1993).
88. Y.-Y. Chang and C.-I. Lin, *J. Chem. Eng. Jpn.*, **34**, 1 (2001).
89. S.-P. Lin, C.-I. Lin, and H.-K. Chen, *J. Chem. Eng. Jpn.*, **34**, 737 (2001).
90. P. Budowski, F. G. T. Menezes, and F. G. Doller, *J. Amer. Oil Chem. Soc.*, **27**, 377 (1950).
91. Y. Fukuda, M. Isobe, M. Nagata, T. Osawa, and M. Namiki, *Heterocycles*, **24**, 923 (1986).
92. M. Beroza, *J. Amer. Oil Chem. Soc.*, **78**, 5082 (1956).
93. P. Budowski, *J. Amer. Oil Chem. Soc.*, **41**, 280 (1964).
94. F. Shahidi, R. Amarowicz, H. A. Abou-Gharbia, and A. Adel Y. Shehata, *J. Amer. Oil Chem. Soc.*, **74**, 143 (1997).
95. K. Asakura, E. Honda, S. Matsumura, S. Osanai, and K. Kawada, *J. Oleo. Sci.*, **51**, 97 (2002).
96. S. Shimizu, K. Akimoto, H. Kawashima, Y. Shinmen, and H. Yamada, *J. Amer. Oil Chem. Soc.*, **66**, 237 (1989).
97. S. Shimizu, K. Akimoto, Y. Shinmen, H. Kawashima, M. Sugano, and H. Yamada, *Lipids*, **29**, 512 (1991).
98. M. Sugano, T. Inoue, K. Koba, K. Yoshida, N. Hirose, Y. Shinmen, K. Akimoto, and T. Amachi, *Agric. Biol. Chem.*, **54**, 2669 (1990).



99. N. Hirose, F. Doi, T. Ueki, K. Akazawa, K. Chijiwa, M. Sugano, K. Akimoto, S. Shimizu, and H. Yamada, *Anticancer Res.*, **12**, 1259 (1992).
100. Y. Fujiyama-Fujiwara, R. Umeda-Sawada, and O. Igarashi, *J. Nutr. Sci. Vitaminol.*, **38**, 353 (1992).
101. R. Umeda-Sawada, N. Takahashi, and O. Igarashi, *Biosci. Biotech. Biochem.*, **59**, 2268 (1995).
102. R. Umeda-Sawada, M. Ogawa, and O. Igarashi, *Lipids*, **33**, 567 (1998).
103. H. O. Bang, *Lancet*, **1**, 1143 (1971).
104. S. Satchithanandam, M. Reicks, R. J. Calvert, M. M. Cassidy, and D. Kritchevsky, *J. Nutr.*, **123**, 1852 (1993).
105. S. Satchithanandam, R. Chanderbhan, A. T. Kharroubi, R. J. Calvert, D. Klurfeld, S. A. Tepper, and D. Kritchevsky, *Int. J. Vit. Nutr. Res.*, **66**, 386 (1995).
106. N. Hirose, T. Inoue, K. Nishihara, M. Sugano, K. Akimotok, S. Shimizu, and H. Yamada, *J. Lipid Res.*, **32**, 629 (1992).
107. A. Nakabayashi, Y. Kitagawa, Y. Suwa, K. Akimoto, S. Asami, S. Shimizu, N. Hirose, M. Sugano, and H. Yamada, *Int. J. Vit. Nutr. Res.*, **65**, 162 (1995).
108. F. Hirata, K. Fujita, Y. Ishikura, K. Hosoda, T. Ishikawa, and H. Nakamura, *Atherosclerosis*, **122**, 135 (1996).
109. N. Hirose, T. Inoue, K. Nishihara, M. Sugano, K. Akimoto, S. Shimizu, and H. Yamada, *J. Lipid Res.*, **32**, 629 (1991).
110. L. Ashakumary, I. Rouyer, Y. Takahashi, T. Ide, N. Fukuda, T. Aoyama, T. Hashimoto, M. Mizugaki, and M. Sugano, *Metabolism*, **48**, 1303 (1999).
111. T. Ide, L. Ashakumary, Y. Takahashi, M. Kushiro, N. Fukuda, and M. Sugano, *Biochimica Biophysica Acta*, **1534**, 1 (2001).
112. M. Kushiro, T. Masaoka, S. Hageshita, Y. Takahashi, T. Ide, and M. Sugano, *J. Nutr. Biochem.*, **13**, 289 (2002).
113. M. Namiki, K. Yamashita, and T. Osawa, in K. Yagi, ed., *Active Oxygens, Lipid Peroxides, and Antioxidants*, Japan Scientific Society Press, Tokyo/CRC Press, Boca Raton, Florida, 1993, p. 319.
114. K. Yamashita, Y. Kawagoe, Y. Nohara, M. Namiki, T. Osawa, and S. Kawagishi, *J. Jpn. Soc. Nutr. Food Sci.*, **43**, 445 (1990).
115. K. Yamashita and M. Namiki, in T. Takeda, ed., *The SAM Model of Senescence*, Excerpta Medica Amsterdam, London-New York-Tokyo, 1994, p. 153.
116. B. Loo, R. Labugger, C. P. Aebischer, J. N. Skepper, M. Bachschmid, V. Spizer, J. Kilo, L. Altwegg, V. Ullrich, and T. F. Lüscher, *Circulation*, **105**, 1635 (2002).
117. I. R. Peake and J. G. Bieri, *J. Nutr.*, **101**, 1615 (1971).
118. J. G. Bieri and R. P. Evarts, *J. Nutr.*, **104**, 850 (1974).
119. G. G. Duthie, B. M. Gonzalez, P. C. Morrice, and J. R. Arthur, *Free Radic. Res. Commun.*, **15**, 35 (1991).
120. T. Saldeen, D. Li, and J. L. Mehta, *J. Amer. Coll. Cardiol.*, **34**, 1208 (1999).
121. K. Yamashita, Y. Nohara, K. Katayama, and M. Namiki, *J. Nutr.*, **122**, 2440 (1992).
122. K. Yamashita, Y. Iizuka, T. Imai, and M. Namiki, *Lipids*, **30**, 1019 (1995).
123. A. Kamal-Eldin, D. Pettersson, and L.-Å. Appelqvist, *Lipids*, **30**, 499 (1995).
124. S. Ikeda, K. Toyoshima, and K. Yamashita, *J. Nutr.*, **131**, 2892 (2001).

125. M. Lemcke-Norojärvi, A. Kamal-Eldin, L.-Å. Appelqvist, L. H. Dimberg, M. Öhrvall, and B. Vessby, *J. Nutr.*, **131**, 1195 (2001).
126. R. V. Cooney, L. J. Custer, L. Okinaka, and A. A. Franke, *Nutr. Cancer*, **39**, 66 (2001).
127. Y. Matsumura, S. Kita, S. Morimoto, K. Akimoto, M. Furuya, N. Oka, and T. Tanaka, *Biol. Pharm. Bull.*, **18**, 1016 (1995).
128. S. Kita, Y. Matsumura, S. Morimoto, K. Akimoto, M. Furuya, N. Oka, and T. Tanaka, *Biol. Pharm. Bull.*, **18**, 1283 (1995).
129. Y. Matsumura, S. Kita, Y. Tanida, Y. Taguchi, S. Morimoto, K. Akimoto, and T. Tanaka, *Biol. Pharm. Bull.*, **21**, 469 (1998).
130. D. Steinberg, *J. Biol. Chem.*, **272**, 20,963 (1997).
131. R. M. Dieber, H. Puhl, G. Waeg, G. Striegl, and H. Esterbauer, *J. Lipid Res.*, **32**, 1325 (1991).
132. J. Regnstrom, G. Walldius, L. A. Carlson, and J. Nilsson, *Atherosclerosis*, **82**, 43 (1990).
133. C. P. Sparrow, T. W. Doebber, J. Olszewski, M. S. Wu, J. Ventre, K. A. Stevens, and Y. S. Chao, *J. Clin. Invest.*, **89**, 1885 (1992).
134. M.-H. Kang, H. Katsuzaki, and T. Osawa, *Lipid*, **33**, 1031 (1998).
135. M.-H. Kang, M. Naito, K. Sakai, K. Uchida, and T. Osawa, *Life Sci.*, **66**, 161 (2000).
136. M.-H. Kang, M. Naito, N. Tsujihara, and T. Osawa, *J. Nutr.*, **128**, 1018 (1998).
137. B. Halliwell and O. I. Aruoma, *DNA and Free Radicals*, Ellis Horwood, Chichester, U.K., 1993.
138. P. Budowski, *J. Amer. Oil Chem. Soc.*, **27**, 264 (1950).
139. N. H. Baba, Z. Ghossoub, and Z. Habbal, *Nutr. Res.*, **20**, 1113 (2000).
140. S. Sirato-Yasumoto, M. Katsuta, Y. Okuyama, Y. Takahashi, and T. Ide, *J. Agric. Food Chem.*, **49**, 2647 (2001).
141. H. Katsuzaki, M. Kawasumi, S. Kawakishi, and T. Osawa, *Biosci. Biotechnol. Biochem.*, **56**, 2087 (1992).
142. H. Katsuzaki, S. Kawakishi, and T. Osawa, *Phytochemistry*, **35**, 773 (1994).
143. M.-H. Kang, Y. Kawai, M. Naito, and T. Osawa, *J. Nutr.*, **129**, 1885 (1999).
144. F. S. Facchini, M. H. Humphreys, C. A. DoNascimento, F. Abbasi, and G. M. Reaven, *Am. J. Clin. Nutr.*, **72**, 776 (2000).
145. S.-F. Kang, M. S. Thesis, Grad. Inst. of Food Sci. and Tech., National Taiwan University, Taipei, Taiwan, 2001, p. 72.
146. B. Halliwell, R. Aeschbach, J. Löliger, and O. I. Aruoma, *Food Chem. Toxicol.*, **33**, 601 (1995).
147. T. Osawa, M. Namiki, and S. Kawakishi, in Y. Kuroda, D. M. Shankel, and M. D. Waters, eds., *Antimutagenesis and Anticarcinogenesis Mechanisms II*, Plenum, New York, 1990, p. 139.
148. A. Mimura, K. Takebayashi, M. Niwano, H. Tokuda, H. Nishino, A. Iwashima, and Y. Takahara, *Proc. Jpn. Cancer Assoc.*, **57** (1991).
149. A. Mimura, K. Takebayashi, M. Niwano, Y. Takahara, T. Osawa, and H. Tokuda, in C.-T. Ho, T. Osawa, M.-T. Huang, and R.-T. Rosen, eds., *Food Phytochemicals in Cancer Prevention II*, American Chemical Society, Series 547, American Chemical Society, Washington, D.C., 1994, p. 281.
150. T. Ogawa, T. Makino, N. Hirose, and M. Sugano, *Carcinogenesis*, **13**, 1663 (1994).

151. S. Tamano, M. Hirose, H. Tanaka, E. Asakawa, K. Ogawa, and N. Ito, *Jpn. J. Cancer Res.*, **83**, 1279 (1992).
152. K. Akimoto, Y. Kitagawa, T. Akamatsu, N. Hirose, M. Sugano, S. Shimizu, and H. Yamada, *Ann. Nutr. Metab.*, **37**, 218 (1993).
153. M. Nakamura, H. Nagai, M. Nakagawa, and M. Sugano, 45th Annual Meeting of the Japanese Society of Nutrition and Food, 1991, p. 168.
154. M. Nonaka, K. Yamashita, Y. Iizuka, M. Namiki, and M. Sugano, *Biosci. Biotech. Biochem.*, **61**, 836 (1997).

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## *Soybean Oil*

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### **1. INTRODUCTION**

The amounts of soybeans and total vegetable oil crops have been rising for a number of years. World production of soybeans in 2003 was estimated to be 184.49 million MT out of 317.89 million MT total for vegetable oil crops, making soybeans the world's largest oilseed crop, rivaled only by palm oil (1). The 2003 crop of soybeans was expected to yield 29.85 million MT of soybean oil out of a total of 91.79 million MT of vegetable oil worldwide. The U.S. production of soybean oil was estimated at 8.59 million MT for 2002, of which 7.86 million MT was consumed domestically. During 2002–2003, Brazil produced 4.90 million MT and Argentina 4.12 million MT of soybean oil (2). The U.S. price of crude soybean oil has varied from \$0.24/kg to \$0.62/kg over the past 5 years with the lower prices being more recent (1).

Soybeans owe their dominance of the oilseed market to the value of their protein, which is much greater than that of other oilseeds. Of the oilseed meals produced in 2003, 129.58 million MT out of a total of 185.69 million MT was soybean meal (1). Of the money made on extracting soybeans, the meal accounted for between 51% and 76% of the total in the last 10 years. Soybean oil of typical composition performs well as a salad oil, but it is usually hydrogenated for use as a margarine stock or frying oil. Soybean oil's stability to oxidation also is limited by its content

of linolenic acid. Recent decades have witnessed numerous attempts to manipulate the fatty acid composition of soybean oil to help it compete better in various uses, but the cost of growing, segregating, and testing special varieties and resistance to genetically modified oils have limited the appeal of these altered varieties.

## 2. COMPOSITION OF SOYBEANS

Table 1 shows the average composition of soybean seed (oil, protein, and some amino acids) grown in the United States during recent years (3). Aside from varietal differences, the composition is affected by various geographic/environmental factors. According to Hurburgh (5), "oil is much more variable than protein from year-to-year. States most distant from the center of the Corn Belt (probably those with the greatest weather extremes) experience the most variability in composition." Table 2 lists some of the environmental and cultivation practices that have an

**TABLE 1. Typical Composition (wt%  $\pm$  std. dev.) of Soybeans (dry weight basis) (3).**

Protein	40.69 $\pm$ 0.51
Lysine	2.56 $\pm$ 0.11
Methionine	0.57 $\pm$ 0.03
Cysteine	0.72 $\pm$ 0.06
Tryptophane	0.52 $\pm$ 0.05
Threonine	1.54 $\pm$ 0.07
Oil	21.38 $\pm$ 0.64
Ash	4.56 $\pm$ 0.34 (4)
Carbohydrate	29.4 $\pm$ 3.29 (4)

**TABLE 2. Soybean Protein and Oil Responses to Various Environmental and Cultivation Practices (3).**

Variable	Protein	Oil
High temperatures	? <sup>a</sup>	
Early season drought	-	+
Late season drought	+	-
Early frost/cold temperature	-	- <sup>b</sup>
Additional soil nitrogen	+	-
Increased fertility (P,S)	+	+
Late planting	+	-
Insect defoliation	-	-
Insect depodding	+	?
Rhizobium inoculation	+	-

<sup>a</sup>? = inconclusive; + = increase; - = decrease.

<sup>b</sup>Oil is reduced because of refining loss to remove chlorophyll.

observable effect on soybean protein and oil percentages. Maestri et al. (6) grew soybean cultivars in several regions of Argentina and concluded that the protein and oil contents were positively correlated with altitude. Protein was negatively correlated with latitude and precipitation, and oil was negatively correlated with temperature and precipitation. Oil content in soybeans tends to be negatively correlated with protein, but breeding soybeans for high protein while maintaining oil content has been a priority of the U.S. soybean producers, and some progress has been achieved (7, 8). The variety Prolina reportedly produces 22.7% oil and 45.5% protein on a dry weight basis. There also has been interest in reducing the oligosaccharides that cause flatulence and reduce the digestibility and nutritive value of soybeans.

Isoflavones are minor constituents of soybeans whose consumption is believed to have beneficial effects (9–11). The benefits of isoflavones have encouraged the direct consumption of soy protein in the United States. The concentration of isoflavones changes with variety and growing conditions and has been reported to be 1.2–2.5 mg/g in U.S. beans (9), 0.5–2.3 mg/g in Korean beans (10), and 0.2–3.5 mg/g in Japanese beans (11).

Table 3 shows the typical composition of the lipid phase of soybeans. Triacylglycerols are the primary component. The 3.7% phospholipids content in the soy beans is higher than that usually found in hexane-extracted oil, which is typically

**TABLE 3. Typical Composition of Crude Soybean Oil.**

Component	%	Std. Dev.
Triacylglycerol	94.4 <sup>a</sup>	1.4
Phospholipids	3.7 <sup>b</sup>	1.2 (12)
Unsaponifiable matter (13–15)	1.3–1.6	
Sterols <sup>c</sup> (16)	0.236	0.053
Campesterol	0.059	0.018
Stigmasterol	0.054	0.013
$\beta$ -Sitosterol	0.123	0.027
$\Delta$ 5-Avenasterol (17)	0.005	
$\Delta$ 7-stigmasterol (17)	0.005	
$\Delta$ 7-avenasterol (17)	0.002	
Tocopherols (16)	0.123	0.040
Alpha	0.0093	0.0044
Beta	0.0018	0.0028
Gamma	0.0834	0.036
Delta	0.029	0.010
Hydrocarbons (14, 15)	0.38	
Free fatty acids (18)	0.3–0.7	
Trace metals (18)	ppm	
Iron	1–3	
Copper	0.03–0.05	

<sup>a</sup>By difference.

<sup>b</sup>Based on 23 varieties chosen to represent a wide fatty acid composition.

<sup>c</sup>Based on 13 varieties chosen to represent a wide range of composition.

1.85–2.75% (19). Of the unsaponifiable matter of soybean oil, typically about 1.45% of the oil, 16% is sterols, 8.5% is tocopherols, and 26% is hydrocarbons. The remaining 50% of the unsaponifiable matter consists of other minor and unidentified products. The sterols are about 52%  $\beta$ -sitosterol, 25% campesterol, and 23% stigmasterol. Maestrl et al. (4) reported similar proportions on the three major sterols but also reported  $5.4\% \pm 0.82$  of  $\Delta 5$ -avenasterol,  $3.8\% \pm 0.76$  of  $\Delta 7$ -stigmasterol,  $1.3\% \pm 0.42$  of  $\Delta 7$ -avenasterol, and traces of cholesterol in the total sterols. The tocopherols are about 7.6%  $\alpha$ , 1.5%  $\beta$ , 67.8%  $\gamma$ , and 23.6%  $\delta$ . There is considerable variation among plant varieties in the amounts and proportions of molecular species of sterols and tocopherols (4, 16, 20). Vlahakis and Hazebroeck (21) also have investigated the effects of planting locations and temperature on the sterol and total tocopherol contents of a number of soybean varieties. They found that growth temperature can cause as much as a 2.5-fold difference in sterol content, with higher temperatures favoring higher amounts of sterols, increasing the campesterol/ $\beta$ -sitosterol ratio and decreasing total tocopherols. McCord et al. (22) examined a number of soybean lines with low and normal contents of linoleate. The low-linolenate lines averaged about 6% lower in tocopherol than the high-linolenate lines, but some reduced-linolenate lines were not significantly different from normal-linolenate lines in tocopherols. The  $\alpha$ - and  $\gamma$ -tocopherols tend to be concentrated in the soybean germ, whereas  $\delta$ -tocopherol is concentrated in the endosperm (23). The hydrocarbon fraction of soybeans consists of n-hydrocarbons of chain length 14 to 33 plus squalene and small amounts of hexahydrofarnesylacetone (14, 15). The squalene content is reported to be about 0.014% of the oil. There seems to be considerable variation in the distribution of the hydrocarbon chain lengths with plant variety, judging from the two examples in the literature. Free fatty acids vary considerably with the age and soundness of the beans but are seldom lower than the 0.1% of the crude oil (18). Damaged beans can contain 1–8% free fatty acid as well as elevated iron and copper, 3–7 ppm and 0.08–0.18 ppm, respectively.

Refined oil usually retains little phospholipid, but damaged beans can have a significant content of phosphatidic acid, and the amount of iron in the oil is related to the amount of phosphorus (24). During deodorization, considerable amounts of sterol and tocopherol may be removed from the oil. The proportion removed depends on deodorization conditions, but a 30% to 40% decrease is not unusual (25). Much of the hydrocarbons and squalene are lost to the deodorizer distillate as well. Free fatty acids in fully refined oil are required to be  $<0.05\%$  and unsaponifiable matter  $<1.5\%$  (26).

Table 4 shows the percentages and standard deviations of the methyl esters of 21 typical refined soybean oil samples. This composition is typical of most presently commercial soybean varieties. The typical composition probably has been selected through plant breeding because it is associated with good yield and other important agronomic properties. It has been possible to change the composition of soybean oil considerably, and Table 4 also shows the ranges of percentages that have been reported for each methyl ester. Many of the changes in composition can be achieved without great losses in yield or oil content, but lines with high or low palmitate

**TABLE 4. The Averages and Standard Deviations of Methyl Esters from Typical Soybean Oils and the Range Reported for each Methyl Ester.**

Methyl Ester	Typical Value % <sup>a</sup> (27)	Range Achieved %
Myristate	0.04 ± 0.5 (27)	trace–0.03 (4)
Palmitate	10.57 ± 0.43 (27)	3.2–26.4 (33, 34)
Palmitoleate	0.02 ± 0.04 (27)	trace–0.7 (29)
Stearate	4.09 ± 0.34 (27)	2.6–32.6 (33, 35)
Oleate	22.98 ± 2.01 (27)	8.6–79.0 (36, 37)
Linoleate	54.51 ± 1.54 (27)	35.2–64.8 (35–37)
Linolenate	7.23 ± 0.78 (27)	1.7–19.0 (38, 39)
Arachidate	0.33 ± 0.14 (27)	trace–0.7 (28)
Gondoate	0.18 (28)	trace–0.6 (4)
Behenate	0.25 ± 0.20 (27)	trace–1.0 (4)
Lignocerate	0.1 (29)	—
Furanoid II <sup>b</sup>	0.014 ± 0.0086 (30)	0.0033–0.0290 (30)
Furanoid III <sup>c</sup>	0.015 ± 0.0076 (30)	0.0084–0.0272 (30)
Saponification Value	190.4 (31, 32)	188.5–201.6 (31, 32)
Iodine Value	132.7(31, 32)	114.0–138.5 (31, 32)

<sup>a</sup>Based on 21 commercial samples.

<sup>b</sup>10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoate.

<sup>c</sup>12,15-epoxy-13,14-dimethyloctadeca-12,14-dienoate.

percentages tend to have reduced oil contents (33, 40, 41). Lines with high stearate percentages suffer from low yields and sporadically from poor germination. Wang et al. (42) tested lines with elevated palmitate or stearate in a number of tests of germination and seedling vigor at three temperatures and found that, although the high-saturate seed did well in these tests, vigor was negatively correlated with saturate percentage. Most of the changes reported in Table 4 were attained by traditional plant breeding or use of mutagenic agents. The high-oleic mutant is an exception and was attained by direct genetic manipulation (37). High-oleate lines developed by traditional plant breeding have been reported, but their oleate percentage varies widely with growth environment, which limits their commercial value (38).

The fatty acid composition of soybean oil changes considerably with maturity and with seed oil deposition (15, 35, 43, 44). In typical soybean triacylglycerols, the palmitate and linolenate tend to decrease with maturity, whereas linoleate increases. Oleate tends to increase to a maximum and then decline slightly. Soybeans selected for atypical fatty acid compositions show quite different patterns of change with maturity from typical soybeans.

Seitz (31) and Wesolowski (32) measured the saponification and iodine values of a number of samples from various geographic locations, and their ranges and typical values are shown in Table 4.

Harp and Hammond (45) explored the stereospecific distribution of acyl groups on the three positions of the glycerol molecule for soybean triacylglycerols with a wide range in fatty acid composition. They found that the amount of an acyl group



on a particular position was linearly related to the amount of that acyl group in the whole triacylglycerol. At low concentrations of palmitate, stearate, oleate, and linoleate in the total triacylglycerols, the amounts on the *sn*-1 > *sn*-3, but the reverse was true at higher total concentrations. Palmitate and stearate were confined to the *sn*-1 and *sn*-3 positions, whereas the oleate concentration was similar on all three positions. Linoleate concentrations at the *sn*-2 position were generally greater than those at the *sn*-1 and *sn*-3 positions, but the amount of linoleate on the *sn*-2 position seemed to be strongly and negatively correlated with the amounts of saturates on the *sn*-1 and *sn*-3 positions. Plots of linolenate concentrations at particular positions versus the concentrations in the whole triacylglycerol showed considerably more scatter than plots for the other acyl groups, but they generally showed the amount of linolenate on *sn*-2 > *sn*-1 > *sn*-3. The saturate percentages also seemed to influence the amounts of linolenate on the *sn*-2 position positively and on the *sn*-3 position negatively. Table 5 shows the stereospecific distribution of typical soybean triacylglycerols.

Theoretically, stereospecific data can be used to predict the acylglycerol structure using the 1-random-2-random-3-random distribution theory (47), if one assumes the fatty acid composition of the three glycerol positions are individually controlled but that the combinations of the three positions are random. However, the change in fatty acid composition with maturity, described in the previous paragraph, shows that the triacylglycerol composition is unlikely to be truly random in the combination of the three glycerol positions. In addition, soybeans from the same plant or pod can have slightly different acyl group compositions, so pooled oil from many seeds and plants is unlikely to be exactly random in its glycerol position combinations. Thus, such a calculation can lead only to approximate compositions.

Neff et al. (48, 49) partially separated the triacylglycerols of soybean oils with a wide range of fatty acid compositions using high-performance liquid

**TABLE 5. Stereospecific Distribution of Acyl Groups in the Triacylglycerols, Phosphatidylcholine, Phosphatidylethanolamine, and Phosphatidylinositol of a Typical Soybean (45, 46).**

Compound/Acyl group	16:0	18:0	18:1	18:2	18:3
Triacylglycerols	11.8	4.6	29.4	47.1	7.2
<i>sn</i> -1	19.3	7.5	25.4	39.6	7.8
<i>sn</i> -2	2.9	0.8	27.9	61.1	7.4
<i>sn</i> -3	14.9	6.4	34.8	38.9	5.0
Phosphatidylcholine	11.2	11.9	8.6	58.6	9.9
<i>sn</i> -1	16.0	22.6	7.3	38.3	6.0
<i>sn</i> -2	4.1	3.7	9.9	71.1	11.2
Phosphatidylethanolamine	16.0	8.3	6.8	57.3	11.7
<i>sn</i> -1	28.5	17.1	5.0	42.4	7.1
<i>sn</i> -2	3.5	2.4	8.7	73.8	11.7
Phosphatidylinositol	22.2	19.3	6.1	43.4	9.3
<i>sn</i> -1	45.1	35.2	5.3	17.1	2.4
<i>sn</i> -2	4.6	3.4	5.9	70.9	15.3

chromatography, and their results are shown in Table 6. These data show how the amounts of the triacylglycerol species change with fatty acid composition.

The primary phosphatides of soybean oil are phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol, which generally make up 55.3%, 26.3%, and 18.4% of the total phosphatides, respectively (50). The stereospecific distribution of the acyl groups in these phospholipids for a typical soybean lipid is shown in Table 5. In all the phospholipids, the saturated acyl groups are concentrated in the

**TABLE 6. Acyl and Triacylglycerol Composition in mol% of Soybean Oils Having a Wide Range of Fatty Acyl Compositions (48, 49).**

Acyl group	Sample Number				
	1	2	3	4	5
Palmitate (P)	3.9	21.4	23.6	28.2	8.5
Stearate (S)	3.3	3.3	19.0	3.9	26.5
Oleate (O)	28.5	23.6	9.3	13.9	18.0
Linoleate (L)	61.8	49.0	38.0	43.8	38.9
Linolenate (Ln)	2.5	2.7	10.0	10.2	8.2
Triacylglycerol Species					
LnLL	2.0	1.2	1.4	3.2	2.6
LnLnO	—	0.1	0.1	0.2	0.1
LnLnP	—	—	0.4	0.6	0.1
LLL	30.0	11.5	3.7	9.6	6.5
LnLO	1.7	1.5	0.8	2.0	1.9
LnLP	0.4	1.7	6.9	9.4	2.2
LLO	26.9	14.4	3.6	8.7	7.1
LnOO	0.4	0.5	0.1	0.3	0.3
LLP	6.4	20.7	17.5	21.4	11.6
LnOP	0.1	1.0	1.4	2.1	0.4
LnPP	0.1	—	1.5	2.0	0.1
LOO	13.9	7.3	1.3	3.1	2.5
LLS	3.6	2.6	7.7	2.0	13.0
LOP	3.7	16.3	7.4	12.2	6.4
PLP	0.8	8.6	13.8	14.8	2.0
OOO	4.6	2.1	1.0	0.8	1.1
LOS	2.6	2.3	3.9	1.3	11.8
POO	0.9	3.2	0.6	1.0	0.5
SLP	0.8	2.2	—	—	—
LnSS	—	—	16.0	3.0	8.8
POP	0.2	1.7	1.2	1.5	0.3
PPP	—	—	0.1	—	—
SOO	0.7	0.5	0.3	0.2	2.1
SLS	0.3	0.2	6.5	0.3	12.3
SOP	0.1	0.4	1.3	0.2	1.4
PPP	—	—	0.6	—	0.6
SOS	—	—	0.6	—	3.4
PSS	—	—	0.2	—	0.1
SSS	—	—	—	—	0.1

*sn*-1 position and the unsaturated acyl groups, especially linoleate, on the *sn*-2 position. Phosphatidylinositol tends to be richest in palmitate and stearate, whereas phosphatidylcholine has the least palmitate. Wang et al. (51) reported the stereospecific distribution of the acyl groups in the various phospholipids types and the amounts of particular acyl combinations for soybean lipids with a wide variety of fatty acid compositions. Some phosphatidic acid and lysophospholipids also may be present as a result of hydrolysis of the phospholipids (52). The amounts of the hydrolytic products usually increase with age and damage to the beans (53).

Soybeans also contain  $170 \pm 47$  ppm of cerebrosides in which the sugar is glucose and the chief fatty acid is 2-hydroxy palmitic acid (54). Traces of ceramides also are present. These are believed to play a role in cell signaling in the soybean plant.

Crude soybean oil contains about 1.9 ppm of Vitamin K<sub>1</sub> or phylloquinone (55). This vitamin plays a role in blood coagulation and bone metabolism. During refining, some Vitamin K<sub>1</sub> may be lost (56, 57), especially during deodorization. Hydrogenation of the fat converts some of the Vitamin K<sub>1</sub> to 2',3'-dihydrovitamin K<sub>1</sub> (58).

Wilson et al. (7, 8, 39, 59) have reviewed the genetic control of fatty acid biosynthesis in soybeans and discussed the advantages of soybean oil with special compositions. Oil with reduced palmitate is available presently in a limited market. The commercial introduction of low-linolenate soybeans has been inhibited by the availability of corn oil, which has a composition like very low-linolenate soybean oil. The price differential between these oils often is smaller than the costs of contract growing, segregating, and processing low-linolenate soybeans. High-oleate soybean oil is stable under frying conditions, but this trait alters the flavor of the fried products (60). The acceptance of high-oleate soybean oil also suffers from public concern about the growth and consumption of plants produced by direct genetic modification.

There are small amounts of two acyl groups containing furan rings in soybeans (30). These oils are reported to be the sources of the odorous compound 3-methyl-2,4-dione by photo-oxidation (61), but Kao et al. (62) were not able to find differences in flavor of photo-oxidized varieties with high and low content of these acyl groups.

### 3. PHYSICAL PROPERTIES OF SOYBEAN OIL

The physical properties of fatty acids vary with their chain length, unsaturation, and other substituents and change with temperature. Numerous attempts have been made to develop equations that will predict these properties. Soybean oil's properties should reflect its constituents and, especially, its fatty acid composition, and physical properties have frequently been measured for typical soybean oils, but there have been fewer measurements of soybean oils with modified fatty acid compositions.

Table 7 shows the values of physical properties of soybean oil of typical composition. Seitz (31) examined 77 samples of soybean oil from various parts of the

TABLE 7. Some Physical Properties of Typical Soybean Oil.

Density 20°C	0.9165 to 0.9261 g/mL (31, 32) Decreases 0.000643 to 0.000668 g/mL°C (63–67)
Specific Heat Capacity 20°C	0.448 cal/g°C Increases 0.000616 cal/g°C (68)
Melting Point	0.6°C (35)
Cloud Point	–9°C (69)
Pour Point	–12 to –16°C (69, 70)
Heat of Combustion	9450–9388 cal/g (71) 9135 ± 91 cal/g (72)
Heat Transfer Coefficient	269.7 watts/°K M <sup>2</sup> at 180°C (73)
Surface Tension 30°C	27.6 dyne/cm Decreases 0.077 dyne/cm°C (63, 64)
Viscosity 20°C	58.5–62.2 cP (31)
Refractive Index n <sub>D</sub> 20°C	1.4733–1.4760 (32)
Vapor Pressure	1μ at 254°C (74)
Heat of Vaporization	44,200 cal/mol (74)
Electrical Resistivity 24°C	
Dry	23.7 Tohm · cm (75)
Water Saturated	7.25 Tohm · cm (75)
Smoke Point	~245°C (76)
Flash Point	~324°C (76)
Fire Point	~360°C (76)

world over a seven-year period and reported densities at 20°C ranging from 0.9165 g/mL to 0.9210 g/mL. Wesolowski (32) examined the density of 53 Polish soybean oils at 19.9°C and reported values ranging from 0.9202 g/mL to 0.9165 g/mL. The following correlations of density and other variables were found: with refractive index 0.62, with iodine value –0.64, with saponification value 0.34, and with acid value 0.59. Yokota and Tachimori (77, 78) also reported a close relation between density and iodine value. Halvorsen et al. (79) and Rodenbush et al. (80) developed equations to predict the density of vegetable oils that took their fatty acid compositions into account and predicted densities of soybean oils with <0.1% error. The density of vegetable oils changes approximately linearly with temperature, and Kravchenko et al. (65, 66) found the density decreased 0.000668 g/mL°C between 0°C and 100°C, whereas Alvarado (63, 64) found a value 0.000643 between 20°C and 70°C, and Nouredini et al. (67) found 0.0006674 between 23.9°C and 110°C.

The densities of soybean oil-solvent mixtures at various temperatures are important for engineering calculations and have been reported for hexane, ethylene dichloride, and trichloroethylene at 25°C, 37.8°C, and 50°C (81); Skellysolve B at –20°C, –10°C, 0°C, 10°C, 25°C, and 40°C (82); dichloromethane at 25°C (83); and hexane at 25°C (84).

The specific heat capacity of soybean oil was measured by Clark et al. (85) and varied from 0.448 cal/g°C to 0.666 cal/g°C between 1°C and 271°C. Specific heat increased linearly with temperature at 0.00070 cal/g°C. Tochitani and Fujimoto (68) measured the specific heat capacity of soybean oil from about the

approximate melting point to 150°C and found a linear increase that fit the following equation:

$$\text{Sp. Heat Capacity in cal/g}^\circ\text{C} = 0.4353 + 0.000616 T, \quad (1)$$

where T is the temperature in °C. Their data agreed closely with those of Clark et al. (85) but were slightly higher than those reported by Kasprzycka-Guttman et al. (86), who made measurements between 70°C and 140°C. Wang and Briggs (87) estimated the heat capacities of soybean oils of various compositions based on an equation by Morad et al. (88). They calculated that high-oleate oils should have a slightly higher heat capacity and low-saturate oils a slightly lower heat capacity than typical soybean oil, and the change with temperature should be 0.00057 cal/g°C. Their equation agreed with their experimental values within  $\pm 5\%$ .

Miller et al. (73) determined the heat-transfer coefficient for soybean oil at frying temperatures and found that they varied from 261.3 watts/°KM<sup>2</sup> to 276.2 watts/°KM<sup>2</sup> between 170°C and 190°C, where M<sup>2</sup> is square meters of surface.

The melting of natural fats and oils usually occurs over a considerable temperature range, and soybean oil's typical melting range is below 0°C. The availability of differential scanning calorimetry (DSC) at low temperatures has made information on melting of soybean oil available, and interest in using vegetable oils as fuels has also sparked measurements of their cloud and pour points. Table 8 (41) gives the temperatures of onset, maximum, and end of melting for various types of soybean oil. Table 7 gives the cloud and pour points of typical soybean oil. Wang and Briggs (87) also gave DSC curves for the melting of high-oleate, low-saturate, and low-linolenate soybean oil. Hagura and Suzuki (89, 90) used the change in electrical capacitance of oil samples to obtain the melting range of soybean oil and found the results agreed with those obtained by DSC.

Seitz (31) measured the viscosity at 20°C of 77 soybean oils from four geographic locations, and the range of variation was 58.1cP to 62.2cP (Table 7). Viscosity decreases with temperature, and the relation is not linear. Kinematic values (viscosity/density) have been reported at 20°C and 80°C by Chioffi (91) and by Miller et al. (73) at frying temperatures (170–190°C); dynamic viscosities have been reported between 0°C and 100°C by Kravchenko et al. (65, 66), between 23.9°C and 110°C by Nourreddini et al. (67), between 20°C and 70°C by Alvarado

**TABLE 8. The Onset, Maximum Rate, and Termination of Melting Temperatures of Soybean Oil with Various Fatty Acid Compositions % (41).**

Class	Onset	Maximum	Termination	16:0	18:0	18:1	18:2	18:3
Typical	-39.6	-9.4	-0.6	11.4	4.2	26.1	50.3	7.9
18:0 ↑	-13.7	18.3	20.7	10.1	22.8	17.3	42.2	7.7
16:0 & 18:0 ↑	-17.1	16.8	18.9	24.6	18.7	8.6	37.5	10.7
16:0 ↑	-21.8	8.4	11.6	28.0	4.7	13.8	42.1	11.4
16:0 ↓	-46.1	-13.8	-8.1	3.4	2.6	18.0	64.8	11.2

(63, 64), and between 1°C and 60°C by Arissen (92). Dahlberg et al. (93) were able to predict the viscosity of soybean and other oils from the Fourier transform infrared spectra. Rodenbush et al. (80) calculated the viscosity of oils by relating viscosity to a function they termed the reduced density, which they could calculate from the fatty acid composition.

Several authors have fit their viscosity-temperature data to equations (63–67, 87, 94). Some of these come with a claim of theoretical significance, but all have enough variables to fit the data well. One of Alavarado's equations (63, 64) is

$$\ln \mu = \ln \mu_0 + E/RT, \quad (2)$$

where  $E/R$  was 3262 and  $\ln \mu_0$  was  $-6.997$  for soybean oil. Wang and Briggs (87) reported graphically the change of viscosity with temperature from 10°C to 90°C for soybeans with altered fatty acid compositions. They found the viscosity of high-oleic soybean oil higher and low-saturated soybean oil lower than that of typical soybean oil.

Miller et al. (73) determined the kinematic viscosity of soybean oil at temperatures of 170°C, 180°C, and 190°C, and obtained values of 3.151 cm<sup>2</sup>/sec, 2.880 cm<sup>2</sup>/sec, and 2.614 cm<sup>2</sup>/sec, respectively. The viscosities of soybean oil-hexane (Skellysolve B) mixtures at temperatures between  $-20^\circ\text{C}$  and  $40^\circ\text{C}$  were investigated by Magne et al. (84). Ibemesi and Igwe (95) examined the reduced viscosity (viscosity/concentration) of solutions of soybean oil in toluene, xylene, cyclohexane, and tetrahydrofuran. They found an anomalous reduced viscosity increase at concentrations below about 0.12 g/mL that they attributed to clustering of the fat molecules in the solvent. Erhan et al. (96) determined the kinematic viscosity of blends of typical soybean oil with polyalphaolefins and isobutyl oleate and high-oleic soybean oil with isotridecyl adipate and mineral oil to achieve viscosities suitable for lubricants.

The surface tension of soybean oil at 20–70°C was reported by Alvarado (63, 64) and is given in Table 7. The surface tension decreased linearly with temperature at 0.077 dyne/cm°C.

Wesolowski (32) examined the refractive index of 53 samples of soybean oil from Poland, and the range is given in Table 7. Sietz (31) reported average values for samples from several geographic locations, and these values (1.4747–1.4752) fall near the mean of Wesolowski's samples. Refractive index depends on chain length and unsaturation (97) and often has been used to follow hydrogenation (98–102). Refractive index also has been used to follow autoxidation (103). A closely related quantity, the dielectric capacitance also has been used to assess the quality of frying oil (104). Perry et al. (74) measured the vapor pressure of soybean oil at various temperatures and found that the data fit the equation:

$$\log P = 18.3 - 9650/T, \quad (3)$$

where  $P$  is the pressure in microns and  $T$  is in K. The also estimated the heat of vaporization (Table 7).

Tomoto and Kusano (105, 106) measured the solubility of carbon dioxide, nitrogen, hydrogen, and oxygen in soybean oil between 0.2 atm and 1 atm and between 30°C and 70°C. The Bunsen coefficient (volume of gas at standard conditions / volume of soybean oil at 760 mm) at 30°C was 1.018 for carbon dioxide, 0.086 for nitrogen, and 0.048 for hydrogen. The Bunsen coefficient of oxygen at 30°C was 0.141 but increased with temperature, probably because of oxidation during the measurement. The Bunsen coefficient decreases linearly to zero at zero gas pressure. The natural logarithm of the Bunsen coefficient versus  $1/T$  in K is linear, and the constant is the heat of solution of the gases divided by the gas constant. These heats of solution are  $-2.42$  kcal/mol for carbon dioxide,  $-2.58$  kcal/mol for nitrogen, and  $-3.86$  kcal/mol for hydrogen. From this relation, one can calculate the solubility at any temperature and pressure in the range of the study. Comparison of the values for soybean oil with olive and linseed oil suggested that the Bunsen coefficients are influenced by the degree of unsaturation of the oil. The viscosity of soybean oil decreased with the amount of carbon dioxide dissolved, but dissolved nitrogen slightly increased the viscosity.

Loncin (107) reviewed the data on the solubility of water in fats and oils. For typical soybean oil, the solubility of water was 0.11% by weight at 22°C and rose to 0.19% at 60°C. The solubility of water decreases with fatty acid chain length and increases with the percentage of free fatty acids.

The vapor pressures of soybean oil-hexane mixtures between 75°C and 120°C were reported (108, 109), and similar data for soybean oil with commercial hexanes was reported by Smith (110). Arnold and Breuklander (83) measured the boiling point of dichloroethylene-soybean oil mixtures and found the log (V.P.) was a linear relation of the mole fraction of oil. Kusano (111, 112) measured the vapor pressure (P) of soybean oil-solvent mixtures that included hexane, benzene, and carbon tetrachloride between 20°C and 50°C and found linear relations between log P and  $1/T$ . Anikin et al. (113, 114) measured the vapor pressure of mixtures of soybean oil with the khladon 113 (trichlorotrifluoroethane) between 30°C and 100°C. Aeberhard and Spekuljak (115, 116) measured the vapor pressure of hexane in hexane-soybean oil mixtures and found the vapor pressure at 25°C could be predicted by the equation

$$P = 9128x - 0.2807x^2 + 0.004695x^3, \quad (4)$$

where P is the vapor pressure in Torr and x is the weight percentage of solvent in the mixture.

Tekin and Hammond (75) measured the resistivity of soybean oil and found it decreased logarithmically with temperature from about 100 Tohm·cm at  $-5^\circ\text{C}$  to 0.251 Tohm·cm at 100°C. The resistivity was decreased by saturating the oil with water and the addition of oleic acid,  $\alpha$ -tocopherols,  $\beta$ -carotene, phospholipids, and monoacylglycerol.

The smoke, flash, and fire points of soybean oil have been determined by the Cleveland Cup method and show considerable variation. Dickhart (117) reported a smoke point of 138°C while Detwiler and Markley (76) reported 241–250°C.

Detwiler and Markley (76) found that the smoke point varied considerably with the degree of refining, especially the removal of free fatty acids, and also with the mode of oil extraction. Yen et al. (118) found a smoke point of 191°C, which was raised several degrees by the addition of phenolic antioxidants. The flash point of soybean oil, the temperature at which vapors coming from the oil will catch fire from an ignition source, were reported as 304°C (117), 326–331°C (76), 174°C (69), 318°C (70), and 320°C (119). The low value reported by Ali et al. (69) was obtained by using a Pensky-Martens closed tester and ASTM method 093-90. The flash points of hexane-soybean oil mixtures were determined and correlated with headspace gas chromatography data (120).

Fire points or self-ignition temperatures (SITs) for soybean oil by using the Cleveland Cup method, which uses a brass cup, were reported to be 356–363°C (76) and 400°C using a stainless-steel cup apparatus (71). The burning rate of soybean oil was 4.3 g/m<sup>2</sup>sec, flame height 129 mm, and irradiance 0.153 kW/m<sup>2</sup> (71). Kowalski (119) studied the self-ignition temperature in a differential scanning calorimeter heated at rates of 40–90°C/min and under 800–2800 kPa of oxygen pressure and found values of 260–290°C for soybean oil. He found the addition of copper wire to the sample decreased the self-ignition temperature by 5–15°C. The self-ignition temperature was inversely related to oxygen pressure. Wakakura (121, 122) used a scanning calorimeter at an oxygen pressure of 980 kPa with soybean oil spread on glass wool and in bulk and found self-ignition temperatures of 147°C and 376°C, respectively.

#### 4. GRADING

To facilitate soybean marketing, the U.S. Federal Grain Inspection Service (FGIS) established grading standards for soybeans (Table 9) (123), and the FGIS website (124) provides much more detailed information than can be provided here (124).

**TABLE 9. Official Grades and Grade Requirements of the Federal Grain Inspection Service, United States Department of Agriculture.**

Grade	Minimum Test Weight per Bushel (lbs)	Maximum Limits				
		Damaged Kernels			Foreign Material (%)	Soybeans of Other Colors (%)
		Heat Damaged (%)	Total (%)	Splits (%)		
U.S. No. 1	56.0	0.2	2.0	1.0	10.0	1.0
U.S. No. 2	54.0	0.5	3.0	2.0	20.0	2.0
U.S. No. 3	52.0	1.0	5.0	3.0	30.0	5.0
U.S. No. 4	49.0	3.0	8.0	5.0	40.0	10.0
U.S. Sample Grade						



Soybeans are classified into two classes based on color, Yellow Soybeans and Mixed Soybeans. There are four numerical grades (U.S. No. 1, 2, 3, and 4) and a U.S. Sample Grade for each class. Sample Grade designates those soybeans that do not meet the requirements of any of the numerical grades. Six factors are considered in assigning a grade designation: test weight, amounts of beans that are damaged or heat damaged, and amounts of foreign material, splits, and soybeans of other colors. Although important to processors because they affect yields and qualities of finished products, the FGIS official grades do not consider moisture, protein, and oil contents, but these factors may be specified on contracts in some markets. Near infrared transmission (NIT) spectroscopy is widely used to rapidly estimate (within less than 2 min after sampling and without any sample preparation required) moisture, protein, and oil contents. Brumm and Hurburgh (125) developed a computer program to estimate the process value of soybeans based on their composition and selling prices of oil and meal. In some cases, price premiums are offered for soybeans high in oil content or high in both oil and protein contents, and details of the program are available on the Internet (126).

Beans low in test weight may contain less oil. Test weight is the weight in pounds of grain per Winchester bushel (35.2 L) and is determined by using an Official Test Weight Apparatus and a 1 1/4-quart (1.18 L) sample before removing foreign material. All other grading factors are measured as percentages of total sample weight. Foreign material, which is other grains, weed seeds, pods, leaves, stems, etc., reduces oil and protein contents and storage life. Foreign material is determined by sieving a sample. All materials, including soybeans and soybean pieces that readily pass through an 8/64-inch (3.2-mm) round-hole sieve and all material other than soybeans remaining on the sieve after sieving are considered to be foreign matter. Split soybeans, which result from mechanical damage during handling and over drying, reduce storage life and oil yield, and increase losses during oil refining. Splits (typically the cotyledon splits into two halves) and broken beans (more than two pieces) increase free fatty acid (FFA), phosphatides, iron, and peroxide contents of the crude oil. Heat-damaged beans have high-FFA content and darken the oil color, both changes in oil quality increase refining loss (127). Splits are defined as beans with more than one-fourth of the bean removed and are not damaged. Splits are determined by sieving a portion of the grain after removing the foreign material. Damaged beans reduce the storage life of the beans and oil yield in processing, cause the oil to be dark-colored and poor in flavor, and increase losses during oil refining (128). Soybeans and soybean pieces that are badly damaged by the ground, weather, frost, heat, insects (stinkbug-stung kernels are considered at one-fourth the actual percentage), mould, or sprouting are considered to be damaged. Damaged beans are determined by hand picking after removing foreign material. Soybeans of other colors may affect oil color by contributing undesirable pigments and are those beans that are green, black, brown, or have multiple colors.

Almost 27 million MT of soybeans were exported from the United States during the 2002 crop year, of which 4.8% was U.S. No. 1, 94.6% was U.S. No. 2, 0.4% was U.S. No. 3, and 0.1% was U.S. No. 4. By comparison, Brazilian soybeans are

typically slightly higher in oil content (6-yr average of 1.2% higher oil content), foreign matter, damage, free fatty acid, and moisture contents and lower in test weight (129).

## 5. RECOVERY OF OIL FROM SOYBEANS

Soybeans are economically important because of their high qualities and quantities of oil and protein. From one bushel of soybeans (60 lb, 27.2 kg), crushers typically recover 11.1 lb (5.0 kg) of crude oil, 44.3 lb (20.1 kg) of meal (48% protein), and 3.3 lb (1.5 kg) of hulls with the remainder being shrinkage. According to the U.S. Department of Agriculture statistics, the oil accounts for about one-third of the returns in processing soybeans with the protein in the form of meal accounting for the remainder (130). Over the past five years, the meal (48% protein) has ranged in yearly average prices of \$153–289/MT (6.9–13.1 cents/lb), whereas the oil has ranged \$311–569/MT (14.1–25.8 cents/lb). Hulls have limited outlets, mostly in cattle feeds, and sell for about \$66/MT (3 cents/lb) and return \$4.04/MT of soybeans (\$0.11/bu). During the same period, the average price of soybeans in the United States ranged from \$167–270/MT (\$4.54–7.35/bu) and crushing margins, the difference in soybean price and crusher returns, averaged \$23.1–56.2/MT (\$0.63–1.53/bu).

Farmers often store their soybeans in metal bins on the farm or in concrete silos at local elevators for a fee. This allows farmers to sell their crop later in the year when prices usually increase. Soybeans should be stored at less than 13% moisture to assure safe storage and preservation of the quality. This moisture content is usually achieved by drying in the field before harvesting. Lower moisture contents increase the tendency of soybeans to split during handling to form two half pieces of cotyledon. Higher moisture content during storage can lead to mold damage or heating damage due to seed respiration (131). These forms of damage can affect soybean grade and oil quantity and quality when processed.

The processing of soybeans has been described in more detail elsewhere than can be done here (132–134). Oil is recovered today by either mechanical means or through the use of organic solvents. In the preindustrial revolution period, soybeans were merely pressed with lever or animal-driven screw-operated batch presses. Around the turn of the Twentieth Century, when soybeans became a viable commercial crop in the United States, steam-powered hydraulic batch presses were used. Today, electric-powered continuous screw-presses, often referred to as expellers (but this is a trademarked name for screw presses manufactured by one supplier), or continuous countercurrent solvent extractors are used.

In either case, soybeans are pretreated prior to oil recovery to either make oil recovery easier or more complete, or to increase the value of the defatted solids known as meal. Usually, soybeans arriving from the farm or elevator are cleaned to remove stems, leaves, pods, broken grain, dirt, stones, and extraneous seeds using shaker screens and aspirators. It is usually advantageous to remove the major portion of the hulls because they are low in oil (<1%) and protein. The hulls of

soybeans account for 7–8% of the weight. Dehulling reduces the material going downstream into costly operations and increases the protein content of the meal. Dehulling raises the meal protein content by about four percentage points (i.e., from 44% for undehulled solvent-extracted soybean meal to 48–49%) and reduces fiber content (from 7.0% to <3.3%). The formulated feed market prefers high-protein and low-fiber meal, especially in manufacturing swine and poultry feeds. The hulls are relatively easy to remove from soybeans compared with those of other oil-seeds, simply cracking the bean into 6–8 pieces to free the hull using corrugated roller mills and aspirating the hulls away from the oil- and protein-rich cotyledon, known as meat, is effective. Consistent bean size is important to proper cracking and drought-caused shrinking and wrinkling make dehulling much more difficult and less efficient (135). Often, the aspirated hulls go to gravity tables to scavenge any small meats aspirated with the hulls. Usually, cleaned soybeans are conditioned prior to cracking to improve dehulling efficiency by heating and drying the beans to about 9.5% moisture and allowing the moisture to equilibrate for 1–7 days within the bean to loosen the hull. Various hot-dehulling schemes have also been devised to increase dehulling efficiency, and are often used in northern latitudes where the protein contents of soybeans, and, consequently, meal protein levels, may be lower and specified protein levels cannot be achieved without more complete hull removal.

In the 1930s, soybeans were widely processed by screw pressing after cooking the seed. A typical process diagram for screw pressing soybeans is shown in Figure 1 and a plant photo is shown in Figure 2. The beans are heated and the oil is squeezed out. The pressed oil usually goes to settling basins to reduce fine

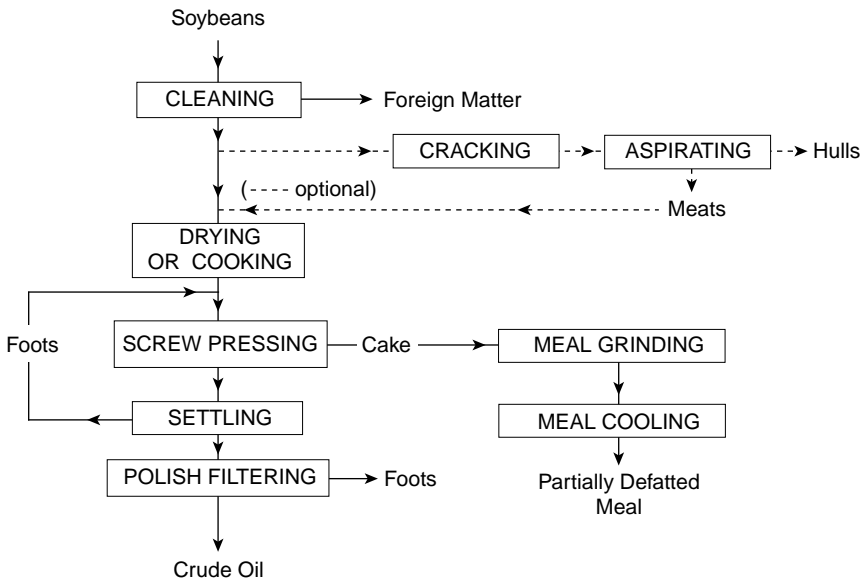


Figure 1. Process flow diagram for screw pressing soybeans.



**Figure 2.** Photograph inside a modern soybean screw-press plant (courtesy of West Central Cooperative, Ralston, IA). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

solids content, with the fines being recycled to the screw press. The oil then goes to polish filters before being placed into storage for shipment to a refinery. Today, in the United States, there are less than a half-dozen traditional screw press plants (excluding extrusion-expelling, which will be discussed later). Only one screw-press plant crushing more than 800 MT/day exists, and it is located in Ralston, IA. Under optimum processing, the meal can contain as low as 4–6% residual oil, which contributes metabolizable energy to livestock consuming screw-pressed meal. As a result of the heat treatment during cooking and screw pressing, increased rumen-bypass characteristics improves feed efficiency in high producing dairy cattle. Thereby, the meal may sell for premium prices over solvent-extracted meal when adequate numbers of dairy animals are located nearby. As this meal is used to feed ruminants, the beans are not usually dehulled.

Direct solvent extraction is the most widely used oil-recovery method for soybeans, but it also requires considerable capital and large scale to compete. In actual practice, solvent extraction is used to crush over 98% of the soybean processed in the United States. Process flow diagrams are shown in Figures 3 and 4. Most soybean solvent-extraction plants process more than 2,500 MT/day (Figure 5), and some are capable of processing as much as 5,000 MT/day (especially newly constructed plants in Brazil). Direct-solvent-extraction plants smaller than 1,000 MT/day have difficulty competing in the United States. At various times, soybeans have been extracted commercially with petroleum distillate fractions that resemble gasoline, acetone, carbon disulfide, ethanol, trichloroethylene, and even water,

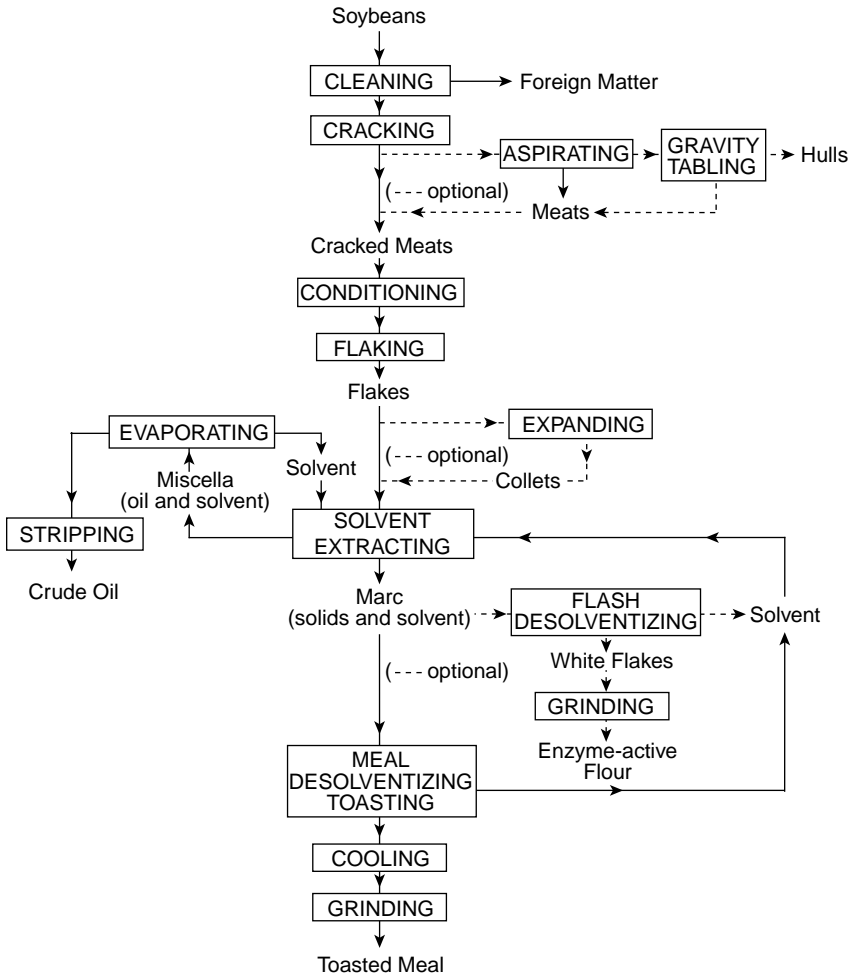
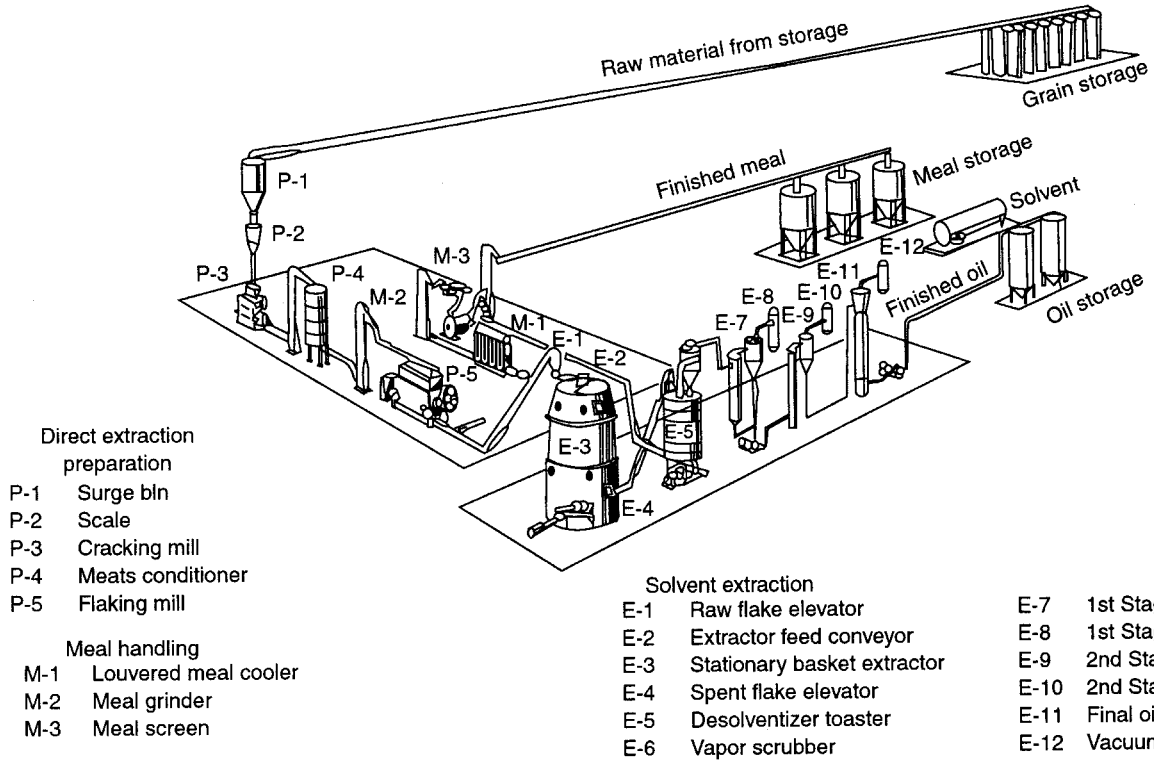


Figure 3. Process flow diagram for direct solvent-extracting soybeans.

which is not a true solvent but facilitates oil separation by creaming. A petroleum distillate containing a mixture of hexane isomers having a typical boiling range of 65°C to 71°C is the only solvent used today. These products typically contain 45% to 70% *n*-hexane. *n*-Hexane is considered a neurotoxin in the United States and has proven toxicity at high concentrations. The U.S. Occupational and Safety Administration has set the maximum workplace exposure level at 500 ppm and a time-weighted average not to exceed 50 ppm (136). In recent years, there has been considerable interest by the soybean industry in alternative solvents to hexanes because of increasing environmental and safety concerns. Alternative solvent technologies have been extensively reviewed (137–139).



**Figure 4.** Depiction of equipment and process flow diagram for direct solvent-extracting soybeans (courtesy of French Oil Mill Machinery Co., Piqua, OH).



**Figure 5.** Photograph of a modern soybean-extraction plant (courtesy of Bunge North America, Council Bluffs, IA). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

Cleaned and dehulled soybeans are conditioned by heating to  $74^{\circ}\text{C}$  to soften the meat prior to flaking using smooth roller mills. Proper cracking and conditioning are important to achieve the desired cell distortion or cell rupture that is necessary for efficient extraction and to prevent production of excessive amounts of fine meat particles that impede proper flaking or extraction. Highly distorted cells are desired (140) so that cell walls and pseudo-membranes around oil bodies are sufficiently ruptured, and the oil can be easily contacted by the solvent and leached out. Soybeans are typically flaked to 0.25 mm (10–12 thousandths of an inch) to achieve the desired distortion (141). The flakes may be conveyed directly to the extractor or to an expander. In recent years, expanders have been adopted to achieve increased cell distortion and to produce an easily extractable porous pellet (collets) that is more dense than flaked soybeans. Thereby, more mass of material can be placed into the fixed volume of the extractor, the oil is more quickly extracted reducing extraction time, and the solvent drains more completely reducing the load on meal desolventizing equipment. All of these factors increase plant throughput capacity (142–144). Plants vary in the amounts of flakes that are expanded, typically about one-third of the flake production, but in a few cases, all flakes are expanded. Although there is not universal agreement, expanding may also improve oil quality by quickly inactivating phospholipases, which cause phospholipids to become nonhydratable. In the author's opinion, adoption of expanders is the most significant change in solvent extraction during the past quarter century.



**Figure 6.** Photograph inside a modern direct solvent-extraction plant processing soybeans (courtesy of Crown Iron Works, Minneapolis, MN). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

Soybeans are exclusively extracted in the percolation mode as opposed to the immersion mode used during early days of soybean extraction. A photograph of a modern chain extractor is shown in Figure 6. The percolating solvent flows by gravity through the bed. Solvent is always passed countercurrent to the transport of meal solids. There are several different types of extractors, including chain and basket types, and shallow- and deep-bed types. Soybean flakes or collets are extracted for 30–45 min in six or more stages.

The best quality oil, low in non-triacylglycerole components, is extracted first, and with more exhaustive extraction, poorer quality oil is recovered. Thus, at low-residual oil levels, the proportions of phosphatides, free fatty acid, and pigments that are extracted are greater and so is the refining loss. However, the current industry practice strives for the most complete extraction possible, typically in the range or 0.5% to 1.25% residual oil. For this reason, exhaustive laboratory devices, such as a Soxhlet extractor with ground material, are not very useful when trying to achieve oil that is representative of that produced by a commercial extractor, and, for best results, the solvent should be percolated in stages through a bed of flaked material.

The full miscella (oil-rich extract) containing 20–30% oil drains from the freshest flaked or expanded meats and is sent to solvent-recovery operations. The operations include two-stage evaporators and an oil stripper. The oil content exiting the first-stage evaporator is 65–70% oil and is heated with vapors from the desolventizer-toaster. After the second-stage evaporator, the oil content is 90–95% oil. The oil stripper uses steam-injection vapor, high heat, and high vacuum to remove the



solvent to less than 0.2% remaining in the oil. The temperature of the oil in the stripper should not exceed 115°C to prevent scorching the oil and causing dark color. Flash point determination is an easy method to assure that the solvent-evaporation equipment is operating as it should and the flash point should exceed 150°C. All evaporated solvent is recycled to the extractor. The oil should be sent to a vacuum dryer to remove any residual stripping steam condensate and the dry oil immediately cooled prior to placing into storage.

As a result of natural antioxidants (i.e., phosphatides, tocopherols), crude soybean oil can be stored for a long time in large tanks provided the oil is first cooled to ambient temperature and has limited access to air. The crude oil should be low in moisture to prevent hydrolysis. Gummy deposits of phosphatides may spontaneously form in the bottoms of storage tanks and tank cars used for shipping crude oil.

There has been much speculation about using supercritical carbon dioxide because using this technology eliminates safety issues as carbon dioxide is not flammable and the oil is better quality (139), but no such plants have been constructed to process soybeans. This is due to the absence of a commercially feasible means of continuously feeding soybean flakes into a high-pressure vessel and removing the spent flakes. Recently, one company has developed a screw press in which supercritical carbon dioxide is injected into the barrel. This equipment has been successfully used to produce soybean meal with lower residual oil contents than typically produced by screw pressing and with little heat denaturation of the protein.

The spent flakes or collets are sent to a meal desolventizer-toaster (DT). Newer equipment incorporates countercurrent steam usage. The Schumacher-type desolventizer/toaster/dryer/cooler has become widely accepted in the soybean industry, and, with this equipment, residual levels of hexane should be less than 500 ppm. Both indirect and direct steam heating are used. Steam vapor and a modest vacuum carry away the solvent vapors for condensing. Condensed solvent is recycled to the extractor after separating water from the hexane. A desolventizer-toaster is a series of trays through which the meal flows. Soybean meal is unique in that it must be toasted to inactivate protease inhibitors (especially trypsin inhibitor) that would reduce feed efficiency if not denatured and inactivated. Urease activity is used as a measure of adequate heating. The toasted meal typically has low-protein solubility as measured by protein dispersibility index (typically 45 PDI). The meal is then sent to a dryer-cooler to reduce the meal temperature for safe storage. The moisture content should be about 12% and the residual fat content less than 1.5%. The free extractable oil after extraction is less than 1.0%, but heating during desolventizing-toasting frees some bound fat that previously was not extractable with hexane. Overtoasting may reduce digestibility and nutritional value of the meal. The meal is then ground with a hammer mill to produce meal with uniform particle size.

If dehulling is employed, as is typical for plants in the United States, the meal will contain around 48% protein. Additionally, dehulling reduces the fiber content of the meal by over 50%. In some plants, a portion of the soybean hulls may be added back to the meal prior to grinding to adjust and precisely control meal protein content. Livestock feeders are concerned about having uniform protein and fiber contents in order to formulate minimum-cost feeds for maximum feed efficiency.

The meal is generally ground so that 95% passes a U.S. 10-mesh screen and a maximum of 3% to 6% passes through a U.S. 80-mesh screen.

Some plants divert part of their spent flake production away from a desolventizer-toaster to a flash desolventizer, which is designed to produce white flakes with high-protein solubility (PDI 70–90). White flakes are used as the starting material for producing protein isolates or concentrates, which contain >90% and 65% protein, respectively, and are used as food ingredients.

Some soybean extraction plants also degum their oil before shipping to centralized refineries. There is not sufficient market to make it profitable to recover all of the soybean phosphatides and market them as soy lecithin. The gums are added back to the meal in the toaster to evaporate the water. The gums contribute to the metabolizable energy content of the meal and the soybean crusher can get meal prices for crude phosphatides.

Quality standards and trading rules for solvent-extracted soybean meal and oil are designated by the National Oilseed Processors Association and are available at a website (145). Soybean products are remarkably uniform in their quality characteristics compared with alternative sources of oil and meal.

Recently, a third process, known as extruding-expelling (or Express Systems as trademarked by the equipment manufacturer), was developed (Figures 7 and 8) (146, 147). In this process, a dry extruder, which generates heat solely through friction of the beans in the extruder, replaces steam generating and steam heating the beans. The heated beans then go to a screw press and the rest of the process is the same as in screw pressing. The plants typically process 5–50 MT/day.

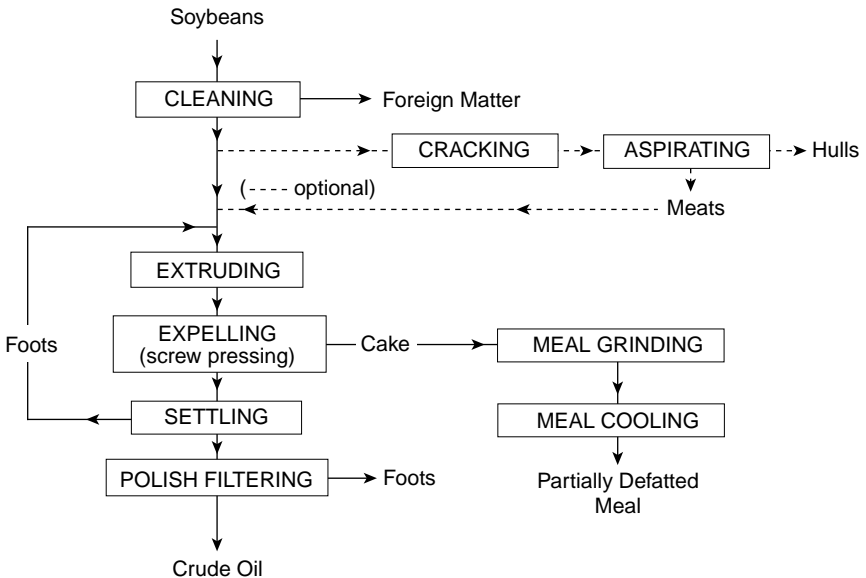


Figure 7. Process flow diagram for extruding-expelling soybeans.



**Figure 8.** Photograph inside a modern extruding-expelling plant processing soybeans. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp.>)

Approximately 70 extruding-expelling plants have been built over the past 10 years for crushing soybeans. Usually, these plants are farmer-owned and provide meal to nearby livestock feeders (148). The oil is sold to the large oil refineries, often at a discount despite the oil being of excellent or superior quality because high costs are incurred in handling small lots of oil. These plants are ideally suited to identity-preserved processing. There are niche opportunities for these plants to market certified organic or nonGMO soybean oil, for which there is a lucrative market in some countries. Other opportunities reside with genetically enhanced soybean oils and meals, such as low-linolenate, high- and low-saturates, and high-oleate oils. This process has even been proposed for producing soybean products during interplanetary exploration (149). NASA plans to grow soybean in space because some missions, such as Mars exploration, cannot be supported without growing food in space.

## **6. QUALITIES OF SOYBEAN OILS AND MEALS EXTRACTED BY DIFFERENT METHODS**

Wang and Johnson (150) compared the qualities of soybean oils and meals obtained by the three processing methods. Soybean oil and meal samples were collected at three times within a one-year period from 13 extruding-expelling plants, eight

**TABLE 10. Quality Characteristics of Soybean Meals Produced by Different Oil-Extraction Processes.**

Property	Processing Method		
	Solvent Extraction	Screw-Pressing	Extruding-Expelling
Moisture, %	11.65	11.03	6.94
Residual oil <sup>1</sup> , %	1.2	6.3	7.2
Protein <sup>1</sup> , %	48.8	43.2	42.5
Urease, ΔpH	0.04	0.03	0.07
Protein solubility in KOH, %	89.1	61.6	88.1
Protein dispersibility index	44.5	10.6	18.1
Rumen-bypass protein, %	36.0	48.1	37.6
Hunter "L" color	69.1	51.5	65.8
Trypsin inhibitor, mg/g	5.46	0.3	5.52
Trypsin inhibitor, TIU/g	5280	2000	12,250

<sup>1</sup> Reported at 12% moisture basis.

solvent-extraction plants, and one continuous screw-press plant. Their results are shown in Tables 10 and 11. Solvent extraction is by far the most efficient method of recovering oil from soybeans, typically only about 1.2% residual oil is left in the meal. Screw-pressing is slightly more efficient in recovering oil than is extruding-expelling, leaving 6.3% oil in screw-pressed meal compared with a mean of 7.2% for extruded-expelled meals. Most solvent-extraction plants dehull soybeans to produce soybean meal with 48% or more protein and carefully control the moisture content at 12%. Solvent-extracted soybean meal is highly uniform, often much more so than either screw-pressed or extruded-expelled meal. The high-protein and low-fiber contents of solvent-extracted soybean meal are desired when feeding poultry and swine, which consume 46% and 25% of the soybean meal produced, respectively. Most extrusion-expelling and screw-press plants have not invested in dehulling equipment, as their meal generally goes into feeding ruminant animals.

Protein dispersibility indices, a measure of protein denaturation that is used in the food industry, are lower for extruded-expelled and screw-pressed meals. Protein

**TABLE 11. Quality Characteristics of Soybean Oils Recovered by Different Processes.**

Property	Processing Method		
	Solvent Extraction	Screw-Pressing	Extruding-Expelling
FFA, %	0.31	0.33	0.21
Phosphorus, ppm	277	463	75
Tocopherols, ppm	1365	1217	1257
Moisture, %	0.08	0.05	0.08
PV, meq/kg	0.96	1.76	1.73
AOM stability, h	39.8	36.2	23.9
Lovibond color, red	11.1	17.5	10.2

solubilities in potassium hydroxide solution, a measure of protein denaturation and an indicator of overcooking that is used in the feed industry, are similar for extruded-expelled and solvent-extracted meals, but higher than that of screw-pressed meal (62%). Rumen-bypass protein values are higher for the screw-pressed meals, indicating that more protein escapes the rumen and is not converted to microbial protein that has a lower nutritive value than the original soybean protein. All meals examined by Wang and Johnson (151), regardless of the processing method employed, had low-trypsin-inhibitor activity, which is important to proper protein digestion. Soybean trypsin inhibitors, especially in unheated soybeans, can inhibit the protease enzymes trypsin and chymotrypsin, reducing protein hydrolysis during digestion. There are two trypsin inhibitors in soybeans, Kunitz inhibitor and Bowman-Birk inhibitor. The Kunitz inhibitor is relatively easily inactivated by moist heat, comprises about 85% of the inhibitory activity, and acts only on trypsin; the Bowman-Birk inhibitor is much more stable to heat (due to six disulfide cross linkages) and acts on both trypsin and chymotrypsin. The activity of the enzyme urease (easily measured as pH change) is often used as a quick and easy indicator of adequate cooking. A valuable resource for characteristics of soybean meal is <http://www.stratsoy.uiuc.edu/epv/>.

Oil properties vary considerably between different types of plants (Table 11) and among plants of the same type and sampling times. The free fatty acid (FFA) content, a measure of hydrolytic degradation during seed storage and oil extraction, of extruded-expelled oil is significantly lower than that of solvent-extracted oil, which may be due to the rapid inactivation of lipases during extrusion. Screw-pressed soybean oil typically contains 0.33% FFA, which is similar to that of typical solvent-extracted oil. The amounts of phospholipids in the oils after settling are much lower in extruded-expelled oil (75 ppm phosphorus) than in solvent-extracted oil (277 ppm phosphorus). Screw-pressed oil has much higher phospholipid content (463 ppm phosphorus) than does solvent-extracted oil. The phospholipid in extruded-expelled oil is readily hydratable and easy to settle, which are attributed to the rapid heat inactivation of the phospholipases. The tocopherol contents of crude extruded-expelled oils are slightly lower than those of crude solvent-extracted oil.

Peroxide values (PVs), a measure of primary lipid oxidation products, are significantly higher for crude extruded-expelled oil than for crude solvent-extracted oil, which is attributed to the high temperature used in extruding-expelling, the long period typically allowed for oil cooling, or the often poor oil-storage conditions and longer storage times at extruding-expelling plants. Oxidative stability, as measured by the Active Oxygen Method (AOM), of extruded-expelled oil is significantly lower than that of solvent-extracted oil, probably because of the higher PV value and lower contents of phosphorus (phosphatides) and tocopherols in crude extruded-expelled oil. The colors of extruded-expelled and solvent-extracted oils are significantly different. Although solvent-extracted oil tends to be slightly darker than extruded-expelled oil, screw-pressed oil is much darker in color than are the other two types, probably because of the more severe heat treatment of the screw-pressed oil before pressing.

## 7. SOY PROTEIN INGREDIENTS

Defatted soybean meal (white flakes) may be heated to produce a variety of solubility and enzyme-activity characteristics, ground and sized to produce grits or flour, and used as a food ingredient in bakery products, soymilk, and meat products. A historical accounting of the development of these products was published by Johnson et al. (151, 152). Soy flour may be relecithinated or refatted with refined, bleached, and deodorized oil to achieve desirable functional properties. Soy flour can also be texturized by using an extruder to produce meat-like products called TVP (texturized vegetable protein) that are often used to extend ground meat. Enzyme-active soy flour is used in bread at 0.5% of the wheat flour. Lipoxigenase in the soy flour bleaches the carotenoids of wheat flour to produce a whiter crumb and improves dough-mixing properties. White flakes may be processed into soy protein isolates or concentrates (132, 153). Soy protein is poorly soluble in water at pH 4.5, the isoelectric point, and highly soluble at pH >8.0. These solubility characteristics can be used to isolate or concentrate soy protein.

Untoasted and flash-desolventized meal in which the protein is undenatured and highly soluble (>70 PDI and preferably >90 PDI) is the preferred starting material in manufacturing soy protein isolates. Under some conditions, extruded-expelled meal can be used, but the yield of soy isolate is reduced. The meal is ground in water adjusted to pH 8.0 with sodium hydroxide and centrifuged to remove insoluble fiber. The soluble fraction is acidified to pH 4.5, and the protein precipitates. The precipitated protein curd is separated from the soluble sugars by centrifuging. The protein curd may be washed, neutralized, and spray-dried.

High protein solubility is not needed for protein concentrates and heating to insolubilize the protein and facilitate extracting the solubles (mostly sugars) with water is one way that has been used to prepare soy protein concentrates. Concentrates today, however, are normally made by extracting the sugars with either acid (pH 4.5) or aqueous ethanol (60–80%). Aqueous ethanol is most frequently used because it produces the blandest product, but ethanol denatures the protein and leaves the protein with reduced functional properties unless the product is refunctionalized by jet cooking (154, 155) or by homogenizing under alkaline conditions (156). Soy protein concentrate must contain >65% protein on a dry basis.

The soybean storage proteins glycinin and  $\beta$ -conglycinin, which often are recognized in the older literature as 11S and 7S proteins, respectively, based on their sedimentation during ultra centrifuging, comprise 65–80% of the protein. Methods have even been developed to separate soy protein into fractions rich in individual proteins (157, 158). Some believe  $\beta$ -conglycinin has greater health benefits than glycinin.

Soy protein isolates are used in dairy analogs (milk replacers and beverage powders), meat-pumping solutions, luncheon meats, and infant formulas, whereas soy protein concentrates are used in dairy analogs (milk replacers, beverage powders, cheeses, coffee whiteners, frozen desserts, whipped toppings), baked goods, and meat products (156). These protein products are used for their functional properties such as solubility, water absorption and binding, viscosity control, gelation,

cohesion-adhesion, elasticity, emulsification, fat absorption and binding, foaming, and color control. The solubility and thermal properties of these products were recently compared by Lee et al. (159). Some products have high solubility even though they were largely denatured.

Many health benefits have been attributed to soy protein products, either because of the proteins or accompanying phytochemicals, such as isoflavones, saponins, etc. There is a growing body of evidence that soy protein products may impact hypertension and heart disease, osteoporosis and bone health, and certain cancers. The perception of such nutritional benefits is driving an increased interest by food companies in the incorporation of soy protein products. In October 1999, the U.S. Food and Drug Administration (FDA) authorized a health claim for soy protein in cardiovascular disease. U.S. food labeling laws now permit a statement on the label that "Diets low in saturated fat and cholesterol that include 25 grams of soy protein a day may reduce the risk of heart disease. One serving of (name of food) provides (list number) grams of soy protein." The health claim allowance is reported in the Federal Register (160) and is posted on the FDA website (161).

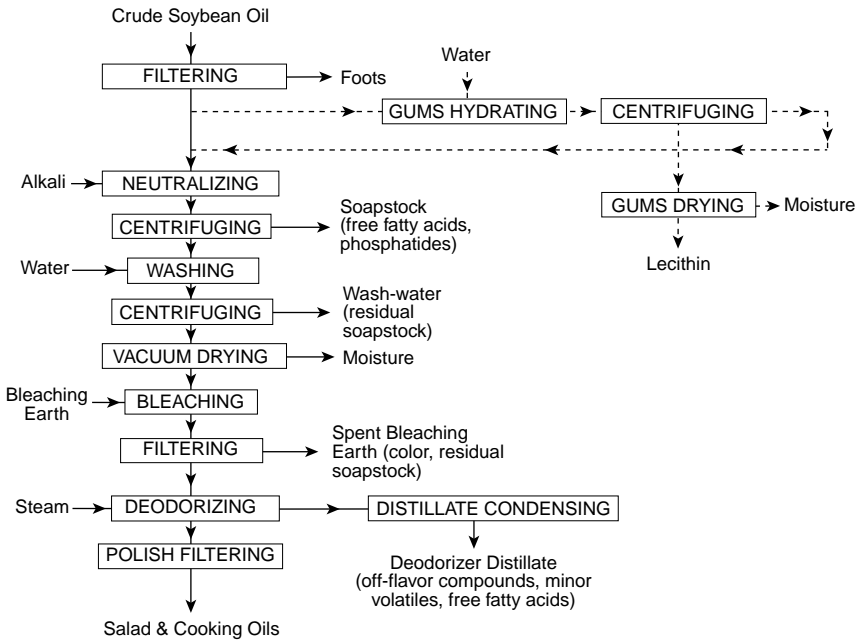
## 8. BASIC PROCESSING OPERATIONS

As discussed in the previous section on soybean oil composition and Table 11, crude soybean oil can contain phospholipids, free fatty acids, lipid oxidation products, and unsaponifiable matter, which includes chlorophyll and carotenoid pigments, tocopherols, sterols, and hydrocarbons. Some of these components negatively affect oil quality, and some may play positive roles in nutrition and functionality. The goal of oil refining is to remove the undesirable components so that a bland, stable, and nutritious product can be obtained. The basic processing operations in oil refining are (1) degumming, (2) neutralization, (3) bleaching, (4) hydrogenation, (5) deodorization, and (6) winterization or crystallization. These steps are outlined in a flow chart as shown in Figure 9.

### 8.1. Degumming

Crude soybean oil contains a relatively high concentration of phospholipids compared with other vegetable oils. Degumming is a process of removing these components from crude soybean oil to improve its physical stability and facilitate further refining. Phospholipids can lead to dark-colored oils and they can also serve as precursors of off-flavor (162) compounds. Free fatty acids, pigments, and other impurities are also partially removed by degumming. Soybean oil can also be neutralized directly without degumming if gum or lecithin recovery is not desired. Conventional belief holds that the loss of neutral oil in refining crude oil by direct neutralization is less than the combined losses of degumming and caustic refining of the degummed oil.

The quality of crude soybean oil influences the efficacy of degumming. Phospholipids can exist in a hydratable form, which can be readily removed by addition



**Figure 9.** Diagram of conventional soybean oil refining.

of water, or in a nonhydratable form, which cannot be easily hydrated and removed. The nonhydratable phospholipids are considered to be the calcium and magnesium salts of phosphatidic acids, which are formed by enzymatic hydrolysis of the original phospholipids. This degradation can result from seed damage during storage and improper handling. List et al. (53) studied the factors promoting the formation of nonhydratable phospholipids in soybeans and showed that they are promoted by four interrelated factors: (1) moisture content of beans or flakes, (2) phospholipase D activity, (3) heat applied to beans or flakes prior to and during extraction, and (4) disruption of the cellular structure by cracking or flaking. These results suggest that a nonhydratable-phosphatide formation can be minimized by control of the moisture of beans or flakes entering the extraction process, inactivation of phospholipase D, and optimizing the temperature during conditioning of cracked beans or flakes. Normal quality soybean oil from the conventional solvent extraction contains about 90% hydratable and 10% nonhydratable phospholipids. Phosphoric or citric acid can be used as a pretreatment to achieve more complete removal of nonhydratable phospholipids, but their presence in the gum will darken it and reduce its quality. The total phospholipid content in crude soybean oils ranges from 1.85% to 2.75% (19) and partially depends on the seed preparation and extraction methods employed. Use of an expander or the Alcon process to cook the flakes prior to extraction will increase total phospholipids content in the crude oil and the phosphatidylcholine percentage in the gum (163).



Degumming can be achieved in a batch or continuous fashion. In batch degumming, soft water at the same percentage as total phospholipid is added to oil heated to 70°C and mixed thoroughly for 30–60 min, followed by settling or centrifuging. In continuous water degumming, heated oil is mixed with water by an in-line proportioning and mixing system and the mixture is held in a retention vessel for 15–30 min before centrifugation. The phosphorus content is typically lowered to 12–170 ppm (164). A well-degummed soybean oil should contain less than 50 ppm of phosphorus, which is well below the 200 ppm level specified in the National Oilseed Processors Association (165) trading rules for crude degummed soybean oil. Degumming for physical refining, as opposed to alkali refining of soybean oil, requires more complete removal of the phospholipids to prevent darkening during fatty acid distillation. For more complete phospholipid removal, several modified degumming methods can be employed (166, 167).

Recently, polymeric ultrafiltration membranes were used for degumming crude soybean oil and removing phospholipids from the crude oil/hexane miscella (168). Crude soybean oil also can be de-acidified by methanol extraction of the free fatty acids and the extract separated into fatty acids and solvent by a membrane filter (169). A surfactant-aided membrane degumming also has been applied to crude soybean oil, and the degummed oil contained 20–58 ppm of phosphorus (170). Supercritical carbon dioxide extraction was shown to be an effective means of degumming (171). In this process, soybean oil countercurrently contacted supercritical carbon dioxide at 55 MPa and 75°C. The phosphorus content of the oil was reduced from 620 ppm to less than 5 ppm. Ultrasonic degumming was also successfully used to reduce the gum content of soybean oil (172).

## 8.2. Neutralization

Neutralization is also referred to as de-acidification and alkali or caustic refining. Neutralization is achieved by treating the soybean oil with aqueous alkaline solution (most commonly, sodium hydroxide) to neutralize the free fatty acids in a batch or continuous system. The soap formed in the reaction also adsorbs natural pigments, the gum and mucilaginous substances not removed by degumming. Natural settling or centrifugation is used to remove the soap. Crude soybean oil also can be neutralized directly without degumming. When this is practiced, the oil commonly is pretreated with 300–1000 ppm of 75% phosphoric acid to facilitate removal of phospholipids. The percentage of excess sodium hydroxide solution required for crude oil is higher than that for degummed oil (173).

The quality changes, such as lipid oxidation and reduction of tocopherols and phytosterols during neutralization, are considerable compared with the other processing steps as shown by Wang and Johnson (174), and also as presented in Table 12. The further phospholipid removal (below 2 ppm phosphorus) also reduces the oxidative stability of soybean oil (175) due to the antioxidant property of these phospholipids.

One of the new developments in neutralization is the use of silica-based adsorbent to remove the residual soap instead of using water washing. Water usage and

**TABLE 12. Effect of processing on content of tocopherols, sterols, and squalene in soybean oil (25).**

Processing Step	Tocopherols		Sterols		Squalene	
	ppm	% Loss	Ppm	% Loss	ppm	% Loss
Crude	1132	—	3870	—	143	—
Degummed	1116	1.4	3730	3.6	142	0.7
Neutralized	997	11.9	3010	22.2	140	2.1
Bleached	863	23.8	3050	21.2	137	4.2
Deodorized	726	35.9	2620	32.3	89	37.8

waste generation is greatly reduced by this practice. Sodium silicate also was used as a mild neutralizing agent to refine specialty oils (176). Its agglomerating tendency allowed the removal of the soap by filtration, and its low alkalinity minimized saponification of neutral oil and loss of minor nutrients. Other adsorbents, such as magnesium silicate, also were shown to be effective in reducing free fatty acids, as well as reducing primary and secondary oxidation products in the treated oil (175, 177).

Physical refining or steam refining is a process similar to steam deodorization. Steam distillation is typically used for oil with a high free-fatty acid content to reduce the refining loss, which would be significant if caustic refining was used. Acid-aided degumming produces soybean oil with very low phosphorus content and makes the distillation of free fatty acids possible. Nevertheless, the relatively difficult task of removing sufficient phospholipids from soybean oil has prevented extensive use of this technique in the United States. Physical refining, however, has virtually replaced caustic refining of palm oil in Malaysia.

### 8.3. Bleaching

Bleaching is a process designed not only to remove the oxidation-inducing pigments such as chlorophylls, but more importantly to decompose the peroxides produced by oxidation into lower molecular weight carbonyl compounds that can be removed by subsequent deodorization. Bleaching also removes other impurities such as soap and metal ions. In soybean oil refining, color reduction occurs at each step, nevertheless, the most significant reduction of chlorophylls occurs in the bleaching step. Acid-activated bleaching clay is most effective in adsorbing chlorophylls and decomposing peroxides, and it is commonly used for soybean oil. The chlorophyll content in normal crude soybean oil (1–1.5 ppm) can be reduced by 25% by alkali refining, and bleaching with acid earth further reduced chlorophylls to 15 ppb (178). The subsequent hydrogenation and deodorization remove or degrade red and yellow pigments more than chlorophyll, so incomplete chlorophyll removal by bleaching will cause the refined oil to appear greenish. The refined and bleached oil is particularly susceptible to oxidation and is less stable than the crude, degummed, refined, or deodorized oils (178).

The desired bleaching endpoint is typically zero peroxide, although a color specification is often used as an important measure. The amount of bleaching earth should be adjusted based on the quality of oil to be bleached, and it usually ranges from 0.3% to 0.6% for a typical soybean oil. Low contents of phosphorus (5–10 ppm P) and soap (10–30 ppm) in the neutralized oil are essential to maximize the bleaching effect. Successful bleaching can be achieved by atmospheric batch bleaching, vacuum batch bleaching, or continuous vacuum bleaching at temperatures between 100°C and 120°C for 20–30 min. More details of soybean oil bleaching are described by Erickson (179).

Recently, silica-based synthetic materials have been used in bleaching. The natural bleaching earth, fuller's earth, a hydrated aluminum silicate, mostly has been replaced by acid activated clays, which are sulfuric- or hydrochloric-acid-treated bentonites or montmorillonites. Manufacturers continuously improve the quality and develop new bleaching earths to meet the market's needs. Higher activity and filterability are the main focuses of such development.

#### 8.4. Hydrogenation

The high degree of unsaturation, particularly the relatively high content of linolenate, of soybean oil significantly limits its food applications because of low oxidative stability. Hydrogenation is used to improve oxidative stability as well as to increase the melting temperature of soybean oil. A great proportion of soybean oil is hydrogenated to produce cooking oil, bakery/confectionery fats, and shortening.

When oil is treated with hydrogen gas in the presence of a catalyst (typically nickel) and under appropriate agitation and temperature conditions, it becomes more saturated and forms a semisolid or plastic fat that is suitable for many food applications. Selectivity is a term used to describe the relative reaction rate of the fatty acids from the more unsaturated to the more saturated forms. Perfect selectivity would provide sequential elimination of linolenate, linoleate, and then oleate. To completely hydrogenate linolenate while minimizing changes in the other acyl groups, a high ratio of the reaction rates of linolenate to linoleate compared with linoleate to oleate is desirable. Generally, selectivity increases with temperature and catalyst concentration and with decreases in hydrogen pressure and agitation rate (180). The effect of pressure on hydrogenation selectivity of soybean oil was reported by List et al. (181), who found that the linoleate-containing triacylglycerols were reduced at a slower rates than the linolenate-containing triacylglycerols under selective condition. At higher pressures (500 psi), the reaction was truly non-selective; whereas at 50 psi, the reaction became selective. Impurities in soybean oil, such as phosphorus, oxidation products, carotene, and metal ions can poison the catalyst and cause slower hydrogenation (182). A particular limitation with nickel catalyst is its low selectivity for linolenate over linoleate, and copper-containing catalysts have greater selectivity for linolenate acid than the conventional nickel catalysts (183). The use of copper catalyst can produce soybean oil that has a low degree of hydrogenation (iodine value of 110–115) but has less than

1% linolenate. However, copper catalysts are not as active as nickel catalysts; they are also easily poisoned (184). Furthermore, any trace of residual copper in the fully processed oil will promote lipid oxidation.

The most common tests for degree of hydrogenation are congeal point and the iodine value as determined by refractive index. Refractive index is a valuable tool for iodine values above 95, but when the oil is further hydrogenated, refractive index becomes an inadequate measurement for melting prediction because increased amount of *trans*-isomers results in harder oil than the refractive index would indicate (185). For margarine or shortening, the solid fat index (SFI), as determined by dilatometry, or solid fat content (SFC), determined by nuclear magnetic resonance, is the most appropriate method to measure the consistency of the hydrogenated oil. These indices predict the workability and creaming ability at a particular temperature.

Double-bond isomerization or *trans*-fatty acid formation is the most important side-reaction that occurs during hydrogenation, and it has a strong impact on the physical and possibly the nutritional properties of the products. *Trans*-double bonds are thermodynamically a more favorable configuration than their *cis*-counterpart; so *trans*-bonds are produced in significant quantities if the hydrogenation does not go to completion. The *trans*-fatty acids have a much higher melting point than their *cis*-isomers, therefore a fat product with considerable *trans*-acyl groups will have an elevated melting point, which is desirable in shortening and margarine applications. A partially hydrogenated soybean oil can have at least 30 different one-, two-, and three-double-bond isomers that will result in more than 4000 different triacylglycerol molecules. This complexity allows the production of a great variety of oils, margarines, and shortenings that have a wide range of physical and functional properties. However, the established relationship between *trans*-fat consumption and health has prompted research to minimize *trans*-double formation in fats and oils.

Hydrogenation of soybean oil may be carried out in a batch or a continuous system. In the United States, batch operations are typical. More comprehensive reviews on hydrogenation and formulation can be found in Erickson and Erickson (180), Hastert (186), and Kellens (187).

## 8.5. Deodorization

Deodorization is usually the last step in conventional oil processing. It is a steam-stripping process in which good quality steam (1–3% of oil) generated from de-aerated and properly treated feed water is injected into soybean oil under high temperature (252–266 °C) and high vacuum (<6 mm Hg) to decompose peroxides and vaporize the free fatty acids and odorous compounds. Deodorization relies on the large differences in volatility between the triacylglycerols and other undesirable components under certain conditions. The musty and earthy odor produced from bleaching and the hydrogenation odor and flavor are effectively removed by deodorization. The free fatty acids, typically ranging from 0.1% to 0.5% in neutralized oil and 0.5% to 5% in oil to be physically refined, are also reduced to below 0.03%, a value used as an indicator for deodorization efficiency. Zero peroxide

value is another indicator for effective deodorization. Heat bleaching is achieved by holding the oil for 15–60 min at high temperature to ensure considerable decomposition of carotenoid pigments.

During the deodorization process, many desirable reactions take place, but some undesirable reactions, such as lipid hydrolysis, polymerization, and isomerization, also occur. Therefore, the deodorization temperature is carefully controlled to achieve optimum quality of the finished soybean oil product. The effect of refining condition on *trans*-fatty acid content in refined vegetable oils was investigated by Okamoto et al. (188). *Trans*-fatty acid contents of deodorized oils increased with prolonged exposure to high temperature, and *trans*-formation was higher in oils containing greater proportions of polyunsaturates. The isomerization rate of linolenate was 6.5- to 16.3-fold higher than that of linoleate in soybean oil. Kemeny et al. (189) studied kinetics of the formation of *trans*-linoleic acid and *trans*-linolenic acid in vegetable oils deodorized at temperatures from 204–230°C for 2–86 h. Their data can give good estimates of the *trans*-level of refined oils for given deodorization conditions. Deodorization has also been modified to retain more nutrients and prevent other undesirable reactions. Mathematical models have been established describing the influence of different process parameters such as time, temperature, steam rate, and pressure on tocopherol stripping, production of oxidized and polymeric triacylglycerols, and *trans*-fatty acid formation during physical refining of soybean oil (190). Tocopherol removal was mainly influenced by processing temperature and steam rate, whereas oxidized and polymerized triacylglycerols were not significantly affected by any of the investigated process parameters.

There are three types of deodorization operations. The batch process is the least common because of its low efficiency and inconsistent product quality. The semicontinuous and continuous deodorizers have improved processing efficiency. There are several configurations of the continuous deodorizer, including the single-shell cylindrical vessel type, the vertically stacked-tray type, and the thin-film packed-column type. The thin-film system provides excellent fatty acid stripping with minimum use of steam, but it does not achieve the desired heat bleaching or effective deodorization because of its relatively short retention time. A retention vessel held at high temperature has to be used after the column distillation to achieve bleaching (191).

The overall oil quality change during refining of soybean oil was examined by Jung et al. (178), and their results are shown in Table 13. A study of oxidative

**TABLE 13. Effect of Processing Steps on Quality of Soybean Oil (178).**

Refining Step	Phosphorus (ppm)	Iron (ppm)	Chlorophyll (ppm)	Peroxide Value (meq/kg)	Tocopherol (ppm)	Free Fatty Acid (%)
Crude	510	2.9	0.30	2.4	1670	0.74
Degummed	120	0.8	not available	10.5	1579	0.36
Refined	5	0.6	0.23	8.8	1546	0.02
Bleached	1	0.3	0.08	16.5	1467	0.03
Deodorized	1	0.3	0.00	0.0	1138	0.02

stability of soybean oil at different stages of refining indicated that crude oil was the most stable and highly purified oil was the least stable (192). The influence of the refining steps on the distribution of free and esterified phytosterols in soybean and other oils was reported by Verleyen et al (193). A significant reduction in free sterols was found after neutralization. Deodorization removed free sterols and also promoted steryl ester formation when the oil was physically refined due to a heat-promoted esterification reaction between free sterols and free fatty acids.

## 8.6. Fractionation and Winterization

Fractionation or winterization is a process in which the more saturated molecular species in the oil are solidified and removed by a low-temperature treatment, which increases the cold storage physical stability of the oil. Partially hydrogenated soybean oil with 110–115 iodine value (IV) that is intended for use as salad and cooking oil should be fractionated. By doing so, the more saturated molecules and some high-melting *trans*-isomers are removed to produce clear oil that meets low-temperature storage requirements. The formation of large and easily filterable crystals and the removal of the crystallized fraction from the liquid oil can be challenging tasks. The temperature of the oil should be lowered slowly to prevent small crystal formation. Nucleation occurs when the oil is supercooled to a temperature that is much lower than the thermodynamic equilibrium temperature. Heterogeneous nucleation, i.e., the formation of nuclei on to foreign substances, typically takes place around dust particles or on the walls of the crystallizer. The crystal growth rate depends on the degree of supercooling and polymorphic form. In order to have continuous and uniform crystallization, an intense but nondestructive agitation is required. To produce salad oil with good cold stability, soybean oil is usually hydrogenated to an iodine value of 100–110 (linolenate content of 2–3%) and winterized at 2–3°C. To produce a cooking and frying oil, hydrogenation to an iodine value less than 90 (linolenate content of less than 0.5%) is more desirable, and the stearine fraction obtained from winterization of such oil is a good shortening and margarine base. Crystal separation can be done by filtering, centrifuging, or decanting. More details about these systems are presented by Krishnamurthy and Kellens (194).

## 9. ALTERNATIVE REFINING METHODS

Although oil extraction by mechanical pressing of soybeans accounts for a very small percentage of soybean processing, it is used by many farm cooperatives or family-owned on-farm operations in the United States, primarily for using protein meals as animal feed. There is an increasing use of extrusion-expelling technology to produce identity-preserved soybean oil and protein products for niche market. The advantages of small tonnage requirement, no flammable solvent used, low initial capital investment, and unique products have made this processing technology very appealing for many soybean growers and processors.

Alternative techniques are being developed for refining soybean oil produced by mechanical means. Simple refining methods were explored to process extruded-expelled (E-E) soybean oils with various fatty acid compositions (174, 177). E-E oils can be easily water degummed to very low phosphorus levels. Free fatty acid content was reduced to 0.04% by adsorption treatment with Magnesol®<sup>®</sup>, a commercial magnesium silicate product from Dallas Group of America (Jeffersonville, IN). This material also adsorbed primary and secondary oil oxidation products. A mild steam deodorization as the last processing step produced good-quality soybean oil. This adsorption refining procedure was much milder than conventional refining, as indicated by little formation of primary and secondary lipid oxidation products and less loss of tocopherol during refining.

## 10. COPRODUCTS AND UTILIZATION

### 10.1. Lecithin

Soybean lecithin is the predominant source of food and pharmaceutical lecithin because of its availability and outstanding functionality. The composition of crude soy lecithin is shown in Table 14. As a result of the presence of a large amount of neutral oil, crude lecithin is usually de-oiled to improve its functionality. De-oiling is based on the solubility difference of neutral and polar lipids in acetone, in which the phospholipids are precipitated and separated. Alcohol fractionation of de-oiled lecithin can further separate lecithin into an alcohol-soluble fraction that is enriched with phosphatidylcholine and an alcohol-insoluble fraction enriched with phospho-

**TABLE 14. Composition of Commercial Soy Lecithin in Comparison with Egg Lecithin, wt % (195).**

Compounds	Soy Lecithin	Egg Lecithin
Phosphatidylcholine	10–15	65–70
Phosphatidylethanolamine	9–12	9–13
Phosphatidylinositol	8–10	–
Phosphatidylserine	1–2	–
Phosphatidic acid	2–3	–
Lysophosphatidylcholine	1–2	2–4
Lysophosphatidylethanolamine	1–2	2–4
Phytoglycolipids	4–7	–
Phytosterines	0.5–2.0	–
Other phosphorus-containing lipids	5–8	–
Sphingomyelin	–	2–3
Carbohydrate	2–3	–
Free fatty acids	max 1	max 1
Mono-, diacylglycerols	max 1	Trace
Water	max 1.5	max 1.5
Triacylglycerols	35–40	10–15

**TABLE 15. Typical Composition (%) of Commercially Refined Soy Lecithin Products (196).**

	Lecithin Oil-Free	Lecithin Alcohol-Soluble	Lecithin Alcohol-Insoluble
Phosphatidylcholine	29	60	4
Phosphatidylethanolamine	29	30	29
Phosphatidylinositol and glycolipid	32	2	55
Neutral oil	3	4	4
Others	7	4	8
Emulsion type favored	w/o or o/w	o/w	w/o

tidylinositol. The phosphatidylcholine-enriched fraction is an excellent oil-in-water emulsifier, and the phosphoinositol-enriched fraction is a good water-in-oil emulsifier that is often used in the chocolate industry. The typical composition of de-oiled and fractionated lecithin products is shown in Table 15.

Supercritical CO<sub>2</sub> extraction also has been used to selectively extract phosphatidylcholine from de-oiled soybean lecithin (197). The effects of temperature, pressure, and amount of ethanol on phosphatidylcholine extraction were examined, and a high-purity product could be produced with optimized conditions.

Lecithin recovered from solvent-extracted soybean oil had different phospholipid class compositions from those produced by mechanical pressing (198). The percentage of phosphatidylcholine was considerably higher in lecithin recovered from extruded-expelled oil than from solvent-extracted oil. The phosphatidylcholine- and phosphatidylinositol-enriched fractions produced by ethanol extraction of the crude lecithin also showed different functional properties (199).

Soybean lecithins can be chemically altered to modify their emulsifying properties and improve their dispersibility in aqueous systems. Phospholipids may be hydrolyzed by acid, base, or enzyme to achieve better hydrophilic and emulsification properties. Hydroxylation of lecithin improves its oil-in-water emulsification property and water dispersibility. Acetylation creates improved fluidity and emulsification, water dispersion properties, and heat stability (200).

## 10.2. Deodorizer Distillate

Deodorizer distillate is the material collected from the steam distillation of oils. It is a mixture of free fatty acids (especially during physical refining) tocopherols, phytosterols and their esters, hydrocarbons, and lipid oxidation products. The quality and composition of deodorizer distillate depends on the feedstock oil composition and processing conditions. Tocopherols and sterols are the most valuable components that can be recovered from the distillate, and they are used in the nutrition supplement and pharmaceutical industries (201). Typical soybean deodorizer distillate contains about 33% unsaponifiable matters, of which 11% is tocopherol and 18% sterol (202).



Soybean tocopherols are the major source of natural fat-soluble antioxidants and Vitamin E. The Vitamin E activity of natural d- $\alpha$ -tocopherol is much greater than that of synthetic Vitamin E, which is a mixture of eight stereoisomers (203). Phytosterols are used as raw materials for over 75% of the world's steroid production. The more recent application of phytosterol, phytostanol, and their fatty acid esters in margarine and table spreads is based on the blood cholesterol-lowering effect of these compounds (204, 205). The recent development of functional foods containing phytosterols has been reviewed by Hollingsworth (206) and Hicks and Moreau (207).

The preparation of high-purity tocopherols and phytosterols involves steps such as molecular distillation, adduct formation, liquid-liquid extraction, supercritical fluid extraction, saponification, and chromatography (175). The extraction of tocopherols from soybean oil deodorizer distillate by urea inclusion and saponification of free fatty acids resulted in good recovery of tocopherols (208). To improve the separation of sterols and tocopherols, Shimada et al. (209) used a lipase to esterify sterols with free fatty acids. Then the steryl esters and tocopherols were separated better by molecular distillation. Chang et al. (210) used supercritical fluid CO<sub>2</sub> extraction to recover tocopherols and sterols from soybean oil deodorizer distillate. A patent by Sumner et al. (211) advocated treatment of the distillate with methanol to convert free fatty acids and other fatty acid esters to methyl esters that can then be removed by a stripping operation. Then separation of sterols and tocopherols could be carried out by molecular distillation.

### 10.3. Soapstock

Soap is recovered from alkaline neutralization of the crude or degummed soybean oil. Soap consists of water, free fatty acids, neutral oil, phospholipids, unsaponifiable matter, proteins, and mucilaginous substances. Its composition depends on seed quality and oil extraction and refining conditions. Soapstock is the least valuable byproduct from oil processing, and it is generated at a rate of about 6% of the volume of crude soybean oil refined (212), amounting to as much as 0.8 million MT in the United States annually. The majority of the soap or acidulated soap is used as a feed ingredient contributing metabolizable energy. Soybean oil can be refined using potassium hydroxide and acidulated with sulfuric acid, followed by neutralization with ammonia rather than sodium hydroxide to produce a fertilizer (213). Soybean oil methyl esters can also be produced from soapstock (214–218) for biodiesel applications.

## 11. FOOD AND BIOBASED PRODUCT USES OF SOYBEAN OIL

### 11.1. Distribution of Soybean Oil Utilization

In 2001–2002, when 8.32 million MT (18,300 million pounds) of soybean oil was used in the United States, over 97% (8.09 million MT, 17,800 million pounds) was used for food, with the remainder used in nonfood products (219). Among the food

uses, about 48% (3.89 million MT, 8,570 million pounds) was for shortening, 43% (3.58 million MT, 7,897 million pounds) for cooking and salad oils, 7% (0.56 million MT, 1,237 million pounds) for margarine, and 1% (0.06 million MT, 125 million pounds) for other food uses. Soybean oil is used to produce about 95% of the total margarine and 83% of the total shortening consumed in the United States.

Among the 0.24 million MT (519 million pounds) used in nonfood products, about 16% (0.04 million MT, 85 million pounds) was for resins and plastics, 12% (0.03 million MT, 60 million pounds) for paint and varnish, 13% (0.03 million MT, 68 million pounds) for fatty acids, and 59% (0.14 million MT, 306 million pounds) for a myriad of other inedible uses. The use of soybean oil in lubricants (220), oleochemicals (221), and bioplastics (222), and the production of methyl soyate for environmentally friendly solvents (223, 224) and for blending with diesel fuel to produce biodiesel (20% methyl soyate/80% diesel fuel) (225) are significant parts of the soy oil used in nonfood applications (226). Usage of soybean oil to make biodiesel is likely to increase in future years because several new plants are planned for construction as a result of the recent Farm Bill of 2002 providing financial incentives for producing biodiesel. Some states, notably Minnesota, have enacted legislation that provides biodiesel tax incentives. Biodiesel interests have become organized as the National Biodiesel Board (Jefferson City, MO) and the Renewable Fuels Association (Washington, DC), and exercise considerable political influence. During 2002, 57 million liters (15 million gal) of biodiesel were produced in the United States (227), almost three times that which was produced in 2001.

The usage of soybean oil in food products is similar to other oils, and these uses and products are discussed in more detail for all oils in other chapters of this edition. This chapter will focus on specifics of soybean oil in those uses. The major products in which soybean oil is consumed are cooking and salad oils, frying oils and fats, baking shortenings, and margarine. Only minor amounts of soybean oil are used in vegetable dairy products and confectionery products.

## 11.2. Trading Rules for Crude and Refined Soybean Oils

As the U.S. government does not have trading rules, the National Oilseed Processors Association (NOPA, Washington, D.C.) has established them, including quality specifications, to facilitate trade and marketing of three types of oils: crude degummed, once-refined, and fully refined soybean oils (Table 16). These rules are also available on the Internet (228). Factors that impact grade of crude degummed and once-refined soybean oils are moisture and volatile matter content, flash point, free fatty acid content, smoke point, unsaponifiable matter content, green color, phosphorus content, and refined bleached color. The flash point reflects the presence of residual hexane, and the other factors reflect expected refining loss. For fully refined soybean oils, the flavor, cold test values, peroxide value, and AOM (Active Oxygen Method) are additional considerations that reflect crystallization at low temperatures and stability to oxidation. Crude soybean oil is sold as degummed oil because the gums tend to spontaneously hydrate and settle out during

**TABLE 16. Trading Specifications for Crude Degummed, Once-Refined and Fully Refined Soybean Oils (228).**

Factor	Crude Degummed	Once-Refined <sup>a</sup>	Fully Refined <sup>a</sup>	Methods of Analysis <sup>b</sup>
Moisture and volatile matter and insoluble impurities (%)	0.3 max. <sup>c</sup>	0.10 max.  (up to 0.15 with discount)	0.10 max. <sup>d</sup>  (up to 0.15 with discount)	Ca 2d-25  Ca 3a-46
Flash point (°C)	121 min.	121 min.		Cc 9c-95
Free fatty acids (% as oleic)	0.75 max.  (up to 1.25 with discount)	0.10 max.  (up to 0.15 with discount)	0.05 max.	Ca 5a-40
Unsaponifiable matter (%)	1.5 max.	1.5 max.	1.5 max.	Ca 6a-40
Presence of fish and marine animal oils	Neg.			28.121
Phosphorus (%)	0.02 max. (up to 0.025 with discount)			Ca 12-55
Refined bleached color (Lovibond)		3.5 Red max.	20 Yellow, 2.0 Red, max.	Cc 8e-63 Cc 13b-45
Green color		None		
Flavor			Bland	
Cold test (hr)			5.5 min.	Cc 11-53
Peroxide value (meg/kg)			2.0 max.	Cd 8-53
AOM Stability (hr to 35 PV)			8 min.	Cd 12-57

<sup>a</sup>The oil shall be clear and brilliant in appearance at 21–29°C (70–85°F) and free from settlings in this temperature range.

<sup>b</sup>Analyses in accordance with the *Official and Tentative Methods of the American Oil Chemist's Society* except for presence of fish and marine animal oils in accordance with Association of Official Analytical Chemists methods.

<sup>c</sup>Includes insoluble impurities as determined by AOCS Method Ca 3-46.

<sup>d</sup>Oil shall be free of settlings or foreign matter of any kind.

transportation and storage, which cause numerous handling problems. Once-refined soybean oil is seldom traded anymore because most buyers do their own refining or purchase fully refined oil. End-users typically have their own specifications for fully refined soybean oil and use the NOPA values as bases for their more stringent specifications (136).

### 11.3. Cooking and Salad Oils

In most parts of the world, both cooking and salad oils from soybeans are refined to have bland taste and light color. For other oils, distinct flavors and dark colors may be acceptable. Important distinctions between salad oils, cooking oils, and frying

oils, however, reflect their differences in oxidative and thermal stabilities. Cooking and frying oils need to be more stable to oxidation than salad oil because of the higher temperatures to which cooking oils are exposed. Temperature stability is especially required in fats and oils used in deep-fat frying. Salad oils must be physically stable so that they do not crystallize at refrigerated temperatures.

As soybean oil contains relatively great amounts of the polyunsaturates, notably unstable linoleate (61%) and linolenate (7.8%), partial hydrogenation is customary to make cooking or salad oils more stable to oxidation. Typical specifications for different cooking and salad oils are shown in Table 17.

Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), ascorbyl palmitate, and tertiary-butylhydroquinone (TBHQ), are used in soybean cooking oils and frying fats (230). These antioxidants are typically added at 0.01% for one antioxidant and 0.02% total for two or more. Natural antioxidants, derived from sage, rosemary, and green tea, are increasingly popular because of consumer preferences for natural food ingredients (231).

Salad oils differ from cooking oils in their tolerance to cold temperatures without crystallizing. Salad oils must not crystallize, cloud, or leave deposits of any kind when stored at refrigerator temperatures (4.4°C) and are defined as such. Soybean oil used as a salad oil should not cloud or produce any visible crystals and remain brilliant and clear for a minimum of 5.5 hr at 0°C. Fully refined soybean oil can be directly used as salad oil because it will normally meet this specification, whereas other oils, such as sunflower and corn, must be dewaxed before they can meet typical salad oil specifications. Soybean oil may be partially hydrogenated and then winterized to achieve greater oxidative stability and still not crystallize nor lose proper emulsion properties when refrigerated, although most of the soybean oil used in commercial dressings is not hydrogenated.

New nutrition-oriented salad and cooking oils have been developed in recent years. LoSatSoy is an oil low in saturated fatty acids that was developed at Iowa State University, licensed to Pioneer Hybrid International (Johnston, IA), and commercialized as a salad or cooking oil. This specialty soybean oil has one-half the amount of saturated fatty acids in normal soybean oil (7% versus 15%); therefore, it is promoted as having improved nutritional and health benefits.

Other specialty soybean oils, low (<2% or <1%) in linolenate and with improved oxidative stabilities in salad and cooking oil applications, are comparable with typical soybean oil that is partially hydrogenated. Today, low-linolenic-acid soybean oil is an attractive alternative to hydrogenated oil that contains *trans*-fatty acids. Beginning in 2006, labeled food products must disclose both the grams of saturated fat and grams of *trans*-fat per serving (232). This is inducing food companies to eliminate or significantly reduce *trans*-fatty acid contents of their products.

All specialty soybean oils require identity-preserved soybean production, crushing, and refining systems. As financial incentives are needed all along the production process to compensate for increased costs of identity preservation, specialty soybean oils command premium consumer prices and have been slow to impact soybean oil markets.

TABLE 17. Trading Specifications for Soybean Cooking and Salad Oils (229).

Factor	Cooking and Salad Oil				Analytical Method <sup>c</sup>
	Refined, Deodorized	Fully, Refined <sup>a</sup>	Fully, Refined <sup>a</sup>	HW <sup>b</sup> Soybean	
Source of specifications	Fed <sup>d,e</sup>	NSPA	ASCS <sup>f</sup>	Fed <sup>d</sup>	
Moisture and volatile matter (max) (%)	0.06 max.	0.10 max.	0.10 max. (0.14 with discount)	0.06 max.	Ca 2d-25
Unsaponifiable content (%)	—	1.5 max.	—	—	Ca 6a-40
Flash point, °C	—	—	228 min.	—	Cc 9b-55
Free fatty acids (wt%) as oleic	0.05 max. <sup>g,h</sup>	0.05 max.	0.05 max.	0.05 max. <sup>g,h</sup>	Ca 5a-40
Red color (Lovibond)	4 max.	2.0 max.	2.0 (2.6 with discount)	2.0 max.	Cc 8b-52
					Cc 8e-63 Cc 13b-45
Yellow color (Lovibond)	35 max.	20 max.	20 max.	20 max.	Cc 8b-52
					Cc 8e-63 Cc 13b-45
Peroxide value (meg/kg)	1.0 max. <sup>h</sup>	2.0 max.	0.5 max. (1.0 with discount)	1.0 max. <sup>h</sup>	Cd 8-53
Fat stability by AOM method <sup>i</sup>					
(a) Peroxide value after 8 hr	—	35 max.	35 max.	—	Cd 12-57
(b) Peroxide value of 100 or less at indicated no. of hr	15 min. <sup>h</sup>	—	—	25 min. <sup>h</sup>	Cd 12-57
Cold test (hr)					
Free from sediment and foreign matter of any kind	Yes <sup>j</sup>	Yes	Yes	Yes <sup>j</sup>	Ca 3-46
Clear and brilliant at 21-29°C	Yes	Yes	Yes	Yes	—
Fish oil and marine animal oil test	—	Neg.	—	—	k
Iodine value	—	—	—	105-115	Cd 1-25
Linolenic acid (wt%)	—	—	—	3.0 max. by or 3.5 max. by	Cd 7-58 Cd 1-62
Odor and flavor	1	1	1	1	m

**TABLE 17.** (Continued)

Additives	n	o	p	n, q	—
/preservative					
Permitted/required					

<sup>a</sup>Typically a refined, bleached, and deodorized oil.

<sup>b</sup>Refined, bleached, partially hydrogenated, winterized, and deodorized, pure soybean oil.

<sup>c</sup>Analyses in accordance with the *Official and Tentative Methods of the American Oil Chemist's Society* Champaign, Illinois, unless indicated otherwise.

<sup>d</sup>Federal specifications No. JJJ-S-30G dated March 24, 1978, issued by U.S. General Services Administration, Washington, D.C.

<sup>e</sup>The salad oil may contain properly refined and deodorized cottonseed, corn, peanut, soybean, sesame, sunflower, or safflower vegetable oils or a mixture of these oils. Olive oil shall not be used. Edible vegetable oils not specified may also be used provided they are in accordance with good commercial practice.

<sup>f</sup>Specifications per announcement PV-50-1 dated June 17, 1976, issued by Agricultural Stabilization and Conservation Service, U.S. Department of Agriculture, Shawnee Mission, Kansas.

<sup>g</sup>0.05% will be acceptable if propyl gallate has been added as an antioxidant or as a component in an antioxidant.

<sup>h</sup>Determination will be made within 7 days after packaging each lot.

<sup>i</sup>Active oxygen method.

<sup>j</sup>Exclusive of particles of resinous flux material from can manufacture.

<sup>k</sup>Association of Official Analytical Chemists' Method No. 28.107.

<sup>l</sup>The oil after heating shall be bland and free from beany, rancid, painty, musty, soapy, fishy, metallic, and other undesirable or foreign flavors and odors when tested by the method prescribed in footnote m within 7 days after packaging each lot.

<sup>m</sup>Approximately 50 g of the finished product shall be placed in a clean 150-mL Pyrex glass beaker and heated to a temperature  $177 \pm 3^\circ\text{C}$ . The oil shall be examined for odor at this temperature, and for flavor, each cooling to approximately  $38^\circ\text{C}$ . From Federal Specification JJJ-S-30G.

<sup>n</sup>Heavy metal scavengers, antifoaming agents, and antioxidant materials may be added to improve the keeping quality and use performance of the oils. The ASCA specifications also permit the addition of oxystearin. Such additives should be of a kind and at levels permitted in edible oil products under the federal Food, Drug, and Cosmetic Act and regulations promulgated thereunder.

<sup>o</sup>Preservatives "generally recognized as safe" are permitted.

<sup>p</sup>During the cooling stage of deodorization, 0.005% of citric acid or 0.006% of monoisopropyl citrate shall be added to the oil.

<sup>q</sup>The packaging gas shall be of food-grade quality and may consist of pure nitrogen or a mixture of nitrogen and approximately 10% of carbon dioxide plus other inert gases in the atmosphere, but it shall contain no more than 0.005% oxygen. Maximum permissible oxygen content of the headspace gas within 15 min after the oil is packaged is 0.50% as measured at standard temperature and pressure. Measurement shall be made at time of packaging or within 15 min thereafter. For method of analysis, see Bulletin 916, issued in 1963 by American Dry Milk Institute, Chicago, IL.

## 11.4. Frying Oils and Fats

In addition to its use as a common household cooking oil, soybean oil is used widely in home and commercial deep-fat frying procedures. The popularity of fried foods among U.S. consumers has created a large market for stable frying oils and for fast-food establishments. Typical untreated cooking and salad oils, including soybean oil, are not suitable for frying applications because they oxidize too quickly. Thus, the oils must be altered to make them stable to the frying treatment.

Heat treatments, such as commercial and household frying, accelerate autoxidation. The heat itself causes oxidation and breakdown of the fat. In addition, when

fats are heated in the presence of moisture, as often is the case in food applications, fatty acids are released via hydrolysis of the ester linkages (233). The free fatty acids, in turn, can accelerate oxidation of the oil. Decomposition and condensation of hydroperoxides also produces a multitude of nonvolatile monomeric products, including di- and tri-oxygenated esters, and dimeric and polymeric materials, especially at elevated temperature. Many of these dimers and polymers are known to be rich sources of volatile carbonyl compounds and decrease the flavor and oxidative stability of soybean oil (234). These high-molecular-weight materials also can produce a series of physical and chemical changes to the oil and food products, including increased viscosity, polarity, free-fatty acid content, development of dark color, and an increased tendency of the oil to foam (233).

A typical soybean oil shortening is generally hydrogenated to enhance its stability, making it suitable for frying procedures. In addition, polydimethylsiloxane is routinely added at a level of 0.02–2 ppm as an antifoaming agent, which greatly extends the frying life of soybean oil (235). The antioxidants mentioned in the subsection on Cooking and Salad Oils provide oil stability prior to frying and can enhance the oxidative stability of the fried food. Even though most antioxidants are volatile at frying temperatures, with their concentration decreasing during frying, some antioxidant is transferred to and retained in the food (carry through), thus providing antioxidant protection in the food during storage. In tests, heated palm olein with no frying lost 70% of its original BHT and 60% of the original BHA after 8 hr (236). TBHQ being the highest molecular weight (lowest volatility) of the typical antioxidants, provides the greatest carry-through benefit (237).

Extensive hydrogenation produces flaked fats or shortening-like products for frying applications, which offer convenience in filling fryers and excellent frying stability. Unfortunately, the process of hydrogenation creates *trans*-fatty acids as byproducts of the reaction. As noted elsewhere in this chapter, recent concerns about the presence of *trans*-fatty acids in our diets, and the subsequent new labeling requirements for *trans*-fatty acids (232), have prompted food manufacturers and oil producers to explore alternative treatments to create soybean oil that is stable to frying.

One procedure to increase stability without creating *trans*-fatty acids involves adding a small amount of a fully hydrogenated oil (hardstock) to a typical soybean oil. The blended oil is then interesterified to create a stable frying oil without *trans*-fatty acids. In a recent study, the low-linolenate soybean oil noted in the subsection on Cooking and Salad Oils, when blended with 5% of a soybean oil hardstock, was as stable as a traditional *trans*-fat-containing soybean oil that had been stabilized for deep-fat frying, while still retaining excellent flavor characteristics (238). Another approach to enhance frying stability of soybean oils is to increase the oleate concentration in the soybean oil created by the plant, either through traditional plant breeding or biotechnological methods. The resulting oil, however, when used in frying, creates a fried food with a stale, waxy-like flavor that lacks the desirable flavor components typical of a fried food (239, 240).

## 11.5. Mayonnaise and Salad Dressing

In the United States, mayonnaise, salad dressing, and French dressing are defined by Standards of Identity issued by the U.S. Food and Drug Administration (FDA; Code of Federal Regulations, Section 21, 169.140) (241). The Food, Drug and Cosmetic Act of 1930 and later revisions and amendments were promulgated to prevent adulteration and misrepresentation of certain food products by establishing Standards of Identity.

Mayonnaise is defined as a semisolid food prepared with not less than 65% vegetable oil, and egg yolk and vinegar. Most mayonnaise in the United States, however, contains 75–82% oil, to get the proper texture (242). Soybean oil is usually used in mayonnaise but winterized cottonseed, corn, and canola and hydrogenated soybean oil also can be used. Mayonnaise is an oil-in-water emulsion with oil droplets measuring 1–2  $\mu\text{m}$  in diameter. The higher the oil content, the more tightly the oil droplets are packed in the continuous water phase and thus, the greater the viscosity and rigidity. Mayonnaise production is partly an art because of the difficulty of producing an oil-in-water emulsion in which the dispersed phase has seven times more volume than the continuous phase. The protein in the egg yolk solids is the only emulsifier allowed and processing conditions play critical roles in achieving high-quality and high-stability mayonnaise.

Salad dressings are also oil-in-water emulsions and were developed as alternatives to mayonnaise. The Standard of Identity (21 CFR, 1699.150) requires that salad dressings contain not less than 30% vegetable oil (but most contain 35–50% oil), vinegar,  $\geq 4\%$  egg yolk, and starch. For texture and viscosity, salad dressings rely on starch, in contrast to mayonnaise, which depends on greater oil content. The oils used in salad dressings are selected using the same criteria for mayonnaise.

The qualities of mayonnaise and salad dressing are determined by the physical and oxidative stability of its lipid components. Phase separation or emulsion breakdown is caused by mechanical shock, agitation, extreme temperatures, or fat crystallization. Oxidation of vegetable oil and egg lipid also can occur. As the quality of oil plays a major role in the flavor stability of these products, only the best quality salad oil should be used. It is particularly important to use salad oils with long cloud point times (high cold test hours). If fat crystals form during storage at refrigerated temperatures, the emulsion will break and the product will become unsightly with visible free oil. Crystal inhibitors, such as oxystearine, lecithin, and polyglycerol esters, are allowed to prevent crystallization and emulsion breakdown.

Although mayonnaise and salad dressings are spoonable products due to their high viscosity, French dressing is a pourable oil-in-water dressing. French dressing must contain  $\geq 35\%$  oil as defined by a Standard of Identity (21 CFR, 169.115). Egg products are optional. Other dressings, such as Thousand Island, are not subject to Standards of Identity, and any ingredients can be used. Pourable dressings can be in two different finished forms; emulsion or two phases depending on whether the product is homogenized. The oil used in these products is predominantly soybean salad oil in the United States. In Canada and Europe, other salad oils are often used, depending on the availability and costs of those vegetable oils in each specific region.



As the oil contents of mayonnaise, salad dressings, and French dressing are high, it is important to prepare them from salad oils that taste bland and are relatively stable to oxidation. Peroxide values of the oil should be  $<2$  meq/kg. Even early stages of oxidation can be detected in mayonnaise and salad dressings as “grassy” and “beany” flavors. Packaging with an inert headspace is important to prevent oxidation during distribution, retailing, and consumer storage. Storage under refrigeration is important once the package is opened and the headspace gas becomes replaced with air.

### 11.6. Margarine

Margarine was first produced in 1869 by the French chemist Hippolyte Megge Mouriés. During the Franco-Prussian War, he was awarded a prize and patent for his invention of a butter substitute. It was not until the 1940s, however, that margarine became widely used. Until then, the powerful dairy industry in the United States prevented the sale of colored margarine in many states, and consumers did not readily accept white table margarine. Today, more than twice as much margarine is consumed as butter per capita in the United States, and margarine is no longer considered a cheap imitation of butter. Unlike butter, margarine can be formulated from a variety of fats and oils to give a variety of physical and functional properties, which are needed in many food applications today.

In the United States, margarine or oleomargarine is also controlled by an FDA Standard of Identity (21 CFR, 166.110), requiring at least 80% fat. Soybean oil is predominantly used in the United States, followed by cottonseed and corn oils. The other 20% of the margarine formulation may be made up of water and other optional ingredients, including milk products, soy protein isolate, salt, selected emulsifiers (up to 0.5%), mold inhibitors, antioxidants, color additives, flavorings, and acidulants. Margarine is a water-in-oil emulsion.

The traditional retail form of margarine is stick margarine, but margarine is now also marketed as pourable and soft tub products. Margarine may also be sold as a whipped product in which air or an inert gas is incorporated. Still other margarine-like forms, including polyunsaturated and low-fat spreads, have been developed to satisfy consumer demands for improved convenience and reduced saturated fat and calories. In addition to the traditional use as a table food, margarine is also widely used in baking applications such as in cookies and as roll-in fats for puff and Danish pastries.

A significant recent consumer trend is increased demand for margarine-like spreads that are not controlled by a Standard of Identity and that contain much less fat. Most spreads contain 40–60% fat with 40% fat spreads being more popular in Europe and 60% in the United States. During the past 15 years, however, very low-fat spreads containing less than 20% fat have been introduced. As a result of these trends, there are significantly fewer 80%-fat margarine products available in the United States today than in the previous decades. Stabilizing these high levels of aqueous phases in such a small amount of fat as the continuous phase requires special equipment to generate the necessary shear and higher amounts of emulsifiers.

Moustafa (243) reports that the aqueous droplets must no longer be spherical but rather polyhedral when loading levels of the aqueous phase exceed 74%.

Margarine processing includes blending the fats separately from the aqueous phase ingredients and water, dispersing and emulsifying the aqueous phase within the fat phase, chilling to solidify the fats, pin working the solidified mass, resting, forming, and packaging. The ingredients are emulsified before being fed into a swept-surface heat exchanger for crystallizing. The mass emerging from the cooling tubes is partially solidified, and it is further crystallized in the working unit. The texture of the product is further modified in the resting tube before the margarine is packaged.

Margarine and shortening have fat crystal networks in which liquid oil is entrained. As a result, they exhibit a yield stress that must be exceeded before the product begins to flow as a viscous fluid. The yield stress is related to spreadability. The rheological properties of margarine have been discussed by Segura et al. (244).

In North America, margarines may be composed of blends of hydrogenated soybean oil and palm oil, partially hydrogenated soybean oil and cottonseed oil, liquid soybean oil and partially hydrogenated soybean oil, liquid corn oil and hydrogenated corn oil, or simply hydrogenated soybean oil. Most oil blends contain high levels of soybean oil to keep costs competitive. Table 18 shows some typical compositions and properties of margarine.

The most important functional properties of margarines and spreads are spreadability and hardness, oiliness, and melting characteristics. These properties relate to fat level, proportion of solid fat, fat melting point, and crystal form. Diverse textures and functionalities can be achieved by varying the extent of hydrogenation. Consistency and emulsion stability depend on the amount and type of crystallized fat. Spreadability and hardness can be predicted by the solid fat index and penetration measurements. A cone penetrometer is typically used to determine margarine hardness (245). Typical margarines should be spreadable at refrigeration temperatures, remain semisolid at ambient temperatures, and melt at less than body temperature. Oil-off refers to the separation of liquid fat when the fat crystals no longer form a network able to hold the liquid oil.

**TABLE 18. Compositions and Properties of Hydrogenated and Interesterified Soybean Margarine Oils (187).**

Soybean Oil	Type	Melting Point				Trans (%)	IV (calc)
		10°C	21.1°C	33.3°C	(°C)		
Hydrogenated	Stick margarine	28.6	18.9	5.3	46	31.0	92.1
Hydrogenated	Tub margarine	15.6	8.8	1.3	46	23.2	108.0
Hydrogenated	Tub margarine	7.1	4.5	2.0	46	12.9	121.8
Interesterified	90:10 <sup>a</sup>	1.7	1.3	0.2	40	1.7	123.8
Interesterified	85:15 <sup>a</sup>	4.3	2.2	0.9	46	2.1	116.6
Interesterified	80:20 <sup>a</sup>	8.0	3.5	2.2	47	1.6	109.4

Fats exhibit polymorphism in which they can exist in different crystalline forms depending on how the triacylglycerols pack in the crystal and  $\alpha$ ,  $\beta'$ , and  $\beta$  polymorphs are known. The preferred polymorphic form for margarine is  $\beta'$ , which gives a smooth, pleasing mouthfeel and proper spreadability. Despite hydrogenated soybean oil's tendency to form  $\beta$  crystals, it is used in over 90% of all margarines and table spreads in the United States. The less heterogeneous the fatty acid composition of the hydrogenated fats, the more it is  $\beta$  tending. Hydrogenated fats richer in *trans*-isomers are less  $\beta$  tending and tend to produce margarines with smoother textures. Blending small amounts of  $\beta'$ -tending base fats (palm and cottonseed oils) or different soybean base oils increase fatty acid heterogeneity favoring  $\beta'$  crystal stability. Blending unmodified oils with oils that have been hydrogenated to various degrees allows the production of margarines with desirable texture. The greater the number of base stocks available, the greater the flexibility to produce a wide range of products and the higher the tolerance to processing conditions. Different procedures for designing good margarine from various base stocks were evaluated by Cho et al. (246).

Base oils for margarine must be hydrogenated to achieve the desired solid-fat content with the consequential isomerization of some fatty acids. The new regulations requiring reporting of *trans*-fats content on labels may dissuade some consumers from using traditional margarine. Emken (247) reported that some traditional margarines may have as much as 21% *trans*-fatty acids while Kellens (187) found as much as 31%, and D'Souza et al. (248) reported that the high-melting acylglycerols contained in hydrogenated base stocks used for formulating North American margarines have 33.1–45.0% *trans*-fatty acid content in stick margarine and 22.4–30.1% *trans*-fatty acid content in soft margarine. *Trans*-acyl groups contribute to the firmness of margarine. A recent comprehensive review concluded that consuming more than 4% of total calories as *trans*-fatty acids may raise plasma lipid levels (249) and may cause heart disease (250, 251).

Some companies are producing low-*trans*- or zero *trans*-margarines by random (252) or directed interesterification of mixtures of unhydrogenated and fully hydrogenated soybean oils and other fats (253). To produce these products, a liquid oil and completely hydrogenated hardstock are interesterified, so that proper plasticity can be obtained. Oils that contain considerable amounts of palmitic acid favorably influence crystallization and polymorphic form of the interesterified fat blends (254).

Chemical interesterification is conveniently achieved by using alkali metal methylates as a catalyst. Microbial lipases are also used as biocatalysts in enzymatic interesterification. In contrast to the chemical process, the enzymatic process can be more selective if an enzyme with positional specificity is used, but this reaction is usually much slower and more sensitive to reaction conditions. Recent developments in lipase-catalyzed interesterification have resulted in new industrial applications of this process (255). Nevertheless, the high costs of enzymes and process equipment may limit widespread adoption of this process.

In developing *trans*-free fat, various methods for laboratory-scale, pilot plant, and commercial batch reaction were described by Erickson (256). List et al.

**TABLE 19. Example of Combined Hydrogenation, Interesterification, and Fractionation to Produce Low *Trans*-Margarine Fat (187).**

	Iodine Value	Melting Point (°C)	Solid Fat Content (% at °C)			
			10	20	30	40
Soybean oil (SBO) feedstock	134	-7	0	—	—	—
Fully hydrogenated SBO (FHSBO)	1	71	95	94	94	93
Blending SBO and FHSBO (60:40)	81	63	44	42	39	35
Random interesterification of SBO and FHSBO (60:40)	81	53	38	33	20	11
Fractionation of the interesterified oil						
Soft fraction	91	24	25	1	0	0
Hard fraction	63	58	60	58	45	32

(252) developed a *zero-trans* margarine by interesterifying 80% refined, bleached, and deodorized (RBD) soybean oil with 20% fully hydrogenated soybean oil. The resulting product has a solid fat index comparable with that of conventional products. The randomly interesterified low- [zero-] *trans*-soybean margarines crystallize in the more favorable  $\beta'$  crystal form (252) but tend to crystallize slowly after chilling and result in a product that is harder than desired (257). Addition of 20% liquid soybean oil to the interesterified oil yielded a softer, more desirable product. Table 19 presents a typical example of the combined use of hydrogenation, interesterification, and fractionation to produce low-*trans* fats with physical properties comparable with partially hydrogenated soybean oil with high *trans* content.

Alternatively, recent research has focused on soybeans bred for high contents of saturated fatty acids, some with as much as ~43% saturates, 23% palmitate, and 20% stearate compared with the normal ~15% saturates, 11% palmitate, and 4% stearate. Soybeans only produce *cis*-fatty acids and, thus, there are no sources of *trans*-fatty acids in the blends. List et al. (258, 259) showed that soybean oil from soybeans bred to produce 30–40% saturates was not sufficiently solid to make good margarine, but soybean oil with elevated saturated fatty acid contents (17–38%) could be blended with high-melting oils, such as palm oil, interesterified palm oil, interesterified palm and soybean oils, and cottonseed and soybean hardstocks, to make a good margarine. Kok et al. (260) used blends (50:50) of traditional soybean oil and oil from soybeans bred to produce oil high in saturated fat (~43% saturates, 23% palmitate, 20% stearate). The blend was then interesterified to produce oil that was made into soft tub margarines. The small differences in sensory properties observed in comparisons with other tub margarines indicated the interesterified product should be quite acceptable to most consumers. List et al. (259) also report randomly interesterifying (randomizing) neat soybean oil high in saturated fatty acids (10% palmitate, 18% stearate) gave good margarine without graininess (SFI values of 5–8 at 10°C, 2–3 at 21.1°C, and 1–2 at 33.3°C).

## 11.7. Shortenings

Shortenings are fats of vegetable or animal origin used in baking, but the term shortening also has been accepted as a term to describe semisolid fats for frying and cooking. Just as in margarine, the solid fat exists as a tight network of small crystals, which trap liquid oil. Plastic shortenings differ from margarine in that shortening is not an emulsion; it is all lipid material and may contain emulsifiers. Prior to the development of hydrogenation, lard and tallow were the principle shortening fats, but these fats lack the diversity of texture and functionality required for many products. Today, most shortenings contain at least some soybean oil, largely because it is the least expensive oil that can confer adequate functionality. Shortening is available in many forms: plastic and semisolid (cubed, sheeted, and printed), pourable fluid (with suspended solids), encapsulated powder, and flaked. Most plastic shortenings are produced by blending oils with hydrogenated fats and often emulsifiers and solidifying or crystallizing and plasticizing the blend. The shortening is packaged and tempered by holding it in a quiescent state for several days at 30°C. During solidification, 10–25% air is often incorporated to improve the color and texture. Pourable and fluid shortenings are produced by blending appropriate oils and emulsifiers. They are crystallized by cooling the fluid mass and stirring the suspended crystals for 4–6 hr at precise temperatures so that large crystals do not develop, and the fluid becomes stabilized.

Shortenings are added to baked goods to shorten or tenderize them by interrupting the gluten structure. Shortenings improve mouthfeel and eating qualities, add lubricity, improve dough-handling properties, contribute flavor and structure, and promote desirable crumb grain and texture (261). Shortening and tenderizing effects are especially important in cakes, piecrusts, pastries, cookies, and crackers. Generally, solid fat indices that change little with temperature are desired for most shortening applications. Table 20 shows plasticity and melting properties of different commercial shortenings. Typical shortening levels are 2–5% in bread, 5–25% in cake, 20–30% in sweet goods, 30–40% in puff pastry, and 20–35% in piecrusts.

Many plastic shortenings are packaged in 50-lb polyethylene-lined boxes, primarily for use in retail bakeries, e.g., in grocery stores. These are difficult to handle in large, automated wholesale bakeries. Sometimes, 190-kg drums are used, but are still difficult to manage and use in the bakery where large amounts are needed. Pourable and pumpable fluid shortenings were developed to avoid these problems and are based on soybean oil. However, liquid oils do not cream and aerate well. The addition of small amounts of hardfats, known as stearine, and various emulsifiers can impart good functional properties to the liquid shortening.

Although adequate quality bread and rolls can be produced without shortening by using the sponge-and-dough or straight-dough methods, the inclusion of shortening increases volume by as much as 25% compared with breads with no shortening. This volume increase often is referred to as oven spring, and it reduces firmness throughout the products storage life. The largest volume of bread is made by the continuous-mixing method in the United States and shortening is critical to good quality bread manufactured when using this method. Shortening

**TABLE 20. Typical Compositions and Properties of Baking Shortenings.**

Type	Composition	Solid Fat Index			Melting Point		
		10°C	21.1°C	26.7°C	33.3°C	37.8°C	(°C)
Cookie and pie dough shortening	Partially hydrogenated soybean and palm oils (unemulsified)	26–30	18–22	16–20	12–15	9.5–13	46–48
Cake and icing shortening	Partially hydrogenated soybean and cottonseed oils (mono and diglycerides)	23–27	16–19	15–18	12–15	9–12	48–50
Yeast-raised sweet goods	Partially hydrogenated soybean and palm oils (mono and diglycerides)	24–29	14–18		9–12		44–47
Fluid cake shortening	Partially hydrogenated soybean oil (mono and diglycerides, triglycerol monostearate, sodium stearyl 2-lactylate)						
High volume cream filling and icing	Partially hydrogenated soybean and palm oils (mono and diglycerides, polysorbate 60)	25–28	19–22	18–21	14–17	11–14	47–49
Biscuit shortening	Partially hydrogenated soybean and palm oils (unemulsified)	25–30		16–20	7.5–11.5		44–47
Roll-in margarine for yeast-raised sweet goods	Partially hydrogenated soybean and palm oils (mono and diglycerides)	25–30		15–19	6–9		41–42
Fluid bread shortening	Partially hydrogenated soybean oil (mono and diglycerides)						
Fluid bread shortening	Partially hydrogenated soybean oil (mono and diglycerides, sodium stearyl 2-lactylate, ethoxylated mono and diglycerides)						

delays starch gelatinization and allows the dough to expand more before the structure is set. Maximum loaf volume, which is a desirable trait in the United States, is achieved with 6% of emulsified shortening, based on flour weight, but, in practice, 3–5% is normally used. Hardfats in bread shortenings are important in reducing collapse of the loaf's sidewall. At least 4% hydrogenated lard stearine is desired in many bread shortenings. Refined, bleached, and deodorized soybean oil is used in most commercial white pan breads.

Bread shortenings should crystallize in the  $\beta$  form. The base fat of a typical plastic bread shortening is comprised of 90% partially hydrogenated soybean oil (70 IV) and 10% lard stearine (<5 IV); whereas the base fat of a typical fluid bread shortening is comprised of 95% partially hydrogenated soybean oil (95 IV) and 5% lard stearine. Mono- and diglycerols, are added to reduce staling rate and more functional emulsifiers, such as sodium steryl-2-lactylate or ethoxylated or succinylated mono- and diglycerols, are added as dough conditioners to impart greater mixing tolerance to enable the bread to withstand abuse without loss of loaf volume (262).

Using emulsified shortening in layer cakes, cake doughnuts, and muffins increases volume and reduces air cell size and produces a fine internal grain. Creaming is defined as the mixing of the shortening over wheat flour particles and incorporating of air nuclei into the fat. The air nuclei can become sites for gas bubble formation, which is important in cakemaking. The large number of minute air bubbles incorporated into shortening improves the leavening in baked goods. For the shortening used in cakes and icings, small ( $\sim 1 \mu\text{m}$ ) needle-like  $\beta'$  crystals are preferred to the larger (5–15  $\mu\text{m}$ )  $\beta$  crystals because the  $\beta'$  shortenings appear smooth, provide good aeration, and have better creaming properties (263).

Typically, partially hydrogenated soybean oil is blended with cottonseed or palm oil hardstock to obtain  $\beta'$  crystals. Most cake shortenings contain mono- and diglycerides to decrease the size of entrained air cells during creaming, to produce finer air cells and grain in the cake crumb, and obtain a larger volume per unit weight of batter (specific volume). To achieve proper aeration of fluid cake shortenings, however, partially hydrogenated soybean oil with  $\beta$ -tending soybean hardstock is balanced with  $\alpha$ -tending emulsifiers, which are typically mono- and di-glycerides and glyceryl-lacto fatty esters.

Generally, plastic baking shortenings should be firm and plastic, but not brittle or too soft and oily. Hardfat is added to soybean oil to achieve proper texture, plasticity, and creaming properties. Plastic shortenings should be soft and plastic at low temperatures and still remain semisolid at body temperature.

Soybean oil is excellent for preparing hydrogenated base stocks from which a wide array of shortenings is made. Up to 50% soybean hardfats are blended with partially hydrogenated soybean oil in some shortenings. Soybean hardfats, however, crystallize in the  $\beta$  polymorph unless blended with an equal or greater amount of  $\beta'$  hardfat, such as hydrogenated palm or cottonseed oil. Partial hydrogenation of the base soybean oil improves the oxidative stability of the shortening. The amount of hardstock is varied to achieve the desired texture for the specific product application. Various kinds of baked goods need varied shortening functionalities and

plasticities to produce optimum quality. Plasticity is controlled by achieving the proper solid fat content or solid fat index. Typical plastic shortenings should have a relatively flat solid fat index, with solids content in the range of 15% to 30% over the temperature range of 15°C to 32°C (264). One means of getting these properties is blending 10% hardstock from two sources to get the proper crystal structure with 90% partially hydrogenated soybean oil (IV 65–80).

### 11.8. Confectionery and Imitation Dairy Products, and Low-Calorie Fat Substitutes

Very little soybean oil is used to manufacture the hard butters used in confectionery products or imitation dairy products. For imitation chocolate, enrobing fats, coffee whiteners, whipped toppings, imitation cheese, frozen desserts, and filled milk, coconut and palm kernel oils are preferred because of their sharp melting points. It is important in these applications to have very low solids at body temperature to prevent a waxy mouthfeel. A few fractionated specialty blends of hydrogenated soybean oil and hydrogenated cottonseed oils (265) or soybean oil that has been hydrogenated by using sulfur-treated nickel catalysts to achieve high selectivity (266) occasionally may be used. These fats, however, are also high in *trans*-fatty acids (>40%) and new *trans*-fat labeling requirements discourage their use. The advantages of imitation dairy and chocolate products are improved functionality compared with natural products. Thus, freeze-thaw stability in whipped toppings and melting properties can be customized for specific applications (267).

As a result of widespread concern about weight control, the production of lipid materials with reduced or zero calories has been of special interest recently. The lipid-based fat replacers are esters that resist enzymatic hydrolysis, are poorly absorbed, have relatively low-energy content, or have different modes of metabolism. Many of these materials can be made from soybean oil or contain soybean oil fatty acids. Sucrose polyester or other synthetic esters and diacylglycerol oils are examples of these low-calorie fat substitutes (268–274).

## 12. OXIDATIVE QUALITY OF SOYBEAN OIL

The oxidative stability of soybean oil is affected by its composition, handling of beans prior to extraction, processing conditions, and additives. Important compositional factors in soybean oil stability include its fatty acid composition and the presence of free fatty acids, phospholipids, natural antioxidants, and pigments (275). Important handling and processing factors include excessive bean moisture, damage, and temperature; exposure to oxygen; contamination by pro-oxidant metals; and exposure to light (276).

### 12.1. Flavor Reversion

Soybean oil has poor oxidative stability, which is a major problem for the soybean industry. Crude soybean oil has a characteristic “green-beany” flavor, which is



eliminated during refining, bleaching, and deodorization, to produce a bland-tasting, light-colored oil. During storage, however, refined soybean oil develops a characteristic flavor that often is called "reversion flavor" (277). Prior to the 1940's, some believed that soybean oil "reverted" to its unrefined flavor after being refined and deodorized. Soybean oil was considered extremely light sensitive, and it was believed to revert if one carried the freshly deodorized oil past the light of a north window. This reversion was not considered an oxidative phenomenon (278). Actually, the term "reversion" is a misnomer, because (1) soybean oil does not revert to its original crude-oil flavor, (2) the effect of light is real but was greatly exaggerated, and (3) the off-flavor development is indeed an oxidative reaction (278). Procedures available for following oxidation prior to the 1940's involved an iodometric titration to obtain a peroxide value, but this method was too insensitive to measure the low degree of oxidation that could be detected in soybean oil by sensory examination. With the support of more sensitive methods, we now know that upon oxidation, soybean oil develops "beany and grassy" flavors at the early stages (i.e., peroxide value 10 or below), rancidity at higher levels of oxidation (peroxide value of 10 or more), and "fishy" or "painty" flavors at the more advanced stages. These flavor deterioration characteristics are common to all unsaturated oils containing significant amounts of linolenate (279). It is now widely accepted that flavor deterioration of soybean oil is an oxidative phenomenon, and that linolenate is the most important precursor of flavor reversion of soybean oil.

The technology to handle soybean oil's off-flavor was discovered by an interesting set of circumstances. Near the end of World War II, Warren Goss, who was commissioned to learn the secrets of the German oilseed industry, found that a Dr. Tassusky and his daughter Ilona had patented a process involving multiple washes of crude soybean oil with water or sodium silicate solution and the addition of 0.01% citric acid to the deodorizer (278). This process worked, not because of the washings, but because of the addition of citric acid. Now we know that trace metals accelerate flavor deterioration and that treatment with citric acid or other metal deactivators is a practical and effective means of improving flavor stability (274).

## 12.2. Studies on Oil Oxidation

Extensive work has been done to clarify the mechanism of oil oxidation. It is a free-radical chain reaction catalyzed by light, heat, and metals, in which molecular oxygen reacts with unsaturated fatty acids to produce hydroperoxides. (280). An important factor in initiating the oxidation of unsaturated fats is by exposure to light in the presence of oxygen and a sensitizer. The activation of ordinary triplet oxygen in this way forms singlet oxygen, which reacts readily with unsaturated fatty acids (281). Oxygen is quite soluble in soybean oils (282), which frequently contain natural photosensitizers, such as chlorophylls or pheophytins. Singlet oxygen readily reacts with the double bonds of unsaturated fatty acids; for example, singlet oxygen reacts with methyl linoleate at a rate of at least 1500 times faster than normal triplet oxygen (282). Once oxidation is initiated by singlet oxygen, the hydroperoxides

that result can decompose to yield free radicals, and the reaction mode quickly becomes autocatalytic in the presence of triplet oxygen. A study by Carlsson et al. (283) found that the photo-oxidation of various unsaturated vegetable oils was not retarded by known free-radical scavengers, but was retarded by compounds known to quench singlet oxygen. Furthermore, the degree of retardation apparently paralleled the singlet oxygen-quenching ability of these compounds.

Commonly, the fatty acids in food lipids are exposed to heat during oil processing and food manufacture. Once peroxides are formed, they can decompose and generate free radicals, and the rate of peroxide decomposition increases with temperature. Such reactions are of extreme importance to both consumers and processors, because of their flavor significance, and under frying conditions they can affect the physical, nutritional, and toxicological properties of the fried food.

Enzymes native to plants and animals can initiate oxidation reactions. The most important and best known of these enzymes is lipoxygenase (linoleate:oxygen oxidoreductase, E.C. 1.13.11.12) (LOX) (284, 285). Enzymatic oxidations in plant systems are mediated by lipoxygenases that use molecular oxygen to catalyze the oxidation of lipids containing a *cis*, *cis*-1,4-pentadiene moiety, such as linoleate and linolenate. The reaction leads to the formation of hydroperoxides, giving the same isomers as those formed during autoxidation of linoleate and linolenate. Soybeans are a rich source of lipoxygenase isozymes known as LOX-1, LOX-2, and LOX-3, and their activity is associated with the development of off-flavors, especially green-beany flavors, in soybean products (285).

Monohydroperoxides are the primary products of lipid oxidation. A variety of hydroperoxides with positional and geometrical isomers are formed depending on the position and number of double bonds of the unsaturated fatty acids and the oxidation mechanism. A number of reviews have been published on the composition of isomeric hydroperoxides formed from oxidation of oleate, linoleate, and linolenate (286, 287–291). The hydroperoxides formed are odorless, but they are relatively unstable and are the precursors of a variety of volatile and nonvolatile scission products that are important to the oxidized flavor.

Secondary volatile scission products from primary hydroperoxide decomposition include aldehydes, alkanes, alkenes, alkynes, alcohols, and hydrocarbons. There are considerable differences, however, in the flavor significance of these volatile compounds. When estimating the impact of volatile oxidation products on flavor, it is necessary to know not only their relative concentration, but also their relative threshold values. One way of evaluating flavor impact is to divide the concentration by the threshold concentration, although the relative flavor impact may change with absolute concentration (292). Also, interactions among flavor compounds in the olfactory response may be important. The relative volatility also may play a role if a compound must be in the gas phase to reach the olfactory organ. Lee et al. (293) created equations to relate the flavor impact of individual volatiles, dispersed in an oil-water emulsion, to a specific concentration of 2-heptanone (Table 21). By this method, in a fresh and oxidized soybean oil, nonanal contributed the greatest individual effect on the flavor intensity, followed by *trans*, *trans*- and *trans*,

**TABLE 21. Concentrations (ppb in emulsion) of 2-Heptanone Perceived to Have the Same Flavor Intensity as the Components Isolated from Commercial Soybean Oil Oxidized at 35°C Under Fluorescent Light for up to 11 Days (293).**

Component	Day			
	0	4	7	11
1-Penten-3-one	0.21	0.46	1.14	1.00
Pentanal	0.27	0.27	0.34	0.35
<i>t</i> -2-pentenal	0.27	0.18	0.24	0.35
Toluene	0.80	0.44	0.37	1.24
Hexanal	2.57	4.67	6.21	6.42
Heptanal	12.35	15.50	17.47	16.19
<i>t</i> -2-Heptenal	5.37	16.02	28.55	38.86
1-Octen-3-one	1.58	1.86	2.43	2.47
1-Octen-3-ol	1.41	3.98	8.74	12.60
<i>t,c</i> -2,4-Heptadienal	17.09	29.30	42.66	48.05
2-Pentylfuran	3.54	4.51	5.26	5.52
<i>t,t</i> -2,4-Heptadienal	20.80	34.27	45.10	48.18
2-Octenal	5.14	8.07	10.82	12.09
Nonanal	76.58	108.18	113.70	101.90
<i>t,c</i> -2,4-Decadienal	none	none	15.62	26.24
<i>t,t</i> -2,4-Decadienal	none	none	25.09	43.30
Total	148.0	227.8	323.8	364.8

*cis*-2,4-heptadienal, and 2-heptenal. Hexanal produced a large GC peak, but its effect on flavor intensity was relatively small. More recently, Kao et al. (294) suggested that particles formed in the oral cavity could transport entrained triacylglycerols to the olfactory epithelium, allowing the triacylglycerols themselves to impart flavor, thus implying that compounds in oxidized soybean oil do not need to be volatile to contribute to flavor. They noted that the nutty flavor of fresh soybean oil could only be observed when the lips were parted or the tongue drawn away from the palate, both being conditions that generated particles. Liu and Hammond (295) did further work to support the hypothesis that oral particles strongly influence flavor perception of ketones typically found in oxidized soybean oils and of flavor compounds in other foods.

Numerous studies have shown that the off-flavor intensity of soybean oil is correlated with its concentration of linolenate. Although the concentrations of both linoleate and linolenate, which can reach 60–65% in typical soybean oil, undoubtedly contribute to soybean oil's instability, it is not clear why the much smaller amount of linolenate has such a strong effect on soybean oil flavor. Linolenate is expected to oxidize about twice as fast as linoleate, but there is seven to eight times more linoleate than linolenate in typical soybean oil. The flavor compounds produced by linolenate do not seem to have much lower thresholds than those produced from linoleate. Possibly flavor interactions in olfaction may account for these effects.

### 12.3. Control/Stabilization Measures

Selective hydrogenation to lower the concentrations of linolenate or linolenate and linoleate has been practiced to improve the oxidative stability of soybean oil. The linolenate concentration of soybean oil also can be altered by mutation breeding and genetic engineering (296).

Autoxidation can be inhibited or retarded by adding low concentrations of chain-breaking antioxidants that interfere with either chain propagation or initiation (286). Chain-breaking antioxidants include phenolic and aromatic compounds hindered with bulky alkyl substituents. Common synthetic chain-breaking antioxidants used in food lipids include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and propyl gallate (PG). This class of antioxidants react with peroxy free radicals to terminate reaction chains. The antioxidant radical (A•) formed in Equation 5 should be relatively stable and unable to initiate or propagate the oxidation chain reaction.



The phenolic antioxidants achieve stability by forming resonance hybrids (Figure 10) (297). A radical intermediate, such as semiquinone, can undergo a variety of reactions, including dismutation, to form a stable quinone and can regenerate the original hydroquinone (Figure 11). However, these antioxidants generally lose their efficiency at elevated temperatures, and they are most effective during the induction period. Once the antioxidant is consumed, oxidation accelerates (297).

Preventive antioxidants reduce the rate of the chain initiation. The most important initiation suppressors are metal deactivators that chelate metal ions. Metal deactivators used for stabilizing edible fat and lipid-containing foods include citric, phosphoric, tartaric acids, and phospholipids. Peroxide destroyers also are preventive

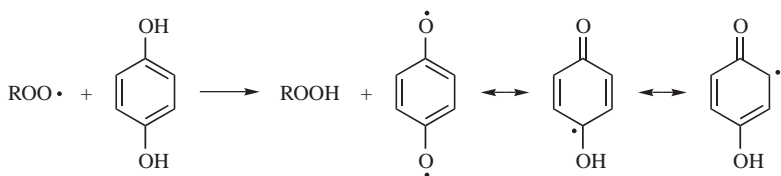


Figure 10. The formation of resonance hybrids by phenolic antioxidants.

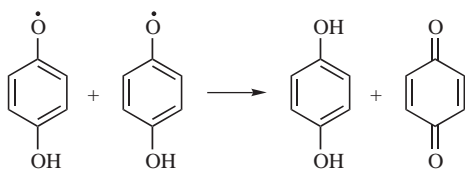


Figure 11. This dismutation of a semiquinone radical intermediate.

antioxidants; for example, the sulfur compounds, phosphates, and phosphines reduce hydroperoxides to more stable alcohols (286).

Ultraviolet light deactivators can prevent oxidation by absorbing irradiation without the formation of radicals. Examples include pigments such as carbon black, phenyl salicylate, and  $\alpha$ -hydroxybenzophenone. A significant synergistic antioxidative effect can be achieved when chain-breaking and preventive antioxidants are used together, because they suppress both initiation and propagation. The synergistic effect of common antioxidants in combination with metal inactivators in foods has been known for some time (33). Loliger (298) showed that the tertiary antioxidant system of Vitamin E, Vitamin C, and phospholipids provided the best protection against oxidative degradation when compared with the two antioxidants used alone or in combination.

Light deterioration is also an important factor in the storage stability of soybean oils. Refining and bleaching remove not only natural photosensitizers, but also singlet oxygen quenchers such as carotenoids. The restoration of the removed carotenoids may protect lipids effectively against singlet oxygen deterioration, but the resulting yellow coloration may be objectionable to consumers. Another approach to protecting stored oils from light is the use of a package or container that absorbs the light necessary for photosensitization or that prevents light from reaching the oil.

Avoiding metal contamination is also very important, as metals such as copper and iron are strong pro-oxidants for soybean oil. Copper or iron-containing alloys, except stainless steel, should never be used for equipment involved in direct contact with soybean oil. Soybean oil may be stored in containers made from carbon steel that is coated on the interior with an epoxy or polyurethane lacquer, in stainless steel, or in fiberglass-reinforced polyester.

Displacement of oxygen in container headspaces by nitrogen or carbon dioxide to  $\leq 2\%$  has been shown to reduce oxidation effectively in vegetable oil (299). Therefore, nitrogen or other inert gas protection should be considered whenever the oil is to be stored for an extended period or held in the hot, liquid state.

## 12.4. Evaluation of Finished Oil Quality

Regardless of the official specifications for soybean oil and its products, the ultimate “proof of the pudding is in the eating”; that is, sensory evaluation of the odors and flavors of soybean oil and its products is the ultimate method to assess oil quality and stability. Sensory evaluation cannot be replaced fully by any chemical or instrumental analysis, although some methods can correlate fairly well with sensory results. Sensory evaluation of oils usually is done by a panel of experts or a trained panel, and often the method recommended by the American Oil Chemists’ Society (300) is used. During the evaluation, the panel is asked to score the overall flavor quality, as well as the intensity of many individual off-flavors. Although chemical and physical tests are more reproducible and less time consuming than sensory evaluations, oxidative rancidity and off-flavor evaluation of soybean oils are best done by sensory tests. Correlations established between sensory evaluation scores and

various chemical tests, however, can be used to predict the sensory quality of finished oil products.

Peroxide value, expressed as milliequivalents of peroxide per kilogram of oil, measures the primary oxidation products of oils—the hydroperoxides. The peroxide value has shown a particularly good correlation with sensory flavor scores of soybean oil, and its use during storage is quite common. The peroxide value is an index to the oxidative state of an oil. Soybean oil is considered “fresh” with a peroxide value  $<1.0$  mEq/kg, to have low oxidation with  $1.0$ – $5.0$  mEq/kg, to have moderate oxidation at  $5.0$ – $10.0$  mEq/kg, to have high oxidation at  $>10.0$  mEq/kg, and to have poor flavor quality at  $>20$  mEq/kg (6). Several methods (300–303) can be used to measure the peroxide value of an oil depending on the specific circumstance.

One of the first steps in the oxidation of polyunsaturated fatty acids is a shift in the position of double bonds, resulting in the formation of conjugated hydroperoxides. The conjugated structure absorbs strongly at a wavelength of  $232$ – $234$  nm. The conjugated diene value (300) is expressed as the percentage of conjugated dienoic acid in the oil and is an indication of initial or primary oxidation products. Conjugated diene value can be used as a comparative method only when the oils have the same initial fatty acid composition, because the greater the amount of polyenoates in an oil, the greater the potential rise in the conjugated diene value. As a result, this method should be used as a relative measurement of oxidation in an oil only if the fatty acid composition is known (303).

As aldehydes and some ketones have long been identified as oxidation and breakdown products of fats, their determination also has been common in soybean oil quality control. The p-anisidine value (300) measures light absorbance of aldehydes, primarily 2-alkenals, and 2,4-dienals at  $350$  nm. However, this measure is not entirely specific, because the color intensity developed depends not only on the concentration but also on the structure of the aldehyde. Therefore, the results are comparable only within oils of similar type and treatment (304).

Free fatty acid (305), polar compounds (300), viscosity, and color analyses are often performed to determine the degree of abuse that oils receive during heating or frying. They are important indicators of frying oil quality, because these components affect the quality of the fried food. The free fatty acid increase during frying indicates released from triacylglyceride ester linkages via hydrolysis (233). Thus, it is an important marker for oil quality. Abused frying oil should be discarded if it contains  $>27\%$  total polar compounds, according to a German standard of frying oil quality (306). Changes in viscosity and color of the frying oil also are used as indicators of the extent of frying oil degradation.

There are many other methods for measuring lipid oxidation and quality by chemical means. Among the best-known procedures are the thiobarbituric acid (TBA) test, carbonyl value, and headspace oxygen analysis. These methods have been reviewed and discussed elsewhere (287, 307).

The volatile carbonyl compounds formed during oxidation of fats and oils are major contributors to off-flavor development. Therefore, there have been significant efforts at identifying and quantifying these compounds. It is difficult to analyze these compounds in fats and oils for several reasons. First, it is difficult to remove

them quantitatively from the fats and oils. Second, widespread contamination by carbonyls in solvents, glassware, and other laboratory materials may cause artifacts. Finally, hundreds of volatile compounds may be formed in fats and oils during oxidation causing difficulties in the interpretation. Today, the use of efficient gas chromatography (GC) columns and proper means of identification has made reliable volatile compound analysis become possible.

Three basic GC procedures are generally employed (300), including static headspace, dynamic headspace, and direct injection. Static headspace involves equilibration of gases from the area above a liquid sample; a set volume of the headspace gas from the sample is then injected directly into the GC for separation and quantification. The dynamic headspace method, also known as purge and trap, employs a sorbent, such as Tenax GC, Chromosorb, or Porapak Q, to collect volatile compounds that are swept from a heated sample with an inert gas such as helium or nitrogen. After trapping, the sorbent may be extracted with solvent, or transferred directly to the GC inlet port. In direct injection, an oil sample may be injected directly into the port of the GC through a silanized glass wool plug. Each of these methods has their own advantages and disadvantages (287).

Recently, the method of gas chromatographic solid-phase microextraction (GC-SPME) has been developed (308–310). This method uses fibers coated with various polymers to extract volatile compounds from a food system. The method can be used in solid, liquid, and gaseous systems. It is fairly easy to evaluate volatile compounds by this analysis and to maintain consistent conditions.

Evans et al. (311) and Scholz and Ptak (312) used GC analysis of n-pentane as a measurement of rancidity of vegetable oils. Dupuy et al. (313, 314) determined the volatile carbonyl compounds from soybean oil using a modified gas chromatographic inlet tube and found good correlations between the volatile profile analysis and sensory scores. The Flavor Quality and Stability Committee of the AOCS evaluated GC volatile profiling as a standard method of flavor evaluation (275). As a result, they wrote two Recommended Practices, entitled “Volatiles in Fats and Oils by Gas-Liquid Chromatography” Cg 4-94, 1997 (300) and “Correlation of Oil Volatiles with Flavor Scores of Edible Oils” AOCS method Cg 1-83, 1997 (300). These AOCS methods were validated in an AOCS collaborative study on sensory and volatile analyses, in which three methods of volatile compound analyses were compared with sensory analyses by using the AOCS flavor scales (315). Despite agreement on the usefulness of these methods, the committee stressed that only humans can measure flavor, thus these volatile GC methods measured features such as oxidative stability and compound breakdown—not sensory perceptions per se.

Not surprisingly, heat treatment, such as commercial and household frying, accelerates autoxidation. In addition to undergoing autoxidation, when fats are heated in the presence of moisture, as often is the case in food applications, fatty acids are released via hydrolysis of the ester linkages (233). The free fatty acids can accelerate oxidation of the oil. During heat treatment, the formation of dimeric and cyclic compounds seems to be the predominant thermolytic reaction of unsaturated fatty acids. In the presence of oxygen during heat treatment, however, oxidative

polymerization also can occur (233). Obviously, temperature, heating time, availability of oxygen, etc. can largely influence the extent to which these thermal and oxidative polymerization reactions occur.

Decomposition and condensation of hydroperoxides also produces a multitude of nonvolatile monomeric products, including di- and tri-oxygenated esters, and dimeric and polymeric materials, especially at elevated temperature. Many of these dimers and polymers are known to be rich sources of volatile carbonyl compounds and to decrease the flavor and oxidative stability of soybean oil (316). These high-molecular-weight materials also can produce a series of physical and chemical changes to the oil and food products, including increased viscosity, polarity, free acid content, development of dark color, and an increased tendency of the oil to foam (233).

## 12.5. Storage and Handling

Production of good quality soybean oil requires close control from harvesting of the soybeans, during bean storage, during and after oil processing, through consumption of the finished oil products to guard against oxidative, enzymatic, and microbiological deterioration. Good processing measures include careful control of refining temperature, vacuum bleaching, and inert gas blanketing. Heat accelerates the reaction of atmospheric oxygen with edible oils, therefore, localized overheating is detrimental to final oil quality. After processing, soybean oil should be stored at as low a temperature as possible and practical, and with protection from light.

Vacuum conditions are very important during bleaching, because oxidation can readily occur by exposure of a large surface area to air at elevated temperatures. During storage, a package containing the maximum amount of oil is preferable, because oxygen availability is lower with a lower headspace-to-oil ratio. Peroxide formation also is a linear function of surface-to-volume ratio (275). According to List (317), in field storage tanks, the oil is also subjected to conditions that cause development of sizable temperature gradients that can produce considerable internal oil movement. Such movement would be expected to increase the quantity of oil available at the surface and to accelerate oxygen diffusion. Therefore, soybean oil stored in filled tanks should be at as low a temperature as possible to avoid such conditions.

## 12.6. Special Processing for Off-Specification Oil

Oils from field-, frost-, moisture-, and storage-damaged beans usually have higher levels of free fatty acids and iron, lower levels of phosphorous, darker colors, and poorer flavor and oxidative stability in the finished products than do oils from undamaged beans. Such beans are difficult to process, and standard processing methods usually do not produce finished oils that can meet soybean oil specifications for trading or domestic consumption.

The National Soybean Processors' Association (318) trading rules specify that prime crude oils, after refining and bleaching by an official method (300), must



meet a Lovibond color level of 6.0. Frost-damaged oils often will not meet this requirement. Oils from frost-damaged beans tend to have an undesirable green color in the crude oils caused by compounds related to, but not identical to, chlorophyll (includes pheophytin) or some of its derivatives, according to Stern and Grossman (319). When bleaching such oils, acid-activated clays are more efficient than neutral clays and increased amounts of bleaching earth make the removal of the green color more effective. According to Stern and Grossman (319), pretreatment with charcoal (0.4–1.0%) at 90°C or treatment of a cold hexane-oil mixture with charcoal is effective in partly removing the green pigment. When charcoal pretreatment is combined with additional treatment from sugars and activated bleaching clays, complete removal of green pigments is possible. Hydrogenation can also be used to remove green color from soybean oil. According to Beal et al. (175), a green oil (IV 132) hydrogenated to IV 110 in the presence of 1% copper chromate catalyst was no longer green after cooling and filtration. However, the use of copper chromate is not a common practice.

When soybeans are exposed to rain or humid weather in the field, the beans tend to sprout and decay, and the oil from these beans develops a dark-brown color and chalky texture (312). Drought stress affects protein and oil content of soybeans but seldom damages oil quality significantly. According to List (317), off-specification oils from field-, frost-, heat-, and moisture-damaged soybeans result in high refining losses during processing, poor refined-bleached color, and lowered flavor and oxidative stability. High refining losses may be partly overcome by use of phosphoric acid or acetic anhydride degumming. Color problems of oils from damaged beans may be alleviated, in part, by use of acidic bleaching earths, increased amounts of bleaching earths, and higher bleaching temperatures. Overall, however, the best practice for producing high-quality oil is to segregate the bad beans and not include them in the processing.

## 13. DIETARY FATTY ACIDS AND THEIR HEALTH EFFECTS

### 13.1. Cholesterol and Heart Disease

Heart disease is still the number one cause of death for both men and women in the United States. High-blood-cholesterol levels increase the risk of getting heart disease (319), so, generally, serum (blood) cholesterol is measured to determine a person's risk of developing heart disease. Although some cholesterol is essential in forming the body's cell membranes and synthesizing hormones and bile acids, too much cholesterol is associated with heart disease. The fat eaten can affect the blood-cholesterol level. In addition to monitoring total blood cholesterol, the ratio of high-density lipoproteins (HDL) to low-density lipoproteins (LDL) of the blood also is important in predicting heart disease. As cholesterol, a waxy substance, does not mix with water, it needs help circulating through blood, which is mostly water. Lipoproteins transport cholesterol throughout the body. Low-density lipoproteins carry cholesterol from the liver to the body and leave deposits on artery walls. High-density lipoproteins carry cholesterol back to the liver for

elimination. If the ratio of low/high-density lipoproteins becomes too large, it is likely that more cholesterol will be deposited in the arteries than is removed. So the low/high-density lipoprotein ratio also may be used to predict a person's chances of developing heart disease. A ratio greater than 3 can indicate above average risk. The most important dietary influences on blood cholesterol levels are saturated fat, total fat, and dietary cholesterol.

### **13.2. Saturated Fat and Health Effects**

Saturated fat has more impact on raising blood cholesterol levels than anything else in the diet. The most effective way to reduce the blood cholesterol level is to reduce the amount of saturated fat in the diet. Animal products are a major source of saturated fat in the average American diet. A very few vegetable oils, including coconut, palm kernel, and palm oils, are rich in saturated fat. Other vegetable oils, including soybean oil, can become saturated by hydrogenation. Consumption of too much saturated fat has been associated with the development of heart disease, some cancers, and other health problems. As soybean oil is the major edible oil consumed in the United States, lowering its saturated fat could help reduce heart disease in this country, even though its total saturated fatty acid composition is only about 15% to 16%. As noted, the major saturates in soybean oil are palmitate and stearate. Palmitate is responsible for about 70% of the total saturated fat in soybean oil. Substitution of palmitate for carbohydrates or monounsaturates in the diet increased levels of serum low-density lipoproteins and total cholesterol (320). Stearate has been found to be relatively neutral in its effects on blood lipids, and some researchers (321, 322) showed that dietary stearate actually lowered serum low-density lipoproteins and total cholesterol levels; thus, many people recommend that this saturate not be included in the category of hypercholesterolemic acyl groups. It was for these reasons that Iowa State University scientists developed LoSatSoy™, a soybean oil with half the saturated fat of conventional soybean oil, with reduction of palmitate to  $< \sim 3\%$ .

### **13.3. Unsaturated Fat and Health Effects**

Unsaturated fats, classified as either monounsaturated or polyunsaturated, can help lower the cholesterol levels in blood when substituted for saturated fats. Sources of monounsaturated fat include nuts, olive oil, and canola oil. Sources of polyunsaturated fat include corn, safflower, sesame, soybean, and sunflower oils.

Soybean oil contains about 21% of the monounsaturate oleate. Studies have shown that the oxidation rate of oleate is much slower than that of the polyunsaturates, linoleate and linolenate, which oxidize quickly and are the major contributors to the poor stability of soybean oil (287, 323). A diet high in monounsaturates may help to reduce elevated levels of total plasma cholesterol without reducing the high-density lipoprotein-cholesterol level (324). Therefore, high-oleate soybean oil is not only more stable than conventional soybean oil (275), but also has enhanced nutritive value.

In both clinical trials and population studies, polyunsaturated fats in the diet have been shown to actively lower serum cholesterol levels. Soybean oil is considered to have good nutritive value mainly because of its high concentration of essential polyunsaturates. As noted previously, it contains about 55% linoleate and 8% linolenate, both recognized as essential fatty acids. Ingestion of approximately 1–2% of daily calories as linoleate is widely accepted as the amount needed to meet the essential fatty acid requirement of rodent species and humans (325). The physiological effects of linoleate have been well characterized. Various deficiency symptoms include depressed growth, scaly dermatoses, increased skin permeability, fatty liver, kidney damage, and impaired reproduction. The 8% linolenate of soybean oil, makes it not only an excellent source of essential fatty acids, but also a member of the n-3 fatty acid group (the third carbon atom from the terminal end of the hydrocarbon chain is involved in a double bond). A number of health benefits have been associated with the consumption of foods or oils that contain n-3 fatty acids. These associations originally derived from epidemiological studies of Eskimos who consumed high levels of n-3 fatty acid from seals and coldwater fish (326). Compared with Danish counterparts, these Eskimos were found to have a low incidence of heart disease and immune system diseases, although a somewhat higher level of hemorrhagic stroke. Still today, large-scale epidemiological studies suggest that individuals at risk for CHD benefit from the consumption of plant- and marine-derived n-3 fatty acids (327).

### 13.4. *Trans*-Fatty Acids and Their Health Effects

The process of catalytic hydrogenation of vegetable oils was discovered in 1897 to reduce the polyunsaturates and to improve flavor stability, versatility, and performance of vegetable oils in salad dressings, during cooking, in deep-fat-frying, and for the manufacture of margarines, shortenings, and other baking and snack food applications (328). A side reaction that occurs during hydrogenation is the formation of positional and geometrical isomers of the unsaturated sites that are left unsaturated. Formation of *trans*-isomers is rapid and extensive (320). Although hydrogenation can improve soybean oil oxidative stability and performance versatility, the presence of the *trans*-fatty acids may make hydrogenated oils nutritionally undesirable. In particular, the role of partially hydrogenated soybean oil in nutrition has been under scrutiny because of the health concerns over the presence of *trans*-acyl groups in our diets (329); however, the biological significance of these *trans*-acyl groups is unclear. The formation of *trans*-acyl groups in vegetable oils also can occur, to a small extent, during deodorization (330, 331) and during frying (332, 333). The 9-*cis*,12-*trans*-linoleate is present in most vegetable shortenings in much greater quantities than the 9-*trans*,12-*trans*-linoleic acid (334). In heated vegetable oils, the isomers just mentioned have been reported, plus *trans*-, *cis*-isomers of linolenate (330, 332, 335). *Trans*-isomers are essential fatty acid antagonists, especially when the linoleate and linolenate are limited in the diet. For example, the *cis*, *cis*, *trans*-isomer of 18:3 is elongated and desaturated to form n-3 *trans*-isomers of 20:5n-3 and 22:6n-3 in rats (336); isomers that also have

been found in human platelets (337). The 9-*cis*,12-*trans*-linoleic acid can be converted to 20:4n-6 containing a *trans*-double bond. Unfortunately, this *trans*-isomer of 20:4n-6 inhibited the formation of prostaglandins from all-*cis*-20:4n-6 (338). Mensink and Katan (250) reported that a diet high in *trans*-acyl groups raised total and low/high-density lipoprotein cholesterol ratio compared with a diet high in *cis*-acyl groups may be more cholesterolemic than saturates (339), and were linked to an increased risk of breast cancer development (340).

The estimated *trans*-acyl group intake by typical U.S. consumers is 11.1–27.6 g/person/day (341). A comprehensive review concluded that *trans*-acyl groups consumed at 4.0% or more of total calories may raise plasma lipid levels (342). As a result of health concerns over the presence of *trans*-acyl groups in our diet, modifying fatty acid composition of soybean oil to improve its oxidative and flavor stability in ways similar to that obtained by hydrogenation, but without *trans*-formation, has become an objective of plant breeders.

### 13.5. Total Fat and Its Health Effects

Excessive intake of any fat is not healthy. According to Klurfeld and Kritchevsky (342), the enhancement of tumor growth by dietary fat may result, in part, from the caloric contribution of this nutrient. Significant reduction of tumor incidence with consumption of 25% less energy was seen consistently in rat tumor systems induced by chemicals. Currently, most American children get about 34% of their calories from fat (318). It is recommended, however, that healthy children's intake of fat average no more than 30% of calories. Experts also suggest lowering children's saturated fat intake to less than 10% of calories. Similar recommendations have been made for adults (343).

## REFERENCES

1. Economic Research Service of United States Department of Agriculture. (October, 2002). Oil Crop Situation and Outlook Yearbook. Available: <http://usda.mannlib.cornell.edu/reports/erssor/field/ocs-bby/ocs2002.pdf>.
2. *2004 Soya & Oilseed Bluebook*, Soyatech, Inc., Bar Harbor, Maine, 2003.
3. T. J. Brumm and C. R. Hurburgh, Jr., Quality of the 2002 Soybean Crop from the United States, Report to the American Soybean Association.
4. D. M. Maestri, J. M. Mariles, and C. A. Guzmán, *Grassas Aceites*, **49**, 395–399 (1998).
5. C. R. Hurburgh, Jr., *J. Amer. Oil Chem. Soc.*, **71**, 1425–1427 (1994).
6. D. M. Maestri, D. O. Labuckas, J. M. Meriles, A. L. Lamarque, J. A. Zygadlo, and C. A. Guzman, *J. Sci. Food Agric.*, **77**, 494–498 (1998).
7. R. F. Wilson, *Agro-Food Industry Hi-Tech.*, **12**(6), 17–22 (2002).
8. R. F. Wilson, *Lipid Technol.*, **11**, 107–110 (2000).
9. J. A. Hoeck, W. R. Fehr, P. A. Murphy, and G. A. Welke, *Crop Sci.*, **40**, 48–51 (2000).
10. J.-S. Choi, T.-W. Kwon, and J.-S. Kim, *Food Biotechnol.*, **5**, 167–169 (1996).

11. C. Tsukamoto, S. Shimada, K. Igita, S. Kudou, M. Kokubun, K. Okubo, and K. Kitamura, *J. Agric. Food Chem.*, **43**, 1184–1192 (1995).
12. T. Wang, Phospholipid fatty acid composition of modified soybeans and the effect of saturated fatty acid content on seed performance, Ph.D. Dissertation, Iowa State University, Ames, Iowa, 1998.
13. T. Gutfinger and A. Letan, *Lipids*, **9**, 658–663 (1974).
14. M. Basić, L. J. Basić, J. A. Javanović, and G. Spiteller, *J. Amer. Oil Chem. Soc.*, **55**, 886–891 (1978).
15. J. D. Weete and R. C. Manley, *J. Ala. Acad. Sci.*, **50**, 35–46 (1979).
16. T. L. Mounts, S. L. Abidi, and K. A. Rennick, *J. Amer. Oil Chem. Soc.*, **73**, 581–586 (1996).
17. J. L. Weihrauch and J. M. Gardner, *J. Amer. Diet. Assoc.*, **73**, 39–47 (1978).
18. C. D. Evans, G. R. List, R. E. Beal, and L. T. Black, *J. Amer. Oil Chem. Soc.*, **51**, 444–448 (1974).
19. G. R. List, A. J. Heakin, C. D. Evans, L. T. Black, and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **55**, 521–522 (1978).
20. G. O. Almonor, G. P. Fenner, and R. F. Wilson, *J. Amer. Oil Chem. Soc.*, **75**, 591–596 (1998).
21. C. Vlahakis and J. Hazebroek, *J. Amer. Oil Chem. Soc.*, **77**, 49–53 (2000).
22. K. L. McCord, W. R. Fehr, T. Wang, G. A. Welke, S. R. Cianzio, and S. R. Schnebly, *Crop Sci.*, **44**, 772–776 (2004).
23. S.-N. Ko, C.-J. Kim, H. Kim, C.-T. Kim, S.-H. Chung, B.-S. Tae, and I.-H. Kim, *J. Amer. Oil Chem. Soc.*, **80**, 585–589 (2003).
24. L. H. Wiedermann, *J. Amer. Oil Chem. Soc.*, **58**, 159–166 (1981).
25. S. Ramamurthi, A. R. McCurdy, and R. T. Tyler, in S. S. Koseoglu, K. C. Rhee, and R. F. Wilson, eds., *Proc. World Conf. Oilseed Edible Oils Process*, vol. 1, AOCS Press, Champaign, Illinois, 1998, pp. 130–134.
26. *Yearbook and Trading Rules*, National Oilseed Processors Association, Washington, D.C., 1993.
27. H. Klein, *Ernährung/Nutrition*, **23**, 452–460 (1999).
28. D. M. Maestri, D. O. Labuckas, C. A. Guzmán, and L. M. Glorda, *Grasas Aceites*, **49**, 450–453 (1998).
29. J. Siebrecht and J. Hazebroek, *J. Amer. Oil Chem. Soc.*, **74**, 989–998 (1997).
30. X. Wu, E. G. Hammond, P. J. White, and W. Fehr, *J. Amer. Oil Chem. Soc.*, **74**, 1099–1103 (1997).
31. F. G. Sietz, *Fette Scifen Anstrichm.*, **67**, 411–412 (1965).
32. M. Wesolowski, *Food Sci. Technol.*, **95**, 377–383 (1993).
33. X. Ndzana, W. R. Fehr, G. A. Welke, E. G. Hammond, D. N. Duwick, and S. R. Cianzio, *Crop Sci.*, **34**, 646–649 (1994).
34. S. R. Schnebly, W. R. Fehr, G. A. Welke, E. G. Hammond, and D. N. Duwick, *Crop Sci.*, **36**, 1462–1466 (1996).
35. G. L. Graef, L. A. Miller, W. R. Fehr, and E. G. Hammond, *J. Amer. Oil Chem. Soc.*, **62**, 773–775 (1985).
36. T. Wang, E. G. Hammond, and W. R. Fehr, *J. Amer. Oil Chem. Soc.*, **78**, 1139–1144 (2001).

37. A. J. Kinney, *J. Food Lipids*, **3**, 273–292 (1996).
38. W. R. Fehr and E. G. Hammond (to Iowa State University Research Foundation), U.S. Patent 5,534,425, July 9, 1996.
39. R. F. Wilson, J. W. Burton, V. R. Pantalone, and R. E. Dewey, *Lipid Biotechnology*, Marcel Dekker, New York, 2002, pp. 95–113.
40. T. F. Horejsi, W. R. Fehr, G. A. Welke, D. N. Duvick, E. G. Hammond, and S. R. Cianzio, *Crop Sci.*, **34**, 331–334 (1994).
41. R. B. Hartmann, W. R. Fehr, G. A. Welke, E. G. Hammond, D. N. Duvick, and S. R. Cianzio, *Crop Sci.*, **36**, 1466–1470 (1996).
42. T. Wang, T. Harp, E. G. Hammond, J. S. Burriss, and W. R. Fehr, *Seed Sci. Res.*, **11**, 93–97 (2001).
43. W. R. Fehr, J. C. Thorne, and E. G. Hammond, *Crop Sci.*, **11**, 211–213 (1971).
44. G. Ishikawa, H. Hasegawa, Y. Takagi, and T. Tanisaka, *Plant Breeding*, **120**, 417–423 (2001).
45. T. K. Harp and E. G. Hammond, *Lipids*, **33**, 209–216 (1998).
46. T. Wang, unpublished results, September 2003.
47. C. Litchfield, *Analysis of Triglycerides*, Academic Press, New York, 1972, pp. 233–264.
48. W. E., Neff, G. R. List, and W. C. Byrdwell, *J. Liq. Chromatogr. Related. Technol.*, **22**, 1649–1662 (1999).
49. W. E. Neff and W. C. Byrdwell, *J. Amer. Oil Chem. Soc.*, **72**, 1185–1191 (1995).
50. T. Wang, E. G. Hammond, and W. R. Fehr, *J. Amer. Oil Chem. Soc.*, **74**, 1587–1594 (1997).
51. T. Wang, E. G. Hammond, J. L. Cornette, and W. R. Fehr, *J. Amer. Oil Chem. Soc.*, **76**, 1313–1321 (1999).
52. T. L. Mounts and A. M. Nash, *J. Amer. Oil Chem. Soc.*, **67**, 757–760 (1990).
53. G. R. List, T. L. Mounts, and A. C. Lanser, *J. Amer. Oil Chem. Soc.*, **69**, 443–446 (1992).
54. E. Gutierrez, *Method Development for Sphingolipid Quantification in Soybeans and Soy Products*, M. S. Thesis, Iowa State University, Ames, Iowa 2003.
55. V. Piironen, T. Koivu, O. Tammisalo, and P. Mattila, *Food Chem.*, **59**, 473–480 (1997).
56. T. Yamagami, M. Aoyama, T. Tsutsumi, S. Tokairin, H. Ehara, T. Maruyama, and I. Niiya, *Yukagakkaishi*, **48**, 1271–1274 (1999).
57. T. Yamagami, M. Aoyama, T. Tsutsumi, S. Tokairin, H. Ehara, T. Maruyama, and I. Niiya, *Chem. Abstr.*, **132**, 77768 (2000).
58. T. Koivu, V. Piironen, A.-M. Lampi, and P. Mattila, *J. Food Chem.*, **64**, 411–414 (1999).
59. R. F. Wilson, J. W. Burton, W. P. Novitzky, and R. E. Dewey, *J. Oleo Sci.*, **50**, 353–358 (2001).
60. K. Warner, P. Orr, and M. Glynn, *J. Amer. Oil Chem. Soc.*, **74**, 347–356 (1997).
61. H. Guth and W. Grosch, *Fat Sci. Technol.*, **93**, 249–255 (1991).
62. J.-W. Kao, X. Wu, E. G. Hammond, and P. J. White, *J. Amer. Oil Chem. Soc.*, **75**, 831–835 (1998).
63. J. deD. Alvarado, *Grasas Aceites*, **46**, 264–269 (1995).
64. J. deD. Alvarado, *Chem. Abstr.*, **124**, 200581 (1996).

65. V. V. Kravchenko, A. K. Pugach, and V. Z. Geller, *Izv. Vyssshikh, Uchebnykh, Zavedenii, Pishchevaya Tekhnol.*, **1987**, 79–81 (1987).
66. V. V. Kravchenko, A. K. Pugach, and V. Z. Geller, *Chem. Abstr.*, **108**, 54638 (1987).
67. H. Noureddini, B. C. Teoh, and L. D. Clements, *J. Amer. Oil Chem. Soc.*, **69**, 1184–1188 (1992).
68. Y. Tochitani and M. Fujimoto, *Netsu Bussei*, **15**, 230–236 (2001).
69. Y. Ali, M. A. Hanna, and S. L. Cuppett, *J. Amer. Oil Chem. Soc.*, **72**, 1557–1564 (1995).
70. R. Sh. Kuliev, F. R. Shirinov, and F. A. Kuliev, *Chem. Technol. Fuels Oils.*, **35**, 235–237 (1999).
71. H. Koseki, Y. Natsume, and Y. Iwata, *J. Fire Sci.*, **19**, 31–44 (2001).
72. E. W. Washburn, ed. *International Critical Tables*, vol. 5, McGraw-Hill, New York, 1929, pp. 162–169.
73. K. S. Miller, B. E. Farkas, and R. P. Singh, *J. Food Process. Preservation.*, **18**, 461–472 (1994).
74. E. S. Perry, W. H. Weber, and B. F. Daubert, *J. Amer. Chem. Soc.*, **71**, 3720–3726 (1949).
75. A. Tekin and E. G. Hammond, *J. Amer. Oil Chem. Soc.*, **75**, 737–740 (1998).
76. S. B. Detwiler, Jr. and K. S. Markley, *Oil & Soap*, **17**, 39–40 (1940).
77. K. Yokota and M. Tachimori, *Kogyo Kagaku Zasshi*, **40** (Suppl. binding) 426–429 (1937).
78. K. Yokota and M. Tachimori, *Chem. Abstr.*, **32**, 13680 (1937).
79. J. D. Halvorsen, W. C. Mammel, Jr., and L. D. Clements, *J. Amer. Oil Chem. Soc.*, **70**, 875–880 (1993).
80. C. M. Rodenbush, F. H. Hsieh, and D. S. Viswanath, *J. Amer. Oil Chem. Soc.*, **76**, 1415–1419 (1999).
81. H. F. Johnstone and I. H. Spoor, *Ind. Eng. Chem.*, **32**, 832–835 (1940).
82. F. C. Magne and E. L. Skau, *Ind. Eng. Chem.*, **37**, 1097–1102 (1945).
83. L. K. Arnold and L. J. Breuklander, *Iowa Acad. Sci.*, **57**, 157–160 (1950).
84. F. C. Magne, E. J. Hughes, and E. L. Skau, *J. Amer. Oil Chem. Soc.*, **27**, 552–555 (1950).
85. P. E. Clark, C. R. Waldeland, and R. P. Cross, *J. Ind. Eng. Chem.*, **38**, 350–353 (1946).
86. T. Kasprzycka-Guttman and D. Odzeniak, *Thermochimica Acta*, **191**, 41–45 (1991).
87. T. Wang and J. L. Briggs, *J. Amer. Oil Chem. Soc.*, **79**, 831–836 (2002).
88. N. A. Morad, A. A. Mustafa Kamal, F. Panau, and T. W. Yew, *J. Amer. Oil Chem. Soc.*, **77**, 1001–1105 (2000).
89. Y. Hagura and K. Suzuki, *Nippon Shokuhin Kagaku Kogaku Kaishi*, **49**, 272–276 (2002).
90. Y. Hagura and K. Suzuki, *Chem. Abstr.*, **138**, 38393 (2003).
91. V. Chioffi, *Chim. Provinciali*, **12**, 123–136 (1961).
92. G. J. Arissen, *Verfkroniek*, **6**, 9–11 (1996).
93. D. B. Dahlberg, S. M. Lee, S. J. Wenger, and J. A. Vargo, *Applied Spectroscopy*, **51**, 1118–1124 (1997).
94. I. Boyaci, A. Tekin, M. Çizmeçi, and I. Javidpour, *J. Food Lipids*, **9**, 175–183 (2002).
95. J. A. Ibemesi and I. O. Igwe, *J. Amer. Oil Chem. Soc.*, **68**, 147–152 (1991).
96. S. Z. Erhan, S. Asaduaukas, and A. Adhvaryu, *J. Amer. Oil Chem. Soc.*, **79**, 1157–1161 (2002).
97. E. G. Hammond and W. O. Lundberg, *J. Amer. Oil Chem. Soc.*, **31**, 427–432 (1954).

98. K. R. Majors and R. T. Milner, *Oil & Soap*, **16**, 228–231 (1939).
99. Y. Maruta and K. Tenurama, *Kogyo Kagaku Zasshi*, **40** (Suppl), 299 (1937).
100. Y. Maruta and K. Tenurama, *Chem. Abstr.*, **31**, 65303 (1937).
101. J. R. Dafler, *J. Amer. Oil Chem. Soc.*, **52**, 425–426 (1975).
102. C. F. Cole, G. M. Hill, and A. J. Adams, *J. Amer. Oil Chem. Soc.*, **71**, 1339–1342 (1994).
103. Y. H. Chu, *J. Amer. Oil Chem. Soc.*, **68**, 379–384 (1991).
104. C. Inoue, Y. Hagura, M. Ishikawa, and K. Suzuki, *J. Food Sci.*, **67**, 1126–1129 (2002).
105. N. Tomoto and K. Kusano, *Yukagaku*, **16**, 108–113 (1967).
106. N. Tomoto and K. Kusano, *Chem. Abstr.*, **66**, 106141 (1967).
107. M. Loncin, *Fette Seifen Anstrichm.*, **57**, 413–415 (1955).
108. A. S. Smith and F. J. Wechter, *J. Amer. Oil Chem. Soc.*, **27**, 381–383 (1950).
109. A. S. Smith, *J. Amer. Oil Chem. Soc.*, **28**, 360–361 (1951).
110. A. S. Smith, *J. Amer. Oil Chem. Soc.*, **28**, 356–359 (1951).
111. K. Kusano, *Yukagaku*, **8**, 8–14 (1959).
112. K. Kusano, *Chem. Abstr.*, **54**, 2002060 (1960).
113. A. I. Anikin, G. N. Danilova, and V. V. Klyuchkin, *Izvestiya Vysshikh Uchebnykh, Zavedenii Pishchevaya Tekhnologiya*, 43–45 (1987).
114. A. I. Anikin, G. N. Danilova, and V. V. Klyuchkin, *Chem. Abstr.*, **108**, 54636 (1988).
115. C. Aberhard and Z. Spekuljak, *Revista Facultad Ingenieria Quimica*, **48**, 7–12 (1988).
116. C. Aberhard and Z. Spekuljak, *Chem. Abstr.*, **112**, 156952 (1990).
117. W. H. Dickhart, *Amer. J. Pharm.*, **104**, 284 (1932).
118. G.-C. Yen, C. H. Shao, C. J. Chen, and P. D. Duh, *Food Sci. Technol.*, **30**, 648–652 (1997).
119. B. Kowalski, *Thermochimica Acta*, **173**, 117–127 (1990).
120. V. Barquin, *Tecnicas Laboratorio*, **11**, 345–349 (1987).
121. M. Wakakura, *Kenkyu Hokoku-Kanagawa-ken Kogyo Shikensho*, **55**, 76–77 (1984).
122. M. Wakakura, *Chem. Abstr.*, **103**, 217718 (1985).
123. Federal Grain Inspection Service, *Federal Grain Inspection Handbook*, Washington, D.C., 1990.
124. U.S. Department of Agriculture. Available: <http://www.usda.gov/gipsa/reference-library/brochures/soyinsepection.pfd>.
125. T. J. Brumm and C. R. Hurburgh, *J. Amer. Oil Chem. Soc.*, **67**, 302–307 (1990).
126. University of Illinois, Urbana-Champaign, Illinois. Available: <http://www.stratsoy.uiuc.edu/epv/>.
127. T. L. Mounts, G. R. List, and A. J. Heakin, *J. Amer. Oil Chem. Soc.*, **56**, 883–885 (1979).
128. G. R. List, C. D. Evans, K. Warner, R. E. Beal, W. F. Kwiolek, L. T. Black, and K. J. Moulton, *J. Amer. Oil Chem. Soc.*, **54**, 8–14 (1977).
129. C. R. Hurburgh, T. J. Brumm, J. M. Quin, and R. A. Hartwig, *J. Amer. Oil Chem. Soc.*, **67**, 966–973 (1990).
130. E. G. Hammond, L. A. Johnson, and P. A. Murphy, in C. Wrigley, C. Walker, and H. Corke, eds., *Encyclopaedia of Grain Sciences*, Academic Press, London, 2003.
131. P. Golbitz, *2003 Soya & Oilseed Bluebook*, Soyatech, Inc., Bar Harbor, Maine, 2003.



132. D. Erickson, ed., *Handbook of Soybean Processing and Utilization*, AOCS Press, Champaign, Illinois, 1995.
133. L. A. Johnson, in P. J. Wan and W. Farr, eds., *Introduction to Fats and Oils*, AOCS Press, Champaign, Illinois, 2000, pp. 108–135.
134. L. A. Johnson, in C. Akoh and D. Min., eds., *Food Lipids*, 2nd ed., Marcel Dekker, New York, 2002, pp. 223–273.
135. T. J. Brumm, C. R. Hurburgh, and L. A. Johnson, *J. Amer. Oil Chem. Soc.*, **67**, 750–756 (1990).
136. J. M. Guinn, American Soybean Association, St. Louis, MO. (2002). Available: [http://www.asasoya.org/Import/Guinn\\_Quality\\_Standards\\_Trading\\_Rules2002.htm](http://www.asasoya.org/Import/Guinn_Quality_Standards_Trading_Rules2002.htm).
137. L. A. Johnson and E. W. Lusas, *J. Amer. Oil Chem. Soc.*, **60**, 181A–193A (1983).
138. R. J. Hron, *J. Amer. Oil Chem. Soc.*, **59**, 674A–684A (1982).
139. P. J. Wan and P. J. Wakelyn, eds., *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*, AOCS Press, Champaign, Illinois, 1997.
140. R. D. Good, *Oil Mill Gaz.*, **75**(3), 14–17 (1970).
141. F. McDonald, *Oil Mill Gaz.*, **84**(12), 25 (1980).
142. M. A. Williams, *Oil Mill Gaz.*, **91**(5), 24–29 (1986).
143. M. A. Williams, *INFORM*, **6**(3), 289–293 (1995).
144. E. W. Lusas and L. R. Watkins, *J. Amer. Oil Chem. Soc.*, **65**, 1107–1114 (1988).
145. National Oilseed Processors Association, Washington, D.C. (2002). Available: [http://www.asasoya.org/Import/Guinn\\_Quality\\_Standards\\_Trading\\_Rules2002.htm](http://www.asasoya.org/Import/Guinn_Quality_Standards_Trading_Rules2002.htm).
146. A. I. Nelson, W. B. Wijeratne, S. W. Yeh, T. M. Wei, and L. S. Wei, *J. Amer. Oil Chem. Soc.*, **64**, 1341–1347 (1987).
147. N. W. Said, *INFORM*, **9**(2), 139–144 (1997).
148. T. Wang, L. A. Johnson, and W. Wijeratne, in N. T. Dunford and H. B. Dunford, eds., *Nutritionally Enhanced Edible Oil Processing*, AOCS Press, Champaign, Illinois, 2004, p. 219–238.
149. T. Wang and L. A. Johnson, *J. Amer. Oil Chem. Soc.*, **78**, 311–318 (2001).
150. T. Wang and L. A. Johnson, in *Proceedings of 2003 International Conference on Environmental Systems*, Vancouver, British Columbia, Canada, 2003.
151. L. A. Johnson, D. J. Myers, and D. J. Burden, *INFORM*, **3**, 282–290 (1992).
152. L. A. Johnson, D. J. Myers, and D. J. Burden, *INFORM*, **3**, 429–444 (1992).
153. J. C. Endres, *Soy Protein Products: Characteristics, Nutritional Aspects, and Utilization*. AOCS Press, Champaign, Illinois, 2001.
154. C. T. Egger and R. E. Olson (assigned to Grain Processing Corporation, Muscatine, Iowa), U.S. Patent 3,849,391, November 19, 1974.
155. C. Y. Wang and L. A. Johnson, *J. Amer. Oil Chem. Soc.*, **78**, 189–195 (2001).
156. P. A. Howard, M. Campbell, and D. T. Zollinger (assigned to Central Soya Co., Ft. Wayne, Indiana), U.S. Patent Re. 32,725, August 2, 1988.
157. S. Wu, P. A. Murphy, L. A. Johnson, A. R. Fratzke, and M. A. Reuber, *J. Amer. Oil Chem. Soc.*, **76**, 285–293 (1999).
158. S. Wu, P. A. Murphy, L. A. Johnson, M. A. Fratzke, and M. A. Reuber, *J. Agric. Food Chem.*, **48**, 2702–2708 (2000).
159. K. H. Lee, H. S. Ryu, and K. C. Rhee, *J. Amer. Oil Chem. Soc.*, **80**, 85–90 (2003).

160. National Archives and Records Administration, *Federal Register*, **64**, (206), 57699–57733 (1999).
161. Food and Drug Administration. Available: <http://vm.cfsan.fda.gov/%7Elrd/fr991026.html>.
162. M. Kock, *J. Amer. Oil Chem. Soc.*, **60**, 198–202 (1983).
163. C. D. Evans, P. M. Cooney, C. R. Scholfield, and H. J. Dutton, *J. Amer. Oil Chem. Soc.*, **31**, 295–297 (1954).
164. G. R. List, C. D. Evans, L. T. Black, and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **55**, 275–276 (1978).
165. *Yearbook and Trading Rules (1993–1994)*, National Oilseed Processors Association, Washington, D.C., p. 86.
166. J. C. Seger and R. L. K. M. van de Sande, in D. R. Erickson, ed., *World Conference Proceedings Edible Fats and Oils Processing: Basic Principles and Modern Practices*, AOCS Press, Champaign, Illinois, 1990, pp. 88–93.
167. A. J. Dijkstra, in T. A. Applewhite, ed., *Proceedings of the World Conference on Oilseed Technology and Utilization*, AOCS Press, Champaign, Illinois, 1992, pp. 138–151.
168. C. Pagliero, N. Ochoa, J. Marchese, and M. Mattea, *J. Amer. Oil Chem. Soc.*, **78**, 793–796 (2001).
169. L. P. Raman, M. Cheryan, and N. Rajagopalan, *J. Amer. Oil Chem. Soc.*, **73**, 219–224 (1996).
170. S. Subramanian, M. Nakajima, A. Yasui, H. Nabetani, T. Kimura, and T. Maekawa, *J. Amer. Oil Chem. Soc.*, **76**, 1247–1253 (1999).
171. G. R. List, J. W. King, J. H. Johnson, K. Warner, and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **70**, 473–476 (1993).
172. K. J. Moulton and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **67**, 33–38 (1990).
173. D. R. Erickson, in D. R. Erickson, ed., *Practical Handbook of Soybean Processing and Utilization*, AOCS Press, Champaign, Illinois, 1995, pp. 184–202.
174. T. Wang and L. A. Johnson, *J. Amer. Oil Chem. Soc.*, **78**, 461–466 (2001).
175. R. E. Beal, E. G. Lancaster, and O. L. Brekke, *J. Amer. Oil Chem. Soc.*, **33**, 619–624 (1956).
176. E. Hernandez, *Abstracts: 92nd AOCS Annual Meetings and Expo*, Minneapolis, 2001, p. S98.
177. T. Wang and L. A. Johnson, *J. Amer. Oil Chem. Soc.*, **78**, 809–815 (2001).
178. M. Y. Jung, S. H. Yoon, and D. B. Min, *J. Amer. Oil Chem. Soc.*, **66**, 118–120 (1989).
179. D. R. Erickson, in D. R. Erickson, ed., *Practical Handbook of Soybean Processing and Utilization*, AOCS Press, Champaign, Illinois, 1995, pp. 203–217.
180. D. R. Erickson and M. D. Erickson, in D. R. Erickson, ed., *Practical Handbook of Soybean Processing and Utilization*, AOCS Press, Champaign, Illinois, 1995, pp. 218–238.
181. G. R. List, W. E. Neff, R. L. Holliday, J. W. King, and R. Holser, *J. Amer. Oil Chem. Soc.*, **77**, 311–314 (2000).
182. Y. Chu and L. Lin, *J. Amer. Oil Chem. Soc.*, **69**, 880–883 (1992).
183. K. J. Moulton, R. E. Beal, and E. L. Griffin, *J. Amer. Oil Chem. Soc.*, **50**, 450–454 (1973).
184. S. Koritala, *J. Amer. Oil Chem. Soc.*, **54**, 267–268 (1977).
185. T. L. Mounts, in D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soybean Oil Processing and Utilization*, AOCS Press, Champaign, Illinois, 1980, pp. 131–144.

186. R. C. Hastert, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, vol. 4: *Edible Oil and Fat Products: Processing Technology*, 5th ed., John Wiley & Sons, Inc., New York, 1996, pp. 213–300.
187. M. Kellens, in W. Hamm and R. J. Hamilton, eds., *Edible Oil Processing*, Sheffield Academic Press, Sheffield, U.K., 2000, pp. 129–173.
188. T. Okamoto, T. Tsutsumi, S. Tokairin, H. Ehara, T. Maruyama, I. Niiya, and M. Sugano, *Jpn. Inst. Oil & Fat*, **48**, 877–883 (1999).
189. Z. Kemeny, K. Recseg, G. Henon, K. Kovari, and F. Zwobada, *J. Amer. Oil Chem. Soc.*, **78**, 973–979 (2001).
190. W. F. De Greyt, M. J. Kellens, and A. D. Huyghebaert, *Fett/Lipid*, **101**, 428–432 (1999).
191. W. De Greyt and M. Kellens, in W. Hamm and R. J. Hamilton, eds., *Edible Oil Processing*, Sheffield Academic Press, Sheffield, U.K., 2000, pp. 79–128.
192. T. W. Kwon, H. E. Snyder, and H. G. Brown, *J. Amer. Oil Chem. Soc.*, **61**, 1843–1846 (1984).
193. T. Verleyen, U. Sosinska, S. Ioannidou, R. Verhe, K. Dewettinck, A. Huyghebaert, and W. De Greyt, *J. Amer. Oil Chem. Soc.*, **79**, 947–953 (2002).
194. R. Krishnamurthy and M. Kellens, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, vol. 3: *Edible Oil and Fat Products: Products and Application Technology*, 5th ed., John Wiley & Sons, Inc., New York, 1996, pp. 193–223.
195. A. Wendel, in M. Howe-Grant, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, vol. 15, 4th ed., John Wiley & Sons, New York, 1995, pp. 192–210.
196. O. L. Brekke, in D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soy Oil Processing and Utilization*, AOCS Press, Champaign, Illinois, 1980, pp. 71–88.
197. L. Teberikler, S. Koseoglu, and A. Akgerman, *J. Amer. Oil Chem. Soc.*, **78**, 115–119 (2001).
198. Y. Z. Wu and T. Wang, *J. Amer. Oil Chem. Soc.*, **80**, 127–132 (2003).
199. Y. Wu and T. Wang, *J. Amer. Oil Chem. Soc.*, **80**, 319–326 (2003).
200. G. R. List, in B. F. Suzhai, ed., *Lecithins: Source, Manufacture & Uses*, AOCS Press, Champaign, Illinois, 1989, pp. 145–161.
201. M. D. Pickard, T. J. Jones, and R. T. Tyler, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, vol. 4: *Edible Oil and Fat Products: Processing Technology*, 5th ed., John Wiley & Sons, Inc., New York, 1996, pp. 603–630.
202. R. L. Winters, in D. R. Erickson, ed., *Proceedings: World Conference Edible Fats and Oils Processing, Basic Principles and Modern Practices*, AOCS Press, Champaign, Illinois, 1990, pp. 402–405.
203. J. P. Clark and S. S. Frandsen, in S. S. Koseoglu, K. C. Rhee, and R. F. Wilson, eds., *Proc. World Conf. Oilseed Edible Oils Process*, vol. 1, AOCS Press, Champaign, Illinois, 1998, pp. 135–138.
204. M. R. Law, *Western Journal of Medicine*, **173**, 43–47 (2000).
205. L. Normen, P. Dutta, A. Lia, and H. Andersson, *Amer. J. Clin. Nutr.*, **71**, 908–913 (2000).
206. P. Hollingsworth, *Food Tech.*, **55**, 59–62 (2001).
207. K. B. Hicks and R. A. Moreau, *Food Technol.*, **55**, 63–67 (2001).
208. H. Wu, X. Weng, O. Qiu, and L. Li, *Shanghai Daxue Xuebao-Ziran Kexueban*, **7**, 331–333 (2001).

209. Y. Shimada, S. Nakai, M. Suenaga, A. Sugihara, M. Kitano, and Y. Tominaga, *J. Amer. Oil Chem. Soc.*, **77**, 1009–1013 (2000).
210. C. J. Chang, Y. Chang, H. Lee, J. Lin, and P. Yang, *Ind. Eng. Chem. Res.*, **39**, 4521–4525 (2000).
211. C. E. Sumner, Jr., S. D. Barnicki, and M. D. Dolfi (to Eastman Chemical Co.), U.S. Patent 5,424,457, June 12, 1995.
212. P. Golbitz, *Soya & Oilseed Blue Book*, Soyatech, Inc., Bar Harbor, Maine, 2000.
213. R. S. Daniels, Can. Patent 1,256,449, June 27, 1989.
214. R. Stern, G. Hillion, P. Gateau, and J. C. Guibet, in A. R. Baldwin, ed., *Proceeding: World Conference on Emerging Technologies in the Fats and Oils Industry*, AOCS Press, Champaign, Illinois, 1986, pp. 420–422.
215. H. N. Basu, and M. E. Norris (to Agricultural Utilization Research Institute), U.S. Patent 5,525,126, June 11, 1996.
216. M. J. Haas and K. M. Scott, *J. Amer. Oil Chem. Soc.*, **73**, 1393–1401 (1996).
217. M. J. Haas, S. Bloomer, and K. Scott, *J. Amer. Oil Chem. Soc.*, **77**, 373–379 (2000).
218. M. J. Hass, K. M. Scott, P. J. Michalski, and S. Runyon (to Runyon Industries), U.S. Patent 2,003,158,074, August 21, 2003.
219. P. Golbitz, *2004 Soya & Oilseed Bluebook*, Soyatech, Inc., Bar Harbor, Maine, 2004.
220. D. R. Kodali, *INFORM*, **14**, 121–123 (2003).
221. H. Eierdanz, in T. Applewhite, ed., *Proceedings of the World Conference on Oilseed Technology and Utilization*, AOCS Press, Champaign, Illinois, 1992, pp. 221–223.
222. L. Crandall, *INFORM*, **13**, 626–631 (2002).
223. Anonymous, *Oils and Fats*, **17**(6), 18–19 (2001).
224. F. Sliney, *INFORM*, **12**, 10–18 (2001).
225. G. Knothe, *INFORM*, **13**, 900–903 (2001).
226. R. Ohlson, in T. Applewhite, ed., *Proceedings of the World Conference on Oilseed Technology and Utilization*, AOCS Press, Champaign, Illinois, 1992, pp. 221–223.
227. American Soybean Association, St. Louis, MO. (2003). [http://www.soystats.com/2003/page\\_24.htm](http://www.soystats.com/2003/page_24.htm).
228. *Yearbook and Trading Rules 2003–2004*, National Oilseed Processors Association, Washington, D.C., 2003.
229. E. F. Sipos and B. F. Szuhaj, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, vol. 2, 5th ed., John Willey & Sons, Inc., New York, 1996, pp. 497–601.
230. E. R. Sherwin, *J. Amer. Oil Chem. Soc.*, **53**, 430–436 (1976).
231. Q. Chen, H. Shi, and C. Ho, *J. Amer. Oil Chem. Soc.*, **69**, 999–1002 (1992).
232. National Archives and Records Administration, *Federal Register*, **68**, (133), (2003). Available: <http://fda.gov/OHRMS/DOCKETS/98fr/03-17525.htm>.
233. W. W. Nawar, in D. B. Min and T. H. Smouse, eds., *Flavor Chemistry of Fats and Oils*, AOCS Press, Champaign, Illinois, 1985, pp. 39–60.
234. G. Paquette and D. B. Kupranycz, *Can. Inst. Food Sci. Technol. J.*, **18**, 197–206 (1985).
235. I. P. Freeman, F. B. Padley, and W. L. Sheppard, *J. Amer. Oil Chem. Soc.*, **50**, 101–103 (1973).
236. M. A. Augustin and S. K. Berry, *J. Amer. Oil Chem. Soc.*, **60**, 1520–1523 (1983).

237. M. H. Gordon and L. Kourimska, *Food Chem.*, **52**, 175 (1994).
238. P. J. White, C. P. Su, and M. Gupta, in American Oil Chemists' Society annual meeting abstract book, pp. 45–46 (2003).
239. K. Warner and S. Knowlton, *J. Amer. Oil Chem. Soc.*, **74**, 1317–1322 (1997).
240. C. P. Su and P. J. White, in American Oil Chemists' Society annual meeting abstract book, p. 132 (2003).
241. *Code of Federal Regulations*, Food and Drug Administration, Washington, D.C.
242. H. J. Basset, *Cereal Foods World*, **24**(1), 7–9, 24, 25, 29, 30 (1979).
243. A. Moustafa, in D. Erickson, ed., *Practical Handbook of Soybean Processing and Utilization*, AOCS Press, Champaign, Illinois, 1995, pp. 339–362.
244. J. A. Segura, M. L. Herra, and M. C. Anon, *J. Amer. Oil Chem. Soc.*, **72**, 375–378 (1995).
245. A. J. Haighton, *J. Amer. Oil Chem. Soc.*, **36**, 345–348 (1959).
246. F. Cho, J. M. deMan, and O. B. Allen, *J. Food Lipids*, **1**, 25–52 (1993).
247. E. A. Emken, *Ann. Rev. Nutr.*, **4**, 339–376 (1984).
248. V. D'Souza, L. deMan, and J. M. deMan, *J. Amer. Oil Chem. Soc.*, **68**, 153–162 (1991).
249. R. Wood, in C. K. Chow, ed., *Fatty Acids in Foods and Their Health Implications*, Marcell Dekker, New York, 1992, pp. 663–688.
250. R. P. Mensink and M. B. Katan, *N. Engl. J. Med.*, **323**, 439–445 (1990).
251. H. B. Hu, M. J. Stampfer, J. E. Manson, E. Rimm, G. A. Colditz, B. A. Rosner, C. H. Hennekens, and C. W. Willet, *N. Engl. J. Med.*, **337**, 1491–1499 (1997).
252. G. R. List, E. A. Emken, W. F. Kwolek, T. D. Simpson, and H. J. Dutton, *J. Amer. Oil Chem. Soc.*, **54**, 408–413 (1997).
253. H. H. Hustedt, *J. Amer. Oil Chem. Soc.*, **53**, 390–392 (1976).
254. M. A. M. Zeitoun, W. E. Neff, G. R. List, and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **70**, 467–471 (1993).
255. C. Hoy and X. Xu, in F. D. Gunstone, ed., *Structured and Modified Lipids*, Marcel Dekker, Inc., New York, 2001, pp. 209–239.
256. M. D. Erickson, in D. R. Erickson, ed., *Practical Handbook of Soybean Processing and Utilization*, AOCS Press, Champaign, Illinois, 1995, pp. 277–296.
257. G. R. List, T. Pelloso, F. Orthoefer, M. Chrysam, and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **72**, 383–384 (1995).
258. G. R. List, T. L. Mounts, F. Orthoefer, and W. E. Neff, *J. Amer. Oil Chem. Soc.*, **73**, 729–732 (1996).
259. G. R. List, T. Pelloso, F. Orthoefer, K. Warner, and W. E. Neff, *J. Amer. Oil Chem. Soc.*, **78**, 103–104 (2001).
260. L. L. Kok, W. R. Fehr, E. G. Hammond, and P. J. White, *J. Amer. Oil Chem. Soc.*, **76**, 1175–1181 (1999).
261. C. E. Stauffer, *Fats and Oils*, Eagan Press, American Association of Cereal Chemists, St. Paul, Minnesota, 1996.
262. W. M. Smith, *Bakers Dig.*, **53**(8), 8–10 (1979).
263. H. Birnbaum, *Bakers Dig.*, **52**(2), 28–36 (1978).
264. A. E. Thomas, *J. Amer. Oil Chem. Soc.*, **55**, 830–833 (1978).
265. F. R. Paulicka, *J. Amer. Oil Chem. Soc.*, **53**, 421–424 (1976).

266. R. D. O'Brien, in D. Erickson, ed., *Practical Handbook of Soybean Processing and Utilization*, AOCS Press, Champaign, Illinois, 1995, pp. 363–379.
267. J. H. Hetrick, *J. Amer. Oil Chem. Soc.*, **46**, 58A, 60A, 62A (1969).
268. S. A. Schlicker and C. Regan, *Topics Clin. Nutr.*, **6**, 50–60 (1990).
269. T. Nagao and T. Teramoto, *Rinsho Eiyo*, **99**, 414–418 (2001).
270. M. G. Soni, H. Kimura, and G. A. Burdock, *Food Chem. Toxicol.*, **39**, 317–329 (2001).
271. N. Matsuo and I. Tokimitsu, *INFORM*, **12**, 1098–1102 (2001).
272. T. Yasukawa and K. Yasunaga, *J. Oleo Sci.*, **50**, 427–432 (2001).
273. C. Watkins, *INFORM*, **14**, 70 (2003).
274. Anonymous, *Oils and Fats*, **17**(1), 18–19 (2001).
275. C. Su, *Fatty Acid Composition of Oils, Their Oxidative, Flavor, and Heat Stabilities and the Resultant Quality in Foods*, Ph. D. Dissertation, Parks Library, Iowa State University, Ames, Iowa, (2003).
276. Y. H. Hui, in Y. H. Hui, ed., *Bailey's Industrial Oil & Fat Products*, vol. 2, 5th ed., John Wiley & Sons, Inc., New York, 1996, pp. 497–601.
277. T. H. Smouse, *J. Amer. Oil Chem. Soc.*, **56**, 747A–750A (1979).
278. H. J. Dutton, in K. Warner and N. A. M. Eskin, eds., *Methods to Assess Quality and Stability of Oils and Fat-Containing Foods*, American Oil Chemists' Society, Champaign, Illinois, 1995, pp. 1–16.
279. E. N. Frankel, in D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soy Oil Processing and Utilization*, AOCS Press, Champaign, Illinois, 1980, pp. 229–244.
280. E. H. Farmer, G. F. Bloomfield, A. Sundralingam, and D. A. Sutton, *Trans Faraday Soc.*, **38**, 348–356 (1942).
281. M. B. Korycka-Dahl and T. Richardson, *CRC Critical Reviews in Food Science and Nutrition*, **10**, 209–241 (1978).
282. P. J. Ke and R. G. Ackman, *J. Amer. Oil Chem. Soc.*, **50**, 429–435 (1973).
283. D. J. Carlsson, T. Suprunchuk, and D. M. Wiles, *J. Amer. Oil Chem. Soc.*, **53**, 656–660 (1976).
284. J. M. deMan, in C. K. Chow, ed., *Fatty Acids in Foods and Their Health Implications*, Marcel Dekker, Inc., New York, 1992, pp. 17–46.
285. R. J. Hsieh, in C.-T. Ho and T. G. Hartman, eds., *Lipids in Food Flavors*, ACS Symposium Series 558, American Chemical Society, Washington, D.C., 1994, pp. 30–48.
286. E. N. Frankel, *Prog. Lipid Res.*, **19**, 1–22 (1980).
287. P. J. White, in R. D. O'Brien, W. E. Farr, and P. J. Wan, eds, *Fats and Oils Technology*, 2nd ed., AOCS Press, Champaign, Illinois, 2000, pp. 341–353.
288. H. W. Gardner, in H. W.-S. Chan, ed., *Autoxidation of Unsaturated Lipids*, Academic Press, Inc., London, 1987, pp. 51–94.
289. E. N. Frankel, in D. B. Min and T. H. Smouse, eds., *Flavor Chemistry of Fats and Oils*, AOCS Press, Champaign, Illinois, 1985, pp. 1–37.
290. E. N. Frankel, *Prog. Lipid Res.*, **22**, 1–33 (1982).
291. E. N. Frankel, *Prog. Lipid Res.*, **23**, 197–221 (1985).
292. M. D. Dixon and E. G. Hammond, *J. Amer. Oil Chem. Soc.*, **61**, 1452–1456 (1984).

293. I. Lee, S. H. Fatemi, E. G. Hammond, and P. J. White, *J. Amer. Oil Chem. Soc.*, **72**, 539–546 (1995).
294. J.-L. Kao, E. G. Hammond, and P. J. White, *J. Amer. Oil Chem. Soc.*, **75**, 1103 (1998).
295. L. Liu, and E. G. Hammond, *J. Food Quality*, **23**, 521 (2000).
296. K. Liu, *Inform*, **10**, 868–878 (1999).
297. M. H. Gordon, in B. J. F. Hudson, ed., *Food Antioxidants*, Elsevier Applied Science, New York, 1990, pp. 13–18.
298. J. Loliger, in D. B. Min and T. H. Smouse, eds., *Flavor Chemistry of Lipid Foods*, AOCS Press, Champaign, Illinois, 1985, pp. 303–325.
299. S. J. Bishov, A. S. Henick, J. W. Giffie, I. T. Nil, P. A. Prell, and M. Wolf, *J. Food Sci.*, **36**, 532–535 (1971).
300. D. Firestone, ed., *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th ed., AOCS Press, Champaign, Illinois, 1993.
301. D. L. Hamm, E. G. Hammond, V. Parvanah, and H. E. Snyder, *J. Amer. Oil Chem. Soc.*, **42**, 920–922 (1965).
302. C. Paquot, ed., *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th ed., Pergamon Press, New York, 1987.
303. G. Yildiz, R. L. Wehling, and S. L. Cuppett, *J. Amer. Oil Chem. Soc.*, **80**, 103–107 (2003).
304. P. J. White, in K. Warner and N. A. M. Eskin, eds., *Methods to Assess Quality and Stability of Oils and Fat-Containing Foods*, AOCS Press, Champaign, Illinois, 1995, pp. 159–178.
305. I. H. Rukunudin, P. J. White, C. J. Bern, and T. B. Bailey, *J. Amer. Oil Chem. Soc.*, **75**, 563–568 (1998).
306. G. Billek, G. Guhr, and J. Waibel, *J. Amer. Oil Chem. Soc.*, **55**, 728–732 (1978).
307. J. I. Gray, *J. Amer. Oil Chem. Soc.*, **55**, 539–546 (1978).
308. J. Pawliszyn, in J. Pawliszyn, ed., *Solid Phase Microextraction: Theory and Practice*, Wiley-VCH, Inc., New York, 1997, pp. 97–140.
309. H. H. Jelen, M. Obuchowska, R. Zawirska-Wojtasiak, and E. Wasowicz, *J. Agric. Food Chem.*, **48**, 2360–2367 (2000).
310. D. D. Roberts, P. Pollien, and C. Milo, *J. Agric. Food Chem.*, **48**, 2430–2437 (2000).
311. C. D. Evans, G. R. List, R. L. Hoffman, and H. A. Moser, *J. Amer. Oil Chem. Soc.*, **46**, 501–504 (1969).
312. R. G. Scholz and L. R. Ptak, *J. Amer. Oil Chem. Soc.*, **43**, 596–599 (1966).
313. H. P. Dupuy, S. P. Fore, and L. A. Goldblatt, *J. Amer. Oil Chem. Soc.*, **50**, 340–342 (1973).
314. H. P. Dupuy, E. T. Rayner, and J. I. Wadsworth, *J. Amer. Oil Chem. Soc.*, **53**, 628–631 (1976).
315. K. Warner and T. J. Nelsen, *J. Amer. Oil Chem. Soc.*, **73**, 157–166 (1996).
316. G. Paquette and D. B. Kupranycz, *Can. Inst. Food Sci. Technol. J.*, **18**, 197–206 (1985).
317. G. R. List, in D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soy Oil Processing and Utilization*, AOCS Press, Champaign, Illinois, 1980, pp. 267–354.

318. Iowa State University Extension. (2003). Available: <http://www.extension.iastate.edu/Publications/4H956.pdf>.
319. K. G. Stern, and Grossman H. S., (to H. Sobotka), U.S. Pat. 2,842,577 (1958).
320. J. M. DeMan, in C. K. Chow, ed., *Fatty Acids in Foods and Their Health Implications*, Marcel Dekker, Inc., New York, 1992, pp. 17–46.
321. A. Bonanome and S. M. Grundy, *N. Engl. J. Med.*, **318**, 1244–1248 (1988).
322. S. M. Grundy and M. A. Denke, *J. Lipid Res.*, **31**, 1149–1172 (1990).
323. S. H. Fatemi and E. G. Hammond, *Lipids*, **15**, 379–385 (1980).
324. S. M. Grundy, L. Florentin, D. Nix, and M. F. Whelan, *Amer. J. Clin. Nutr.*, **47**, 965–969 (1988).
325. R. T. Holman, S. B. Johnson, and P. L. Ogburn, *Proc. Natl. Acad. Sci. USA*, **88**, 4835–4839 (1991).
326. W. E. M. Lands, in *Fish and Human Health*, Academic Press, Inc., Orlando, Florida, 1986.
327. P. M. Kris-Etherton, W. S. Harris, and L. J. Appel, *Circulation*, **106**, 2747–2757 (2002).
328. J. M. King and P. J. White, in L. Jackson, ed., *Impact of Processing on Food Safety*, Kluwer Academic/Plenum Publishers, New York, 1999, pp. 51–65.
329. K. Almendingen, O. Jordal, P. Kierulf, B. Sandstad, and J. I. Pedersen, *J. Lipid Res.*, **36**, 1370–1384 (1995).
330. R. L. Wolff, *J. Amer. Oil Chem. Soc.*, **69**, 106–110 (1992).
331. R. L. Wolff, *J. Amer. Oil Chem. Soc.*, **70**, 219–224 (1993).
332. J.-L. Sebedio, A. Grandgirard, C. Septier, and J. Prevost, *Rev. Francais Corps Gras*, **34**, 15–18 (1987).
333. R. L. Wolff, *J. Amer. Oil Chem. Soc.*, **70**, 425–430 (1993).
334. K. Almendingen, O. Jordal, P. Kierulf, B. Sandstad, and J. I. Pedersen, *J. Lipid Res.*, **36**, 1370–1384 (1995).
335. A. Grandgirard and F. Juilliard, *Rev. Francais Corps Gras*, **34**, 213–219 (1987).
336. A. Grandgirard, A. Piconneaux, J. L. Sebedio, S. F. O’Keefe, E. Semon, and J. L. Lequere, *Lipids*, **24**, 799–804 (1989).
337. J. M. Chardigny, J. L. Sebedio, and A. Grandgirard, in A. Sinclair and R. Gibson, eds., *Essential Fatty Acids and Eicosanoids*, AOCS Press, Champaign, Illinois, 1992, pp. 148–152.
338. L. Bretillon, J. M. Chardigny, J. P. Noël, and J. L. Sébédio, *J. Lipid Res.*, **39**, 2228–2236 (1998).
339. J. R. Judd, B. A. Clevidence, R. A. Muesing, J. Wittes, M. E. Sunkin, and J. J. Podczasy, *Amer. J. Clin. Nutr.*, **59**, 861–868 (1994).
340. L. Kohlmeier and M. Mendez, *Proc. Nutri. Soc.*, **56**, 369–383 (1997).
341. M. G. Enig, S. Atal, M. Keeney, and J. Sampugna, *J. Amer. College Nutr.*, **9**, 471–486 (1990).
342. D. M. Klurfeld and Kritchevsky, *INFORM*, **3**, 515–516 (1992).
343. U.S. Department of Agriculture and U.S. Department of Health and Human Services, *Nutrition and Your Health: Dietary Guidelines for Americans*, 3rd ed., Home and Garden Bulletin No. 232, Washington D.C., 1990.



# 14

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## Sunflower Oil

Maria A. Grompone

### 1. HISTORICAL REVIEW

Sunflower (*Helianthus annuus* L.), one of the most ancient oilseed species in North America, belongs to the family Compositae (*Asteraceae*) and the genus *Helianthus*. Cultivation of sunflower dates from times earlier than 3000 B.C., as indicated by archeological evidence obtained in sites once inhabited by the Hopi Indians, in the north of Arizona.

According to other archaeological findings and traditional tales, sunflower was cultivated by indigenous people throughout central North America (from New Mexico to the Dakotas) and eastward (Pennsylvania and Ontario). It appears that sunflower was domesticated in America even before corn was. Single-head plants were preferred by the indigenous people, who differentiated them from multiple-head plants growing wildly. Some tribes ate fruits directly, or ground into a meal that was baked in the form of bread. Other tribal practices included boiling of heads, and crushed roots, for the treatment of disease and bites. The oil extracted from seeds was used as body and hair ointment. Seeds, petals, and pollen were used in the preparation of facial and body makeup, and for dyeing cloth and utensils (1–2).

Sunflower was introduced into Europe by the Spanish explorers returning to the continent at the beginning of the 1500s A.D. The first scientific review of American plants was made by Sevillan doctor Nicolás Bautista Monardes (1508–1588), who wrote *Historia medicinal de las cosas que se traen de nuestras Indias Occidentales* (*A medical review of things brought from the West Indies*, published in Seville in

three volumes, in 1565, 1571, and 1574). It was in this study that sunflower was first mentioned. Monardes, who never traveled to America, described those plants that reached him and which he grew in a Botanic Garden designed for this purpose. He also gathered information that he obtained from navy captains, missionaries, and travelers.

There is also a description of sunflower—contemporaneous with the work of Monardes—in a herbarium made by Rembert Dodoens in 1568. Even though this and other later herbaria attribute the origin of sunflower to Peru and Central America, it is now believed to have originated in North America.

Starting from Spain, sunflower crops spread rapidly through France and Italy, and toward the north and east of Europe. In several regions, it was a source of smoking leaves, flowers for consumption in salads, or for the manufacture of paint, edible, and medicinal seed, and cooking oil. But it was, perhaps, the beauty in the inflorescence of sunflowers that interested the first growers, large and bright yellow, always facing the sun. Hence, the name of the genus, *Helianthus*, derived from the Greek *helios* meaning sun and *anthos* meaning flower; and its Spanish, English, French, and German words: *girasol*, *sunflower*, *tournesol* and *Sonnenblumen*.

It was not until the eighteenth century that sunflower seeds were used as oilseed. According to records, the first patent of oil extraction for industrial use was granted to Arthur Bunyan in 1716 in England, where reference is made to an English seed that could be pressed yielding sweet oil of great value for those interested in the manufacture of wool, paint, leather, and so on. Seeds of double- and single-head flowers, known as sunflowers, were indicated for oil extraction (2).

In Russia, it was introduced by Peter I the Great, Czar between 1682 and 1725, who, having seen sunflowers in the Netherlands, took seeds to Russia. It was in Russia where the most important development took place in the use of sunflower as both food and oil source. The Russian Orthodox Church banned the consumption of several foods during Lent and Advent (periods of the religious calendar dedicated to fasting and penitence), including several sources of oil. As the ban did not include sunflower seeds, they were adopted as an oil source.

Rapidly, sunflower spread through Russia, the earliest records of cultivation dating from 1770. The extraction of oil from sunflower seeds was first suggested in 1779, according to Russian Academy proceedings. The cultivated area increased rapidly as a result of the development of the sunflower oil extraction industry, Russia being the world's first and largest sunflower producer until current times. Once the value of the crop had been recognized, commercial production was started in 1880 over 150,000 hectares, a figure that reached one million hectares toward 1910. Pioneering and fundamental research work has been carried out in Russia since 1860 concerning the improvement of seed for oil content.

Toward the end of the nineteenth century, improved Russian cultivars were introduced in the Balkans leading to the expansion of sunflower crops. The crop did not reach northern Europe owing to the lack of cultivars adapted to cold climates.

Sunflower was the main Russian crop already at the beginning of the twentieth century. In 1912, scientist V. S. Pustovoit started research work in the fields of the Kuban region. Krasnodar was Russia's experimental oilseed selection center, since

1924. The Pustovoit All Union Research Institute was founded in 1932 and named after V. S. Pustovoit for his valuable contributions (Pustovoit was in charge of the Breeding Department until his death in 1972). Pustovoit's work led to an improvement in the oil content and seed yield. The average oil content of a Russian cultivar was 330-g oil per kilogram seed in 1940, reaching values as high as 550 g per kilogram in strains developed by Pustovoit in 1965.

Hybridization of sunflower resulting from natural cross-breeding, performed in seed-producing fields with parents planted in alternating lines, led to major advances in research. It enabled improvements of yield in USSR cultivars and rapid disease control, as well as increases of oil content and other issues of agronomical interest. Most remarkable among these open-pollinated varieties was the "Peredovik," named after the Russian agronomist. Two major events in the 1960s had a marked effect on the sunflower industry worldwide: the introduction of USSR cultivars of high oil content, and the discovery of cytoplasmic male sterility and fertility-restoring genes. Male sterile sunflowers were obtained in 1968 by Leclercq from the offspring of an interspecific hybrid between the cultivated sunflower and wild sunflower *Helianthus petiolaris*. The identification of fertility-restoring genes of several breeders led to hybrids of special characteristics. Open-pollinated cultivars were rapidly replaced by hybrids of higher yield, uniformity, and disease resistance. Currently, hybrid seeds are widely used for cheap and efficient production throughout the world (3).

Sunflower crops cultivated in North America are derived from seeds introduced by eastern European immigrants toward the end of the nineteenth century; hence, the name "Russian Peanuts." Russian emigrants in the United States and Canada grew strains such as Giant or Mammoth Russian in gardens for the production of edible seeds. These served as a base for the development of improved cultivars for commercial production. The cultivated area in the United States reached 200,000 acres in 1968; most of which was destined to the production of seed for manufacture of food for human consumption, and to the bird meal market (4).

An open-pollinated Russian-bred cultivar of high oil content (Peredovik variety, 40–45%) was introduced in the United States in 1966 (3). Commercial production of oilseed-type sunflower was started with the Peredovik variety among other cultivars, and since 1966, several research programs in the United States have sought to improve sunflower hybrids for oil yield.

Around 1960, the USSR interrupted the supply of sunflower oil to Europe, because of the high internal demand, including satellites, thus leaving an unattended sector in the European market, where consumption of tallow and butter were then indicated as causes of coronary disease. The high content of polyunsaturated fatty acids (PUFA) of sunflower oil naturally interested many American and Canadian oil industries, with the consequent increase in sunflower production in the late 1970s (1).

Russian immigrants carrying sunflower seeds introduced the crop into Argentina in the nineteenth century, for human consumption of seeds. Cultivation of the crop was performed at small-scale initially, and it was not until the world economic crisis of 1930 that it was first sown intensively to supply the internal market, in

replacement of imported oils. Around 1500 metric tons (MT) of sunflower oil were produced in Argentina in 1930, a figure that reached 5000 MT in 1945. New, disease-resistant varieties were developed as a result of the work of Experimental Stations of Argentinean National Institute of Agrarian Technology (INTA) and of private seed breeders. The appearance of hybrids characterized the Argentinean market in the period after 1975, although the first hybrid had been launched in 1972. Almost 100% of the cultivated area is currently sown with hybrids. A higher seed and oil production capacity, together with the introduction of specialized upgraded technology, led to an increase in oil yield per hectare in Argentinean plantations. Seed yield levels increased from 0.73 tons per hectare in 1977–1978—2,200,000 hectares of sunflower plantations producing 1,600,000 tons of seed—to 1.38 tons per hectare in 1987–1988—2,117,000 hectares (2,915,000 tons) (1,2).

## 2. SUNFLOWER CROPS

### 2.1. General Characteristics

The genus *Helianthus* comprises 68 known species divided into two major separate groups: the North American and the South American species. North American sunflower species spread throughout the United States, reaching Canada and Mexico. Both groups do not seem to relate to each other; in South America, they appear to have originated by parallel evolution of the genus *Viguiera* (1).

Sunflower is a highly cross-pollinated crop. Wild sunflowers have several flowers or heads and depend on the work of insects for pollination. Wild sunflowers are the genetic base of current commercial sunflowers of a single flower or head per plant.

Sunflower is an annual crop. Plants reach 1–3 m in height. The head is composed of a large number of tiny flowers that are tubular in shape (700 to 4000 single blossoms) forming a disk, those in the outer row having long strap-shaped corollas that form the rays of the composite flower. Plants have a large number of flowers clustered in a capitulum, inflorescence, or head. The back of the head is covered with small green bracts. Radial structures in the shape of petals are displayed over the bracts. These are known as ray flowers, and they do not have a reproductive function other than serving as a signal for bees and other pollinizer insects. Toward the disk center, are a large number of complete tiny flowers known as disk florets. Each of these flowers is capable of bearing an achene or seed (a fruit from a strict botanical viewpoint).

Those flowers that form the rays are generally sterile, and although they have vestigial styles and stigmas, they do not possess anthers. Flowers yielding seed are complete, each with a tubular corolla and an anther. Sunflower heads will follow the sun cycle until practically all flowers comprising the head have been pollinated. After that, they remain in a fixed position, facing eastward. Around 70 days are required from sowing to flowering of the crop. Seeds reach maturity at 130 days and can be harvested 10 days later (4–5).

Sunflower grows in moderate climates (temperate to temperate-hot), especially in America, Europe, and China, predominantly at temperatures between 20°C and

25°C, with an optimum temperature of 27–28°C. It grows well in dry, sunny weather, in deep soils capable of supplying abundant water. The oil content of seeds is lower in regions of extreme heat. The crop has high resistance to temperature fluctuations between night and day varying between 8°C and 34°C (1, 5).

The highest yield in seed is achieved at temperatures between 18°C and 25°C through the period from formation to filling of the seed. Humidity conditions are critical during both 15–20-day periods prior to and after flowering. Improved filling of the seed takes place in periods without rain. Pollination is nearly all cross-type, i.e., from one flower to another. Crossing within one head is scarce. Insects, in particular bees, are the main fertilization agent (2).

Climatic conditions in Argentina are ideal for cultivation of sunflower in view of the varying degrees of influence of the Atlantic Ocean. The buffering of thermal extremes between summer and winter (mean values in winter and summer of 8°C and 28°C), the circulation of east winds (allowing a rainfall range from 500 to 1100 mm annually), and the span of the period free of frost (over 6 months) are major climatic factors contributing to ensure the establishment and success of the cycle of sunflower (6). In Argentina, the sunflower is grown between latitudes 26°S (Chaco) and 39°S (southern Buenos Aires), over an area averaging 2.93 million hectares for 1990–2000 summer seasons. The cultivation environments include subtropical (northern Argentina) and temperate (central and southern) climate (7).

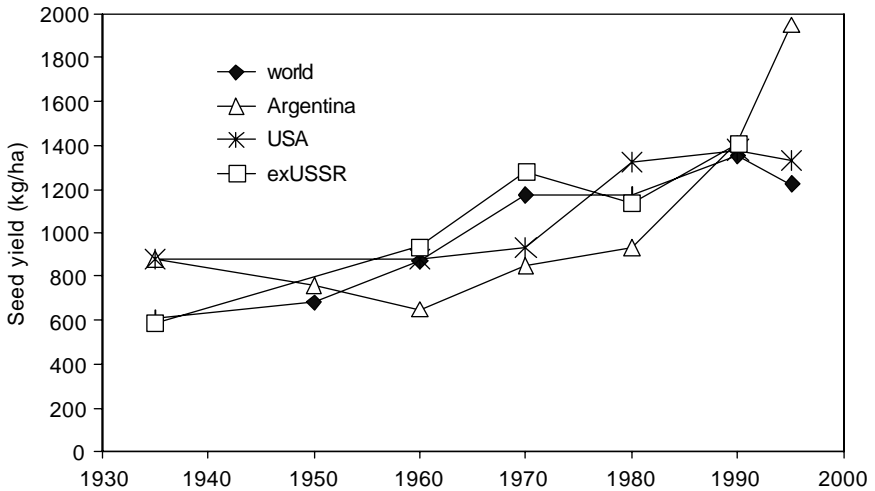
Sunflowers are ripe when the back of the head has turned from green to yellow and the bracts are turning brown. Harvest is done when the seed reaches commercial ripeness, that is, allowing time for the seed to dry from a 35% moisture content of heads at physiological maturity down to 11%. The harvest of seed-loaded, heavy fruits is advanced to prevent seeds falling off (2, 8).

Drying agents such as magnesium chlorate may be used as an aid to advance harvesting. This practice readily reduces the moisture content of heads, stems, and leaves, but seeds retain most of their moisture. Artificial drying of seeds is often necessary prior to storage.

Seeds must be stored with moisture levels lower than 9.5% to avoid undesired enzymatic reactions. Some of these reactions start within 12 hours after harvesting for seeds with moisture higher than 20% (5). Seeding and harvesting periods obviously differ according to hemisphere of producer country. For Argentina and Uruguay, in the South Hemisphere, seeding is in October and harvesting between February and April, and in Australia, harvesting is between January and May. For countries in the Northern Hemisphere, such as ex-USSR, seeding is done in March and harvesting is between August and October; in the United States seeding is in April or May and harvesting is in September or October; in Spain and Italy, harvesting is in August (1, 5).

## 2.2. Yield of Sunflower Crops

Seed yield varies according to region. Maximum yield values in 1994 were obtained in Italy (2596 kg/ha), Greece (2577 kg/ha), and Austria (2544 kg/ha); minimum values were obtained in Tanzania (370 kg/ha). These values have been



**Figure 1.** Evolution of seed yield (kg/ha) in the most important producer countries, as well as world average values (1, 5).

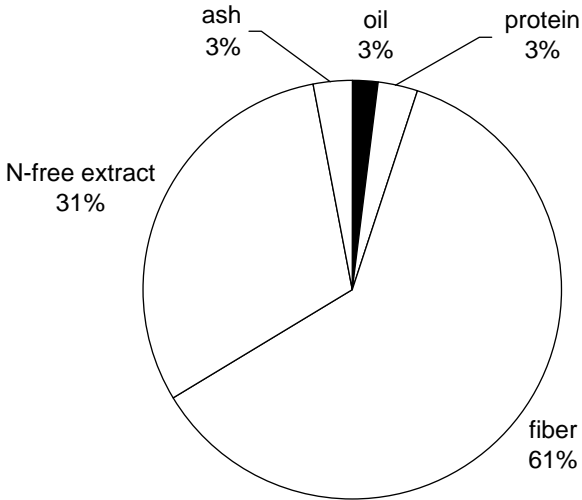
increased in the last years as a result of genetic improvements. Figure 1 shows the evolution of yield levels in major producer countries, as well as world average values (1, 5).

### 2.3. Structure of Sunflower Seeds

An achene, the seed of sunflower, is pointed at the base and rounded at the top. Seed size ranges between 10 and 15 mm in length and between 4 and 12 mm in width, appearing to be four-sided in cross-section. The outer layer, the pericarp or hull, represents 18–45% of the total achene weight. The white papery layer immediately beneath the pericarp, the testa or seedcoat, is made up of three parenchyma layers, the inner layer being spongy in texture. The endosperm comprises a single layer of cells rich in protein, firmly attached to the hull, and an embryo, commonly referred to as kernel. The embryo consists of two cotyledons attached to a protruding radicle (9).

There are two basic types of sunflower: (1) oilseed type and (2) nonoil type, the latter supplying the bird meal and confectionary markets. The first hybrid oilseed types bore small black seeds with a thin hull (representing 20–25% of total seed weight) with a 40% oil content. The non-oilseed type is somewhat different; it has a larger seed with a thicker black-and-white-striped hull (representing 40–45% of total seed weight), which is weakly attached to the kernel and can easily be removed. These seeds contain 30% of oil.

The size of oil-type seeds varies according to cultivar and according to the seed's position in the head, those on the periphery being larger. Besides affecting the oil content, the position of seeds in the head influences the fatty acid composition.



**Figure 2.** Hull composition (1, 5, 11).

Internal seeds contain less oil than those in intermediate or external zones. The content of linoleic and palmitic acids increases, and the content of oleic acid decreases from the perimeter toward the flower head center (10).

One thousand seeds of most currently used hybrids weigh from 30 to 80 g. Hull color ranges from completely white to black, with gray- or brown-striped intermediates. Both hull thickness and structure, as well as other seed characteristics, depend on variety and ambient growth conditions (9).

The hull is mainly composed of fibrous substances, lignin and cellulosic materials in equal proportions. Kernels of oilseed-type sunflower contain nearly all of the oil of seeds, besides proteinaceous substances and carbohydrates. The kernel represents 70% of the seed, with an oil content of approximately 55%, amounting to 40% with respect to the whole seed. The protein content ranges between 20% and 35%, amounting to up to 57% on a water-and-oil-free basis (1, 5, 11).

A commonly occurring hull and kernel composition is shown in Figures 2 and 3, respectively, as well as of sunflower meal in Figure 4. Data correspond to a fully dehulled meal, a condition difficult to obtain in practice (1, 5, 11).

## 2.4. The Influence of Ambient Factors on Sunflower Seed Oil

The oil content of sunflower seeds varies during the development of the seed: increasing from the fourteenth to the thirty-fifth day after flowering, when the seed is physiologically mature. The oil content remains steady after reaching maturity. Oil composition also changes during the formation and ripening stages of the seed. The linoleic acid content increases from the fourteenth day after flowering while the oleic acid content decreases; also, saturated fatty acids decrease slightly (12).

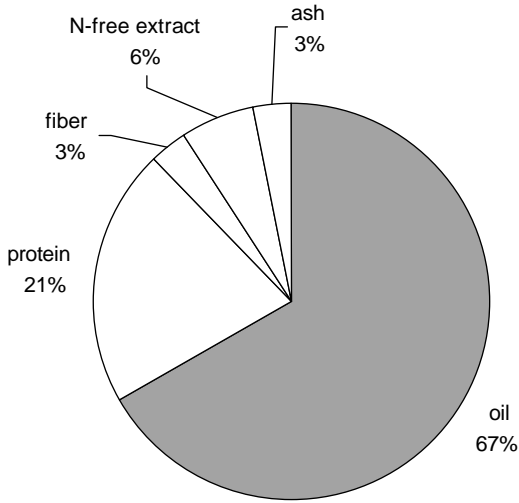


Figure 3. Kernel (dehulled seed) composition (1, 5, 11).

Ambient factors, such as temperature and light, affect the oil composition of sunflower seeds. Robertson and Russell (13) studied the effect of climatic conditions (temperature difference between night and day in Canada, Minnesota, and California) on the composition of sunflower oil, finding that linoleic acid increased proportionally with increasing temperature difference. Robertson and Green (14) studied the effect of sowing time on oil content and composition. Eleven different hybrids

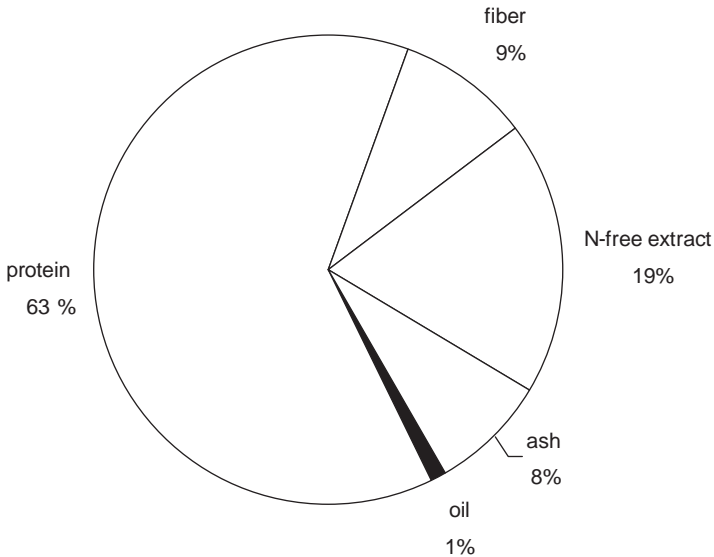


Figure 4. Composition of fully dehulled meal (1, 5, 11).



were used. Sowing was performed in February and August in Florida. The results show that the oleic acid content is lower (average: 19.4%) and that the linoleic acid content is higher (average: 68.4%) for seeds sown in August (lowest mean temperature from flowering to maturity, 18°C). Seeds harvested in plantations sown at the beginning of April (highest mean temperature from flowering to maturity, 27°C) have a lower content of linoleic acid (average: 36.3%) and a higher average content of oleic acid (54.6%). This indicates that adjustments in seeding time in Florida may lead to sunflower oil of different compositions.

Average temperature from flowering to ripening appears to be a major factor affecting the fatty acid composition of sunflower oil (15). The oleic/linoleic ratio was correlated with average temperature for different sunflower plantations across Spain, average temperature being related not only to latitude, but also to other geographic factors that determine a microclimate of the crops. An increase in temperature resulted in a decrease in the linoleic acid content and an increase in the oleic acid content. The linoleic acid content ranged from 48.7% in seeds grown in warmer weather in the south of Spain to 70.2% for colder weather plantations. The addition of the contents of linoleic and oleic acids is not constant with temperature, the increase in oleic acid being greater than the decrease in linoleic acid, with a partial compensation by a reduction in the stearic acid content, suggesting the conversion of stearic acid to oleic acid. The above mechanism is related to the effect of temperature on the activity of desaturase enzymes converting oleic into linoleic acid.

Research carried out with sunflower crops grown in controlled temperature chambers 20/10°C (day/night) and 30/20°C (day/night) with equal light intensity and photoperiod, demonstrated a decrease in the linoleic/oleic ratio with increasing temperature (16). An increase in temperature also leads to a slight decrease in the stearic acid content. Similar results were obtained by authors in different countries, like France, Italy, Japan, the United States, Canada, and so on (17). This is because those enzymes involved in the sequence of steps leading to the formation of linoleic acid are alike in all higher plants; that is, all oilseed crops have a common enzyme base catalyzing the synthesis of fatty acids, producing varying amounts of 16:0, 18:0, 18:1, 18:2, and 18:3.

Among a number of studies carried out in different regions worldwide, the composition of sunflower seeds was determined for six different Argentinean regions (18). For the latitudes considered, according to region, mean ambient temperature during the development period of seed decreased from 28°C to 20°C southward. The linoleic acid content was found to decrease, and the oleic acid content increased with increasing temperature. A similar behavior was observed for sunflower seeds grown in Japan (19).

## 2.5. Sunflower Associations

The International Sunflower Association (ISA), with main offices in Paris aims to enhance international cooperation toward the improvement of cultivation, growth, and technical and nutritional levels, besides promoting and facilitating close

cooperation relations among researchers. ISA holds an International Conference on Sunflower every four years.

In addition, there is a national association in different countries. The U.S. National Sunflower Association (NSA) with main offices in Bismarck, North Dakota, promotes the production and trade of sunflower products both at national and international levels. Founded in 1981, it currently has 20,000 members. The Australian Sunflower Association was established in 1976; the membership of the ASA consists of growers, researchers, and personnel from all facets of the industry. The National Sunflower Association of Canada (NSAC) was founded in 1996, with 105 members in 1999. The Argentinean Sunflower Association (ASAGIR), created in 1980, hosted the Eleventh International Conference on Sunflower in 1985.

### 3. CHEMICAL AND PHYSICAL PROPERTIES OF REGULAR SUNFLOWER OIL

#### 3.1. Composition of Regular Sunflower Oil

Sunflower oil—like most vegetable oils—is composed mainly of triacylglycerols (98–99%), and a small fraction of phospholipids, tocopherols, sterols, and waxes (all of the latter are commonly referred to as the “unsaponifiable fraction”).

**3.1.1. Sunflower Fatty Acids** Regular sunflower oil is characterized by a high concentration of linoleic acid, followed by oleic acid. Saturated fatty acids (mainly palmitic acid and stearic acid) do not amount to more than 15% of the fatty acid content. Table 1 shows the variation range of major fatty acids in regular sunflower oil (9, 20).

Two facts regarding the composition of regular sunflower oil are worth noting from the nutritional viewpoint: It provides an essential fatty acid (linoleic acid), and it has a low content of palmitic acid compared with other oils (palmitic acid is believed to increase LDL-C in blood).

The reported composition of regular sunflower oil has changed with adjustments of analytical methods and the samples considered. This is reflected in the variation ranges approved successively by the Codex Alimentarius Commission. The values approved in 1981 and 1993 (21) are compiled in Table 2, as well as the current

**TABLE 1. Variation Range for Major Fatty Acids (%) of Regular Sunflower Oil (9, 20).**

Fatty Acid	AOCS (20)	Merrien (9)
16:0	5–8	5–7
18:0	2.5–7.0	4–6
18:1	13–40	15–25
18:2	40–74	62–70
18:3	<0.3	<0.2

**TABLE 2. Variation Range for Fatty Acids (%) of Regular Sunflower Oil According to Standards Approved by the Codex Alimentarius Commission in Different Years.**

Fatty Acid	1981	1993	1999
12:0			ND-0.1
14:0	<0.5	<0.2	ND-0.2
16:0	3-10	5.6-7.6	5.0-7.6
16:1	<1.0	<0.3	ND-0.3
17:0			ND-0.2
17:1			ND-0.1
18:0	1-10	2.7-6.5	2.7-6.5
18:1	14-65	14-39.4	14.0-39.4
18:2	20-75	48.3-74.0	48.3-74.0
18:3	0-0.7	0-0.2	ND-0.3
20:0	0-1.5	0.2-0.4	0.1-0.5
20:1	0-0.5	0-0.2	ND-0.3
22:0	0-10	0.5-1.3	0.3-1.5
22:1	0-0.5	0-0.2	ND-0.3
22:2	ND	0-0.3	ND-0.3
24:0	0-0.5	0.2-0.3	ND-0.5
24:1	<0.5	ND	ND

ND = nondetectable, defined as 0.05%.

value of 1999 (Codex-Stan 210-1999). The Codex Alimentarius Commission (Codex) was established in 1962 by two United Nations organizations, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). Codex is the major international organization for encouraging fair international trade in food and protecting the health and economic interests of consumers. The Codex Committee on Fats and Oils (CCFO) was established to elaborate worldwide standards for fats and oils and their products. The Codex Alimentarius is thus taken as reference.

According to the composition indicated by the Codex Alimentarius (Codex-Stan 210-1999), the saturated fatty acid content of regular sunflower oil is lower than that in corn (maximum 22%), cottonseed (maximum 32%), peanut (maximum 28%), and soybean (maximum 20%) oils, and higher than the saturated content of safflower (maximum 12%) and rapeseed (maximum 12%) oils. The linolenic acid content (18:3) of regular sunflower oil is fairly low (always lower than 0.3%), giving the oil a good oxidative stability.

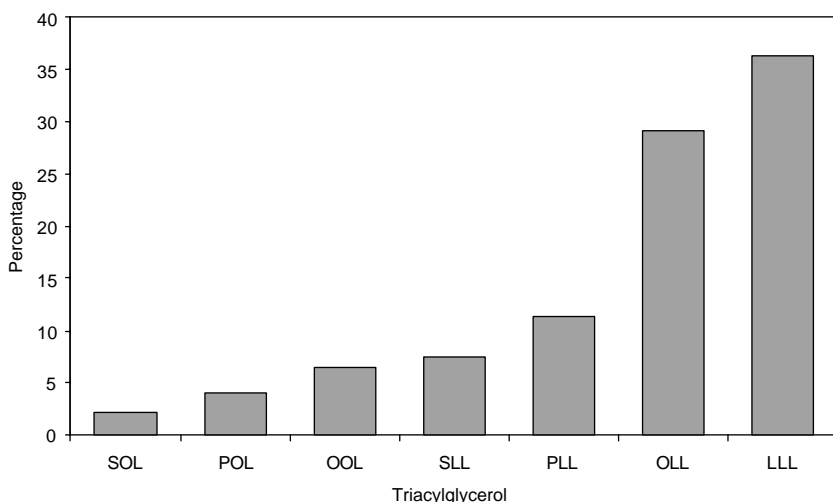
The variation ranges of fatty acids in regular sunflower oil have also changed in several countries. The Canola Council of Canada revised the table of composition of edible oils prepared in 1979, based on a study carried out by the POS Pilot Plant Corporation in Saskatoon, Saskatchewan, Canada. POS analyzed ten vegetable oils and three animal fats supplied by food processing and manufacturing enterprises of Canada and the United States, according to one issue of *Canada Digest* (22). Average regular sunflower oil compositions are shown in Table 3 for Canada and the United States, as well as for Argentina (2, 23).

**TABLE 3. Average Composition (%) of Regular Sunflower Oil for Canada/United States (normalized to 100%) and Argentina, Elaborated in Different Years (2, 22, 23).**

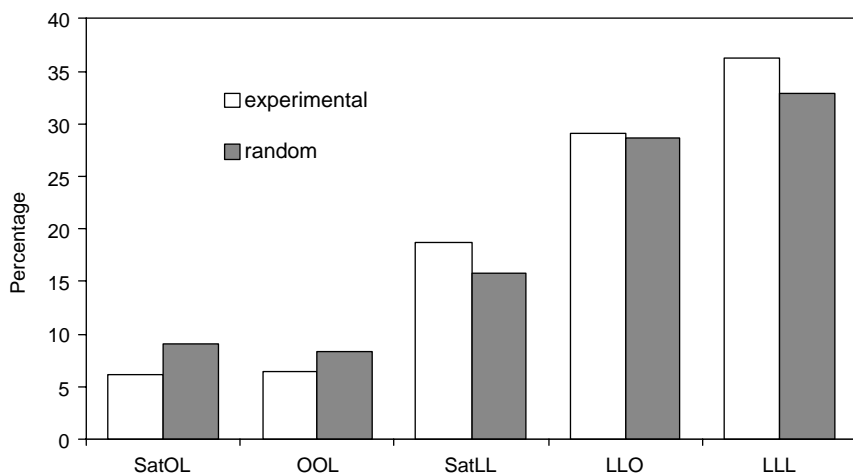
Fatty Acid	Canada/U.S. 1979 (22)	Canada/U.S. 1994 (22)	Argentina 1981 (23)	Argentina 1998 (2)
saturated	11	12	8.7	10.1
18:1	20	16	24.0	26.8
18:2	69	71	66.0	62.2

**3.1.2. Triacylglycerol Composition** Figure 5 shows the composition in major triacylglycerols (above 1%) of regular sunflower oil [based on Prevot (17)]. As expected from its high linoleic acid content, the main triacylglycerol is trilinolein (36.3%), followed by oleo-dilinolein (29.1%); triolein being practically nonexistent (0.6%). Thus, the percentage of triacylglycerols (TAG) with four or more double bonds is higher than 80%. This TAG distribution is responsible for the low solidification point of regular sunflower oil ( $-16^{\circ}\text{C}$  to  $-19^{\circ}\text{C}$ ), allowing, for example, storage of mayonnaise manufactured with regular sunflower oil in a refrigerator without breakage of the emulsion (unlike the case of other oils such as peanut oil).

Rossell et al. (24) analyzed the triacylglycerols of 20 regular sunflower oil samples, regarding the total number of carbon atoms. The content of 54-carbon TAG was 75.1–79.5%. The composition of these triacylglycerols is OOO, SOL, OOL, SLL, OLL, and LLL. Grouping the data provided by Prevot (17) in the same manner, the 54-carbon TAG content would be 82.1%. Thus, the results of both works are to a large extent in agreement.



**Figure 5.** Composition in major triacylglycerols (above 1%) of regular sunflower oil [based on (17)]. (Key: P = palmitic acid, S = stearic acid, O = oleic acid, L = linoleic acid.)



**Figure 6.** Triacylglycerol composition of regular sunflower oil as calculated from random distribution and experimentally determined (17). (Key: Sat = saturated acid, O = oleic acid, L = linoleic acid.)

The 1,2,3-random hypotheses assumes that one pool of fatty acids is randomly distributed to all three positions of the glycerol molecules in an oil. The fatty acid compositions of the *sn*-1, *sn*-2, and *sn*-3-positions would thus be equivalent. Figure 6 shows the theoretical composition of regular sunflower oil as calculated by the equations of random distribution. Calculation of the random distribution was based on the following composition: 11% saturated fatty acids (Sat), 20% oleic acid (O), and 69% linoleic acid (L). The TAG composition of a regular sunflower oil determined experimentally is also shown; there is no indication of the overall fatty acid composition (17). Differences between both compositions are not great, in particular, taking into account the fact that the fatty acid composition may differ for the oils considered.

Fatty acids are not randomly distributed in natural oils. Saturated fatty acids are almost exclusively concentrated in the *sn*-1,3 positions and are practically nonexistent in the *sn*-2 position (taxonomic pattern). Linoleic acid clearly has a higher occurrence in the *sn*-2 position. For example, out of a total 16.2–39.3% linoleic acid in peanut oil, the *sn*-2 position has 27.2–67.8%, clearly showing the concentration of linoleic acid in this position (24).

Table 4 shows the fatty acid distribution in the *sn*-2 position with respect to the composition of the *sn*-1 and *sn*-3 positions (25) or with respect to the overall composition of the sunflower oil samples analyzed (24). Occurrence of 18:2 is slightly higher in the *sn*-2 position than it would be if distributed in equal proportions among all three positions. The occurrence of linoleic acid is also slightly higher in the *sn*-2 position than in the *sn*-1,3 positions by a ratio of 1.27. As the content of linoleic acid is particularly high in regular sunflower oil, the preferential distribution of linoleic acid is less apparent than for other vegetable oils. Saturated fatty

**TABLE 4. Distribution of Fatty Acids in the *sn*-2 Position with Respect to the *sn*-1 and *sn*-3 Positions (25) or with Respect to the Overall Sunflower Oil Composition (24).**

	Álvarez-Ortega et al. (25)		Russell et al. (24)	
	<i>sn</i> -1 + <i>sn</i> -3	<i>sn</i> -2	overall	<i>sn</i> -2
16:0	9.2	0.5	5.7–6.9	0.2–0.4
18:0	6.1	0.4	3.0–6.3	0.1–0.3
18:1	34.0	34.7	14.0–34.4	12.1–31.3
18:2	50.7	64.2	55.5–73.2	66.2–87.4

acids have a tendency to concentrate in the *sn*-1 and *sn*-3 positions, hardly ever occurring in the *sn*-2 position. Oleic acid occurs equally among all three positions. However, differences are small, resulting in an apparent agreement between the TAG profile determined experimentally and the fatty acid distribution calculated on a supposed random distribution (as indicated in Figure 6).

### 3.1.3. Nonacylglycerol Components of Regular Sunflower Oil

**3.1.3.1. Phospholipids** The phospholipid content of crude sunflower oil ranges between 0.5% and 1.2%. Oils extracted by solvent generally have a higher content of phospholipids than those obtained by pressing. Major phospholipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid. Most are hydratable and may be removed from the crude oil through a water-degumming process (See Section 5.3.1.)

**3.1.3.2. Tocopherols** Tocopherols are heterocyclic compounds with a phenolic group and a substituted side chain of branched hydrocarbon. Their high solubility in oil is caused by a long alkyl side chain. The Codex Alimentarius (Codex-Stan 210-1999) indicates levels of tocopherols and tocotrienols in crude regular sunflower oil (mg/kg), compiled in Table 5.

**TABLE 5. Levels of Tocopherols and Tocotrienols in Crude Regular Sunflower Oil (ppm), According to the Codex-Stan 210-1999.**

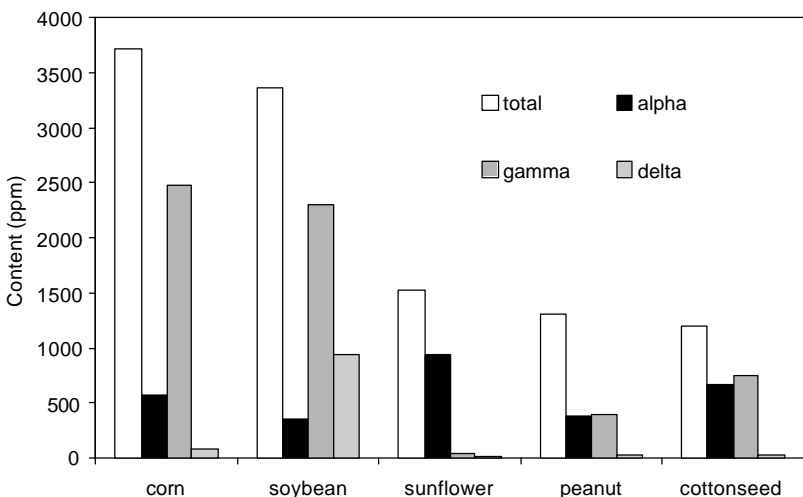
	Content (ppm)
Alpha-tocopherol	403–935
Beta-tocopherol	ND–45
Gamma-tocopherol	ND–34
Delta-tocopherol	ND–7.0
Alpha-tocotrienol	ND
Gamma-tocotrienol	ND
Delta-tocotrienol	ND
Total	440–1520

ND = nondetectable.

The biological value of tocopherols differs according to isomer. Their importance as Vitamin E activity is the following:  $\alpha > \beta > \gamma > \delta$ . Vitamin E functions primarily as an antioxidant, especially in preventing oxidation and peroxidation of polyunsaturated fatty acid units of membrane phospholipid (within and on the plasma membrane of cells). The value of regular sunflower oil as a source of Vitamin E is enhanced by a high content of alpha-tocopherol. Similar conclusions can be found in the literature for tocopherols of Argentinean sunflower oil: 700 ppm of total tocopherols, 91% of which corresponds to alpha-tocopherol (2).

Tocopherols also function as free radical scavengers. The alpha form has the highest Vitamin E activity, and gamma-tocopherol has the highest antioxidant activity. In one study, sunflower tocopherols were added to stripped soybean oil, and soybean tocopherols were added to stripped sunflower oil. The stability pattern of sunflower oil—generally less stable than soybean oil—mimicked that of soybean oil. With the added sunflower tocopherols, the stability pattern of soybean oil resembled that of sunflower oil. The results suggest that gamma-tocopherol is a better antioxidant than the alpha isomer (26). Other authors, however, attribute a higher antioxidant activity to alpha-tocopherol. Disagreement with respect to the relative antioxidant activity of tocopherol homologs may be because of differences in the degree of unsaturation of the substrates used, the degree of oxidation achieved in the measurement, and the method of oxidation analysis.

Figure 7 shows a comparison of maximum values for major tocopherols in crude regular sunflower, peanut, soybean, corn, and cottonseed oils, according to the Codex Alimentarius (Codex-Stan 210-1999). Among these, regular sunflower oil has the highest tocopherol level. In general, gamma-tocopherol is the most widely occurring isomer in vegetable oils.



**Figure 7.** Maximum values of major tocopherols (ppm) of crude sunflower, peanut, soybean, corn, and cottonseed oils, according to the Codex Alimentarius (Codex-Stan 210-1999).

**TABLE 6. Levels of Desmethylsterols in Crude Regular Sunflower Oil, as a Percentage of Total Sterols and Total Sterol Content (ppm), According to Codex-Stan 210-1999.**

Cholesterol (%)	<0.7
Brassicasterol (%)	ND-0.2
Campesterol (%)	7.4-12.9
Stigmasterol (%)	7.0-11.5
$\beta$ -sitosterol (%)	56.2-65.0
$\Delta$ -5-avenasterol (%)	ND-6.9
$\Delta$ -7-stigmasterol (%)	7.0-24.0
$\Delta$ -7-avenasterol (%)	3.1-6.5
Others (%)	ND-5.3
Total sterols (mg/kg)	2400-4600

ND = nondetectable, defined as <0.05%.

**3.1.3.3. Sterols** Sterols are polycyclic alcohols derived from sterane. Sterols constitute most of the unsaponifiable fraction of an oil. The sterol profile is characteristic of each oil. The Codex Alimentarius (Codex-Stan 210-1999) indicates the total sterol content (ppm) and the percentages of each sterol type in regular sunflower oil, as shown in Table 6.

Among vegetable oils, regular sunflower oil is characterized by a medium sterol content. According to the Codex Alimentarius (Codex-Stan 210-1999), the oils with the highest sterol content are rapeseed oil (low erucic acid), with 4800-11,300-ppm sterols; corn oil with 8000-22,100 ppm, and sesame oil with 4500-19,000 ppm.

In regular sunflower oil, the main component is  $\beta$ -sitosterol, followed by  $\Delta$ -7-stigmasterol. The latter may be used as a tracer for detection of adulterations in sunflower oil, as most vegetable oils (except safflower oil) have fairly low amounts of  $\Delta$ -7-stigmasterol (less than 7%).

**3.1.3.4. Other Components of the Unsaponifiable Matter** The unsaponifiable matter in a crude regular sunflower oil is usually in the range of 0.5-1.5% (9, 17), or lower than 15 g/kg according to the Codex-Stan 210-1999. In addition to sterols (around 2.4-4.6 g/kg) and tocopherols and tocotrienols (0.4-1.5 g/kg), there are minor components of sunflower oil. Aliphatic compounds and terpenoids occur naturally in oils. Of the terpenoid family, squalene is the most widely occurring compound. The occurrence of squalene in regular sunflower oil is fairly low: 0.008-0.019% (5) or 15-20 mg/100 g (9). The aliphatic alcohol content is 100-mg/100-g oil (9).

Carotenoids and chlorophylls are the major lipochromes of vegetable oils. Crude regular sunflower oil is not particularly rich in carotenoids (as palm oil is) or in chlorophylls (like rice bran, rapeseed, olive, and avocado oils). This gives crude regular sunflower oil its light-amber color, turning to pale yellow upon the bleaching operation.



### 3.2. Chemical Characteristics of Regular Sunflower Oil

The Codex Alimentarius (Codex-Stan 210-1999) indicates the characteristics of crude regular sunflower oil: (1) saponification value = 188–194-mg KOH/g oil; (2) iodine value (calculated from the fatty acid composition) = 118–141. However, Merrien (9) reports an iodine value of 120–134, and Bockisch (5) reports a value in the range of 110–143 (Wijs method).

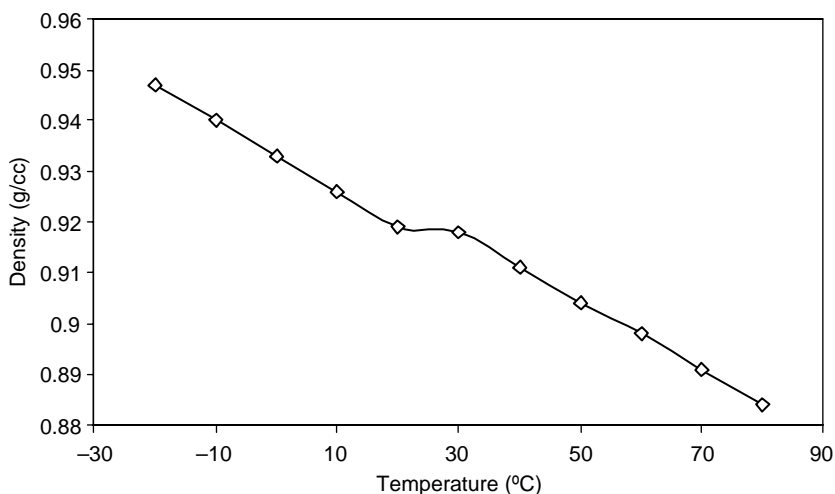
### 3.3. Physical Characteristics of Regular Sunflower Oil

**3.3.1. Refractive Index** The refractive index is a characteristic property of fats and oils and may be used as a fast measurement of the advance of a hydrogenation operation. The Codex Alimentarius (Codex-Stan 210-1999) indicates a refractive index ( $n_D$ ) of regular sunflower oil in the range of 1.461–1.468 at 40°C; Merrien (9) reports the range 1.474–1.476 at 20°C.

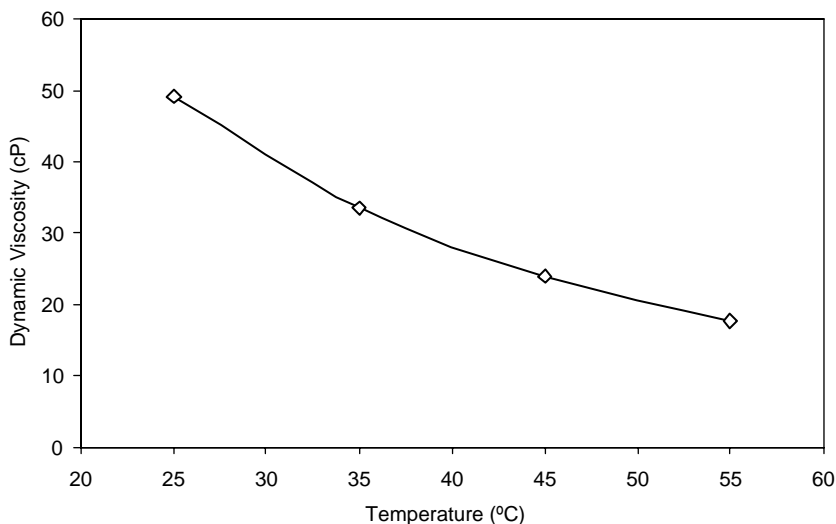
**3.3.2. Density** Determinations of the content of tanks or flow rates are usually based on methods of volumetric dosing. These methods are used to facilitate equipment automation. However, mass determinations based on volume measurements will depend on the nature and temperature of an oil.

The Codex Alimentarius (Codex-Stan 210-1999) indicates a relative density of regular sunflower oil in the range of 0.918–0.923 (20°C/water at 20°C). The values suggested by the Codex do not differ appreciably from the expected values for most vegetable oils.

Figure 8 shows the temperature dependence of the density (g/mL) of an Indian edible sunflower oil [based on Subrahmanyam et al. (27)].



**Figure 8.** Temperature dependence of the density (g/cc) of an Indian edible sunflower oil [based on (27)].



**Figure 9.** Temperature dependence of the dynamic viscosity (cP) of refined sunflower oil [based on (28)].

**3.3.3. Viscosity** The viscosity of an oil is a fundamental parameter when pumping is required. The viscosity of a vegetable oil will depend on the fatty acid composition. Oils with hydroxylated fatty acids (like castor oil and lesquerelle oil) have a particularly high viscosity.

Figure 9 shows the temperature dependence of the dynamic viscosity (cP) of refined sunflower oil. The curve corresponding to the crude oil is along the same curve [based on Abramovic and Klofutar (28)].

**3.3.4. Specific Heat and Combustion Heat** The specific heat of sunflower oil at constant pressure is 2.197 J/kg °C (29). The energy content or combustion heat of an oil is a major parameter when used as an energy source. The gross heat contents of all vegetable oils are fairly close to each other. Ali and Hanna (30) report a gross heat content of regular sunflower oil of 39,575 kJ/kg, and Bhattacharyya and Reddy (31) a value of 39,486 kJ/kg.

**3.3.5. Smoke Point, Flash Point, and Fire Point** The smoke point, flash point, and fire point of an oil are relevant parameters in deep-fat frying processes. The fatty acid composition of the oil is not relevant (unless the oil has short-chain fatty acids, as is the case of butter or coconut oil). The most important effect is generally that of free fatty acids (FFA) in the oil. The following values have been reported for fully refined sunflower oil (with 0.10% free fatty acids): smoke point = 209°C; flash point = 316°C; fire point = 341°C (5).

The flash point is also an important parameter when considering the possibility of using an oil as an alternative diesel fuel in ignition engines. The flash points of all vegetable oils are far above that of diesel fuel, reflecting the nonvolatile nature

of vegetable oils. Ali and Hanna (30) report a value of 274°C for the flash point of regular sunflower oil.

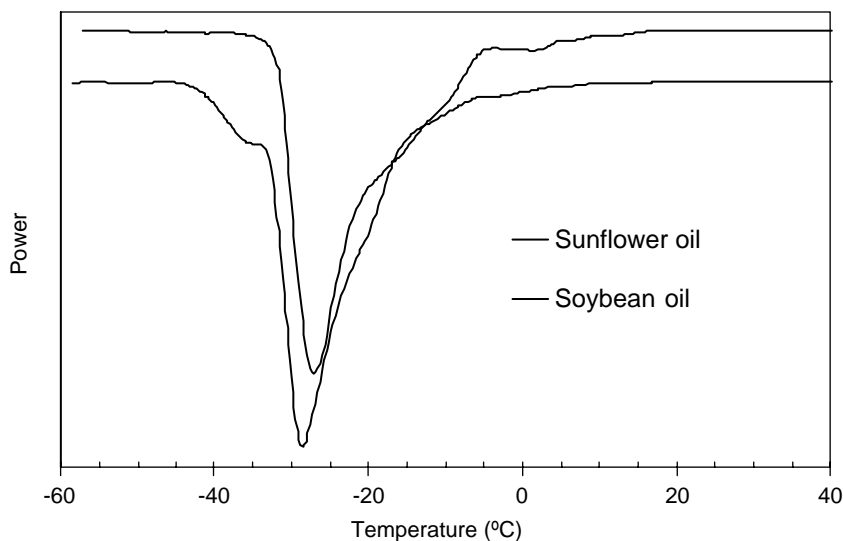
### 3.3.6. Melting Characteristics

**3.3.6.1. Cloud Point and Pour Point** The cloud point is the temperature at which solids first become visible when an oil is cooled. The pour point is the temperature at which the amount of solids out of solution is sufficient to gel the liquid; thus, it is the lowest temperature at which the oil is fluid.

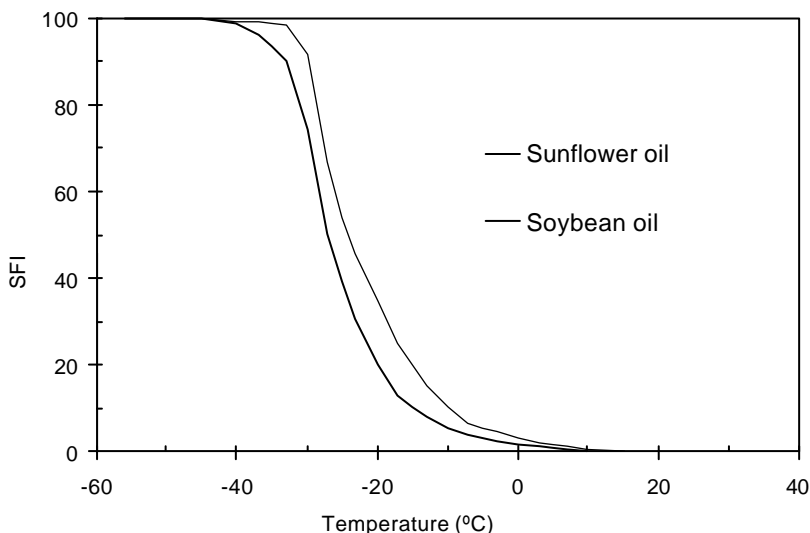
The above parameters are relevant when pumping oils at low temperatures or for their use as alternative diesel fuel in ignition engines. The cloud points and pour points of the vegetable oils are higher than for diesel fuel.

Widely varying values have been reported in the literature for regular sunflower oil. For example, Bockisch (5) reports a cloud point of  $-10^{\circ}\text{C}$  (and a solidification point in the range  $-16$  to  $-18^{\circ}\text{C}$ ). Ali and Hanna (30) report a cloud point of  $7.2^{\circ}\text{C}$  and a pour point of  $-15.0^{\circ}\text{C}$ . Differences between reported cloud points are possibly caused by a varying degree of winterization of the oils considered.

**3.3.6.2. Thermal Behavior of Regular Sunflower Oil** Crude regular sunflower oil is a liquid at room temperature. The refined oil resists refrigerator temperatures without the appearance of turbidity. These characteristics make it suitable as salad oil. The thermal behavior of an oil may be determined within wide temperature ranges through methods of nuclear magnetic resonance (NMR) or through differential scanning calorimetry (DSC). Both methods allow the evaluation of indices related to the solid content as a function of temperature.



**Figure 10.** Thermograms of a refined sunflower oil and of a refined soybean oil (32).



**Figure 11.** Solid fat content (SFI) of a refined regular sunflower oil and a refined soybean oil (32).

The thermogram of an oil determined by DSC allows the study of thermal behavior, and the evaluation of the solid percentage from the area of peaks. This information is characteristic of the fatty material considered. The thermograms of a refined regular sunflower oil and a refined soybean oil (32) are compared in Figure 10. It is clear that melting of sunflower oil is practically complete above  $-15^{\circ}\text{C}$ . Soybean oil, however, has a second, smaller peak between  $-5^{\circ}\text{C}$  and  $5^{\circ}\text{C}$ , which corresponds to a higher content of saturated fatty acids. Figure 11 shows the solid content as a function of temperature for both oils, as determined by partial integration of peaks in the above thermograms (32).

#### 4. SUNFLOWER SEED OF MODIFIED FATTY ACID COMPOSITION

Until two decades ago, the fatty acid composition of vegetable oils was closely related with their origin. The fatty acid profile of sunflower oil was thus defined within natural variation ranges. Current practices, however, are widely based on the production of oilseed of modified fatty acid composition. Several methods have been developed to this end.

The genetic diversity of wild sunflower allowed researchers to obtain a number of varieties of defined characteristics. The North Central Regional Plant Introduction Station (Iowa), which gathers dozens of species, has distributed materials to researchers and companies interested in either the study or use of these materials. The sunflower germplasm collection of the U.S. Department of Agriculture (USDA) is the largest and most complete collection worldwide, including wild materials from 48 U.S. states, as well as samples from Canada and Mexico. The

program of the U.S. Department of Agriculture/Agricultural Research Service (USDA/ARS) evaluates wild material according to oil content and composition.

Most breeding programs are aimed at the development of hybrids, although other projects are for the improvement of open-pollinated varieties and synthetic cultivars. In view of the improvements in yield, disease resistance, uniformity, and self-compatibility achieved in some modifications, open-pollinated varieties have been replaced by hybrids. Varieties have been produced with increased oil content of seeds and/or improvements in oil composition.

Inbreeding has been used since 1920 for the improvement of sunflower, the most common method consisting in the self-pollination of those phenotypically desirable plants within the existing cultivars. The progeny of the best plants are sown in the following season, and the selection procedure is continued among the resulting progeny. A variation of recurrent selection, the method of reserves, developed by Pustovoit in the USSR, consists of the evaluation of progeny and subsequent cross-pollination among superior progenies.

In order to obtain hybrid seeds, self-pollination and pollination by a sibling plant must be avoided. It is sought to develop a female parental line accepting pollen from other lines (cross-pollination), without reproduction of its own line. This can be achieved directly through emasculation, i.e., elimination of the pollen-bearing organ, usually at the expense of large labor requirements. Other methods can be used to induce male sterility.

Modifications in the characteristics of sunflower are obtained through crosses to recombine genes from two sexually compatible parents. Both for self pollination and for controlled crosses, it is important that heads be isolated before flowering to avoid natural cross-pollination.

Emasculation of the female parent is used for the production of artificial hybrids. Acid-induced male sterile plants pollinated without emasculation are also used. These hybrids were produced by natural crossing in seed production fields (breeding nursery), with the two parents planted in alternating groups of rows. The first hybrid cultivars were introduced in Canada for commercial production in 1946 (4).

Cytoplasmic male sterility of sunflower was discovered in 1958, whereby one factor in the cell cytoplasm leads to the male-sterility in all plants of the second generation. However, as a result of the pollination of fertile ordinary lines, plants will still bear some fertile progeny. Patrice Leclercq, in France, in 1969, reported on cytoplasmic male sterility obtained in the progeny of a cross of *Helianthus petiolaris* Nutt and *Helianthus annuus*. In 1970, fertility restorer lines were found. Two years later, hybrid seeds produced by this system were made available to farmers. In 1976, 80% of the U.S. sunflower harvest had been produced on hybrid seed.

The production of single crosses or three-way hybrids using cytoplasmic male sterility and nuclear fertility restorer system is widely used. Genic male sterility was used for the production of hybrid seeds in the early 1970s in France and Romania. The first hybrids produced in this way were introduced in the United States in 1972 (4).

Various genetically different types of cultivar are commercially available. In open-pollination varieties, each individual is genetically different from another

among the population. This results in a high level of adaptation to different environment conditions on the one hand, but it may also cause handling difficulties because of the lack of phenotypical uniformity. Open-pollination varieties may be recovered yearly by the producer, the regular purchase of new seed being necessary only for maintenance of purity and type.

Commercial hybrid cultivars include single and three-way hybrids. The former type is produced by crossing two lines, resulting in genetically identical individuals; the latter are developed by crossing a single hybrid with a homozygote line. Seed must be procured yearly by the producer in both cases.

The production of high-quality hybrid seeds does not only depend on the use of parental lines of superior class, but also on the degree of isolation of the cultivation field from other sunflower plantations, including wild varieties. The isolation conditions cannot be accurately established, in view of the role of insects as pollination agent, and the long viability period of pollen; yet, recommendations have been made.

Genetic variation occurring naturally within crop species is scarce. Additional genetic modification strategies are required to generate “variants” of a certain fatty acid profile. Such variants may be included in crossing programs for the development of cultivars or strains of interest. Modifications in the fatty acid profile of an oil may be achieved through techniques of mutagenesis. Stable genetic mutations may be induced by use of chemical mutagens, such as ethyl methyl sulfonate. This procedure was used in the USSR to develop sunflower seeds of improved oleic acid content.

#### **4.1. Modification of the Saturated Fatty Acid Content**

The consumption of fat of high saturated fatty acid content has been associated with increased risk of coronary heart disease. Traditional sunflower oil contains around 11–12% saturated fatty acids, a considerably low value among vegetable oils. Canola oil has 7% and safflower oil less than 10% of saturated fatty acids, both being strong competitors of the edible oil market.

Since 1992, the National Sunflower Association (NSA) has supported a cultivation program developed by researchers of the USDA/ARS for a reduction of the saturated content of sunflower oil. New germplasm stocks with reduced content of palmitic and stearic acids were made available. They were developed through continuous selection starting from a sunflower accession collected in Egypt around 1950. Several private companies have also carried out investigations aiming at obtaining hybrids of low saturated fatty acid content (6% or lower). Cultivators of Pioneer Hi-Bred International Incorporation managed to reduce the stearic acid content to 1.5% for commercial products. SVO Enterprises and Triumph Seed Company developed research lines aiming at reducing the saturated quantity in high-oleic sunflower oil (33–34).

With a view to finding new industrial uses of vegetable oils, the saturated fatty acid content may also be increased. Three high stearic acid sunflower mutants, having as much as 28%, 15%, and 14% of stearic acid in the seed lipids have been

biochemically characterized (35). An increased solid content of the oil could be obtained in the oil without requiring hydrogenation, although such an increase may not meet nutritional standpoints.

## 4.2. High-Oleic Sunflower

The typical sunflower oil composition is 66–72% linoleic acid, 12% saturated acids (palmitic and stearic), 16–20% oleic acid, and less than 1%  $\alpha$ -linolenic acid. An increase in low-density lipoprotein cholesterol (LDL-C) and a decrease of high-density lipoprotein cholesterol (HDL-C) are believed risk factors of coronary heart disease (CHD). Diets rich in saturated fat increase plasma total and LDL-C. Traditional high-linoleic sunflower oil has always been regarded as healthy because of its high content of polyunsaturated fatty acids (PUFA) and relatively low content in saturated fatty acids.

The substitution of saturated fatty acids by PUFA in a diet leads to a reduction of total cholesterol and LDL-C. It has been suggested that a high intake of polyunsaturated fatty acids leads to a decrease of HDL-C. Some authors have proved this theory based on rather unrealistic high PUFA in the diet (PUFA/MUFA > 3). Others have found statistically nonsignificant decrease values for HDL-C in more realistic high PUFA diets. An increased intake of monounsaturated fatty acids (MUFA) also leads to a decrease in total cholesterol and of LDL-C levels without reducing HDL-C even with fairly high MUFA intake values. As oleic acid is more stable against oxidation than linoleic acid, consumption of MUFA has further advantages over PUFA. It is recommended to avoid foods containing peroxidized lipids, as these might be initiators of pathologic processes. In view of the considerations above, new genetic strategies were started toward a high-oleic sunflower oil (HOSO).

There is also controversy over the importance of MUFA over PUFA from the metabolic viewpoint. Great emphasis is placed on the distinction between the n-3 PUFA and those of the n-6 family. An increased intake of n-3 and a reduced intake of n-6 are recommended in light of the competitive metabolism of both families of fatty acids. As linoleic acid is a n-6 parent, a reduction of its intake favors the n-3/n-6 ratio; on the other hand, it is also an essential fatty acid.

High-oleic sunflower oil, with very low PUFA levels, may well suit the requirements of processors, but it does not support the work of nutritionists who recommend n-6/n-3 ratios within the range 5 to 10. In addition, HOSO does not represent an increased intake of family n-3 fatty acids as recommended by nutritionists, the linolenic acid content being very low for all types of sunflower oil.

K. I. Soldatov, in Russia, developed high-oleic sunflower seeds through the treatment of normal seed with a chemical mutagen (dimethyl sulfate). Through programs of selected breeding, a number of plants containing seed with as much as 80–90% oleic acid were obtained. L. N. Kharachenko, also in Russia, studied the standard Peredovik progeny and the Pervenets progeny—obtained from treatment of seeds of the former variety with a chemical mutagen. It seems that modifications in the seed genotype of high-oleic Pervenets are responsible for an irreversible blockage of the desaturating enzyme system. G. N. Fick developed progenies of

cultivar Pervenets in plantations in the United States, Argentina, and Chile and incorporated the dominant genes into hybrids that were suitable for commercial production. High-oleic seeds were first grown commercially in the United States in 1984 (36–37).

The Lubrizol Corporation obtained U.S. patents (granted to Sigco and inventor Gerhardt N. Fick) for sunflower seeds and oils of oleic acid content of 80% or higher and linoleic/oleic ratios lower than 0.09—Patent 4,627,192 for seed granted on December 9, 1986, and Patent 4,743,402 for oils granted on May 10, 1988. SVO Enterprises, a division of Agrigenetics Company, which is a part of The Lubrizol Corporation, has produced high-oleic sunflower oil trademarked under the name Trisun in the United States (33–34, 38).

High-oleic sunflower oil is sold in Australia under the name Sunola—a registered trademark of Meadow Lea Foods. The seed variety was bred by Australian farmers through traditional selective breeding techniques. The first Sunola crop was developed in Queensland. The oil's fatty acid composition is 85% monounsaturated, 8% polyunsaturated, and 7% saturated. The composition of oil extracted from Sunola seed in the first stages of ripening resembles that of regular sunflower varieties. Only when the synthesis of oil has actually started (some three weeks after flowering) does the oleic acid content start to increase considerably and the linoleic content start to decrease rapidly (39).

A further approach to modified sunflower oils was made by the Agriculture Canada Research Station in Saskatoon (Saskatchewan) from two different types of dwarf early-ripening sunflower trademarked under the name Sunola (Western Grower Seed Corp. was created for commercialization and further improvement of Sunola). One of these types was regular high-linoleic Sunola, which was first produced commercially in 1993. The hybrid was specially developed for farmers in regions of the west of Canada where cultivation of sunflower was nonexistent because of a short growing season. The fatty acid composition of this oil is 72–74% linoleic acid (owing to the colder growth conditions), 14% oleic acid, and 12% saturated acids. The other hybrid is high-oleic Sunola sunflower, whose production started in 1995. The ripening time of high-oleic Sunola is about 100 days (three weeks shorter than for most sunflower crops). The fatty acid composition is 87% oleic acid, 5% linoleic acid, and 7–8% total saturated acids. Seeds of this type are smaller than regular sunflower seeds, the hull being slightly lighter and bearing a narrow stripe (33–34, 40).

As both Sunola crops are special, care must be taken against contamination with traditional sunflower or canola. However, this is rarely the case, as Sunola is grown in northern areas of the United States, where regular sunflower is not grown and in areas of southern Canada that are too hot and dry for the development of canola crops.

It is worth noting that the name Sunola for modified oils is used in Australia for high-oleic sunflower oil, whereas, in Canada, it is a registered trademark of two oils of different composition: one of higher linoleic acid content than traditional sunflower and another of high-oleic type. Care must be taken that this should not lead to confusion. Canadian Western Grower Seed Corporation has also developed



**TABLE 7. Fatty Acid Composition (%) of High-Oleic Sunflower Oil (37, 41, 43).**

Fatty Acid	Vermeersch (41)	Krawczyk (43)	Purdy (37)
16:0	3	6.7	2.7–4.2
18:0	5		3.4–5.0
18:1	83	80.0	80.5–86.7
18:2	9	12.0	4.0–8.5

lines of Sunola with higher oleic acid content to be introduced in the cosmetics market. One of these has as much as 88% oleic acid.

Purdy (37) reported on the fatty acid composition and other analytical characteristics of high-oleic sunflower oil of the Pervenets variety cultivated in three regions of the United States. The oil content of seeds ranged between 43.3% and 47.9% (dry basis), the hull accounting for 24–33% of total seed weight. Table 7 shows the fatty acid composition of high-oleic sunflower oil (37, 41–43).

Table 8 shows the chemical and physical characteristics of crude high-oleic sunflower oil according to the Proposed Draft Amendment to the Codex Standard for Named Vegetable Oils (Alinorm 01/17). The Active Oxygen Method (AOM) value for refined oil extracted from seed of high-oleic Pervenets variety is 51–56 hours, compared with 13 hours for regular oils (37). In another study, Purdy (36) extracted oil from high-oleic Pervenets seed and from high-linoleic seed. The saturated fatty acid content of these oils varies only slightly (8–11%); the main variation occurs in the oleic/linoleic ratio. Table 9 shows AOM values for refined sunflower oils as a

**TABLE 8. Chemical and Physical Characteristics of Crude High-Oleic Sunflower Oil (Codex Alimentarius, Alinorm 01/17).**

Relative density (25°C/water at 20°C)	0.909–0.915
Refractive index (ND 25°C)	1.467–1.471
Saponification value (mg KOH/g oil)	182–194
Iodine value	78–90
Unsaponifiable matter (g/kg)	<15

**TABLE 9. AOM Time (hr) for Refined Sunflower Oils of Different Oleic Acid Content [based on (36)].**

Oleic Acid	AOM (hr)
26 % (regular)	11
51 % (regular)	18
79 % (high-oleic)	38
83 % (high-oleic)	60
89 % (high-oleic)	100

**TABLE 10. Fatty Acid and Triacylglycerol Composition (%) of Regular Sunflower Oil and of High-Oleic Sunflower Oil [based on (25)].**

	Regular Oil	High-Oleic Oil
Fatty Acid:	%	%
16:0	6.8	5.3
18:0	5.0	3.8
18:1	31.4	88.3
18:2	55.4	1.4
Triacylglycerol:	%	%
<i>sn-1 + sn-3</i>		
16:0	9.2	5.1
18:0	6.1	5.8
18:1	34.0	87.4
18:2	50.7	1.6
<i>sn-2</i>		
16:0	0.5	0.3
18:0	0.4	
18:1	34.7	98.6
18:2	64.2	1.1

function of the oleic acid content. Data presented show that the oxidative stability of this oil is enhanced considerably with an increase in the oleic acid content. Table 10 shows the triacylglycerol composition of high-oleic oil (derived from Pervenets) and of regular sunflower oil, with practically no occurrence of saturated fatty acids in the *sn-2* position for either oil (25).

Although the content of oleic acid is high in both high-oleic sunflower oil and olive oil, there is a higher content of saturated acids in olive oil. Their MUFA contents are similar, but the composition of triacylglycerols differs widely (Table 11). Whereas the triacylglycerol OOO (O = oleic) is the main species in both oils, HOSO has a higher content, olive oil, in contrast, having a higher proportion of POO (P = palmitic). Further differences are in the fatty acids occupying those positions other than *sn-2*, which is occupied by oleic acid in both oils. In addition, HOSO has a higher proportion of linoleic acid in position *sn-2*, whereas olive oil has more  $\alpha$ -linolenic acid (44).

Another major difference between olive oil and HOSO is a most distinct flavor of olive oil that characterizes it from HOSO and other oils. Extra virgin olive oil,

**TABLE 11. Composition in Major Triacylglycerols (%) of Olive Oil and High-Oleic Sunflower Oil (HOSO) [Based on (44)].**

Major Triacylglycerols (%)	Olive Oil	HOSO
POO	30.5	12.1
OOO	49.9	65.1
OLL	0.3	3.1

O = oleic, P = palmitic, L = linoleic.

**TABLE 12. Sterol Composition (as Percentage of Total Sterols) of High-oleic Sunflower Oil (According to Codex Alimentarius, Alinorm 01/17) and Olive Oil (20).**

Sterol Composition	High-Oleic Sunflower Oil (Codex)	Olive Oil (20)
Campesterol (%)	5.0–13.0	≤4.0
Stigmasterol (%)	4.5–13.0	<4.0
β-Sitosterol (%)	42.0–70	≥75.0
Δ5-Avenasterol (%)	1.5–6.9	4–14
Δ7- Stigmasterol (%)	6.5–24.0	≤0.5
Δ7-Avenasterol (%)	ND–9.0	
Others (%)	3.5–9.5	
Total sterols (mg/kg)	1700–5200	

ND = nondetectable, defined as <0.05%.

being the preference of so many gourmets worldwide, is considered the finest choice oil.

The similarity in the fatty acid composition of HOSO and olive oil may lead to cases of adulteration or fraud, in view of the price difference between the two oils. These adulterations may be difficult to detect through conventional analytical methods. The nature of an oil can be traced through a study of its sterol composition.

The sterol composition of both oils is compared in Table 12. Clearly, for HOSO, β-sitosterol is the sterol with the highest occurrence (42–70% of total sterols), followed by Δ7-stigmasterol (6.5–24%), campesterol (5–13%), and stigmasterol (4.5–13%). Although there are differences in the sterol composition of both oils, they are not large enough to enable easy analysis.

Several studies have been aimed at the detection of a fraudulent addition of vegetable oils to olive oil. In particular, different analytical methods can be applied to determine blends of regular and high-oleic sunflower oil with olive oil. The minimum sunflower oil detection level depends on the analytical method used. For example, a minimum detectable level of 0.7% of regular or high-oleic sunflower oil may be achieved through methods of sterol analysis, and analysis of the fatty acids will not enable detection of additions below 20% (45).

High-oleic sunflower oil is widely used as salad oil and cooking oil, because of its composition, light flavor. A high content of oleic acid provides enhanced oxidative stability in frying processes. In addition, it does not require partial hydrogenation for an increase in product shelf-life, with the additional nutritional advantages. The effect of *trans*-fatty acids generated as byproducts of hydrogenation processes on the plasma lipoprotein profile is as adverse as that of saturated fatty acids, both increasing the concentration of LDL-C and reducing that of HDL-C (44).

High-oleic sunflower oil is sprayed on cereals, crackers, and cookies to retain freshness and crispness. It is also used in the manufacture of non-dairy creamers, snack foods, and frozen desserts. Special properties of oleic acid make high-oleic sunflower oil a choice ingredient for cosmetic formulations. The AOM value of

Florasun-90 (of International Flora Technologies Ltd.) is higher than 90 hours—a high value compared with less than 40 hours for high-oleic rapeseed oil and about 20 for sesame oil. Research has indicated that the oil is not skin-irritating or sensitizing. It may be used in tanning products and cosmetics with a high content of natural lipids, such as bath oils, massaging oils, skin-care products, lipstick, and cosmetic cream bases (33, 34).

High-oleic sunflower oil is currently used in the manufacture of a lubricant for diesel and gas motors. The product, denominated *Hélianthe*, is commercialized by the Tecno Society (France). It is composed of 70–80% high-oleic sunflower oil and 20–30% additives. *Hélianthe* is a formulation type 5W40, with a high viscosity index and high fluidity at ignition (41).

### 4.3. Mid-Oleic Sunflower

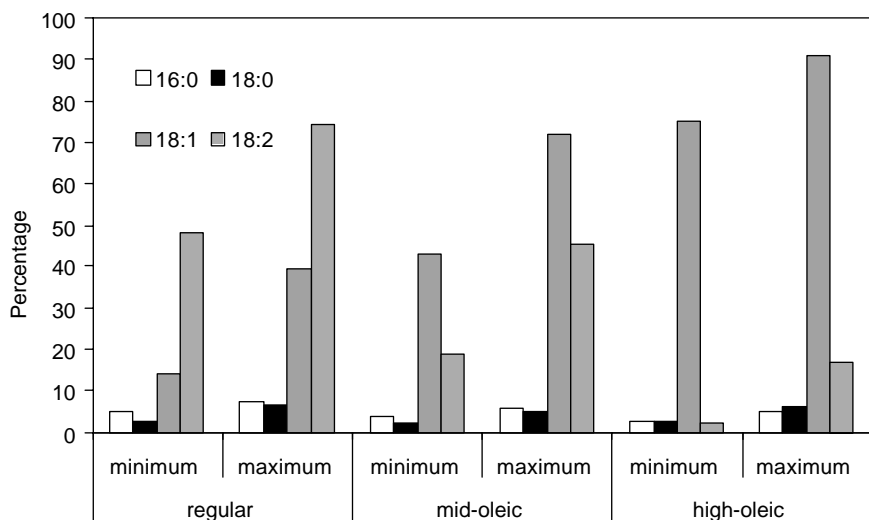
The production of high-oleic sunflower oil with 80% or higher oleic acid content was protected under patents. However, the patent holder agreed to license breeding material for the development of mid-oleic sunflower seed. In July 1995, the NSA decided to redirect efforts toward an increase in oleic acid. It was established that mid-oleic sunflower should contain 65% oleic acid, no higher than 10% saturated acids, and the rest being linoleic acid, a balance that, according to research, provides in-process functionality in frying.

Breeding a mid-oleic sunflower requires at least one oleic parent. The USDA/ARS Northern Crop Science Laboratory in Fargo, North Dakota, provided private companies with crossing lines of mid-oleic sunflower. Hybrid seeds were developed by traditional crossing methods; no hybrids of transgenic sunflower were used. The mid-oleic concentration appears to be controlled by a partially dominant major gene and one or more dominant minor modifier genes (46, 47).

In a market accustomed to HOSO and traditional high-linoleic sunflower, the name “NuSun” seemed suitable and was trademarked by the NSA. Seed and other companies using the name NuSun in their commercial products should have authorization of the NSA. NuSun contains less than 10% saturated, 50–75% monounsaturated, and 30–32% polyunsaturated fatty acids, with less than 1% linolenic acid (46).

Harvests of NuSun were first commercialized in 1999. In 2000, Procter & Gamble chose NuSun for the manufacture of Pringles chips in North America, part of Europe, and Asia, finding a low rate of formation of polar compounds as compared with other oils, an important factor for extending product shelf-life (46, 48).

Figure 12 shows the fatty acid composition (%) of regular, mid-oleic, and high-oleic sunflower oils according to the Proposed Draft Amendments to the Standard for Named Vegetable Oils (Report of the Eighteenth Session of the Codex Committee on Fats and Oils, London, 2003). Table 13 shows the composition in major triacylglycerols of mid-oleic sunflower oil, compared with the composition of regular sunflower oil (49). Clearly, there is a difference in the unsaturated triacylglycerol composition of both oils: mid-oleic sunflower oil has a higher content of OOO, and regular sunflower oil is richer in LLL and LLO (the addition of both contents amounting to 60.3%).



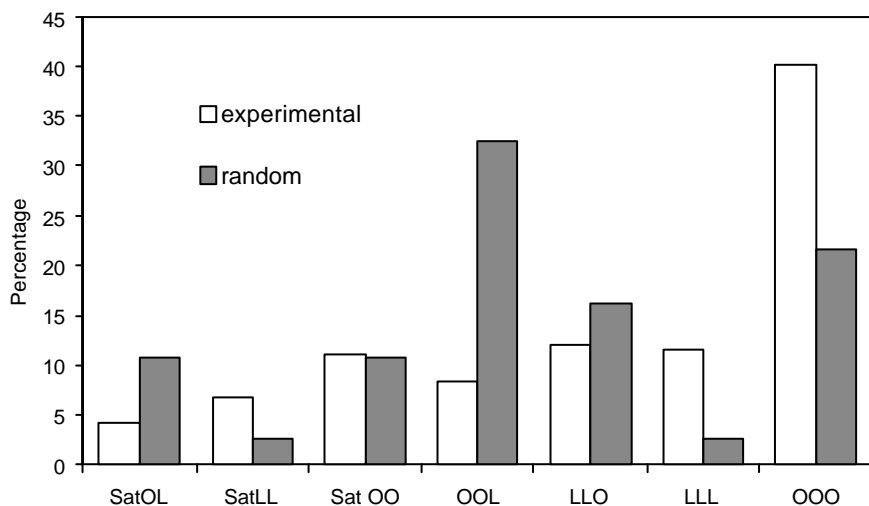
**Figure 12.** Fatty acid composition (%) for regular, mid-oleic, and high-oleic sunflower oil (based on the Proposed Draft Amendments to the Standard for Named Vegetable Oils Committee on Fats and Oils, 2003).

Figure 13 shows the composition in major triacylglycerols of mid-oleic sunflower oil (49), as compared with the calculated composition from a random distribution. The triacylglycerol distribution does not fit the random model, the main differences being in the levels of OOL and OOO, the main triacylglycerols. In contrast, as shown in Figure 6, the fatty acid distribution in regular sunflower oil TAG differs only slightly from the random distribution. Table 14 shows the chemical and physical characteristics of crude mid-oleic sunflower oil according to the Proposed

**TABLE 13. Composition in Major Triacylglycerides (%) of Mid-Oleic Sunflower Oil, as Compared with the Composition of Regular Sunflower Oil [Based on (49)].**

Triacylglycerols	Mid-Oleic	Regular
LLL	11.5	32.4
LLO	12.1	27.9
LLP	4.1	10.7
LOO	8.3	6.7
LLS	2.7	7.4
LOP	2.6	4.8
OOO	40.2	1.7
LOS	1.6	2.2
POO	5.7	0.6
SOO	5.4	0.4

L = linoleic, O = oleic, P = palmitic, S = stearic.



**Figure 13.** Triacylglycerol composition of mid-oleic sunflower oil as calculated from random distribution and experimentally determined (49). (Key: Sat = saturated acid, O = oleic acid, L = linoleic acid.)

Draft Amendments to the Standard for Named Vegetable Oils (Report of the Eighteenth Session of the Codex Committee on Fats and Oils, London, 2003).

Table 15 shows the sterol composition of mid-oleic sunflower oil according to the Proposed Draft Amendments to the Standard for Named Vegetable Oils (Report of the Eighteenth Session of the Codex Committee on Fats and Oils, London, 2003). Clearly,  $\beta$ -sitosterol is the sterol with the highest occurrence (56–58% of total sterols), followed by campesterol (9.1–9.6%) and stigmasterol (9.0–9.3%).  $\beta$ -sitosterol is the main sterol in all three types of sunflower oil (regular, mid-oleic, and high-oleic).

#### 4.4. Semi-Dwarf and Dwarf Sunflower

The search for sunflower varieties of defined characteristics was also aimed at a reduction of plant size and adaptation to other climates. Short-stature cultivars in

**TABLE 14. Chemical and Physical Characteristics of Crude Mid-oleic Sunflower Oil (According to the Proposed Draft Amendments to the Standard for Named Vegetable Oils, 2003).**

Relative density (25°C/water at 20°C)	0.914–0.916
Refractive index (ND 25°C)	1.461–1.471
Saponification value (mg KOH/ g oil)	190–191
Iodine value	94–122
Unsaponifiable matter (g/kg)	<15

**TABLE 15. Sterol Composition (as Percentage of Total Sterols) of Mid-Oleic Sunflower Oil (According to the Proposed Draft Amendments to the Standard for Named Vegetable Oils, 2003).**

Sterol Composition	Mid-Oleic Sunflower Oil (%)
Campesterol	9.1–9.6
Stigmasterol	9.0–9.3
$\beta$ -Sitosterol	56–58
$\Delta$ 5-Avenasterol	4.8–5.3
$\Delta$ 7- Stigmasterol	7.7–7.9
$\Delta$ 7-Avenasterol	4.3–4.4
Others	5.4–5.8

sunflower are classified as semi-dwarf (typical 1.20 m to 1.50 m high) and dwarf (typical 0.80 m to 1.20 m high) types. Dwarf cultivars were developed more recently and include dwarf hybrids and dwarf open-pollinated cultivars.

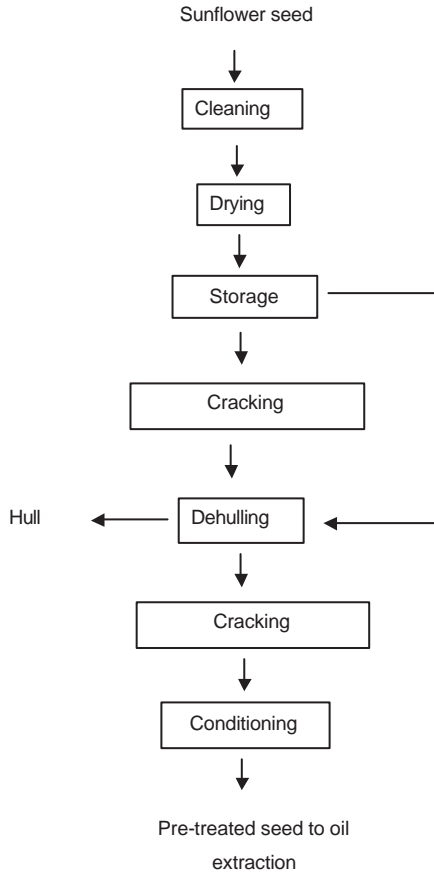
Two types of hybrid denominated Sunola and Sunwheat were developed in Canada to address the handling problems caused by traditional hybrids, requiring special machinery adapted only to the long growing season areas in southeastern Saskatchewan. These new hybrids are 25–35% shorter than regular sunflower (hence the denomination miniature or dwarf), allowing use of the same machinery as is used for cereal or canola production. Both early maturing types offer producers in short-growing-season areas the opportunity to diversify rotations.

Sunola is a miniature type of sunflower developed by the Agriculture Canada Research Station in Saskatoon as a sowing alternative for areas where growth of traditional sunflower is not viable. It is the result of persistent selection of open-pollinated varieties. Plant height is small (60–90 cm), and heads are 8–13 cm in diameter. Ripening time is 99–103 days—three weeks shorter than for most sunflower varieties. Sunola has a high oil content (similar to that of the best hybrids) and a higher content of linoleic acid (72–74%) than any other commercial sunflower.

Sunwheat is a dwarf hybrid of sunflower, having leaves and heads of similar size to other hybrids, but short (96–120 cm). Ripening time is 100–110 days, and the oil content is slightly lower than that of Sunola. It is appropriate for cultivation in barren areas and has a higher resistance to extreme-heat summer periods.

## 5. EXTRACTION AND PROCESSING OF SUNFLOWER OIL

The procedures used for the extraction and processing of sunflower oil are broadly the same as for other seed oils. Focus will be made on those operations or details specific of the production of sunflower oil. Sunflower oil is usually extracted through pressing of seed and later extraction by solvent. The crude oil is usually subjected to traditional refining stages. Otherwise, cold-pressed sunflower oil is currently valued as a new extra virgin oil.



**Figure 14.** Preparatory treatment of sunflower seed for extraction.

## 5.1. Preparation of Sunflower Seeds for Extraction

Figure 14 shows normal stages in the preparation of sunflower seed. Once harvested, sunflower seeds are cleaned, dried, and stored. Seeds must be dehulled prior to pressing and oil extraction stages. Depending on processing plant, seeds may be cracked before the dehulling stage to reduce seed size and help remove the hull. Kernels may be further broken and subjected to two conditioning stages: cooking and flaking. Thermal conditioning or cooking is directed to an adjustment of the moisture content (generally to 3–4.5%) and the temperature (generally 100°C) of meats. The last stage in the preparation of seed is the conversion of the cracked, dehulled, and conditioned meat into a flake. As these stages are common to a number of oilseeds, only those aspects specific of sunflower seeds are considered, namely, in the drying and dehulling stages.



**5.1.1. Drying of Sunflower Seeds** The moisture content of sunflower seed must be reduced to around 8–9% prior to storage. Different authors indicate slightly varying levels: 15% (50), 9–10% (51), 8.5% (52), and 10.5% (5). In order to prevent losses caused by unfavorable climatic conditions, seeds are usually harvested with moisture contents above the recommended levels for storage.

When seed moisture is higher than the recommended value, enormous spoilage of the seeds by microbiological attack is possible. Fungi may also grow explosively over the surface of seeds, with the consequent increase in temperature caused by biological activity. Such temperature increase leads to ideal life conditions for thermophilic bacteria; their metabolism contributes further to a temperature increase. Enzyme and mold activity reduces the quality and the yield of the extracted oil.

Seed moisture is normally expressed as weight percentage of the whole seed. As water is insoluble in seed lipids, the moisture content is concentrated in the nonfatty parts of the seed. The water content calculated on a nonfatty basis is defined as “critical moisture.” The critical moisture of sunflower seeds is 16%, although a maximum 15% is recommended for storage.

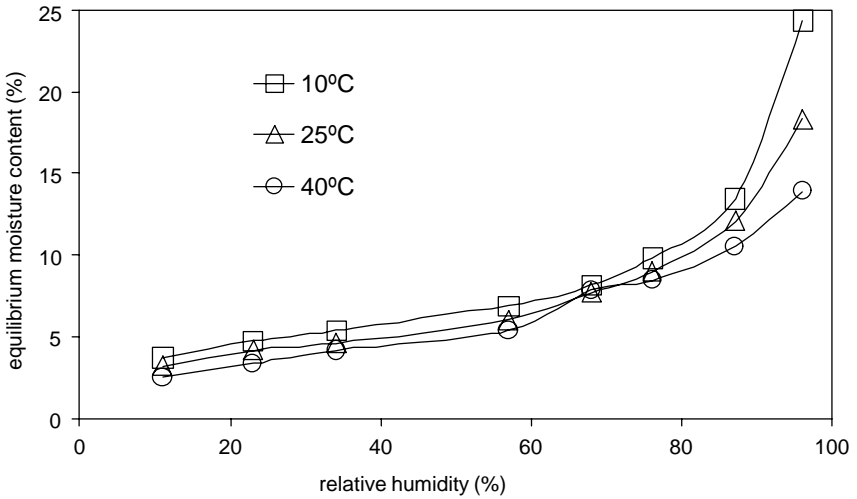
As the content of nonfatty materials in sunflower seeds decreases as the oil content increases, the moisture content corresponding to one critical moisture value is inversely related to the oil content, as shown in Table 16 [based on Muller (50)]. For critical moisture levels above 15%, the rate of respiration of seeds increases. Respiration is accompanied by an exothermic transformation of organic substances of seeds, creating conditions that may lead to spontaneous combustion (50).

Drying of seed may be performed at room temperature with no additional equipment, or with hot-air dryer. The first stage of drying consists of the removal of external moisture from the fresh seeds. Internal water diffuses outward, evaporating in the external part of seeds. After a certain time, seeds reach a hygroscopic equilibrium state at which the moisture content remains constant. The equilibrium depends on ambient temperature and relative humidity of the surrounding air. Figure 15 is a representation of these values for different temperatures: 10°C, 25°C, and 40°C [based on Mazza and Jayas (51)].

The equilibrium moisture of seeds is modified upon dehulling. Equilibrium moisture values for undehulled sunflower seeds, hulls, and kernels are compared as a function of the relative humidity of the surrounding air at 25°C (Figure 16). The initial moisture content of all seeds was 5% (dry basis). Those samples stored at a relative humidity below 33% reached the equilibrium by desorption, and those at a relative humidity above 33% reached the equilibrium by adsorption (51).

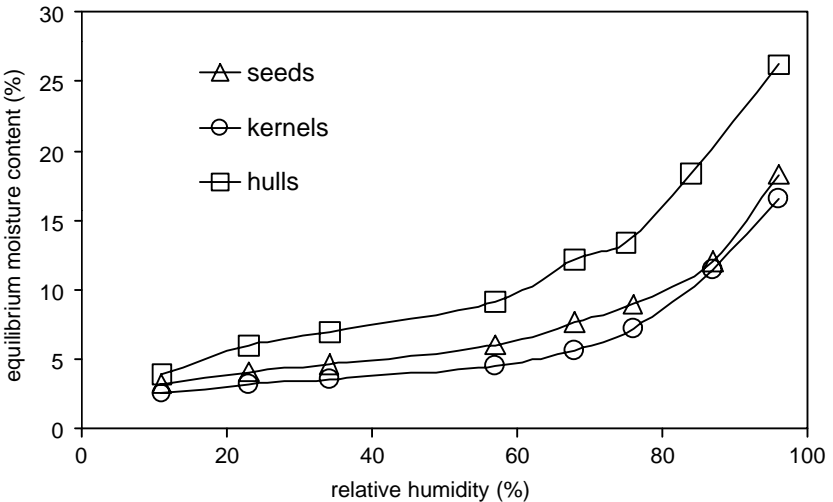
**TABLE 16. Critical Moisture of Sunflower Seeds with Different Oil and Moisture Content [Based on (50)].**

Oil Content (%)	Nonfat Content (%)	Moisture (%)	Critical Moisture (%)
35	65	9.75	15
40	60	9.00	15
45	55	8.25	15
48	52	7.80	15



**Figure 15.** Equilibrium moisture (%) of sunflower seed with hull as a function of air relative humidity (%) for three temperatures [based on (51)].

**5.1.2. Dehulling of Sunflower Seeds** With approximately 30% of hull, sunflower seeds must be dehulled prior to processing. The high wax content of hulls, which would otherwise be transferred to the oil during extraction, is one major reason for dehulling. The wax content of an oil extracted from unde-hulled seed is approximately five times higher than for oils extracted from dehulled seed. However, a small fraction of hull (less than 15%) is left with the seed for easy percolation during the process of extraction by solvent. Seed moisture is usually reduced to values below 8% for hulls to turn more brittle and be easily removed.



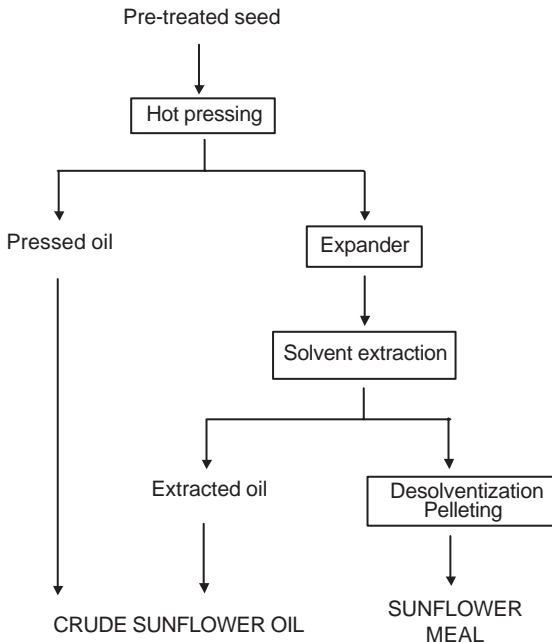
**Figure 16.** Equilibrium moisture (%) of sunflower seeds with hull, hulls, and kernels as a function of air relative humidity (%) at 25°C [based on (51)].

Genetic improvements of sunflower seeds have been aimed at increasing oil content, but also leading to a decrease in the amount of hull. The seed pericarp is thinner and more firmly attached to the kernel in improved varieties. The “hullability” of improved seeds, i.e., the ease with which hulls can be cracked and removed from the seed, is lower than that of seeds with hull of higher thickness.

Dehulling consists of mechanical removal of the pericarp (hull) of seeds. The most widely used method consists of colliding of seeds at high speed against a hard surface by centrifugal effect, leading to the cracking of seeds. Loose hull bits are separated from partially dehulled seed. In addition to the size and shape of seeds, the moisture content is a most relevant parameter in the dehulling process. A decrease in moisture content facilitates hull removal, the effect being greater for hybrids of higher oil content. However, a decrease in moisture also leads to an increase in the amount and composition of fines. Therefore, it is necessary to determine the optimum value of seed moisture for maximum hullability and a reduction in the amount of fines.

## 5.2. Sunflower Oil Extraction

Partially dehulled sunflower seed is generally used for oil extraction, with 8% moisture and 10% residual hull content, approximately. The process employs mechanical pressing followed by hexane extraction. Figure 17 represents a diagram of the unit operations involved.



**Figure 17.** Production of crude sunflower oil.

Extraction of sunflower oil is generally carried out in two stages. The first stage consists of mechanical extraction using screw-presses (expellers). The meal obtained in the pressing stage, containing 15–20% of oil, is subjected to extraction by solvent (normally hexane). The solvent must then be eliminated from both meal and oil. Oils obtained through pressing are of better quality than those obtained by solvent extraction. However, both are blended before storage. Pressed oils are sometimes commercialized separately from solvent extracted oils. The solvent-extracted meal is obtained as a byproduct of this stage.

### 5.3. Treatment of Crude Oil

Figure 18 shows a diagram of alkali refining and physical (steam) refining of sunflower oil. The traditional method, alkali refining, involves degumming, neutralization with alkali, bleaching, dewaxing, and deodorization. A pre-dewaxing stage may be performed after neutralization to reduce the wax content to 100–150 ppm, in addition to a stage of winterization after bleaching for removal of the remaining waxes. Physical refining includes the following stages: degumming, bleaching, dewaxing, and deodorization. Also for physical refining, a combined stage of predewaxing and degumming makes post-dewaxing easier and less costly.

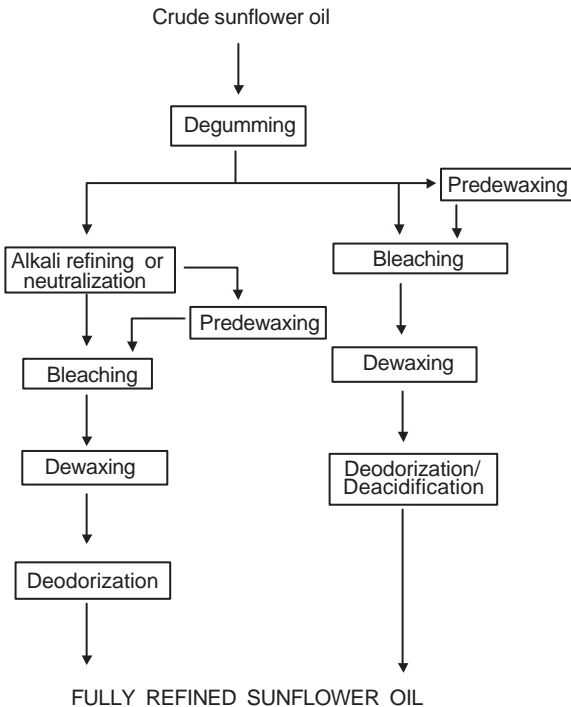
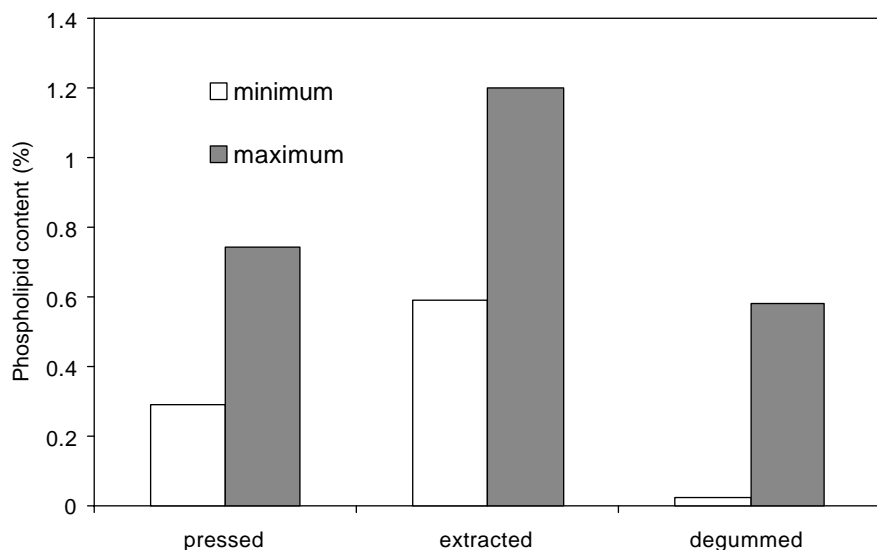


Figure 18. Refining of crude sunflower oil.

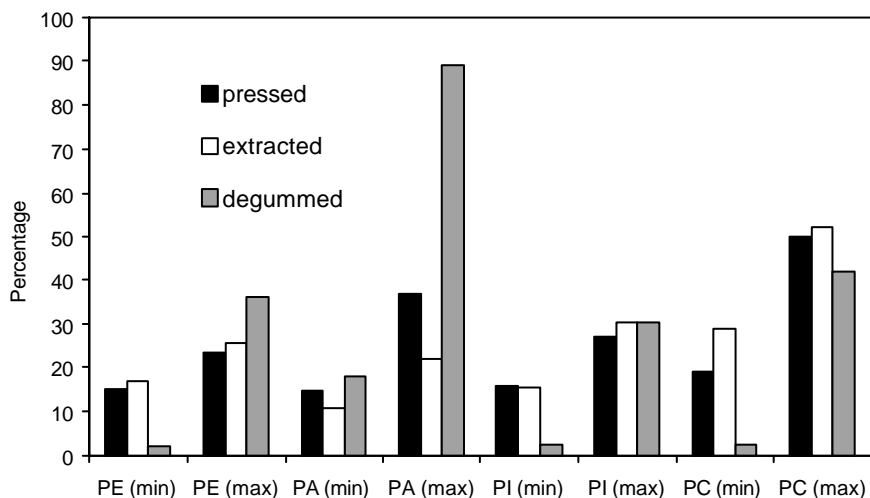
Sunflower oil contains moderate quantities of carotenoids and xanthophylls, but it does not contain chlorophylls. It may be easily bleached with less than 1% bleaching earth. In physical refining, bleaching is carried out for the removal of phosphatides and metals in addition to colored materials. The refining of crude sunflower oil is performed along the same stage sequence as for other oilseeds. Inclusions of a degumming stage and a dewaxing stage are both worth considering separately in detail. Details of physical refining of sunflower oil are also given below.

**5.3.1. Degumming** Major phosphoacylglycerols of sunflower oil are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA). The phospholipid content of solvent-extracted oils is higher than that of hot-pressed oils. Cold-pressed oils contain hardly any phospholipids. Most of the phospholipids in sunflower oil are hydratable and may be removed by water degumming. Figure 19 shows a comparison of the variation in the phospholipid content of two crude sunflower oils, obtained by pressing and by solvent extraction, respectively, and a degummed sunflower oil (53).

Nonhydratable phospholipids (mainly Ca and Mg salts of PA and lysoPA, glycerophosphates, and inorganic phosphates) remain in the oil after water degumming. As a result, a degummed oil may have a significantly higher percentage of PA and a lower percentage of other phospholipids (particularly PC) than the original crude oil—PC being almost fully hydratable and PA is nonhydratable when complexed with Ca or Mg, PC and PI hydrating considerably faster than



**Figure 19.** Minimum and maximum values of the total phospholipid content in two crude sunflower oils, obtained by pressing and by solvent extraction respectively, and in a degummed oil [based on (53)].



**Figure 20.** Minimum and maximum values of the content of each phospholipid in two crude sunflower oils, obtained by pressing and by solvent extraction respectively, and in a degummed oil [based on (53)].

PE and PA (54). Figure 20 shows a comparison of the variation range of each phospholipid in two crude sunflower oils, obtained by pressing and by solvent extraction, respectively, and a degummed sunflower oil (53). The hydratability of these compounds may be increased with the addition of either phosphoric or citric acid, leading to a more efficient degumming process. Nonhydratable phospholipids may also be removed by enzymatic treatment through special biochemical reactions, such as enzyme-catalyzed hydrolytic cleavage of the phospholipid molecules (53).

Smiles et al. (55) studied the effectiveness of six different degumming reagents for crude sunflower oil: water, citric acid, phosphoric acid, oxalic acid, acetic anhydride, and maleic anhydride. All reagents were significantly more effective than water in removing phospholipids, with maleic anhydride and oxalic acid removing 95% and 90% of total phosphorous, respectively. No significant changes were found in the composition of the phospholipids remaining in the oil.

Pan et al. (56) studied the influence of different operation variables (temperature, contact time, type and concentration of degumming reagent, pH, calcium and magnesium content) on both water and acid degumming efficiency for sunflower oil. All degumming solutions (phosphoric acid, citric acid, and a 50:50 blend of both solutions) had a 2.5% concentration. Optimum degumming conditions with phosphoric acid were 60–70°C and addition of 10% of its solution. Optimum degumming conditions with citric acid were 70°C and addition of 10% of its solution, whereas for the blend of both reagents, treatment at 60°C and 8% of the blend was optimum.

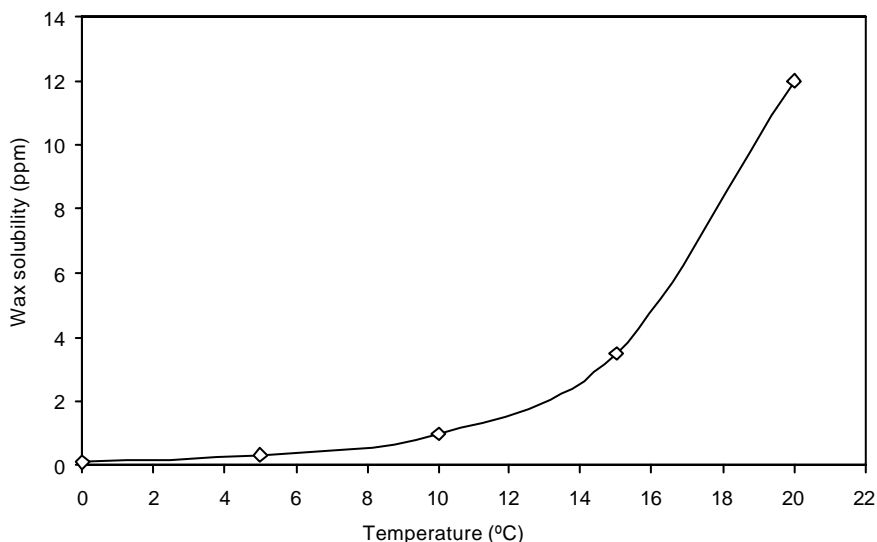
**5.3.2. Dewaxing** Winterization is achieved by cooling an oil with the consequent crystallization of high melting point fractions (waxes and/or triacylglycerols).

These are responsible for the appearance of turbidity in some edible oils during storage at low temperature or even at room temperature.

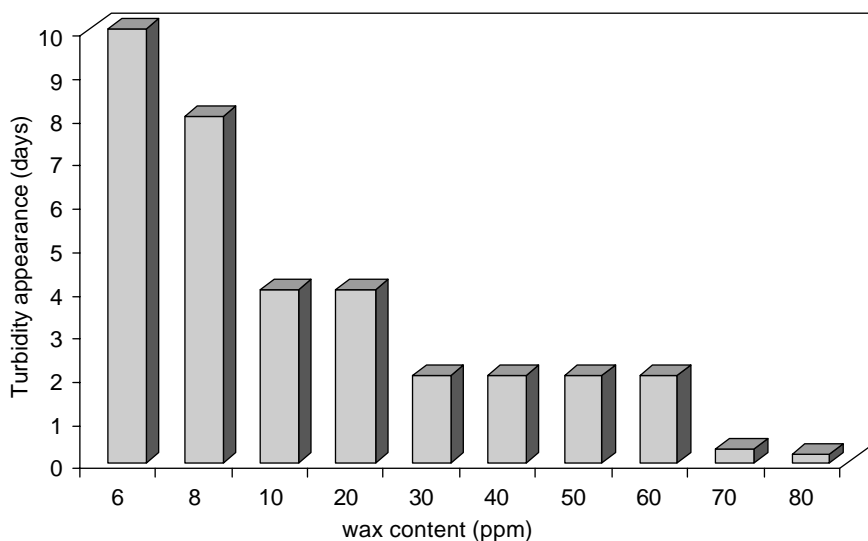
Winterization of sunflower oil is usually referred to as “dewaxing.” Improved techniques have emerged in recent years with the appearance of seed varieties of high oil and additional wax contents. As the improvements in oil yield (higher than 40% for these seeds) have been obtained at the expense of a reduction in hull thickness, the concentration of seed protection substances (waxes) in the hull has also been increased. The concentration of wax in hulls of improved hybrids may be as high as 3–4%, compared with 1% in hulls of the traditional seeds. Around 83% of the wax content is in the seed hull, 17% in the seedcoat and traces are in the seed.

In order to facilitate oil extraction, either through pressing or by solvent extraction, it is necessary to leave a certain amount of hulls in the seed. The wax content is thus considerably higher in these oils than in oils of traditional seed varieties. Crude sunflower oils may contain 2000–3000 ppm of wax, depending on the seed type and the oil extraction method employed.

**5.3.2.1. Cold Stability of Refined Sunflower Oil** Sunflower oil waxes are fatty alcohol esters of fatty acids. Their melting point is around 75°C, and their solubility in the oil is low, leading to the appearance of turbidity in the refined oil with decreasing temperature. An oil’s cold stability is usually assessed by means of the cold test (method AOCS Cc 7-25). Oils passing the cold test will remain clear—without the appearance of turbidity—after 5.5 hours permanence at 0°C. The solubility of waxes in sunflower oil is shown in Figure 21 as a function of



**Figure 21.** Solubility of sunflower oil waxes (ppm) as a function of temperature (°C) [based on (57)].



**Figure 22.** Time required for turbidity appearance in a sunflower oil stored at 0°C as a function of the wax content [based on (58, 59)].

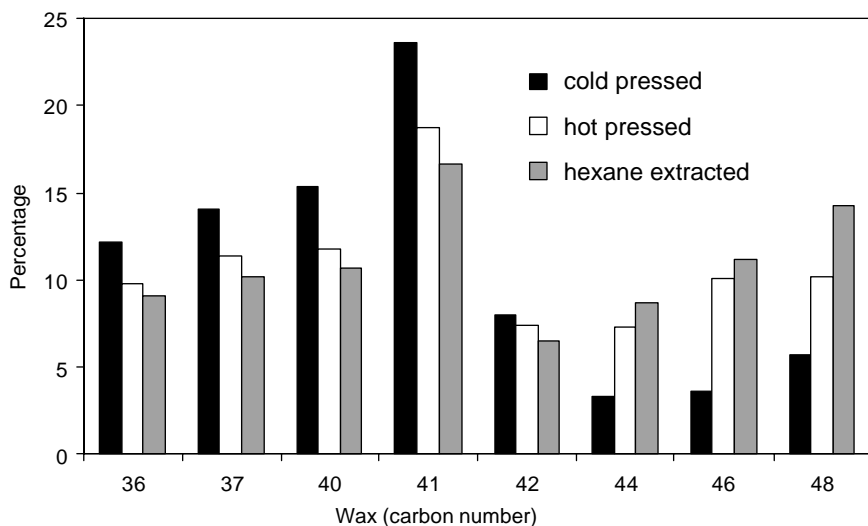
temperature [based on Bloch (57)]. The solubility of waxes is extremely low (in the order of 0.1 ppm) at 0°C and increases to 12 ppm at 20°C. It is possible that oils that are clear at room temperature may develop some cloudiness if stored in a refrigerator.

Another problem concerning refined sunflower oil is that the wax precipitate may appear several days upon elaboration, even for oils successfully passing quality control checks carried out by means of the cold test. The precipitate, although not affecting the nutritional or organoleptic properties of the oil, will be considered as an impurity by the consumer and should be avoided for oils commercialized in transparent bottles.

Consequently, other factors than the wax content influence the time required for the appearance of turbidity. Figure 22 shows the time required for turbidity appearance in sunflower oil stored at 0°C as a function of the wax content. All oils passed the cold test, i.e., remained clear after 5.5 hours at 0°C, except the sample containing 80 ppm of wax (58, 59). Turbidity develops in an oil containing 6 ppm of wax after 10 days, that is, a longer time period than that considered by the cold test.

The phenomenon of turbidity appearance in sunflower oil is complex. The time necessary for turbidity appearance for a given wax content depends on the tempering temperature. Both the time necessary for the appearance of turbidity at temperatures above 0°C and the minimum concentration causing turbidity may be expected to increase with temperature. However, the wax crystallization rate is reported to be the highest at 13°C, i.e., the time necessary for the appearance of visible turbidity in an oil is the shortest at this temperature (58, 59). In view of the above difficulties,





**Figure 23.** Influence of type of extraction on wax composition in sunflower oils [based on (53)].

several analytical methods have been developed for predicting the appearance of turbidity (60–62) or for determining the wax content in sunflower oil (53, 63, 64).

**5.3.2.2. Content and Composition of Sunflower Oil Wax** Both the wax content and composition of sunflower oil depend on the method of oil extraction. According to work carried out by Carelli et al. (53) with sunflower oils extracted from the same seed lot, the crude industrial oil obtained by hexane extraction contained 1073 ppm, the crude industrial oil obtained by hot pressing contained 947 ppm, and the cold-pressed oil obtained in the laboratory had 771 ppm of wax. That is, the oil extracted by hexane had the highest wax content, the hot-pressed oil having a higher content than the cold-pressed oil.

Figure 23 shows the composition of wax esters according to total number of carbons, for the oils obtained by the three above methods. Waxes in the cold-pressed oil were composed predominantly of esters below 42 carbons. Both hot-extracted oils had a similar wax profile. Wax extractability appears to depend largely on temperature, in particular for those waxes containing over 42 carbons, where reductions of 70% can be achieved. Degumming did not lead to any significant reduction in the wax content for either type of industrial oil. In short, the content and composition of sunflower waxes are affected by the extraction method, although degumming does not have a significant effect on the total wax content.

Carelli et al. (53) studied the wax content of crude sunflower oil (995 ppm) and of three commercially refined edible oils (366–624 ppm), finding a high degree of dependence of the wax content on the refining process conditions. The wax composition in the crude oil and in one of the refined oils is shown in Figure 24. Waxes remaining after refining are richer in 40 and 41 carbon esters (less rich in esters of

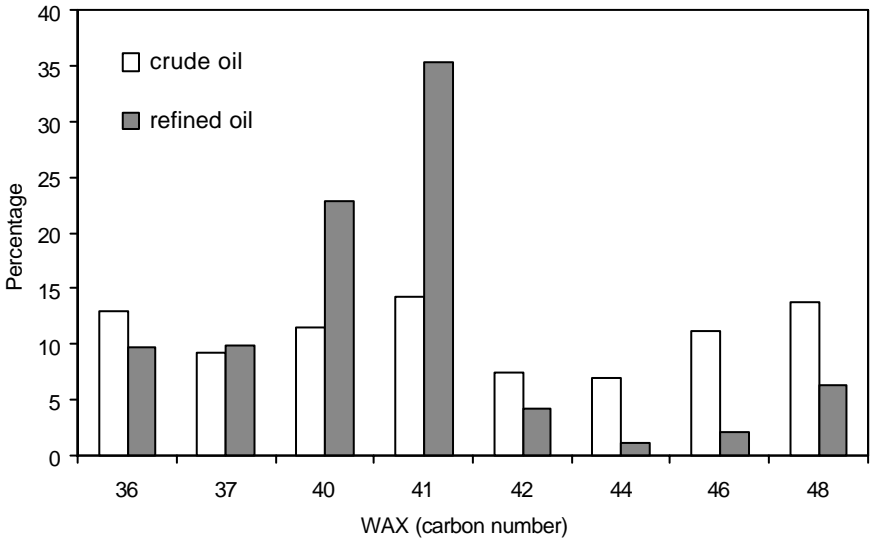


Figure 24. Wax composition in a crude oil and in a commercial refined oil [based on (53)].

over 42 carbons) than the original waxes, indicating a clear tendency of waxes of higher molecular weight (higher melting point) to crystallize during refining; i.e., the cooling stage produces a fractionation of the waxes. Figure 25 shows the composition of waxes precipitating in the dewaxing process of sunflower oil [based on

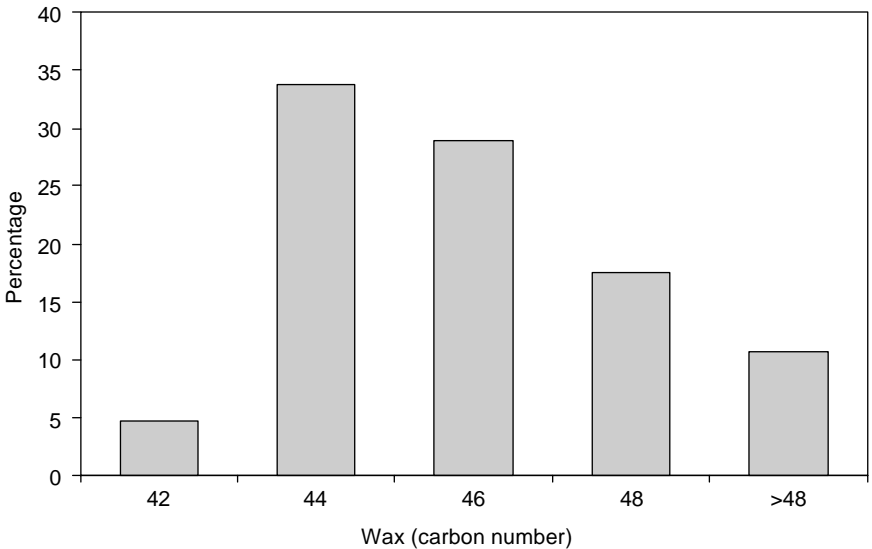
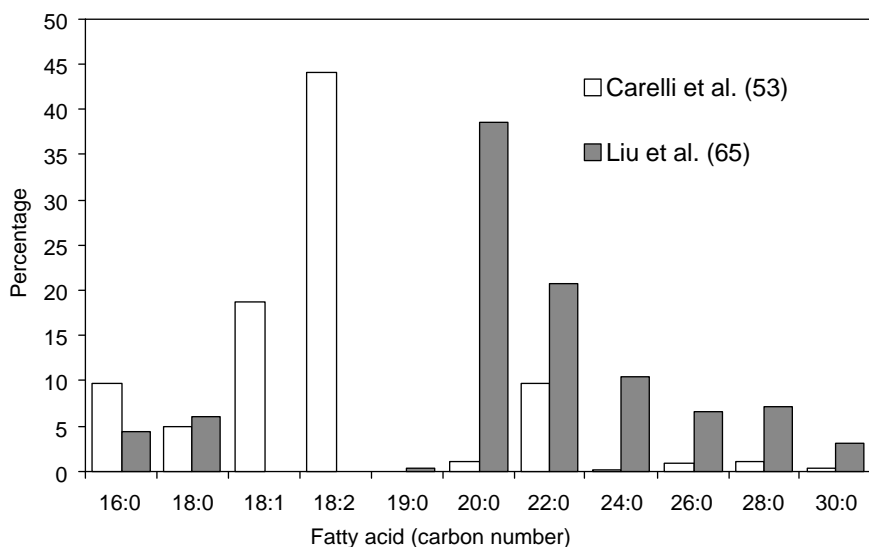


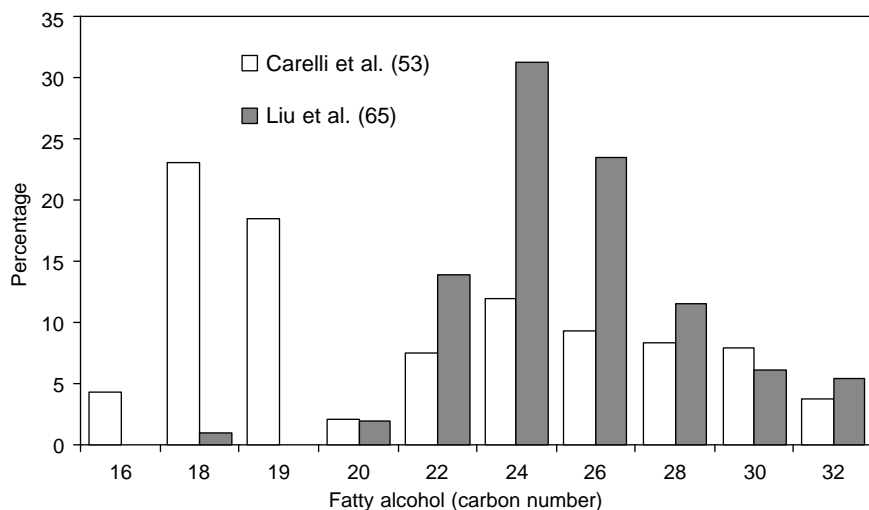
Figure 25. Composition of precipitated waxes in the dewaxing process of sunflower oil [based on (53)].



**Figure 26.** Fatty acid composition of sunflower wax (53, 65).

Carelli et al. (53)]. The predominance of esters of higher number of carbons (over 44) is clear, in accordance with the above.

Because the composition of the wax remaining in an oil differs markedly from that precipitating from it, care must be taken against differences in the sunflower wax profile as reported in the literature. The fatty acid composition and the fatty alcohol composition of sunflower wax (53, 65) are compared in Figures 26 and 27.



**Figure 27.** Fatty alcohol composition of sunflower wax (53, 65).

It is worth noting that Liu et al. (65) studied the wax removed from an oil that had been stored at 0°C for 1 week, allowing enough time for crystallization and fractionation to take place. The wax contained preferentially esters of a higher number of carbons. Carelli et al. (53) extracted quantitatively all the waxes contained in a crude sunflower oil.

**5.3.2.3. Sunflower Dewaxing Procedures** Several methods are used for wax removal. Most widespread are those associated to the refining process, whereas cold degumming and superdegumming, both related to the treatment of crude oils, are scarcely used (57, 66).

Cold neutralization was developed for high-capacity plants and for the processing of oils of high wax content. The crude oil is neutralized at a low temperature, and the wax is removed simultaneously with the soapstock. The processed oil is of good quality, although losses may be considerable, especially with oils containing over 1.5% free fatty acids.

Water dewaxing is the latest method of sunflower oil dewaxing. As the crystallization process of waxes is inhibited or delayed by the phosphatides in the oil, dewaxing is carried out after full degumming has been completed. The process of dewaxing through hot neutralization followed by cold washing has gradually replaced the traditional methods. After conventional hot neutralization is performed, and the soapstock removed, the oil is cooled to 6–8°C and left to settle for 8–10 hours. A small percentage of soda (NaOH) is added; time is allowed for maturation; it is heated to 20–25°C and centrifuged. One final filtration step must be performed on the cold oil to remove the remaining waxes.

Cold filtration may be performed before or after deodorization. Oil from the bleacher or the deodorizer is cooled to 12–15°C, and after settling for 12 hours, it is cold-filtrated with the addition of filter aid (perlite or diatoma) to prevent clogging caused by the wax.

**5.3.3. Physical Refining** The success of the physical refining stage depends largely on the pretreatment of crude oil. Among other compounds, phosphatides must be efficiently removed. The elimination of nonhydratable phosphatides (NHP), mainly Ca and Mg salts of phosphatidic acid and lysophosphatidic acid, poses one major difficulty to the pretreatment of oil for physical refining. NHP may be removed from the oil with the addition of an acid, generally phosphoric or citric acid, or complexation agents for Ca/Mg (preventing the precipitation of insoluble salts). Treatment with these acids is the basis for several oil pretreatment processes prior to physical refining.

The content and ratio of NHP in an oil differs significantly depending on the method applied (Table 17). The NHP content is lower in extracted and mixed oils (67). The NHP content of sunflower oil is low, and degumming may readily be accomplished. Dimic et al. (67) studied a simplified process for pretreatment of sunflower oil with the application of multiple acid degumming stages.

Part of the unsaponifiable matter (such as tocopherols, sterols, and sterolesters) is distilled together with the free fatty acids during deodorization/deacidification, as

**TABLE 17. Content of Total Phosphatides (TP) and Nonhydratable Phosphatides (NHP) of Crude Sunflower Oil [Based on (67)].**

	Pressed Oil	Extracted Oil	Mixed Oil
TP (g/100 g oil)	0.24	1.32	0.70
NHP (g/100 g oil)	0.14	0.04	0.09
NHP*100/TP	59%	3%	13%

well as volatile oxidation byproducts, and flavor components. Ideally, natural oil components should remain in the oil in the highest possible amount. As much as 85% of tocopherols remain in a finished oil upon physical refining operations carried out at temperatures below 240°C.

Sunflower oil processing byproducts depend on the kind of refining, whether chemical or physical. The so-called “deodistillate” of chemical refining of sunflower oil can be used as feedstock for obtaining tocopherols and sterols. The tocopherol composition of sunflower oil (over 90% alpha-tocopherol and only a low proportion of the beta and gamma isomers) makes deodistillates of great value for industrial Vitamin E production. Increased importance has been placed on vegetal sterols because they were found to reduce the risk of cardiovascular disease. As a result, the demand for tocopherols and sterols was increased as food additives.

Deodistillate originated in physical refining, diluted in free fatty acids, is not an attractive feedstock for Vitamin E and sterol producers. Deodistillate originated in chemical refining of sunflower oil may contain 5–7% of total tocopherols, compared with only 1–2% for deodistillate of physical refining (68).

**5.3.4. Deodorization** There are no major differences between deodorization procedures for sunflower oil and other vegetable oils. The loss of tocopherols in the oil is worth noting for sunflower oil, though. The average tocopherol content of sunflower oil is medium (440–1520 ppm, according to Codex-Stan 210-1999), nearly all of which is alpha-tocopherol (403–935 ppm). Table 18 shows the reduction in the tocopherol content upon deodorization of sunflower oil (69). Tocopherols may be recovered from the distillate of deodorization.

**TABLE 18. Total Tocopherol Content (ppm) of Sunflower Oil in Different Refining Stages [Based on (69)].**

Refining Stage	Tocopherols (ppm)
Crude	823
Neutralized	815
Bleached	843
Dewaxed	903
Deodorized	510

## 5.4. Cold-Pressed Sunflower Oil

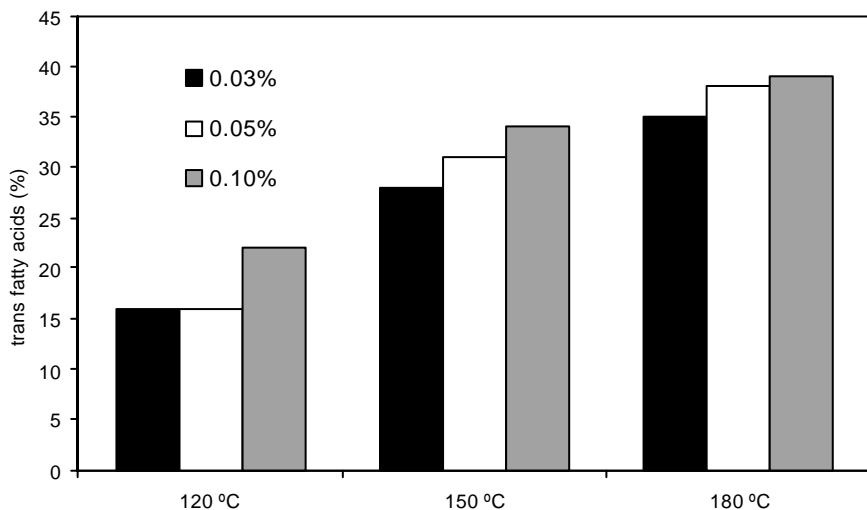
Virgin oils currently available on the market are not restricted to virgin olive oil but include other oils obtained by cold pressing of seed. These oils are appreciated highly by consumers in view of their nutritional characteristics and flavor (particularly those organoleptic notes that are lost in refined oils). The consumer appreciates the “natural” characteristics of these oils, as they are not subjected to chemical treatment. A relatively new market has developed for these oils in the U.K., Germany, France, Italy, and Switzerland, among other markets. Sunflower oil is manufactured and commercialized as “cold-pressed” or “first cold-pressed” oil.

Cold-pressed sunflower oil is obtained through mechanical pressing at a low temperature (30–35°C, for example). It has a clear appearance, an agreeable golden-yellow color, and a typical light flavor. The visible spectrum of this oil indicates a low content of chlorophylls and carotenoids, characteristic of unbleached oils. It may be stored in dark containers at room temperature for 1 year without the appearance of turbidity—characteristic of sunflower oil extracted with solvent from undehulled seed. A high tocopherol content constitutes a natural protection against oxidation, to a higher extent than antioxidants added to commercial oils (70). De Panfilis et al. (71, 72) also analyzed regular cold-pressed sunflower oils manufactured by different European countries. Relatively high acidity values were found for these oils (0.65–1.59 %), characteristic of virgin oils (unrefined).

## 6. HYDROGENATION OF REGULAR SUNFLOWER OIL

Regular sunflower oil contains hardly any linolenic acid, a factor contributing to a high oxidative stability. Light hydrogenation processes (low temperature) are therefore unnecessary to increase the stability of sunflower oil, as is the case for soybean and rapeseed oil—aiming at the elimination of linolenic acid, while avoiding the formation of considerable amounts of *trans*-isomers. Sunflower oil is hydrogenated in producer countries for use in the manufacture of shortenings and margarines. It is a good raw material for the production of hydrogenated fat of relatively flat melting curve, with melting point in the range of 32–36°C. The behavior of sunflower oil in the hydrogenation process is similar to that of soybean oil, and it does not require particularly special or different reaction conditions. It does not contain compounds that may interfere with the reaction, as is the case for rapeseed oil. Mention will be made here only of those aspects that are specific to sunflower oil, without treatment of the general conditions valid for hydrogenation of any vegetable oil. Process control parameters are the refractive index, melting point, iodine value, and solid fat index.

Topallar et al. (73) studied the density and viscosity of hydrogenated regular sunflower oil of iodine value up to 82.4. The density of hydrogenated sunflower oil is slightly higher than that of its nonhydrogenated counterpart, varying linearly with temperature. At a given temperature, oil viscosity was found to double upon hydrogenation.



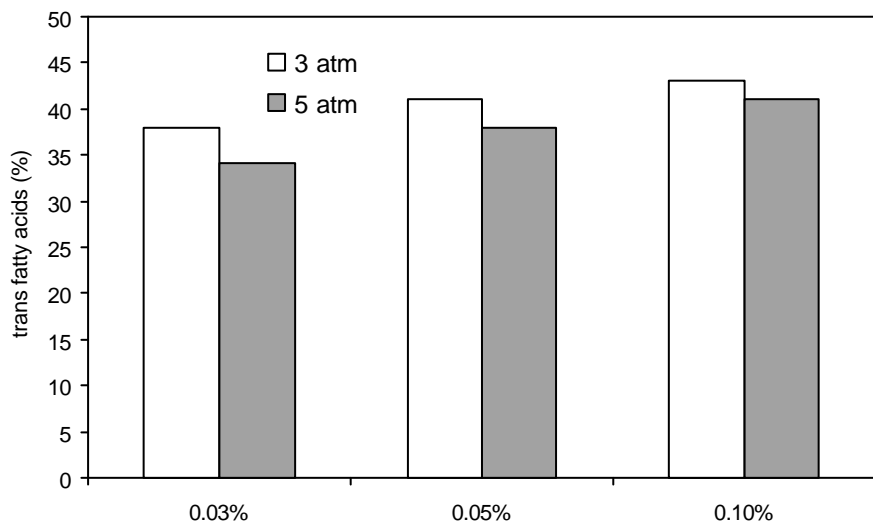
**Figure 28.** Content of *trans*-isomers in a hydrogenated regular sunflower oil at different temperatures (120°C, 150°C, and 180°C) with varying amounts of a nickel catalyst at 20% on silica (0.03%, 0.05%, and 0.10% nickel in the oil) [based on (74)].

Around 40% of *trans*-isomers are formed in the traditional hydrogenation process. Figure 28 shows the amount of *trans*-isomers formed as a function of hydrogenation temperature (120, 150, and 180°C) and the amount of nickel catalyst at 20% on silica (0.03, 0.05, and 0.10% nickel in the oil), for reactions taking place in the laboratory at a pressure of 3 atm and an agitation rate of 1500 rpm (74).

Hydrogenation of vegetable oils at a low temperature (105–120°C) with a high hydrogen concentration on the catalyst results in a minimum amount of *trans*-isomer formation. Medium-temperature hydrogenation (150°C) may be unselective, and high-temperature hydrogenation (180°C) generates the highest content of *trans*-isomers, a value near equilibrium. Sunflower oil shows the expected behavior, with a minimum of *trans*-isomers corresponding to the lowest temperature and the least amount of catalyst, and a maximum at the highest temperature and the highest amount of catalyst.

Variations of the hydrogen pressure and the amount of catalyst, keeping temperature at a constant 180°C and the rate of agitation at 750 rpm, did not result in significant modification in the *trans*-content. Figure 29 shows this effect (74).

The traditional hydrogenation process generates a high proportion of *trans*-isomers. Having considerably higher melting points than their *cis*-isomers, *trans*-isomers contribute to a large extent to an enhancement of product thermal behavior and plasticity. On the other hand, from the nutritional viewpoint, it is recommended to avoid intake of *trans*-isomers; thus, ways have been sought to reduce their content in margarines. One widely used procedure consists full hydrogenation of the oil and later interesterification with nonhydrogenated oil. A new method consists hydrogenation with solvent under supercritical conditions, leading to a reduction



**Figure 29.** Content of *trans*-isomers in a hydrogenated regular sunflower oil at different pressures (3 and 5 atm) and with different amounts of nickel catalyst at 20% on silica (0.03%, 0.05%, and 0.10% nickel in the oil) [based on (74)].

in the *trans*-content below 5%. The rate of hydrogenation is some 1000 times greater owing to an increased transfer of hydrogen toward the catalyst and the lack of blockage of pores in the catalyst produced by stagnant oil (74).

Catalytic transfer hydrogenation (CTH) is one method currently being developed. This method differs from traditional hydrogenation with molecular hydrogen in the use of a hydrogen donor (for example, sodium formate solution) in a catalytic transfer reduction reaction. Naglic et al. (75) used this procedure to hydrogenate regular sunflower oil, among other vegetable oils.

Hydrogenated sunflower oil may suffer from disadvantages for use in the manufacture of margarine spreads. The appearance of sandiness in these products during storage is because of a strong tendency of partially hydrogenated sunflower oil to form  $\beta$  crystals. Under rapid cooling conditions, as used in the manufacture of margarines, the  $\alpha$  phase is the first to form, followed by its rapid transformation into phase  $\beta'$ . This is the desirable crystalline form, because, under certain conditions, it tends to form a fine three-dimensional network capable of immobilizing a large amount of liquid oil. From a thermodynamic viewpoint, phase  $\beta$  is the stable form of hydrogenated sunflower oil. Thus, the solid state transformation of  $\beta'$  into  $\beta$  always takes place, leading to the formation of a coarse, sandy texture detected during storage but not during product elaboration. The transformation of  $\beta'$  to  $\beta$  is slow. The time required for full transformation depends on temperature and crystallization rate of the manufacturing process, as well as storage temperature of the margarine (76–79).

Various procedures have been proposed to solve the problem of graininess of margarines manufactured with hydrogenated sunflower oil. The hydrogenated oil



may be mixed with partially hydrogenated cottonseed oil with the consequent increase of fatty acids of over 16 carbons. Cottonseed oil, crystallizing at a higher temperature than hydrogenated sunflower oil, induces and stabilizes  $\beta'$  crystallization in the rest of the fatty material (76). Food emulsifiers provide another solution to the problem. Addition of saturated and unsaturated fatty acid monoacylglycerols, acting as modifiers of the crystalline structure, aids in preventing the undesired phenomenon. Also, the addition of 0.3% of sorbitan tristearate inhibits the transition from  $\beta'$  to  $\beta$  in a margarine. The same is observed for sucrose polyesters (80).

## 7. STORAGE AND DETERIORATION OF SUNFLOWER OIL

Several general factors affect the oxidative stability of sunflower oil—as well as most vegetable oils—during storage. One of these factors is the degree of unsaturation, i.e., the relative content of oleic and linoleic acids. Product shelf-life is affected by manufacturing conditions such as the type of extraction process (pressing, with solvent, with supercritical fluids), degree of purification (crude, refined, deodorized, etc.), addition of antioxidants, and type of packaging (container material, incorporation of inert atmosphere, etc.). Other major factors influencing the oxidative stability are the particular storage conditions: time, temperature, and light, among others.

### 7.1. Composition of the Refined Oil

Sunflower oil extracted from different types of hybrid may have different compositions. It is expected that the degree of unsaturation will influence the oxidative stability of sunflower oil markedly. AOM time measurements were used to determine the influence of oleic/linoleic ratio on the oxidative stability of sunflower oil (36). Oil samples extracted (refined and deodorized) from three progenies of cultivar Pervenets were analyzed, as well as other oil samples from different regions of the United States. The oleic acid content thus ranged from 18% to 89%, and the linoleic acid content decreased from 69% to 1%; the saturated fatty acid content was nearly constant. AOM values increased from 11 hours for the oil containing the least amount of oleic acid to 100 hours for that with the oleic acid highest content. These results show the importance of monounsaturated fatty acid content on the oxidative stability of sunflower oil.

Inherent stability of an oil may be calculated from the oil composition and the relative rates of oxidation of oleic (defined as 1), linoleic (evaluated as 10), and linolenic (evaluated as 25) acids. The higher this value, the more unstable or susceptible to oxidation an oil is. Values of 6.8 and 1.9 are obtained for regular and high-oleic sunflower oils, respectively, taking into account their standard compositions. The inherent stability of olive oil, calculated as 1.5, is slightly lower than that of high-oleic sunflower oil, whereas even lower values are obtained for saturated fatty materials like tallow (0.86), palm kernel (0.27), and coconut (0.24). The

inherent stability of high-oleic sunflower oil is 3.5 times higher than that of high-linoleic sunflower, and higher than the values for most vegetable oils (81).

## 7.2. Storage Conditions

Three major factors influence the storage conditions of oils: temperature, light, and the presence of dissolved oxygen. The combined effect of temperature and light exposure on the stability of a refined commercial high-linoleic sunflower oil was studied (82). Oil bottled in PET (polyethylene terephthalate)—the head space being filled with nitrogen—was stored at three different temperatures (35, 45, and 60°C), both in a dark chamber and with 12-hour lighting per day (18-W fluorescent tube). Lighting conditions were chosen considering bottles packed in cardboard boxes (darkness), or displayed on supermarket shelves—exposed to artificial lighting.

The criterion for establishing oil deterioration was the rejection of an oil sample by a panel of trained consumers. The corresponding shelf-lives were estimated for the three temperatures (35, 45, and 60°C). These values were used to extrapolate shelf-life at 20°C (taken as room temperature). Storage temperature was found to have a marked influence on oil deterioration, even when bottled under inert nitrogen atmosphere. For example, an oil stored in the absence of light at 45°C is rejected by consumers after 102 days, compared with 1140 days estimated for oils stored at 20°C.

Estimated shelf-lives for those samples stored under conditions of darkness were considerably higher than for the corresponding samples exposed to light. Estimated shelf-life at 20°C was 281 days for oil exposed to light and around 1140 days for oil stored in dark chambers. This value is higher than the 2 years generally established by manufacturers as useful life period of this kind of oil. Exposure to light must be avoided to extend the life-span of sunflower oil, and it must be bottled in containers that prevent the passage of light. PET bottles do not prove efficacious light filters in preventing oil deterioration.

Other researchers (83) have studied the effect of light exposure on regular sunflower oil (iodine value = 132) and on partially hydrogenated sunflower oil (iodine value = 82). Oil samples were stored in containers of glass, PET, and glass covered by tin, and were exposed directly to solar radiation at atmospheric conditions for 30 days (no indication is given of either temperature or exposure time). As expected for each type of container, the increase in the peroxide value was higher for the nonhydrogenated oil than it was for its hydrogenated counterpart. A rapid increase of the peroxide value was reported for those samples stored in glass containers, whereas an intermediate value (yet high) was for PET containers, with practically no deterioration of those samples in metal-covered containers. These results are in accordance with the work of other authors (82).

Values denoting oxidative deterioration increase gradually during the shelf-life of an oil. Determinations of induction times in rapid-aging tests also reflect the oxidative stability of commercial refined oils. Grompone et al. (84) studied the stability of commercial regular sunflower oils. The oil samples were stored at ambient conditions in their original PET bottles for a one-year period. The OSI induction time

at 110°C was found to decrease from 4.6 hours (at the time of purchase of the oil) to 3.3 hours (one year later). A similar behavior was observed for two refined oils produced by the same manufacturer over a one-year time difference. The OSI times of both oils were determined simultaneously: 3.9 hours for the oil procured that year and 3.2 for the sample procured in a previous year (85).

### 7.3. Presence of Free Fatty Acids

The pro-oxidant effect of free fatty acids in vegetable oils is widely known. To determine their influence (86), pure oleic acid was added (up to 3%) to a high-oleic sunflower oil extracted by cold-pressing. The effect intensity was found to be directly related with the concentration of free fatty acids.

### 7.4. Addition of Antioxidants

The effectiveness of antioxidants depends on the type of fatty material; tests are, therefore, required for each case. Stabilization studies have been reported, for example, of refined high-linoleic sunflower oil with the addition of three antioxidants and blends of them (87). Butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroxyquinone (TBHQ) were evaluated as antioxidants through determinations of induction time by Rancimat method at 98°C and the changes in the properties (acidity, peroxide value, anisidine value, polar compounds content) of the oil samples during storage in glass jars without lid, in the dark, at constant temperature: 30 days at 47°C and 30°C and 10 days at 67°C.

The stability of oils containing TBHQ and PG, measured by Rancimat induction time, increased rapidly with increasing antioxidant concentration. An induction time of 12.7 hours for the original oil was increased to 25.7 hours with the addition of 50 ppm of TBHQ and to as much as 42.2 hours with the addition of 200 ppm. PG had a lesser stabilizing effect, with induction times of 18.8 hours at 50 ppm and 32.3 hours at 200 ppm. BHT had a slight stabilizing effect with practically no modification of induction time: increasing only to 14.2 hours at 200 ppm. Thus, TBHQ proved 1.7 times more effective than PG and 22 times more effective than BHT. No cooperative interaction (synergism) was observed between antioxidants through Rancimat method.

Different results were obtained with respect to the effectiveness of antioxidants for oil stored at 47°C in the dark. Effectiveness was evaluated as the time required for a sample to reach a peroxide value of 10 meq/kg, as estimated from curves of peroxide value as a function of storage time. The estimated effectiveness of TBHQ was approximately 9.6 times higher than that of GP and 51 times higher than for BHT.

According to these results, the relative antioxidant effectiveness of BHT increased with decreasing temperature, and it decreased for GP. The Rancimat method underrated the stability of those samples containing BHT, perhaps because of volatilization of antioxidants during Rancimat testing. The relative activity of

antioxidants depends on both temperature and the conditions of the method used to measure such activity.

Independent of the method and test conditions employed, TBHQ proved to be the most effective of all three antioxidants, followed by PG, and BHT had little antioxidant effect on the sunflower oil studied. Further research should focus on evaluation of antioxidant effectiveness through methods of oxidative experimentation in conditions similar to those of storage.

## 7.5. Effect of Heating and Microwaves

Vegetable oils exposed to high temperature undergo rapid deterioration, as can be evidenced both through chemical and sensory analysis. For example, a refined high-linoleic sunflower oil kept at 60°C for one hour showed changes in its ultraviolet absorption spectrum, with the appearance of a peak at 234 nm corresponding to the formation of conjugated dienes generated in the oxidation of linoleic acid. Another peak was observed at 268 nm for oil kept at 180°C for 1 hour, corresponding to oxidation byproducts, especially ethylene diketones (84).

The development of rancidity in sunflower oil is accelerated with increased temperature, the process being even more complex in microwave cooking. To determine the influence of combined factors (88), both a regular sunflower oil and a high-oleic sunflower oil were exposed to different heating conditions: (1) microwave heating at approximately 170°C for 120 min; (2) conventional electric heating at 180°C for 120 min; (3) exposure to microwave radiation for 120 min at temperatures lower than 40°C. Greater degrees of alteration were found for both oil types when microwave-heated than if heated in a conventional oven, and exposure to microwaves without heating did not produce significant alteration. Hence, the increase in temperature was found to be the main deterioration factor for both oil types.

## 7.6. Crude Sunflower Oil

Crude sunflower oils are produced via mechanical pressing or through extraction with hexane, followed by water degumming. Most oils are stored for relatively long periods prior to refining operations. Cold-pressed crude oils, of superior edible quality, are commercialized as such. Whether unrefined or cold-pressed, the oil may be subject to temperature fluctuations.

The influence of composition, storage temperature (30, 47, and 67°C), and oxygen concentration (open and stoppered bottles containing different amounts of oil, and under nitrogen atmosphere) was studied for degummed crude sunflower oils obtained by pressing and by hexane extraction (89, 90). The oil obtained by solvent extraction had a lower rate of oxidation than the cold-pressed oil, although initially at a more advanced step of deterioration. Although all oils had equal amounts of unsaturated fatty acids and the same concentration of natural tocopherols, the solvent-extracted oil had a higher concentration of phosphorous related with the phospholipid content. It appears that differences in oxidative stability may be attributed

to the phospholipid concentration, having synergic activity and a metal scavenger capacity.

OSI induction times were determined at 110°C for three virgin high-linoleic sunflower oils, corresponding to three different manufacturers (85). These OSI times, ranging between 3.8 and 4.7 hours, were equal to or higher than the value obtained for a refined sunflower oil of the same fabrication year (3.9 hours), yet lower than the value for a virgin olive oil (8 hours). Virgin oils, which are not subjected to physical or chemical processes, retain their natural antioxidants, a fact that might explain the high oxidative stability of these oils.

## 7.7. Sunflower Oil Extracted by Supercritical Fluids

Although several studies have been made on the extraction of oil from seed by supercritical fluids, few are about sunflower oil (91). Reports on supercritical carbon dioxide (SC-CO<sub>2</sub>) over a wide range of pressure (20–70 Mpa) and temperature (40–80°C) show a maximum solubility of sunflower oil in supercritical CO<sub>2</sub> at 80°C and 70 Mpa, conditions similar to those obtained for other seed oils. Over 90% of the oil content of seed can be removed under these conditions (92).

One disadvantage of oils obtained by this method is their low oxidative stability (91). To determine the causes of such instability, one study was made of sunflower oil extracted with SC-CO<sub>2</sub>, finding that, although the composition and organoleptic properties of these oils were similar to those of hexane-extracted oils, their oxidative deterioration rate was higher (93). The results of this study suggest that the instability of oils extracted with SC-CO<sub>2</sub> may be attributed to the oxygen contained in the supercritical fluid, the tocopherols in the oil being inefficient in preventing oxidation. Thus, the oxidative stability of sunflower oil decreased when re-extracted with SC-CO<sub>2</sub>. Improvements may be obtained with the addition of traces of ascorbic acid.

## 8. USES OF SUNFLOWER OIL

### 8.1. Food Products

**8.1.1. Regular Sunflower Oil** In view of its light flavor, relatively high oxidative stability, and light golden-yellow color, sunflower oil finds many applications both in domestic and industrial levels. In countries where sunflower oil is a common oil, it is used mainly as a salad oil and as a cooking oil. Industrial applications of sunflower oil include use as frying oil, as well as in the manufacture of mayonnaise and oil-based dressings.

**8.1.2. High-oleic Sunflower Oil Formulations** A high oxidative stability is required by non-dairy coffee creamer formulas for an extended shelf-life without refrigeration. Both high-oleic sunflower oil and monoacylglycerols obtained from it prove stable products for use in this kind of creamer. The oil may also be used in fluid margarine, fluid spreads, dressings, and so on.

**8.1.3. Margarines** Owing to the strong tendency of hydrogenated sunflower oil to crystallize in the  $\beta$  form, precautions must be taken to avoid the problem of sandiness. The addition of crystal-modifying agents delays the transformation from unstable  $\alpha$  phase to  $\beta$  phase, or stabilizes the intermediate meta-stable  $\beta'$  phase. For optimum creaminess conditions, it is generally recommended to add 5–15% of  $\beta'$ -crystallizing hydrogenated oils to formulations of margarines and of some shortenings (2). There are studies in the literature (76) about margarine formulations with blends of partially hydrogenated cottonseed oil, partially hydrogenated sunflower oil, and nonhydrogenated sunflower oil. Hydrogenated cottonseed oil, having a strong tendency to crystallize in the  $\beta'$  form, has a favorable influence in the blend with sunflower oil.

There has been controversy over the consumption of foods rich in *trans*-isomers. As partially hydrogenated oils contain high amounts of *trans*-components, alternative processes have been sought that do not lead to the formation of *trans*-isomers as byproducts. One such alternative is total hydrogenation of an oil (which does not form *trans*-isomers) and later blending with liquid nonhydrogenated oil. The oxidative stability of the blend is determined by the instability of the nonhydrogenated oil. High-oleic sunflower oil appears a most appropriate ingredient in view of its low content in polyunsaturated fatty acids. Products of this kind are produced by some manufacturing companies.

Soft nonhydrogenated margarines containing high-oleic sunflower oil and other oils are available. Parmalat Canada, for example, produces a soft nonhydrogenated margarine trademarked under the name Olivina, containing a blend of refined olive oil, high-oleic and regular sunflower oil, and canola oil, as well as palm, and palm-kernel oils (94).

Another alternative for increasing oxidative stability while improving margarine texture consists in the interesterification of hydrogenated and nonhydrogenated oils. As an example, an outstanding increase of crystals in the  $\beta'$  form was observed upon interesterification of a blend of sunflower oil and fully hydrogenated soybean oil with a strong tendency to crystallize in the  $\beta$  form. This procedure leads to bases for zero-*trans*-margarines of optimum texture (95).

**8.1.4. Interesterified Tallow with Regular Sunflower Oil** Countries rich in livestock resources produce large amounts of tallow as a byproduct of the slaughter industry. Tallow is used in the manufacture of food, mainly bakery products and cookies. Among other reasons, a high hardness texture, and a melting point above the mouth temperature, make foods manufactured with tallow of lower quality. Interesterification of tallow with vegetable oils is an alternative for an improvement in the behavior of tallow.

Studies have been published on chemical interesterification of tallow with regular sunflower oil (96–98). Changes in the proportion of sunflower oil in blends with tallow produce little modification of the solid content or the melting point. However, important modifications take place in the thermal properties of these blends when they are subjected to a process of chemical interesterification. In addition, modifications occur in the crystalline structure. Solid beef tallow—composed

of large spherulites responsible for a sandy mouthfeel—may be interesterified with sunflower oil into a liquid blend (at process temperature), which solidifies in the form of tiny crystals, giving the solid product enhanced consistency and texture.

Lipase catalysis constitutes an alternative to the method of interesterification using chemical catalysts. Products of different physical properties may be obtained, according to lipase specificity (99,100).

**8.1.5. *Frying with Regular and Modified Sunflower Oil*** Deep-fat frying, a common cooking procedure, may be performed in a continuous or discontinuous manner. In repeated discontinuous frying—either in the home or in restaurants—the oil remains hot for long time periods, in contact with the surrounding air, with occasional cooking. The process is carried out with a relatively low rate of fresh-oil supply (turnover).

In continuous frying, generally performed in industrial facilities for processing of fried and prefried foods, turnover is high because of the large amount of oil removed by products during continuous cooking. Contact of the oil with oxygen is limited in this case by the steam protection barrier generated in the cooking process.

The deterioration of deep-frying oil depends on a large number of factors, including frying frequency (discontinuous or continuous), turnover, the time the oil is hot, and type of oil. Oil decomposition products absorbed by fried foods together with the frying oil during the cooking process affect not only the sensory and nutritional quality of these foods but also their shelf-life. They include polar compounds, triacylglycerol polymers and dimers, diacylglycerols, peroxides, volatile compounds, and so on. Storage time and characteristics depend on the type of product. Crisps, for instance, are generally stored at room temperature, whereas prefried french fries are stored in a freezer.

Frozen prefried foods are prepared before ingestion. Deep-fat frying is a most common cooking method for the manufacture of prefried foods. These foods are thus subjected to two different frying processes and a stage in a freezer prior to final cooking and consumption. Several studies have been performed to evaluate the oxidative resistance of sunflower oils when used in frying processes.

**8.1.5.1. *Regular Sunflower Oil*** High-linoleic sunflower oil is of customary use for frying in strong producer countries, as is the case of Argentina and Uruguay. In contrast, consumption and cooking use of olive oil has decreased in traditional consumer countries mainly because of price reasons. In Spain, the consumption of olive oil dropped from 55 g/capita/day in 1964 to 25 g/capita/day in 1987. Olive oil has been replaced by seed oils, especially sunflower oil (101, 102).

Although several studies have been performed to determine the characteristics of regular sunflower oil for use in frying, few were focused on the in situ stability of an oil contained in fried foods. In one study, french fries fried in high-linoleic sunflower oil and crushed to different sizes, were subjected to fast-aging in OSI equipment at 110°C and the induction period was determined (84). The values obtained differed from those for pure sunflower oil. This method is a more realistic

representation of the deterioration phenomenon, as it enables the study of an oil's oxidative stability in the intact food matrix.

Recent publications are based on the simulation of frying operations without the addition of food (103, 104). Others consider the process with the addition of food, generally french fries. The deterioration of sunflower oil was determined in the frying oil during discontinuous frying processes (101, 105), or continuous processes with different rates of turnover (106, 107), as well as in the oil extracted from fried foods (101, 108, 109). Other studies are on the deterioration of a sunflower oil absorbed in foods during storage in normal or fast-aging conditions.

Results show that regular sunflower oil rarely reaches a critical value of 25% of polar compounds in continuous frying processes with frequent turnover, indicating the suitability of sunflower oil for this use. However, use of this oil is recommended only for frying of crisp-type foods with short commercialization periods, ensuring their consumption before detectable levels of deterioration of the absorbed oil are reached (109).

*8.1.5.2. High-Oleic Sunflower Oil* Studies on the use of high-oleic sunflower oil in frying processes have similar characteristics to those performed on regular sunflower oil, and generally they include a comparison of both types (104, 109–119). Several reports have been published on oil deterioration in deep-fat frying processes carried out in intermittent manner (102, 114, 119) or continuously (110, 114, 119). Some studies simulate the frying process (in the absence of food) using Rancimat equipment without air bubbling at 180°C (104), or simply study oils heated in convection oven and on a hot plate (113). Other studies are on oil extracted from fried foods, generally potato crisps and french fries (111), and on the deterioration of oils contained in fried foods during storage in normal and fast-aging conditions (109, 112, 115, 116, 119).

Márquez-Ruiz et al. (119) compared regular and high-oleic sunflower oils in continuous and discontinuous potato frying processes. Oil deterioration was monitored in the fryer as well as in the absorbed oil, during the time of cooking and during 30 days' storage at 60°C. Several conclusions may be reached: (1) high-oleic sunflower oil deteriorated to a lesser extent than regular sunflower oil, in either continuous or discontinuous frying; (2) products fried in high-oleic sunflower oil and stored at 60°C were more stable than those fried in regular sunflower oil; (3) antioxidant protection was essential for fried products requiring storage prior to their consumption.

High-oleic sunflower oil is the most appropriate type for industrial frying, as determined through customary deterioration indices and sensory evaluation of final fried products. Both high-oleic and regular sunflower oils are feasible alternatives for processing of prefried products stored in freezer; i.e., no sensory differences were found in frozen precooked french fries stored for 19 months at -18°C, shelf-life for this kind of product being normally 2 years. However, for crisps stored under ambient conditions, those fried in regular sunflower oil had developed detectable levels of rancidity after 4 months, the process evolution being both more rapid and intense. Despite the above difference, the legal commercialization period for



crisps is usually 3 months, making both oils suitable for manufacture of this kind of product.

Studies have been made on the effect of additions of dimethyl polysiloxane (DMPS; 2 mg/kg) on frying oil performance and the storage of fried potato as crisps or french fries (109–114). DMPS is an antifoaming silicone forming a monolayer over the oil surface, protecting against oxidation by air contact. The addition of DMPS is inefficient for both regular and high-oleic sunflower oil in continuous frying, but it is useful in discontinuous frying where the oil surface is exposed to the atmosphere for extended time periods.

**8.1.5.3. Mid-Oleic Sunflower Oil** With the relatively recent appearance of mid-oleic sunflower oil, researchers have shown an interest in its use in frying processes. Abidi and Warner (120) used the three types of sunflower oil (regular, high, and mid-oleic) in the preparation of french fries, potato crisps, and fresh white corn tortilla chips. However, no general conclusions may be drawn regarding inherent stability as there is no indication of antioxidant type and concentration in the different oils.

Kleingartner and Warner (48) summarized several studies performed with mid-oleic sunflower oil in frying. Mid-oleic sunflower oil was used successfully by several potato chip manufacturing enterprises. In July 2000, Procter & Gamble announced it would use mid-oleic sunflower oil in the production of Pringles potato chips. The U.S. Department of Agriculture's National Center for Agricultural Utilization Research in Peoria also carried out performance evaluations in processes of frying of tortilla chips and of french-fried potatoes. Oxidative stability was evaluated through the appearance of polar compounds during intermittent frying and through flavor evolution during storage. Three years' research showed that mid-oleic sunflower oil was of higher frying quality than other nonhydrogenated oils (soybean, canola, corn, and cottonseed).

Pan-frying is a popular frying method at home and in many restaurants. The pan-fry stabilities of two oils with similar iodine values—mid-oleic sunflower oil (NuSun) and a commercial canola oil—were compared (121). Both oils have similar pan-fry stabilities, with few significant differences in the physicochemical properties during the heating process.

## 8.2. Industrial Products

**8.2.1. Biodiesel** The importance of biodiesel as a partial or total substitute for petroleum-based fuels has increased in the last two decades. Already in the 1980s, studies had been made in the United States for the use of vegetable oils (mustard, canola, corn, soybean, peanut) as fuel, sunflower being the preferred oil. The North Dakota State University, Agriculture Engineering Department, conducted a project to determine the effects of sunflower oil used in diesel engines. The Iowa State University, College of Agriculture, also conducted studies on the use of sunflower oil as fuel. Several short studies were carried out with a tractor by the USDA-SEA-AR Subtropical Texas Area unit in Weslaco (Texas). Other studies

were made outside the United States; for example, sunflower oil was tested on a range of diesel engines in South Africa.

The use of vegetable oils as a substitute for petroleum-based diesel has been replaced by use of their methyl or ethyl esters. These are produced in several countries for use in ignition engines, especially blended with petroleum diesel. Having a high population density and serious pollution problems, the development of biodiesel has been stimulated in European countries. A large number of diesel vehicles constitute a potential market for biodiesel production. In Germany, the first commercial plant was built in 1995 with a capacity of 60,000 ton/year. In 2001, Germany produced some 500,000 ton of biodiesel. Production of methyl ester biodiesel in Italy was 200,000 ton in 2001. In France, several plants currently operate, especially for rapeseed methyl ester production, with a total biodiesel production over 300,000 ton in 2001. Rapeseed and sunflower biodiesels are also produced commercially in Austria.

Sunflower oil, either regular or high oleic, may be used in biocarburants in the form of methyl esters. These products were already in use in Italy in 1998, pure or in blends. In Austria, high-oleic sunflower was used as carburant for tractors. The French oilseed sector launched an experimental program for the incorporation of methyl esters of traditional sunflower in fuels, instead of rapeseed esters, as certain regions of southern France are not suitable for rapeseed cultivation (41). Experiences with sunflower-based biodiesel were carried out in Spain, Greece, and Portugal, among others.

The optimization of biodiesel production by transesterification of sunflower oil was studied (122). The best combination of process parameters was found to be three stoichiometric doses of methanol, 0.28% w/w of KOH, and 70°C temperature. Several reports have been published on the properties of biodiesel manufactured with different fatty materials and on their performance in compression ignition engines, including information about sunflower oil and its esters (30, 31, 41, 123). Table 19 shows major properties of sunflower oil and its methyl esters. The physicochemical characteristics of these esters meet the norm specifications of different countries, even with improvements of some properties, such as the cetane number.

Although the production of methyl esters is the easiest alternative, the production of ethyl esters from ethanol obtained from renewable starch sources, e.g., corn, poses a more interesting challenge. However, the production of ethyl esters through

**TABLE 19. Properties of Sunflower Oil-Based Biodiesel (30, 41, 123).**

	Regular Oil (30)	Methyl Esters of Regular Oil (41)	Methyl Esters of Regular Oil (123)	Methyl Esters of High-Oleic Oil (41)
Cetane number	37	54	49	55
Energy content	39,575 kJ/kg	39,687 MJ/kg	33.5 MJ/l	39,733 MJ/kg
Cloud point (°C)	7.2	1	1	4
Flash point (°C)	183	180	274	185
Density (kg/l)	0.9161	0.8866	0.860	0.8821

basic catalysis is difficult to achieve because of the formation of stable emulsions. Studies have been conducted with a view to finding solutions to the problems associated with the production of ethyl esters of sunflower oil (124), opening new alternatives for its use as biodiesel.

**8.2.2. Lubricants** Mineral-based lubricants lead the lubricant market. However, with the advancement of the need for biodegradable products, vegetable oils have become popular, because of they are also better lubricants. The fatty acid composition of vegetable oils, however, is one major disadvantage. Oils rich in saturated fatty acids have poor low-temperature flow properties, and those rich in polyunsaturated fatty acids are of low oxidative resistance. Vegetable oils rich in monounsaturated fatty acids have optimum oxidative stability and low-temperature properties (125).

In view of the higher oxidative stability of high-oleic sunflower oil, it is used as diesel and gasoline engine lubricant. In France, for example, a product is commercialized containing 70–80% of high-oleic sunflower oil and 20–30% other additives. Several studies have been aimed at the production of polyol esters (propyl glycol, pentaerythritol, trimethylpropane) from high-oleic sunflower oil (41).

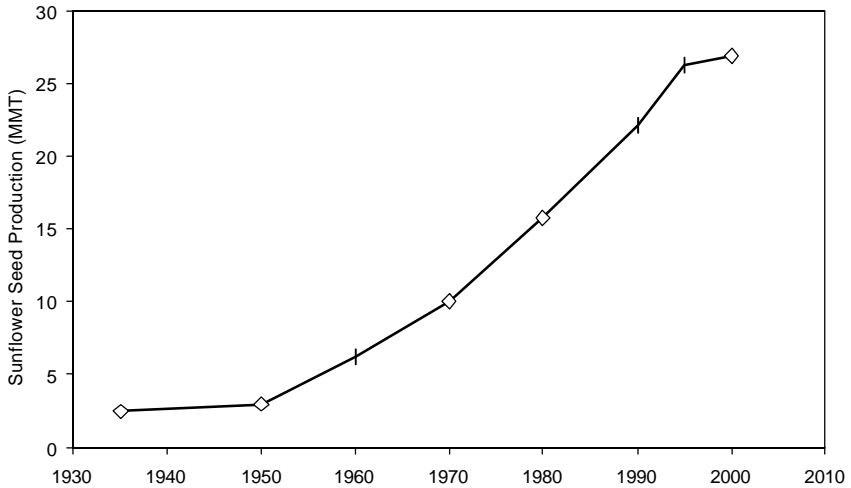
**8.2.3. Vegetable Oil-Based Printing Inks** Lithography and letterpress processes require paste inks. Printing inks that are conventionally used in these applications are multicomponent systems comprising a pigment, a hydrocarbon and/or alkyd resin, a hydrocarbon solvent, and optional additives. Vegetable oil-based non-petroleum inks have been formulated for various specialized applications. These ink formulations cost even less than petroleum oil-based ink formulations. Sunflower oil is used for the manufacture of vegetable oil-based inks (126).

**8.2.4. Other Applications** Containing around 70% linoleic acid, sunflower oil is a semidrying oil. Insofar as economically feasible, sunflower oil may replace soybean oil in the manufacture of resins for paint and press-ink formulations. Through epoxidation of sunflower oil, PVC stabilizers may be obtained, and dimerization would yield products that could be used for lubricant manufacture (41).

Epoxides have received increased attention in view of their interest both as end-products and as chemical intermediates. Epoxidized oils—mainly high-oleic sunflower oil—and their ester derivatives have found important applications as plasticizers and additives for polyvinyl chloride (PVC). Epoxidized esters produced from high-oleic sunflower methyl esters have hydroxyl values of 0, oxirane values of 5.2/4.5, and iodine values of 1.7/1.5 (127)

## 9. WORLD PRODUCTION AND DISTRIBUTION OF SUNFLOWER OIL

The world production of sunflower seed has grown steadily since 1950, at a lower rate toward the last several years. Figure 30 shows the evolution since 1935 (5, 128,

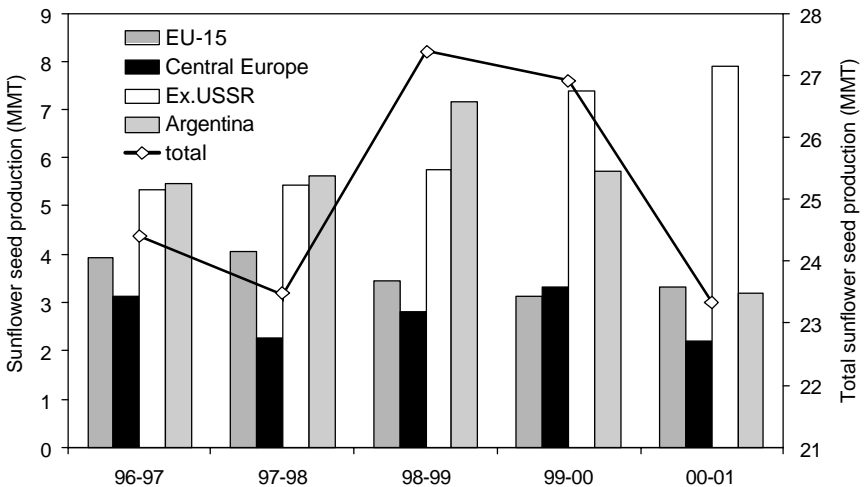


**Figure 30.** World production of sunflower seed in million of metric tons (MMT) (5, 128, 129).

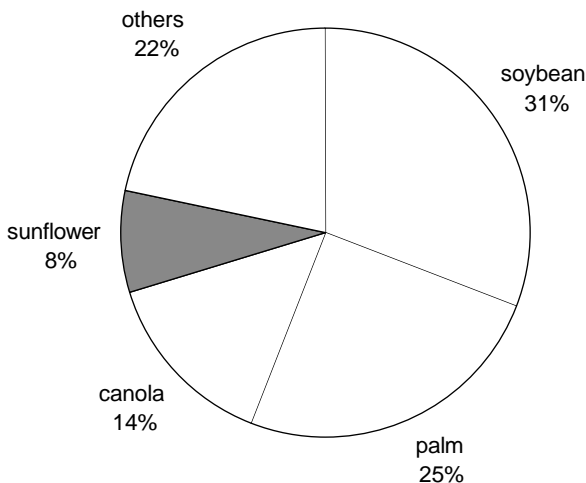
129). World production of sunflower seed in 2000–2001 was of 23.3 MMT, –8.9 MMT of sunflower oil and 10.2 MMT of sunflower meal.

World production of sunflower seed (7%) was third in the world production of oilseeds in 2001–2002, after soybean (57%) and canola seeds (11%). Production of soybean being by far the largest, sunflower seed production does not amount to much of the world total oilseed production (129).

The evolution of the sunflower oil production (MMT) in the last few years is shown in Figure 31 by country/region [based on Gunstone (129)]. The world



**Figure 31.** Evolution of sunflower seed production (MMT) by country/region in the last years compared with total world production [based on (129)].



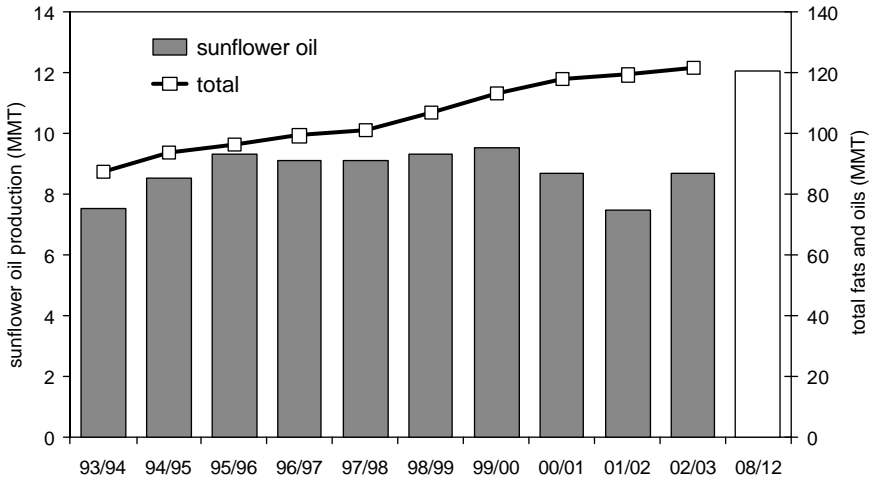
**Figure 32.** Distribution of world production of vegetable oils in 2001–2002 [based on (129)].

production of sunflower oil has changed (in both distribution and amount) in the last decade because of economic and political reasons, such as the facts occurred in ex-USSR and Central Europe. In Argentina, the largest world producer of sunflower oil until 1999, the substitution of sunflower by more profitable crops since that year resulted in a drop in sunflower seed production, making ex-USSR the largest world producer (in this year, the production of sunflower seeds in ex-USSR also increased markedly). In 2000/2001, EU-15 became the second largest producer. In view of the amounts produced in Argentina, the decline in sunflower oil production in this country was reflected in the total world production.

Sunflower oil production is determined by the production of sunflower seed. Sunflower oil is fourth in importance among vegetable oils (including oils extracted from fruits, as is the case of palm oil), as shown in Figure 32. World production of sunflower oil is around 9 million metric tons, amounting to 8% of the total vegetable oil production [based on Gunstone (129)]. Despite its fourth position in the world production of vegetable oils, the participation of sunflower oil is fairly low.

Figure 33 shows the evolution of the world production of sunflower oil in the last several years, compared with the total fat and oil world production (128, 130, 131); including the projected value for 2008–2012, according to the literature (132). The trend in sunflower oil production is for a steady value (declining slightly), and the total fat and oil production tends to increase considerably.

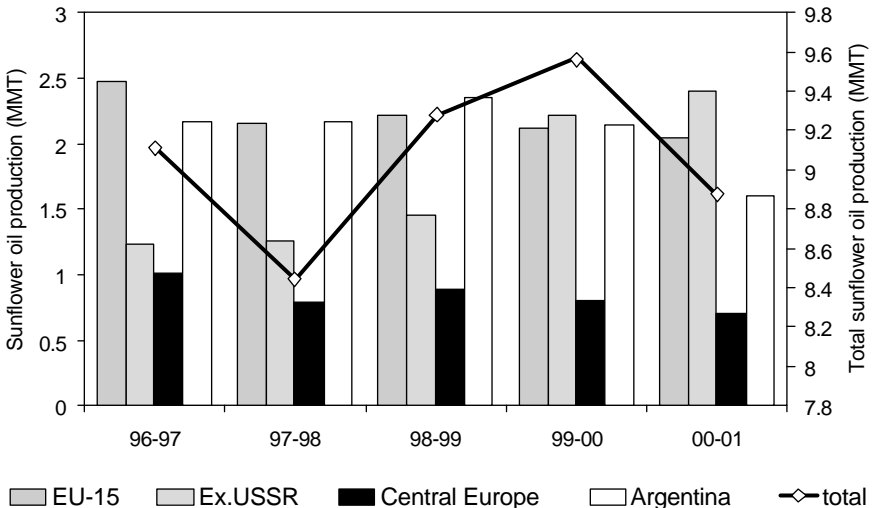
Figure 34 shows the evolution of the sunflower oil production (MMT) by country/region in the last years, compared with the total sunflower oil production [based on Gunstone (129–131)]. The leading position in the producer market varies according to period, with fairly similar amounts for the European Union and Argentina (major producer countries), except for a sharp drop in Argentina in 2000–2001 as a result of the decrease in seed production (showed in Figure 31). As for ex-USSR, it became the world's largest producer country as of 1999–2000. The



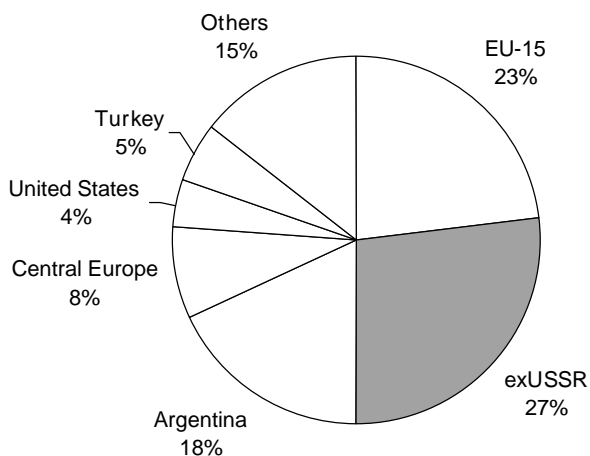
**Figure 33.** World production of sunflower oil (MMT) in the last years, compared with the total fat and oil production (MMT) (Key: Solid box = real production. Open box = estimated production (128, 130–132)).

participation of sunflower oil in the world trade has declined in the last years, as shown in Figures 31 and 33.

Soybeans have a strong participation in the world supply of oilseeds. The supply being oriented mainly to the production of meals rich in protein, an oversupply of soybean oil is commercialized at a lower relative price, a fact that is reflected in the general composition of the vegetable oil trade.



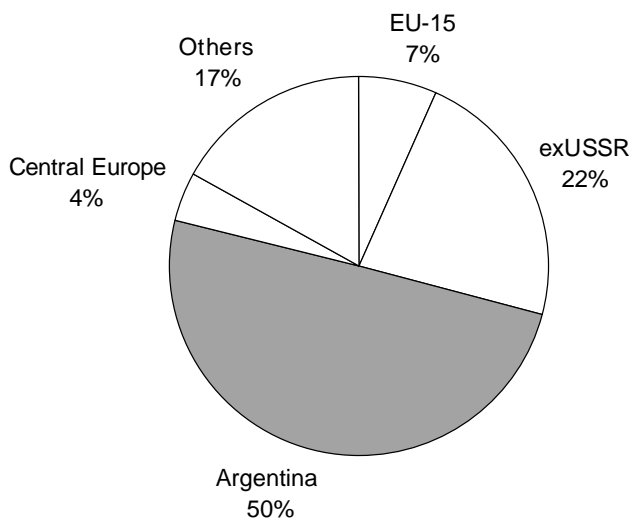
**Figure 34.** Sunflower oil production (MMT) by country/region, compared with total world production of sunflower oil [based on (129–131)].



**Figure 35.** Participation by country/region in the world production of sunflower oil in 2001–2002 (based on (129–131)).

Figure 35 shows the participation (%) by country/region in the world production of sunflower oil in 2000–2001 [based on Gunstone (129–131)]. The largest world producer was ex-USSR for that period (27%), followed by the European Union (23%), and Argentina (18%).

The production of sunflower oil may supply either the internal or external market of a region or country. The world exports distribution does not follow the same pattern as the distribution of production. The participation in sunflower oil exports is shown by country/region in Figure 36 for 2000–2001 [based on Gunstone (129–131)].

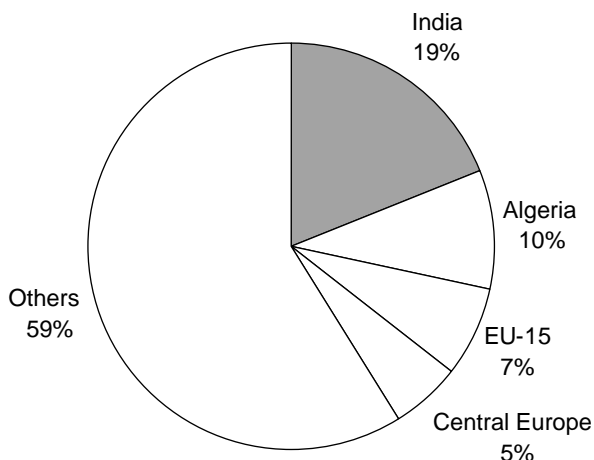


**Figure 36.** Participation by country/region in world exports of sunflower oil in 2001–2002 [based on (129–131)].

For that period, the main exporter was Argentina (50%), with a participation twice as large as ex-USSR (22%) in exports, whereas the latter was the largest producer in the same period. The above figures show how a large proportion of the Argentinean production (1.18 MMT) finds its way into the external market (out of a total 1.60 MMT produced). Most of it is exported as bulk crude oil and as bottled refined oil in a lesser proportion.

The amount of sunflower oil produced by ex-USSR (2.40 MMT) was far larger than the exported amount (0.53 MMT), showing a large consumption (1.87 MMT) in this country of the produced oil. It is exported primarily as processed oil, mainly to the Middle East countries (Algeria, Egypt, Turkey, etc.) for later processing, demand for this type of oil being scarce in the European market. Figure 37 shows the participation by country/region in world imports of sunflower oil for 2000–2001 [based on Gunstone (129–131)]. A comparison of Figures 35 and 37 shows that major importer countries are generally not strong producers of sunflower oil.

In the European Union, exports of sunflower oil (0.16 MMT) were balanced by imports (0.17 MMT), whereas it was a net importer of sunflower seed for processing (1.94 MMT). Likewise, Central Europe exported the same amount of sunflower oil (0.10 MMT) as it imported (0.13 MMT), but it was an exporter of sunflower seed (0.39 MMT). These relationships of the international European market are reflected in the characteristics of the internal market. A number of vegetable oils are available for consumption in the European Union, unlike the case of Argentina. Production of canola oil—the main oil—is closely followed by sunflower, soybean, palm, and olive oils, with regional variations. Olive oil is more important in the south of Europe (Greece, Italy, Spain, and Portugal), amounting to 36% of the total vegetable oil consumption, sunflower oil also being important.



**Figure 37.** Participation in sunflower oil imports by country/region in 2001–2002 [based on (129–131)].



Consumption in the north of Europe is more varied: the market is supplied by regional oilseeds (especially canola), with sufficient sunflower production in France. Soybean, sunflower, canola, and palm oils together constitute nearly 80% of total vegetable oils in northern Europe (133).

In 2000–2001, Argentina produced 1.60 MMT of sunflower oil and exported 1.18 MMT (of that oil), with 0.42 MMT disappearance, 75% of which was destined for direct consumption, whereas the rest was used in the manufacture of margarine and mayonnaise. An analysis of internal demand by oil type in Argentina shows sunflower oil with 70% of the trade, followed by soybean (26%), and low volumes of corn and olive oils. Use of sunflower oil is widespread in this region.

Sunflower oil, as well as canola oil, is produced annually for sale of the oil primarily, unlike the case of soybean oil, mainly related to the demand for its meal. Sunflower oil accounts for 80% of the seed price, being six to seven times higher than the price of meal pellets. The price of sunflower seed is determined by the price of the extracted oil.

## **10. SUNFLOWER OIL EXTRACTION AND PROCESSING BY-PRODUCTS**

Two coproducts are originated in the sunflower-seed oil extraction process: meal and hulls. Sodium soapstock is obtained as a byproduct of alkali refining of the oil, and it may contain phosphatides, depending on process type. Sunflower lecithin is obtained by treatment of the oil. Less important byproducts are waxes, tocopherols, and so on.

### **10.1. Soapstock**

Sodium soapstock obtained from alkali refining of sunflower oil may be used as an ingredient in animal feed meals in view of its high caloric value, in addition to being a good phosphorous source in the case of soapstock containing phosphatides. Use of soapstock is local because of transportation costs and ready fermentation, resulting from a high water content. Dehydration is often carried out prior to transportation, storage, and incorporation of soapstock into other meals.

Sodium soapstock may also be subjected to treatment with mineral acids, freeing the constituent fatty acids upon decomposition of the soaps. The product thus obtained, having a very low water content, is called “acid oil.” Storage and transportation requirements are the same as for crude oil.

Both acid oil and the free fatty acids obtained in the physical refining may be incorporated in the manufacture of soap. In view of its high linoleic acid content (in particular when originating in the refining of regular sunflower oil), soapstock does not make a fatty material of good properties for the manufacture of toilet soap. To this end, it is blended in relatively low proportions with other more appropriate fatty materials. It is used in cattle producer countries also producing sunflower oil as a means to reduce the titer of beef tallow or of the beef tallow/coconut oil blend.

A high titer is one problem of beef tallow in Argentina and Uruguay, for example: 45.4°C mean value, although it may be as high as 48°C (134, 135). Sunflower oil, having a titer of 16–20°C, may be added to this blend to obtain appropriate values for soap manufacture, generally considered around 42°C.

## 10.2. Sunflower Lecithin

Responding to the recommendations of food and nutrition scientists, studies have been made of new phosphoacylglycerols (phosphatides or phospholipid) sources. Oilseeds and cereals are important sources of phospholipids. The phospholipid (lecithin) content of crude sunflower oil ranges from 0.5% to 1.2% (53, 136–138). Oils extracted by solvent generally have a higher phospholipid content than those obtained by pressing. Major phosphoacylglycerols of sunflower oil are PC, PE, PI, and PA. Most are hydratable and may be removed from the crude oil through a water degumming process.

Most published research is into the content and composition of unremoved phospholipids in sunflower oil after different degumming processes. Little research has been done, however, of the separated lecithins. Some conclusions may still be reached about efficiency of production method, as well as the possible composition of these lecithins from the composition of the phospholipids remaining in the oil.

The fact that the phospholipid composition of sunflower depends on the oil extraction method and the degumming treatment used to remove them explains the differences in the reported compositions in the literature. Phospholipid compositions of sunflower oil are shown by type in Table 20 (136, 139–141). The overall fatty acid composition also varies widely for the same reason. Cherry and Kramer (140) report composition ranges of 11.1–31.9% of palmitic acid, 3.0–7.9% stearic acid, 13.3–17.3% oleic acid, and 42.8–68.7% linoleic acid.

Sunflower lecithin is not produced in considerable amounts worldwide. This is mainly because of the low lecithin content of crude sunflower oil as compared with 2.9% for soybean, 1.9% for canola, 2.4% for cottonseed, and 2.0–2.7% for corn oil (normalized at 70% of insolubles in acetone). Lecithin removal from sunflower oil may be justified in strong sunflower producer countries. It may be used as a food additive in view of its high phosphatidylcholine and essential fatty acid content. Upon refining and fractioning stages, the quality of sunflower lecithin may be improved for the manufacture of food products and cosmetics.

**TABLE 20. Phosphoacylglycerol Composition (%) of Sunflower Oil (136, 139–141).**

	PC (%)	PE (%)	PI (%)	PA (%)
Cherry/Kramer (140)	12.7–26.8; 42.2–64.2	9.9–29.4; 46.6	3.7–21.4; 24.0–36.6	
Morrison (136)	52.0	19.7	26.0	2.2
Carelli et al. (141)	44–48	20–21	20–21	12–15
Chapman (139)	48.7	21.2	27.9	2.2

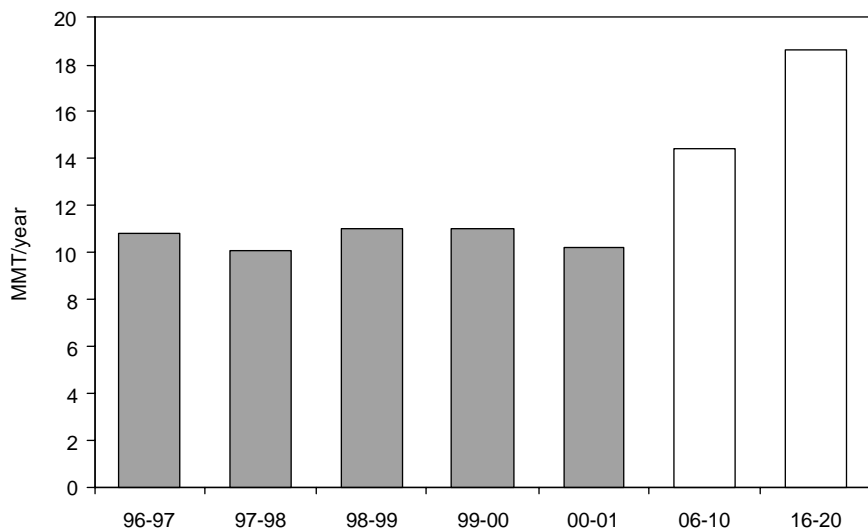
Holló et al. (142) report on studies carried out in Hungary (where sunflower oil represents 80% of the total vegetable oil production) about possible uses of sunflower lecithin. It may be added to sunflower meal by 2.5% or it may be dried, representing an increase to 55–70% in the phospholipid content, prior to blending with animal feed meals. Used in swine feed meals, it leads to an increase of body weight and a shortened fattening period. It is also useful for an adjustment of the energetic level of poultry feed meals, and it replaces the addition of synthetic choline chloride, in view of the high natural choline content of sunflower lecithin. It is also used in the food industry as emulsifier, and as a viscosity reducer agent in the manufacture of chocolates.

### 10.3. Sunflower Meal

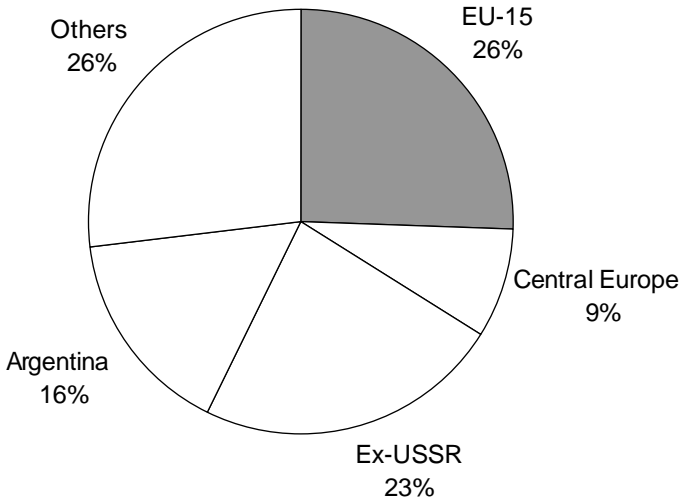
The remaining material from the pressing stage (expeller) of sunflower seed usually contains 10–12% of oil. Sunflower meal is obtained as a byproduct of solvent extraction from this material. In order to facilitate handling and transportation, the meal is often compacted through pressure and temperature treatment, into the shape of sunflower pellets. Sunflower pellets are the fourth important oleaginous raw material used in animal feeds, after soybean, cottonseed, and canola pellets.

Production of sunflower pellets in Argentina, the main world producer and exporter, is around 1,900,000 tons annually, 80–85% of which is exported to the Rotterdam market mainly, and the remaining 300,000 tons are destined to the local market (143).

The evolution of the world production of sunflower meal (MMT/year) in the last years (harvests in 1996/1997 through 2000/2001) is indicated in Figure 38,



**Figure 38.** World production of sunflower meal (MMT/year). (Key: Solid box = actual production. Open box = estimated production) [based on (129)].



**Figure 39.** Production of sunflower meal by major countries/regions in 2001–2002 [based on (129)].

including the estimated projection for the five-year periods 2006/2010 and 2016/2020 [based on Gunstone (129)]. Figure 39 is a representation of the production of sunflower meal (MMT/year) by major countries/regions involved. Argentina is the largest world producer (16% of the world production), although certain regions—as a group of countries—represent larger productions. Exports are usually from Argentina (68%) and ex-USSR (19%), and most of them reach the EU-15 (66%). Soybean meal leads the world market of oleaginous meals, sunflower meal representing only 6% (129).

The sunflower oil extraction industry produces three kinds of meal: meal produced from undehulled seeds, containing around 28% of protein and 25–28% fiber; meal of partially dehulled seed, containing 35–37% protein and 18% fiber; and meal obtained through a two-step dehulling process of seed, containing 40–42% protein and 12–14% fiber. The meal composition thus depends on the efficiency of the dehulling process of sunflower seeds. The oil content of sunflower meals ranges between 1.5% and 2.5%, depending on oil extraction efficiency and raw material (3, 144–146).

Undehulled sunflowerseed meals cannot match soybean meals in the meal market, their use being limited to ruminant feeds. In addition, sunflower hulls contain a large amount of raw fiber (60–65%) of practically no nutritional value, so that they are almost exclusively used as ruminant feed.

The amino acid composition of sunflower meal is generally balanced. The energy content of sunflower meal compares favorably with other oilseed meals. The energy value of a sunflower meal increases with increasing residual oil content and a reduction of the fiber content. Sunflower meal is also valuable as a calcium and phosphorous source and a good source of hydrosoluble B complex vitamins,

mainly nicotinic acid, thiamine, pantothenic acid, riboflavin, and biotin (146). Sunflower meal also contains chlorogenic acid, a polyphenolic compound. For meals extracted in the conventional manner under alkaline conditions, chlorogenic acid reacts with a certain protein fraction, giving the product a dark green color. Several methods have been proposed for the production of protein concentrates obtained from sunflower meal through extraction or inactivation of chlorogenic acid (3, 146). Compared with soybean, cottonseed, and peanut meals, dehulled sunflowerseed meals are substantially less rich in lysine, although richer in methionine and cystine. Sunflower proteins are therefore a good complement for soybean meals when both are blended in animal feeds. Supplementation with lysine must generally be performed for sunflower meals to be used alone as swine and poultry feeds (145).

Sunflower meal (blended with wheat flour) can be used for human nutrition. Despite their dark color, sunflower protein concentrates are of excellent digestibility. A method was proposed (147) for obtaining sunflower protein concentrates from defatted wholemeal sunflower flour, through extraction of these proteins with a basic solution. The process yields a concentrate of 71% protein (dry basis), rich in glutamic and aspartic acids. The supernatant liquid, rich in potassium and phosphorous, can be used as agricultural fertilizer.

#### 10.4. Hulls

Hulls are obtained as a byproduct of sunflower seed processing for oil extraction. The amount of hull represents around 22–28% of seed weight. Hulls may be separated either prior to or upon extraction of the oil. They may also remain in the meal, making a wholemeal product. Hulls contain around 4% of crude protein, 5% of lipidic matter (including waxes, hydrocarbons, fatty acids, sterols, and triterpenic acid), 50% carbohydrates (mainly cellulose and lignin), 26% reducing sugars (mainly xylose), and 2% ash. The high fiber content (60–65%) and the low protein and energy content of hulls reduce their nutritional value; they are used as roughage in certain animal feeds (146).

Hulls may be used as ruminant feed when finely milled and blended with other ingredients, composing the nondigestible part of the meal in view of the high content in cellulose and lignin. They may be used for an additional volume of concentrated meals and to absorb liquids such as molasses. Owing to the high content of nondigestible fiber, the nutritional value of hulls as feed for farm animals is extremely low, so that they are used as livestock or poultry litter.

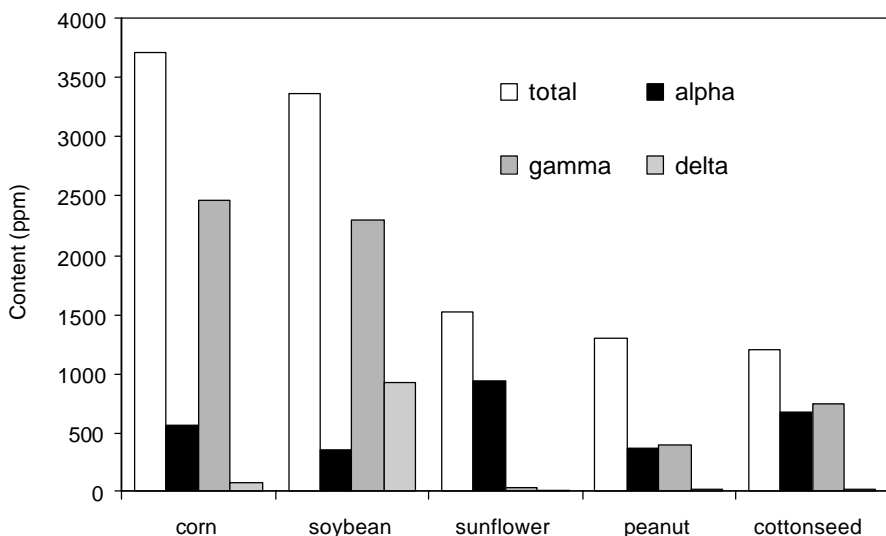
Sunflower hulls are also used as fuel, with a caloric power of 19.2 MJ/kg, and the caloric power of hull and meal is 23.6 MJ/kg, constituting an improved fuel. Hull burning is an alternative to the use of more expensive fuels in some countries. The resulting ashes are rich in potassium and may be used as fertilizers (146).

Sunflower hulls may also be pressed and shaped into fireplace logs, including wood residues. In view of their high content in reducing sugars, sunflower hulls can be used for the production of ethyl alcohol and furfural. Other minor uses include building or insulation board.

## 10.5. Waxes

Sunflower seed oil waxes are fatty alcohol esters of fatty acids. The fatty acids are in the range of 14 to 30 carbons, with a predominance of linoleic (44.0%), oleic (18.6%), behenic (9.7%), and palmitic (9.8%) acids. The fatty alcohols have chain-lengths in the range 16–32 carbons, with a predominance of octadecanol (23.1%), nonadecanol (18.4%), and tetracosanol (11.9%). This fatty acid and alcohol profile leads to esters of 36 to 48 carbons, with a predominance of 41 carbons (14.3%), 48 carbons (13.9%), 36 carbons (13.0%), 40 carbons (11.5%), 46 carbons (11.2%), and 37 carbons (9.2%). Other authors report sunflower waxes to contain fatty acids with 16–30 carbons (highest occurrence of 20 and 22) and fatty alcohols in the range of 20–32 carbons (highest occurrence of 24 and 26). The saponification value of these waxes is 85–90 and the iodine value of 8–12. The melting point is 70–80°C. They behave as nonpolar lipophilic compounds at temperatures below 40°C, and at higher temperatures, they adopt a crystalline state of weak hydrophilic character (53, 57, 64).

These waxes are located mainly in the hull of sunflower seeds (1.5–3%). The concentration will depend on the hybrid or seed variety, as well as on origin and storage factors. They are incorporated in the oil during oil extraction operations. The quantity of extracted wax will depend on the degree of dehulling of seeds, the extraction method (pressing or extraction by solvent), and the temperature and technology used. The wax content of crude sunflower oil is usually in the range 0.02–0.35%, although it may reach values as high as 1%; the wax content of refined sunflower oil can be as high as 60 ppm (53, 64). Sunflower waxes have been used successfully as ingredient in livestock feeds, mixed with grain, silage, and so on.



**Figure 40.** Typical tocopherol levels in deodorizer distillate obtained from crude oils [based on (148)].

## 10.6. Tocopherols

A low percentage of tocopherols is lost during operations of oil neutralization, bleaching, and deodorization of sunflower oil. The tocopherols lost during deodorization may be recovered together other volatile compounds, from the deodorizer distillate. The distillate obtained from sunflower oil is also a good source of phytoosterols. Figure 40 shows typical tocopherol levels in deodorizer distillate obtained from crude oils [based on Walsh et al. (148)].

The distillate obtained from sunflower oil may be sold to pharmaceutical companies for tocopherol and sterol isolation. Tocopherols may be used as natural antioxidants or may be converted to vitamin E by methylating the heterocyclic ring. The interest in phytoosterols is caused by the high potential of some of them to inhibit intestinal cholesterol absorption.

## REFERENCES

1. H. Autino, F. Wnuk, and P. Vacca, *Aceites Grasas*, **3**, 21–30 (1993).
2. M. Melgarejo, *Aceites Grasas*, **8**, 49–52 (1998).
3. D. C. Zimmerman, in E. H. Pryde, L. H. Princen, and K. D. Mukherjee, eds., *New Sources of Fats and Oils*, AOCS Press, Champaign, Illinois, 1981.
4. G. N. Fick, in G. Röbbelen, R. K. Downey, and A. Ashri, eds., *Oil crops of the world*, McGraw-Hill, New York, 1989.
5. M. Bockisch, *Fats and Oils Handbook*, AOCS Press, Champaign, Illinois, 1998, pp. 237–242.
6. F. Ucke, *Aceites Grasas*, **8**, 70–73 (1998).
7. S. C. Chapman and A. J. de la Vega, *Field Crop Res.*, **73**, 107–120 (2002).
8. R. A. Carr, *Aceites Grasas*, **7**, 373–388 (1997).
9. A. Merrien, *Aceites Grasas*, **8**, 75–80 (1998).
10. A. B. Afzalpurkar and G. Lakshminarayana, *J. Amer. Oil Chem. Soc.*, **57**, 105–106 (1980).
11. M. Rataus, *Aceites Grasas*, **5**, 335–338 (1995).
12. J. A. Robertson, G. W. Chapman, and R. L. Wilson, *J. Amer. Oil Chem. Soc.*, **55**, 266–269 (1978).
13. J. A. Robertson and R. B. Russell, *J. Amer. Oil Chem. Soc.*, **49**, 239–244 (1972).
14. J. A. Robertson and V. E. Green, *J. Amer. Oil Chem. Soc.*, **58**, 698–701 (1981).
15. J. R. Lajara, U. Díaz, and R. Díaz Quidiello, *J. Amer. Oil Chem. Soc.*, **67**, 618–623 (1990).
16. E. Martínez-Force, R. Álvarez-Ortega, S. Cantisán, and R. Garcés, *J. Agric. Food Chem.*, **46**, 3577–3582 (1998).
17. A. Prevot, *Rev. F. Corps Gras*, **34**, 183–195 (1987).
18. E. Santalla, I. Riccobene, and S. Nolasco, *Aceites Grasas*, **44**, 175–178 (1993).
19. A. Nagao and M. Yamazaki, *J. Amer. Oil Chem. Soc.*, **60**, 1654–1658 (1983).

20. Physical and Chemical Characteristics of Oils, Fats and Waxes, in *Official Methods and Recommended Practices of the American Oil Chemists' Society*, AOCS Press, Champaign, Illinois, 1997.
21. P. Gillatt, *Inform*, **5**, 981–986 (1994).
22. Anonymous, *Inform*, **5**, 1162–1163 (1994).
23. E. J. Campbell, *J. Amer. Oil Chem. Soc.*, **60**, 387–392 (1983).
24. J. B. Rossell, B. King, and M. J. Downes, *J. Amer. Oil Chem. Soc.*, **60**, 333–339 (1983).
25. R. Álvarez-Ortega, S. Cantisán, E. Martínez-Force, and R. Garcés, *Lipids*, **32**, 833–837 (1997).
26. K. Warner, T. L. Mounts, and G. R. List, *Inform*, **1**, 326 (1990).
27. M. S. R. Subrahmanyam, H. S. Vedanayagam, and P. Venkatacharyulu, *J. Amer. Oil Chem. Soc.*, **71**, 901–905 (1994).
28. H. Abramovic and C. Klofutar, *Acta Chim. Slov.*, **45**, 69–77 (1998).
29. J. N. Coupland and D. J. McClements, *J. Amer. Oil Chem. Soc.*, **74**, 1559–1564 (1997).
30. Y. Ali and M. A. Hanna, *Bioresource Technol.*, **50**, 153–163 (1994).
31. S. Bhattacharyya and C. S. Reddy, *J. Agric. Eng. Res.*, **57**, 157–166 (1994).
32. M. A. Grompone and B. Irigaray, Personal communication.
33. B. F. Haumann, *Inform*, **5**(11), 1198–1210 (1994).
34. B. F. Haumann, *Aceites Grasas*, **8**, 81–92 (1998).
35. S. Cantisán, E. Martínez-Force, and R. Garcés, *Plant Physiol. Biochem.*, **38**, 377–382 (2000).
36. R. H. Purdy, *J. Amer. Oil Chem. Soc.*, **62**, 523–525 (1985).
37. R. H. Purdy, *J. Amer. Oil Chem. Soc.*, **63**, 1062–1066 (1986).
38. B. F. Haumann, *Inform*, **3**, 666–675 (1992).
39. M. Mozzon, F. Bocci, and N. Frega, *J. Agric. Food Chem.*, **46**, 4198–4202 (1998).
40. R. Scarth, in R. Przybylski and B. E. McDonald, eds., *Development and Processing of Vegetable Oils for Human Nutrition*, AOCS Press, Champaign, Illinois, 1995.
41. G. Vermeersch, *Aceites Grasas*, **8**, 112–116 (1998).
42. T. Krawczyk, *Inform*, **10**, 555–561 (1999).
43. T. Krawczyk, *Aceites Grasas*, **9**, 525–534 (1999).
44. M. I. Gurr, *Lipids in Nutrition and Health*, The Oily Press, Bridgwater, 1999.
45. A. Cert, *Aceites Grasas*, **5**, 175–189 (1995).
46. M. K. Gupta, *Inform*, **9**, 1150–1154 (1998).
47. L. W. Kleingartner, in J. Janick and A. Whipkey, eds., *Trends in New Crops and New Uses*, ASHS Press, Alexandria, Virginia, 2002.
48. L. Kleingartner and K. Warner, *Cereal Foods World*, **46**, 399–404 (2001).
49. G. List, K. R. Steidley, and W. E. Neff, *Aceites Grasas*, **10**, 569–573 (2000).
50. S. Muller, *J. Amer. Oil Chem. Soc.*, **60**, 393–394 (1983).
51. G. Mazza and D. S. Jayas, *Trans. Am. Soc. Agric. Eng.*, **34**, 534–538 (1991).
52. D. C. Tandy, in P. J. Wan, ed., *Introduction to Fats and Oils Technology*, AOCS Press, Champaign, Illinois, 1991.
53. A. A. Carelli, L. M. Frizzera, P. R. Forbito, and G. H. Crapiste, *J. Amer. Oil Chem. Soc.*, **79**, 763–768 (2002).



54. M. I. V. Brevedan, A. A. Carelli, and G. H. Crapiste, *Aceites Grasas*, **51**, 417–423 (2000).
55. A. Smiles, Y. Kakuda, and B. E. MacDonald, *J. Amer. Oil Chem. Soc.*, **65**, 1151–1155 (1988).
56. L. G. Pan, R. Harispe, A. Campana, M. C. Tomás, and M. C. Añón, *La Alimentación Latinoamericana*, **238**, 54–60 (2001).
57. S. Bloch, *Aceites Grasas*, **1**, 27–33 (1990).
58. J. Turkulov, E. Dimic, Dj. Karlovic, and V. Vuksa, *J. Amer. Oil Chem. Soc.*, **63**, 1360–1363 (1986).
59. J. Turkulov, E. Dimic, Dj. Karlovic, and V. Vuksa, *Aceites Grasas*, **10**, 256–259 (2000).
60. W. H. Morrison, *J. Amer. Oil Chem. Soc.*, **59**, 284–285 (1982).
61. M. A. Baltanás, H. Molina, and C. Silva, *J. Amer. Oil Chem. Soc.*, **75**, 363–370 (1998).
62. K. J. Moulton, *J. Amer. Oil Chem. Soc.*, **65**, 367–368 (1988).
63. A. A. Carelli, P. R. Forbito, P. Bodnariuk, and G. H. Crapiste, *Aceites Grasas*, **8**, 103–111 (1998).
64. S. Martini and M. C. Añón, *J. Amer. Oil Chem. Soc.*, **77**, 1087–1092 (2000).
65. H. Liu, R. Przybylski, K. Dawson, N. A. M. Eskin, and C. G. Biliaderis, *J. Amer. Oil Chem. Soc.*, **73**, 493–497 (1996).
66. S. Bloch, *Aceites Grasas*, **8**, 53–66 (1998).
67. E. Dimic, Dj. Karlovic, and J. Turkulov, *J. Amer. Oil Chem. Soc.*, **71**, 1357–1361 (1994).
68. K. Kövári, J. Dense, Z. Kemény, and K. Recseg, *OCL*, **7**, 305–308 (2000).
69. R. L. Winters, *Aceites Grasas*, **4**, 54–61 (1994).
70. M. Contini and M. Moresi, *Ind. Aliment.*, **33**, 149–156 (1994).
71. F. De Panfilis, T. G. Toschi, and G. Lercker, *Aceites Grasas*, **7**, 422–429 (1997).
72. F. De Panfilis, T. G. Toschi, and G. Lercker, *Inform*, **9**, 212–221 (1998).
73. H. Topallar, Y. Bayrak, and M. Iscan, *J. Amer. Oil Chem. Soc.*, **72**, 1519–1522 (1995).
74. N. Ajzenberg, *Aceites Grasas*, **53**, 229–238 (2002).
75. M. Naglic, A. Smidovnik, and T. Koloini, *J. Amer. Oil Chem. Soc.*, **75**, 629–633 (1998).
76. G. Rivarola, J. A. Segura, M. C. Añón, and A. Calvelo, *J. Amer. Oil Chem. Soc.*, **64**, 1537–1543 (1987).
77. J. A. Segura, M. L. Herrera, and M. C. Añón, *J. Amer. Oil Chem. Soc.*, **67**, 989–992 (1990).
78. M. L. Herrera, *J. Amer. Oil Chem. Soc.*, **71**, 1255–1260 (1994).
79. M. L. Herrera, *Aceites Grasas*, **5**, 60–67 (1995).
80. M. L. Herrera and F. J. Márquez Rocha, *J. Amer. Oil Chem. Soc.*, **73**, 321–325 (1996).
81. M. D. Erickson and N. Frey, *Food Technol.*, 63–67 (1994).
82. G. Ramírez, G. Hough, and A. Contarini, *J. Food Qual.*, **24**, 195–204 (2001).
83. H. Topallar, Y. Bayrak, and M. Iscan, *J. Amer. Oil Chem. Soc.*, **74**, 1323–1327 (1997).
84. M. A. Grompone, T. Pagano, and Y. Pinchak, in *Proceedings of the International Workshop on Fats, Oils and Oilseeds Analysis (IUPAC)*, 2000, pp. 46–61.
85. T. Pagano and M. A. Grompone, *Revista Ingeniería Química*, **15**, 11–20 (1999).

86. N. Frega, M. Mozzon, and G. Lercker, *Aceites Grasas*, **9**, 580–585 (1999).
87. A. A. Carelli, P. Bodnariuk, and G. H. Crapiste, *Aceites Grasas*, **10**, 227–231 (2000).
88. T. Albi, A. Lanzón, A. Guinda, M. León, and M. C. Pérez-Camino, *J. Agric. Food Chem.*, **45**, 3795–3798 (1997).
89. G. H. Crapiste, M. I. Brevedan, and A. A. Carelli, *J. Amer. Oil Chem. Soc.*, **76**, 1437–1443 (1999).
90. G. H. Crapiste, M. I. Brevedan, and A. A. Carelli, *Aceites Grasas*, **11**, 67–79 (2001).
91. R. Eggers, in J. W. King, and G. R. List, eds., *Supercritical Fluid Technology in Oil and Lipid Chemistry*, AOCS Press, Champaign, Illinois, 1996.
92. F. Favati, J. W. King, and G. R. List, in J. W. King and G. R. List, eds., *Supercritical Fluid Technology in Oil and Lipid Chemistry*, AOCS Press, Champaign, Illinois, 1996.
93. L. Calvo, M. J. Cocero, and J. M. Diez, *J. Amer. Oil Chem. Soc.*, **71**, 1251–1254 (1994).
94. B. F. Haumann, *Inform*, **9**, 6–13 (1998).
95. M. A. M. Zeitoun, W. E. Neff, G. R. List, and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **70**, 467–471 (1993).
96. D. Chobanov and R. Chobanova, *J. Amer. Oil Chem. Soc.*, **54**, 47–50 (1977).
97. M. A. Grompone, *Grasas Aceites*, **42**, 349–355 (1991).
98. A. Rodríguez, E. Castro, M. C. Salinas, R. López, and M. Miranda, *J. Amer. Oil Chem. Soc.*, **78**, 431–436 (2001).
99. T. A. Foglia, K. Petruso, and S. H. Fearheller, *J. Amer. Oil Chem. Soc.*, **70**, 281–285 (1993).
100. I. Jachmanián, M. Gil, and M. A. Grompone, *Aceites Grasas*, **46**, 84–92 (2002).
101. F. J. Sánchez-Muniz, C. Cuesta, M. C. Garrido-Polonio, and R. Arroyo, *Grasas Aceites*, **45**, 300–305 (1994).
102. A. Romero, F. J. Sánchez-Muniz, C. Tulasne, and C. Cuesta, *J. Amer. Oil Chem. Soc.*, **72**, 1513–1517 (1995).
103. D. B. Kupranycz, M. A. Amer, and B. E. Baker, *J. Amer. Oil Chem. Soc.*, **63**, 332–337 (1986).
104. D. Barrera-Arellano, G. Márquez-Ruiz, and M. C. Dobarganes, *Grasas Aceites*, **48**, 231–235 (1997).
105. R. Arroyo, C. Cuesta, C. Garrido-Polonio, S. López-Varela, and F. J. Sánchez-Muniz, *J. Amer. Oil Chem. Soc.*, **69**, 557–563 (1992).
106. C. Cuesta, F. J. Sánchez-Muniz, C. Garrido-Polonio, S. López-Varela, and R. Arroyo, *J. Amer. Oil Chem. Soc.*, **70**, 1069–1073 (1993).
107. C. Cuesta and F. J. Sánchez-Muniz, *Grasas Aceites*, **49**, 310–318 (1998).
108. M. C. Pérez-Camino, G. Márquez-Ruiz, M. V. Ruiz-Méndez, and M. C. Dobarganes, *J. Food Sci.*, **56**, 1644–1650 (1991).
109. M. Martín-Polvillo, G. Márquez-Ruiz, N. Jorge, M. V. Ruiz-Méndez, and M. C. Dobarganes, *Grasas Aceites*, **47**, 54–58 (1996).
110. J. R. K. Niemelä, I. Wester, and R. M. Lahtinen, *Grasas Aceites*, **47**, 1–4 (1996).
111. J. L. Sébédio, M. C. Dobarganes, G. Márquez, I. Wester, W. W. Christie, G. Dobson, F. Zwobada, J. M. Chardigny, Th. Mairrot, and R. Lahtinen, *Grasas Aceites*, **47**, 5–13 (1996).

112. R. M. Lahtinen, I. Wester, and J. R. K. Niemelä, *Grasas Aceites*, **47**, 59–62 (1996).
113. N. Jorge, G. Márquez-Ruiz, M. Martín-Polvillo, M. V. Ruiz-Méndez, and M. C. Dobarganes, *Grasas Aceites*, **47**, 14–19 (1996).
114. N. Jorge, G. Márquez-Ruiz, M. Martín-Polvillo, M. V. Ruiz-Méndez, and M. C. Dobarganes, *Grasas Aceites*, **47**, 20–25 (1996).
115. R. Raoux, O. Morin, and F. Mordret, *Grasas Aceites*, **47**, 63–74 (1996).
116. L. J. Van Gemert, *Grasas Aceites*, **47**, 75–80 (1996).
117. G. Dobson, W. W. Christie, and M. C. Dobarganes, *Grasas Aceites*, **47**, 34–37 (1996).
118. P. Ch. Dutta and L.-A. Appelqvist, *Grasas Aceites*, **47**, 38–47 (1996).
119. G. Márquez-Ruiz, M. Martín-Polvillo, N. Jorge, M. V. Ruiz-Méndez, and M. C. Dobarganes, *J. Amer. Oil Chem. Soc.*, **76**, 421–425 (1999).
120. S. L. Abidi and K. Warner, *J. Amer. Oil Chem. Soc.*, **78**, 763–769 (2001).
121. S. Kiatsrichart, M. S. Brewer, K. R. Cadwallader, and W. E. Artz, *J. Amer. Oil Chem. Soc.*, **80**, 479–483 (2003).
122. G. Antolín, F. V. Tinaut, Y. Briceño, V. Castaño, C. Pérez and A. T. Ramírez, *Bioresource Technol.*, **83**, 111–114 (2002).
123. H. Fukuda, A. Kondo and H. Noda, *J. Biosci. Bioeng.*, **92**, 405–416 (2001).
124. W. Zhou, S. K. Konar, and D. G. B. Boocock, *J. Amer. Oil Chem. Soc.*, **80**, 367–371 (2003).
125. D. R. Kodali, *Inform*, **14**, 121–123 (2003).
126. S. Z. Erhan, M. O. Bagby, and H. W. Cunningham, *J. Amer. Oil Chem. Soc.*, **69**, 251–256 (1992).
127. X. Pagès and C. Alfós, *OCL*, **8**, 122–125 (2001).
128. A. J. M. Rodríguez, *Aceites Grasas*, **8**, 187–193 (1998).
129. F. Gunstone, *Inform*, **13**, 159–163 (2002).
130. F. Gunstone, *Inform*, **13**, 810–812 (2002).
131. F. Gunstone, *Inform*, **14**, 42–43 (2003).
132. Anonymous, *Inform*, **5**, 715 (1994).
133. J. Fry and R. Stringfellow, *Aceites Grasas*, **9**, 43–47 (1999).
134. M. A. Grompone and P. Moyna, *J. Amer. Oil Chem. Soc.*, **60**, 1331–1332 (1983).
135. M. A. Grompone, *Revista Ingeniería Química*, **3**, 14–18 (1991).
136. W. H. Morrison, *J. Amer. Oil Chem. Soc.*, **58**, 902–903 (1981).
137. G. Gil, I. G. de Kuck, and R. A. Macchi, *Aceites Grasas*, **9**, 443–446 (1999).
138. G. I. Dashiell, *Aceites Grasas*, **12**, 197–204 (2001).
139. G. W. Chapman, *J. Amer. Oil Chem. Soc.*, **57**, 299–302 (1980).
140. J. P. Cherry and W. H. Kramer, in B. F. Szuhaj, ed., *Lecithins—Sources, Manufacture and Uses*, AOCS Press, Champaign, Illinois, 1989.
141. A. A. Carelli, M. I. V. Brevedan, and G. H. Crapiste, *J. Amer. Oil Chem. Soc.*, **74**, 511–514 (1997).
142. J. Holló, J. Perédi, A. Ruzics, M. Jeránek, and A. Erdélyi *J. Amer. Oil Chem. Soc.*, **70**, 997–1001 (1993).
143. P. A. Piras, *Aceites Grasas*, **8**, 205–206 (1998).

144. S. P. Clark, P. J. Wan, and S. W. Matlock, *J. Amer. Oil Chem. Soc.*, **57**, 275A–279A (1980).
145. Z. Liebowitz and C. Ruckenstein, *Aceites Grasas*, **3**, 40–44 (1993).
146. M. A. Pickard, T. J. Jones, and R. T. Tyler, *Aceites Grasas*, **8**, 233–250 (1998).
147. C. Ordóñez, M. G. Asenjo, C. Benitez and J. L. González, *Biores. Technol.*, **78**, 187–190 (2001).
148. L. Walsh, R. I. Winters, and R. G. González, *Inform*, **9**, 78–83 (1998).

# *BAILEY'S INDUSTRIAL OIL AND FAT PRODUCTS*

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# 1

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## *Conjugated Linoleic Acid Oils*

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### **1. INTRODUCTION**

The discovery of conjugated linoleic acid (CLA) dates back to 1933, when it was found that treatment of polyunsaturated fatty acids with alkali increased the UV absorbency (1–3). It was later found that the treatment produced a one to one mixture of *cis*-9-, *trans*-11- (9,11-ct) and *trans*-10-, *cis*-12 (10,12-tc) CLA. In 1935, it was noted that UV absorbency at 230 nm of milkfats was higher in milk from cows fed polyunsaturated fatty acids than cows fed saturated fats (4). This phenomenon was shown to be a result of conjugation of the double bonds of the polyunsaturated fatty acids (5). The predominant isomer in dairy products (milk, cheese, butter) or meat is 9,11-ct CLA (6).

Since the time of their discovery, conjugated fatty acids have been the subject of intense investigation. Spectroscopic analysis using ultraviolet light was the major analytical instrument available to researchers in the 1930s. As a result of high absorbance of UV light at 230 nm or higher, conjugated fat became a useful research tool for the study of fat metabolism. The first animal study used naturally conjugated fats of tung oil, but it was poorly tolerated (7). During this period, essential fatty

acids were discovered. Aaes-Jorgensen (8) studied the possibility of using CLA to treat/prevent the symptoms of essential fatty acid-deficiency. They observed that in essential fatty acid-deficient animals, CLA could not prevent deficiency but showed toxicity. Synthetic conjugated fatty acids produced from linoleic acid (LA), usually mixtures of 9,11-ct and 10,12-tc CLA, replaced the natural substances as a preferred biological marker.

Over the last two decades, the conjugated fats and, particularly, conjugated linoleic acid (CLA) have been intensively studied for their biological activity. As a result of the ease of synthesis, blends of two CLA isomers, namely 9,11-ct and 10,12-tc-CLA, have been the focus of most research into biological activity. Recent research, however, has been expanding to include pure or enriched isomer preparations.

## 2. METABOLISM

Early studies using a CLA mixture revealed that animals absorb and incorporate some of the CLA in their tissues in phospholipid, glycolipid, and acylglycerol fractions. In 1950, Reiser (9) observed faster and better absorption and incorporation of CLA when administered as triacylglycerol (TAG) versus free fatty acid. Following administration of CLA as TAG, the maximal levels appear in blood, liver, and organs at 16 hours compared with 24 hours for the free fatty acid form. Incorporation was higher in mesentery fat followed by perirenal and subcutaneous fat (9). Barnes et al. (10), in 1941, reported the kinetics of CLA absorption from the mixture. The absorption rate was highest in the first hour following administration and then gradually declined. Following a single oral dose, over 50% of conjugated CLA was absorbed in neutral mucosal lipids in the first hour followed by a decline, whereas in mucosal phospholipids, the incorporation was much slower, reaching maximum at about 8 hours followed by decline. In 1951, it was found that poultry incorporate CLA into egg lipids (5). Recent studies confirm the earlier finding of preferential incorporation in neutral lipids followed by phospholipids in liver (11) and mammary tissues (11, 12). Incorporation of CLA in tissues is associated with a reduction in the amounts of arachidonic acid and linoleic acid in neutral lipids (11) and in the liver TAG levels with an increase in the levels of 18:0 (13). In pig heart lipids, 11,13-ct isomer was the major CLA isomer followed by 9,11-ct isomer following an oral administration of CLA mixture (14). CLA isomers compete with desaturases and elongases to produce desaturated and elongated products maintaining the geometry of double bonds (15–17). CLA has been shown to inhibit delta-9-desaturase enzyme *in vitro* (18) and *in vivo* (19). CLA feeding also resulted in a decrease in arachidonic acid, and also reduced the desaturation of linoleic acid without affecting the desaturation of alpha-linolenic acid to any significant level. In rat, 9,11-ct isomer was preferentially metabolized to conjugated C20:3, whereas 10,12-tc isomer was metabolized to conjugated C16:2 and 18:3 compounds (19). The CLA mixture and its individual isomers (9,11-ct and 10,12-tc) inhibited basal and calcium ionophore stimulated production of prostaglandin from human saphenous vein endothelial cells in

a dose-dependent manner. The mixture of CLA isomers was reported to inhibit the production of eicosanoids at all doses, whereas 10,12-*tc* isomer was shown to inhibit production at lower dose but stimulate at higher dose (20).

### 3. PHYSIOLOGICAL ACTIONS OF CLA

#### 3.1. Effect on Body Composition

While working with essential fatty acid deficient rats in 1951, Holman observed that CLA-fed rats had significantly less total fat than control rats, and that they lost weight (21). Subsequent studies demonstrated that CLA inhibited fat accumulation and promoted lean muscle mass in growing animals, including pigs and mice (22–24). The results on the effect of CLA on body fat composition in animals are unequivocal, whereas studies in humans are providing mixed results. In a double blind, randomized clinical trial on obese and overweight humans, Blankson et al. (25) observed a clinically significant reduction in body fat mass in groups administered various doses of CLA ranging from 1.7 g per day to 6.8 g per day. Reduction in body fat mass was significant for groups administered 3.4 g CLA per day and 6.8 g CLA per day. Interestingly, this study found that 3.4 g of CLA per day provided maximum reduction in body fat mass; increasing the dose above this level provided no additional effect (25). Lean body mass and body mass index were similar in all the groups, although there was a slight increase in lean body mass in the CLA group. The increase in lean body mass did not achieve significance compared with a placebo group administered olive oil. Additionally, the CLA group presented a significant reduction in total-, LDL-, and HDL-cholesterol (25). In another study, Riserus et al. (26) observed a reduction in sagittal abdominal diameter in abdominally obese humans without affecting total body weight. A reduction in total fat mass in healthy, nonobese, exercising males was observed when CLA was given at a total daily dose of 1.8 g (divided in 3 doses) for 12 weeks. Body weight was not affected in this double blind clinical trial (27). A recent study in type II diabetic patients who were not on any medication found an inverse relationship between plasma CLA levels and weight loss and serum leptin levels (28). The inverse relationship was significant for 10,12-*tc* isomer of CLA and not for 9,11-*ct* isomer. A study in nonobese individuals using a mixture of CLA isomers containing about 20% each of 9,11-*ct* and 10,12-*tc*-CLA isomers, along with 20% to 25% other isomers, did not observe a reduction in body fat mass (29). Another study investigated the effect of CLA on weight regain after weight loss in overweight subjects (30). This study observed no effect of CLA on weight gain after weight loss; however, the weight gain in the CLA group was a result of an increase in fat free mass and was independent of dose (30). Comparison between studies is difficult as these studies differed in the degree of obesity of the subjects, duration of treatment, dose, and the isomer composition of the CLA preparation. Earlier commercial products of CLA contained equal amounts of 9,11-*ct* and 10,12-*tc* isomers with other isomers in small amounts. The other isomers include all *trans*-isomers as well as other



*cis*-, *trans*-isomers, including 11,13-*ct*, 11,13-*tc*, 8,10 *ct*,8,10-*tc*, etc. This illustrates the need for research on specific isomers and standardized protocols.

These reported actions of CLA could be mediated through a number of physiological mechanisms including increased fat oxidation (31) or inhibition of lipid accumulation in fat cells. Recent studies have started to investigate the physiological actions of individual isomers. It appears that the 10,12-*tc* isomer of CLA is mainly responsible for the effect of CLA on adiposity (32–34).

It was demonstrated that 10,12-*tc*-CLA reduces leptin, a hormone involved in regulation of fat deposition, in cultured fat cells (35), and in mice (36). In the latter study, feeding 10,12-*tc*-CLA to mice caused a comparatively small gain in weight with no gain in adipose fat. The 10,12-*tc* isomer of CLA was also shown to inhibit differentiation of preadipocytes in murine (3T3-L1) (37) and human preadipocytes (38). This was associated with decreased accumulation of TAGs in differentiating preadipocytes; inhibition of peroxisome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ ) gene (38) and its downstream gene products including lipoprotein lipase (LPL), GLUT-4 (glucose transporter gene 4), and inhibited expression of fatty acid synthase (FAS) gene. CLA isomer 9,11-*ct*, on the other hand, increased accumulation of TAGs in adiposities and also stimulated GLUT-4 and LPL. This suggests that isomer 10,12-*tc* may be responsible for inhibition of glucose uptake and oxidation in the adipocytes, leading to decreased TAG accumulation. These actions also underlie the effect of 10,12-*tc* isomer in inducing insulin resistance leading to lipotrophic diabetes observed in animal (39, 40) and human studies (41).

### 3.2. Anticancer Properties

In 1985, Pariza and Hargrave discovered an antimutagenic fraction in cooked and raw beef during their studies on identification of carcinogenic compounds present in cooked beef (42). This fraction was identified to be a mixture of 4 isomers of CLA (9,11-*ct*, 9,11-*tt*, 10,12-*tc*, and 10,12-*tt*) (43). Studies in animal models and cell lines demonstrated the antimutagenic activity of a CLA mixture against known chemical carcinogens (7,12-dimethylbenz[a]anthracene, DMBA, and benzo (a) pyrene) (43–47). CLA has been shown to have anticancer effects against breast, colon, and prostate cancer cell lines (48–50). In rat models of breast cancer, CLA was reported to affect the breast structure when given during development stages. In this study, dietary CLA reduced the proliferation of terminal end bud and lobuloalveolar bud structures, whereby breast tissue became resistant to neoplastic transformations associated with cancer at later stages in life (48–50).

The exact mechanism of anticancer effects of CLA is not clear, and several possible mechanisms could underlie the anticancer properties of CLA. These actions may include its ability to interfere with the proliferation of cancer cells, increased apoptotic cell death, inhibition of angiogenesis, or increased oxidative stress. In a study comparing the effects of CLA on estrogen receptor positive human breast cancer cells (MCF-7) and estrogen receptor negative (MDA-MB 231) cells, CLA was shown to selectively inhibit proliferation of estrogen receptor positive cells.

CLA-treated MCF-7 cells selectively remained in G0/G1 phase and the expression of c-myc was inhibited. CLA had no effect on the growth of MDA-MB 231 cells. This study suggests that CLA acts by interfering with the estrogen-mediated second messenger system (51). CLA is also known to interfere with eicosanoids pathway and inhibits production of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) (50, 52). Reduced production of PGE<sub>2</sub> may play a role in anticancer actions of CLA. CLA is also shown to stimulate apoptotic death of cancer cells (50, 53, 54). CLA isomers increased apoptosis by stimulating the expression of caspase 3 and 9 activities and by reducing the expression of Bcl-2, an apoptosis repressor gene. The 10,12-tc isomer of CLA was found to be more potent in mediating these actions than either the 9,11-ct isomer or a mixture of the two (54). The other possible mechanism for anticancer properties of CLA is its ability to inhibit angiogenesis. In a mouse model of breast cancer, both isomers inhibited angiogenesis in mammary fat pad and reduced the concentration of vascular endothelium-derived growth factor (55). The CLA isomer 10, 12-tc also inhibited secretion of leptin and induced apoptosis in white and brown adipocytes, whereas 9,11-ct isomer was without effect on these parameters. CLA was also shown to reduce cell proliferation by reducing the expression of proteins involved in cell cycle regulation (p16 and p27) and DNA synthesis (56).

The above discussion focused on the role of CLA as a chemoprotective agent. Information regarding its effect on cancer treatment is limited. Feeding CLA for four or eight weeks after carcinogen administration was reported to be ineffective in preventing tumor formation, whereas continuous administration protected against tumor development (12, 57). A recent case control study in Finnish women suggested that dietary CLA may be protective against breast cancer (58). The role of CLA in prevention or treatment of cancer in humans is not clear and requires more research.

### 3.3. Insulin Resistance and Diabetes

CLA has been shown to normalize impaired glucose tolerance and improve hyperinsulinemia in prediabetic ZDF rats (59). These actions appeared to be mediated through PPAR- $\gamma$  pathway as CLA treatment induced expression of mRNA for aP2. Recently, it has been observed in Zucker diabetic rats that either a 50:50 mixture of 9,11-ct-CLA and 10,12-tc-CLA isomers or a 90% 9,11-ct-CLA isomer stimulated insulin action in fat and muscle cells (60). These observations suggest that CLA can prevent or delay the onset of diabetes and that the 9,11-ct isomer may contribute much of this activity. Recently, 10,12-tc isomer of CLA was shown to induce insulin resistance and hyperinsulinemia in mice, whereas 9,11-ct isomer had no effect (61). Both these isomers were shown to be equally efficient in stimulating PPAR- $\alpha$  and  $\gamma$  receptors, indicating that the hyperinsulinemia may not be mediated through nuclear receptor pathway. In another study using either high metabolic rate mice or low metabolic rate mice, Hargrave et al. (62) demonstrated that CLA increased the insulin resistance in high metabolic rate mice only, whereas in Zucker diabetic rat, CLA was shown to prevent a rise in insulin and glucose levels that might have been mediated through an increased production of adiponectin, a hormone released by adipose tissues (63). In pigs, dietary CLA had no effect

on plasma glucose or insulin levels or on the ability of insulin to mobilize plasma glucose (64). These studies indicate that the effect of CLA in diabetes is not clear and the reported differences may be species specific. In human subjects, the effects of CLA on insulin resistance and glucose homeostasis are not well studied. In one clinical trial, the 10,12-tc isomer, but not a mixture of 9,11-ct and 10,12-tc isomers, was shown to increase the insulin resistance and blood glucose levels in abdominally obese people (41), whereas another study on normolipidemic subjects failed to observe any effect of either a 50:50 or 80:20 mixture of 9,11-ct and 10,12-tc isomers on blood glucose or insulin levels (65). Belury et al. (28) observed a reduction in fasting plasma glucose levels in type II diabetics when they were treated with 6.0 g of a mixture of CLA for eight weeks. This necessitates the need for controlled studies in human to delineate the effect of CLA and its isomers on blood glucose homeostasis and insulin resistance.

### 3.4. Cardiovascular Actions

An isomeric mixture of CLA was reported to inhibit the development of atherosclerosis in rabbits (66, 67) or hamsters (68) fed a high cholesterol diet. The CLA mixture caused a regression of atherosclerosis in rabbits (67) and Apo E<sup>-/-</sup> mouse (69). In hamsters, CLA was shown to lower the levels of total- and LDL-cholesterol and TAG levels (33, 70). The reduction in plasma levels of cholesterol could be mediated via an increase in LDL receptor expression in the liver leading to increased clearance from the circulation (71). CLA has also been reported to reduce secretion of apolipoprotein B in animals (72). The effect of CLA could be mediated through induction of the PPAR- $\gamma$  pathway. CLA has been shown to inhibit cyclooxygenase enzyme in vitro (69). Its anti-inflammatory actions might be playing a role in prevention of atherosclerosis but does not appear to play a role in regression of atherosclerosis in animals. The 9,11-ct, and 10,12-tc isomers of CLA and the metabolite of 9,11-ct isomer (13-hydroxy-9c,11t- octadecadienoic acid) have been reported to inhibit arachidonic acid and collagen-induced platelet aggregation (73), which could have been mediated through inhibition of thromboxane A<sub>2</sub> formation from arachidonic acid. CLA was also shown to prevent the development of hypertension in Zucker diabetic rats (63) that was associated with increased expression of mRNA for adiponectin, a hormone released by adipose tissues. Similar results on prevention of hypertension were shown in Otsuka Long-Evans Tokushima fatty (OLETF) rats (74). In this rat model, the 10,12-tc isomer of CLA was shown to inhibit angiotensinogen production from adipose tissues. The effect of CLA on plasma cholesterol levels and atherogenic potential in human is not clear. In healthy, normocholesterolemic human subjects, Benito et al. (75) reported no effect of CLA on cholesterol levels, platelet aggregation, or bleeding time, whereas Noone et al. (65) reported a TAG lowering effect of 50:50 mixture of CLA isomers. These differences could be due to differences in the composition of CLA, as Noone et al. (65) used 50:50 or 80:20 mixtures of 9,11-ct and 10,12-tc isomers and Benito et al.'s (75) preparation contained 11.4% 9,11-ct, 10.8% 8,10-tc, 15.3% 11,13-ct, and 14.7% 10,12-tc, with 6.7% c,c and 5.9% tt isomers of CLA. To establish the effects

of CLA on cardiovascular risk factors in humans, more research is needed using pure isomers or standardized mixture of CLA isomers.

### 3.5. CLA and Immune Function

In animal studies, CLA was reported to enhance immune response and attenuate allergic reactions (76, 77). CLA has also been shown to prevent age associated reductions in immune function (78). In Guinea pigs, feeding CLA was shown to reduce the release of histamine and PGE<sub>2</sub> from isolated trachea challenged with antigens (79), suggesting a strong antiallergic action. In mice, feeding CLA dose dependently increased splenic lymphocyte proliferation in response to phytohemagglutinin but not to lipopolysaccharide or concanavalin A, suggesting that CLA has selective actions on immune function. CLA stimulated IL-2 production (80) and also increased the basal and mitogen stimulated natural killer cell activity of splenic lymphocytes in mice that was associated with increased number of NK-cells but no change in the ratio of NK-cells to total splenic lymphocytes (81). CLA has also been shown to reduce the release of proinflammatory mediators, including PGE<sub>2</sub>, IL-1, and IL-6, from macrophages stimulated by interferon gamma (IFN- $\gamma$ ) (82). These actions appeared to be mediated through stimulation of PPAR- $\gamma$  pathway. When fed to pigs, CLA enhanced cellular immunity by modulating white blood cell types that control adaptive and innate immunity (83, 84). Feeding CLA to pregnant and lactating pigs caused an increase in IgG levels of colostrums without affecting the serum IgG levels (85). In the same study, feeding CLA to suckling piglets resulted in increased serum levels of IgG and lysosomes. These results indicate enhancement of immune function in piglets. Following the feeding of CLA to young healthy women, no effects were observed on several measures of immune function including delayed type hypersensitivity response and numbers of circulating white cells and antibodies to vaccine, although an increase in CLA content of mononuclear cells was observed (86, 87). Differences in immune responses may be due to the selection of a young healthy population with optimal immune function, species differences, or dietary CLA isomer composition.

CLA has recently been studied for its actions on peroxisome proliferator-activated receptors (PPARs), most notably of the PPAR $\gamma$ . As PPAR $\gamma$  plays a role in macrophage activity, Yu et al. (82) observed a stimulation of PPAR $\gamma$  in RAW264.7 mouse macrophage (RAW) cells by various CLA isomers. CLA also decreased the production of PGE<sub>2</sub>, TNF- $\alpha$ , nitric oxide (NO), IL-1 $\beta$ , and IL-6 in RAW cells treated with interferon- $\gamma$  (IFN $\gamma$ ) (82). The inhibition of production of these inflammatory mediators was associated with a reduced expression of mRNA for cyclo-oxygenase 2 (COX2), inducible NOS (iNOS), and tumor necrosis factor alpha (TNF- $\alpha$ ). Cheng et al. (88) observed similar inhibition of COX-2 and NOS mRNA in lipopolysaccharide (LPS) stimulated macrophage cell, which was associated with an inhibition of LPS-induced protein expression of the cytoplasmic phosphorylated inhibitor kappaBalpha (I $\kappa$ B $\alpha$ ) and nuclear p65 as well as NF-kappaB nuclear protein-DNA binding affinity. This observation indicate a role of NF-kappaB in regulation of anti-inflamaotry actions of CLA.

#### 4. STRATEGIES TO INCREASE DIETARY INTAKE OF CLA

As the interest in beneficial effects of CLA is increasing, so are the efforts to increase its dietary intake. Various strategies are being employed, which include increasing the content of CLA in eggs, milk, and meat. In 1951, hens were shown to incorporate CLA into egg lipids following dietary administration (5), but the egg production was significantly reduced. Recently, strategies to reduce the population of problem birds, based on feeding CLA to the females to reduce the egg hatchability, have been patented (89). Feeding 0.5% CLA in the diet of hens was shown to increase the CLA content of egg lipids, which was associated with a significant increase in saturated fatty acids and a reduction in monounsaturated fatty acids in the egg lipids. These changes in the egg composition also changed the properties of the egg yolk in that it became hard when stored at cool temperatures (refrigerator). This observation suggests that feeding CLA to poultry may not be an attractive strategy to increase the CLA content of eggs as poultry breeders cannot bear the economic burden of significantly reduced fertility. Strategies are needed to increase the content of CLA without affecting hatchability of eggs. Recently, methods to increase the content of CLA in eggs have been patented (90, 91). These strategies include incorporating CLA along with monounsaturated (91) or mono- and polyunsaturated fatty acids (90) in the diet. Similarly, feeding CLA in a diet to cattle increased CLA content in milk and meat and simultaneously reduced total milkfat content (92) and increased milkfat saturated fatty acids. Other strategies include feeding 11-*trans*-octadecaenoic (C18:1) acid (93) and other sources of polyunsaturated fatty acids to cattle. Feeding either fresh forage or supplementing the cattle diet with high linoleic acid meals/oils is another effective way of increasing the content of CLA in milkfat (94–97) and muscle. Most common sources of polyunsaturated fatty acids include sunflower oil, linseed (flaxseed oil), safflower oil, fish oil, or marine algae (98–101). When CLA is fed to cattle, it is better to protect it from rumen hydrogenation by converting to calcium salts.

#### 5. COMMERCIAL PRODUCTION OF CLA

Industrial conjugated linoleic acid (CLA) is a poorly defined blend of compounds (102). Early commercial syntheses focused on maximizing total CLA content. Many early products were rich in CLA but contained a number of positional isomers. Market demand has now shifted for a product that contains two predominant isomers, specifically 9,11-*c,t*-octadecadienoic acid and 10,12-*tc*-octadecadienoic acid. It is not surprising that alkali isomerization produced some undesirable positional isomers of CLA. In 1970, Mounts and Dutton (103) had shown unequivocally that when potassium *t*-butoxide was used, at least four positional isomers of CLA were produced. It was not until 1997, after the use of CLA as a dietary supplement

**TABLE 1. Linoleic and Linolenic Acid Contents of Some Vegetable Oils (104).**

Oil Source	% Linoleic Acid	% Linolenic Acid	Commercial Oil Available	Commercial Fatty Acid Available
Corn	57	0	Yes	
Cottonseed	53	0	Yes	
Cucumber	72	0	No	
Grapeseed	70	0	Yes	
Linola™ Flaxseed	72	3	Yes	
Poppy	77	0	Yes	
Safflower	75	0	Yes	
Sunflower	64	0	Yes	Yes
Soybean	51	8	Yes	Yes
Squash (pumpkin)	60	0	Yes	
Walnut	62	12	Yes	

began, that Christie et al. (102) elegantly demonstrated that commercial CLA was a blend of positional isomers. In response to this discovery, new commercial CLA products have been introduced that have comparatively high levels of the preferred isomers. In spite of the improvements, all current available commercial CLA products contain some level of the less desirable isomers and other components, which may or may not be desirable.

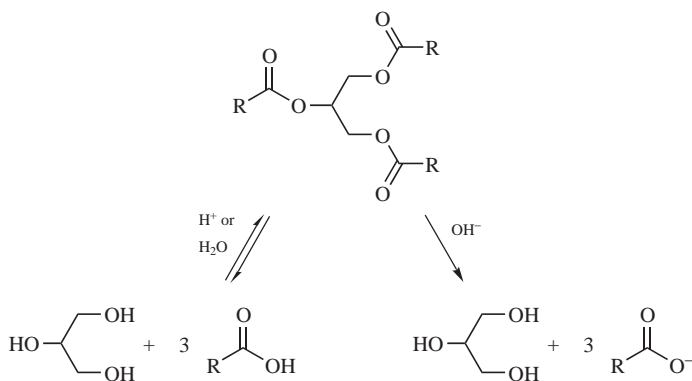
Commercial processes for the synthesis of any compound of economic value is normally proprietary information and the commercial methods of CLA production are no exception. The process by which each brand of commercial CLA is synthesized is not known by the authors of this review. Therefore, this review is directed at the patent literature on CLA synthesis, major problems encountered in CLA synthesis, and analysis of CLA from commercial suppliers.

### 5.1. CLA Production Raw Materials

The raw material for CLA production must be a material that is rich in linoleic acid. This product could be in the triacylglycerol form, fatty acids or fatty acid esters. The concentration of CLA in the final product is directly dependent on the level of linoleic acid in the starting material. The highest level of linoleic acid available from botanical sources is not available in commercial products. Extraction and refining equipment would be required to obtain oils with the highest linoleic acid levels. Table 1 lists the commercial and noncommercial sources of oil and fatty acids that are known to be rich in linoleic acid and their availability as TAGs and fatty acids.

If commercial CLA is to be synthesized from a fatty acid, it must be recognized that commercial fatty acids are generally not intended for use in the production of CLA. Commercial fatty acids are usually produced by the reaction of water (steam) and TAG oil at high temperatures in a continuous reaction (Reaction 1).

Reaction 1: Alkali or acid hydrolysis of acylglycerols.



This reaction is accelerated through the use of a solid phase acid catalyst, which is readily separated from the fatty acid and glycerol products after hydrolysis (105). The disadvantage of this hydrolysis process is that the reaction is reversible and products generated by this process contain appreciable amounts of mono- and diacylglycerols, which may have undesirable side reactions in CLA synthesis. Fatty acids may also be produced by the hydrolysis of TAGs in a pressurized reactor at 200 °C without the addition of a catalyst (105). This reaction may be catalyzed at lower temperatures using zinc oxide in a batch reactor (105). The product of these batch reactions also contains substantial amounts of mono- and diacylglycerols.

Hydrolysis of TAGs is possible using water and strong base to produce soaps (Reaction 1). This reaction proceeds to completion and can be conducted at the modest temperatures required to maintain the reaction mixture as a fluid. More than three moles of potassium hydroxide or sodium hydroxide are required to hydrolyze one mole of TAG oil. As the caustic alkali cannot catalyze the reverse reaction, this process can produce soaps that are virtually free of acylglycerols in a single step (105). The soaps from alkali hydrolysis of TAGs are readily converted to fatty acids by acidification with the addition of citric acid or strong mineral acids, which include HCl, H<sub>2</sub>SO<sub>4</sub>, or H<sub>3</sub>PO<sub>4</sub>. Regardless of the method chosen for production of fatty acids, the acids should be dried under vacuum after washing with brine or by a combination of other acceptable methods (105).

There are commercially available fatty acids suitable for use in CLA production. For example, Henkel Corporation (106) sells a series of fat products including those shown in Table 2. However, none of the products listed in Table 2 would be preferred as starting materials for CLA production for reasons that will be discussed.

## 5.2. Enrichment of Linoleic Acid

A commercial interest may wish to produce CLA at concentrations greater than can be obtained by modifying high linoleic acid plant oils. Several methods exist that will improve the starting material by increasing the concentration of linoleic acid,

**TABLE 2. Henkel Products (106).**

Product	14:0 (%)	16:0 (%)	18:0 (%)	16:1 (%)	18:1 (%)	18:2 (%)	18:3 (%)
Soya fatty acids (Emersol 610)	0.5	16	4	1	25.5	48	5
Linoleic acid (Emersol 315)	0.5	3.5	0.5	Trace	19.5	65.5	10.5
Methyl Linoleate (Emery 2221)	0.5	3.5	0.5	Trace	19.5	65.5	10.5

but only a few methods are used in industrial settings. Industrial separation of fatty acids has been reviewed by others (104, 107). A limited discussion of these methods will be presented.

Crystallization is used to separate saturated fats and oleic acid from linoleic acid. If a highly concentrated product is required, the linoleic acid may be crystallized once or repeatedly as the last step in purification. Crystallization is a mild procedure but usually requires the use of a solvent (108) such as acetone or methanol. The use of low boiling point and flammable solvents raises concerns over plant safety, government regulations on manufacturing, and market acceptance of the product. Furthermore, the removal of oleic acid by crystallization in solvent is only possible by lowering the temperature of the liquor to below  $-40^{\circ}\text{C}$  (108). To crystallize linoleic acid, the temperature must be reduced to  $-75^{\circ}\text{C}$ .

Dry or solvent free crystallization is also possible; but these methods often require the addition of crystal modifiers that become incorporated into the product (108). Losses during crystallization can be very high as the crystals entrain large amounts of fatty acid. However, these losses may be reduced by physically pressing the crystals to remove the entrained solution (109). Linoleic acid-rich products of dry crystallization would be preferred starting materials for CLA production over those of solvent crystallized products; but the losses incurred in dry crystallization may prohibit this method of manufacture. Crystal modifiers may be selected so that they do not adversely affect the quality or acceptance of the final product.

Specific fatty acids may be concentrated by sequentially removing contaminating fatty acids as urea adducts and forming the urea adduct of the desired fatty acid. This process requires dissolving the fatty acids or esters in urea and hot methanol (or other alcohol) and cooling to effect adduct formation. The adduct is filtered from the liquor and, if conditions are carefully controlled, the adducts can be used to sequentially crystallize saturates, monounsaturates, diunsaturates, and triunsaturates. A urea adduct rich in linoleic acid could be produced by first removing adducts of saturates and monounsaturates from a suitable oil and then forming the desired adduct. Once formed, the adduct may then be decomposed by the addition of water to the solid phase. Enriched linoleic acid could be recovered by solvent extraction of the urea:water solution with a nonpolar solvent such as hexane. All problems associated with crystallization in solvent mentioned previously also occur in formation of urea adducts, with the exception of the requirement for



very low temperatures. Typically, urea adducts form between room temperature and 0°C (108).

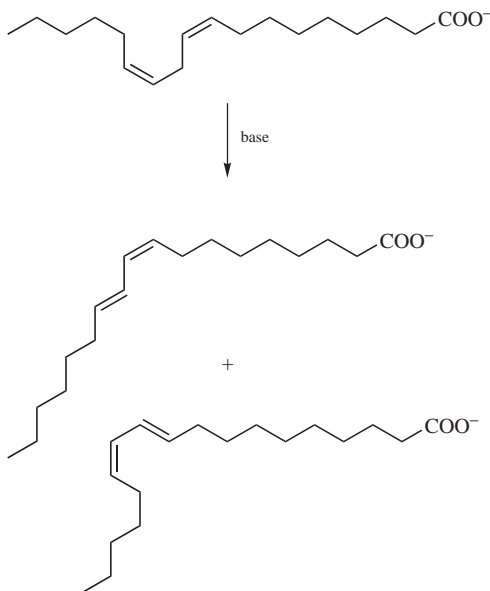
Fatty acids may also be enriched by the use of various absorption media. Molecular sieves can separate saturated fatty acids from unsaturated fatty acids dissolved in acetone (110). Oleic acid and linoleic acid dissolved in blends of solvents, including acetonitrile, tetrahydrofuran, water, and formamide, may be separated using cross-linked polystyrene polymers such as Amberlite™ XAD-2 or XAD-4.

Selective extraction methods using two-phase solvent systems may also be used to enrich fatty acids. Solvent systems such as dimethyl formamide, hexane, and ethylene glycol can form a two-phase system that effectively partitions sunflower oil TAGs rich in linoleic acid from those depleted in linoleic acid (111). TAGs partitioned in this way may contain up to 84.7% linoleic acid. This method would not likely be used in industry because the magnitude of the losses are usually unacceptable.

### 5.3. Approaches to CLA Production

CLA has been produced by the reaction of soaps with strong alkali bases in alcohol, ethylene glycol, and glycerol (112–114) (Reaction 2).

Reaction 2: Isomerization of *cis,cis*-9,12-octadecadienoyl soaps.



The CLA product is generated by acidification of the soap solution with a strong acid (sulfuric or hydrochloric acid) and repeatedly washing the product with brine or an aqueous  $\text{CaCl}_2$  solution.

CLA has been synthesized from fatty acid and soap blends using  $\text{SO}_2$  in the presence of a substoichiometric amount of soap forming base (115). This reaction produced predominantly the all *trans*-configuration of CLA.

Of these methods, alkali isomerization of soaps is the least expensive process for bulk preparation of CLA isomers; however, the use of either monohydric or polyhydric alcohols in alkali isomerization of CLA can be problematic. Lower alcohols are readily removed from the CLA product, but they require that the production facility be constructed to support the use of flammable solvents. Higher molecular weight alcohols and polyhydric alcohols are considerably more difficult to remove from the product and residual levels of these alcohols (e.g., ethylene glycol) may not be acceptable in the CLA product.

Water may be substituted for the alcohols in the production of CLA by alkali isomerization of soaps (116, 117). When water is used in this reaction, it is necessary to perform the reaction in a pressure vessel, whether in a batch (116) or continuous mode of operation (117). The process for synthesis of CLA from soaps dissolved in water still requires a complex series of reaction steps. Bradley and Richardson (118) were able to produce CLA directly from TAGs by mixing sodium hydroxide, water, and oil in a pressure vessel. Their method eliminated the need to synthesize fatty acids followed by soap formation prior to the isomerization reaction. However, the authors reported that they were able to produce an oil with only 40% CLA. Quantitative conversion of the linoleic acid in soybean oil to CLA would have produced a fatty acid mixture with approximately 51% CLA.

#### 5.4. Reaction Kinetics and Production of Positional Isomers

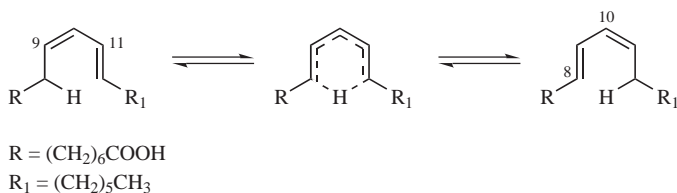
The kinetics of conversion of linoleyl soaps to conjugated linoleyl soaps has been well described by first-order reaction kinetics (119). Total conjugation is readily measured by simple methods such as increases of UV absorbance at 231.5 nm. In industry, it is necessary to allow the reaction to proceed until most of the linoleyl soaps are conjugated but desirable to stop the reaction as soon as possible after that point. The reaction is between 97% and 98.5% complete after 5 half-lives and 6 half-lives respectively. There is little advantage in continuing the reaction longer than this time and, as will be discussed below, undesired reactions may occur with longer reaction times. The reaction constant is readily determined early in the reaction when changes in the level of conjugation are large. The task of determining the rate constant in a large reactor is complicated by the mass of the reactor and its contents. A large batch reactor often requires several hours to reach the optimum heat of reaction, at which time the reaction may be almost complete. Similarly, cooling the contents of a large reactor as a means of stopping a reaction is usually impractical. A reaction that nears 99% completion in 2 hours in a laboratory has a half life of less than 20 minutes. Control of a batch reaction in a commercial operation may use analytical data from periodic sampling, but the analytical method must be very rapid to be an effective tool for decision making.

The authors have found that the half-life of the reaction may vary by approximately 10% per  $1^\circ\text{C}$ . It follows that precise reactor temperature control is essential

to standardize quality control. A reaction planned to continue for 6 half-lives could vary from 5 half-lives to 7 half-lives if the reactor temperature control is  $\pm 2^\circ\text{C}$ .

The isomerization reaction that leads to the production of positional isomers (Reaction 3) has similar first-order kinetics. Using this assumption, we have modeled the sequential conversion of linoleyl soaps to a mixture of 9,11-*cis*-, *trans*-octadecadienoyl and 10,12-*trans*-, *cis*-octadecadienoyl soaps (Reaction 2) and finally, the conversion of these two soaps to 8,10-octadecadienoyl and 11,13-octadecadienoyl soaps. Reaction 3 shows the mechanism of one of these two isomerization processes.

Reaction 3: Isomerization of *cis*-,*trans*-9,11-octadecadienoyl soap to *trans*-,*cis*-8,10-octadecadienoyl soap via sigmatropic rearrangement.



In an earlier review of commercial CLA production, Reaney et al. (120) developed a model of the base catalyzed sequential conversion of unconjugated linoleic acid to a mixture of two isomers, 9,11-*c,t*- and 10,12-*t,c*-CLA, by first-order kinetics. After the formation of the two primary isomers, two additional isomers, 8,10-*t,c*-CLA and 11,13-*c,t*-CLA, were produced by a sequential reaction. It was later reported by Saebo (121) that the second reaction producing the additional isomers was actually a thermal sigmatropic rearrangement as shown in reaction 3. The intramolecular rearrangement is independent of catalyst concentration and, thus, the accumulation of additional isomers is a function of the reaction temperature and the duration of reaction. Saebo (121) reports that prolonged heating results in the accumulation of isomers but that reaction solvent that allows for low-temperature reactions may be used to prevent sigmatropic rearrangement and the consequent formation of isomers.

## 5.5. Solvents Used in Production of CLA by Alkali Catalysts

Research reports describe the use of at least eight solvent systems for the production of CLA using alkali catalysts (112–116) (Table 3). The choice of solvent greatly affects the reaction conditions of CLA production. The choice of solvent by the manufacturer is determined by a number of considerations. Many markets will not accept low levels of ethylene glycol, ethylene glycol monomethylether, *t*-butanol, dimethyl sulfoxide (DMSO), or dimethyl formamide (DMF) in the final product. This limitation could restrict the choice of solvents to only molten alkali, glycerol, propylene glycol, water, and ethanol. The reaction in water and ethanol only proceeds above the boiling temperature of these solvents and, therefore, a pressure reactor would be required to operate using these solvents. Glycerol is

**TABLE 3. Summary of Solvents Used in CLA Production.**

Solvent (reference)	Mol wt.	B.P. (760)	B.P. (Vac)	Temp (°C)	Time (h)	% Reaction	Phase Sep <sup>n†</sup>	Color ‡	Toxicity	Other
Ethylene glycol (148)	62.07	198.00	93 <sup>13*</sup>	180	2.0	100.0	yes	Poor	yes	
Glycerol (148)	92.11	290.00	182 <sup>20</sup>	180	0.75	100.0	yes	Good	none	viscous
Propylene glycol (149)	76.11	189.00	97 <sup>21</sup>	170	2.5	99.1	yes	Good	minimal	
t-butyl alcohol (103)	74.18	82.41	Low	90	4.0	98.5	no	unknown	yes	High Pressure
Water (118)	18	100.00	Low	225	2.5	40.0	yes	Good	none	High Pressure
DMSO (149)	78.14	189.00	83 <sup>17</sup>	30	1.5	78.0	no	unknown	yes	fp** 95
DMF (149)	73.09	153.00	76 <sup>39</sup>				no	unknown	yes	fp** 67

†Phase Separation = Yes if a two-phase system is formed after acidification of the soap.

‡Color = As described in references.

\*fp = flash point.

\*\*Superscript numbers refer to Vacuum in mm of Hg.

DMF, Dimethylformamide; DMSO, Dimethylsulfoxide.

expensive, but it could be recovered from a commercial operation that produces its own fatty acids. The quality of glycerol necessary to produce high-quality CLA has not been investigated, but refining this glycerol stream to remove the salt might prove difficult to a small operation. Recovery would have to be very efficient as fatty acid production only generates 10% of the weight of the oil as glycerol.

## 5.6. Catalyst Selection

Numerous catalysts have been used in the production of CLA. We have found that hydroxides of lithium, sodium, and potassium are all capable of generating CLA in various solvents. As fatty acids neutralize the catalyst, it is necessary to add at least one mole of catalyst for every mole of fatty acid in the reaction to ensure soap is generated. We have found that, on a molar basis, potassium hydroxide has proven to be a more effective catalyst than sodium hydroxide, with lithium hydroxide the least effective and not suited for industrial CLA production. On a weight basis, sodium and potassium hydroxide have similar efficiency of conversion. Although sodium hydroxide is much less expensive than potassium hydroxide, the disposal costs for the waste neutralized alkali should also be considered. Potassium salts are easily used as fertilizer and can be applied to fields, whereas sodium salts cannot be disposed of in a similar fashion.

The effective form of the catalyst is not necessarily determined by the added catalyst itself but rather by the solvent used. When water is used as the solvent and sodium ethoxide is the catalyst, the effective form of the catalyst is likely the hydroxide ion. If t-butanol is used as the solvent and sodium methoxide is added

to catalyze the reaction at 90 °C, the reaction mixture will quickly release methanol vapours and t-butoxide ion will become the effective catalyst.

Water consumes alkoxide catalysts and, in industrial production, maintaining alkoxide catalysts in a water-free environment is difficult. Water is produced by the neutralization of fatty acids with alkali hydroxide. As many alkoxide catalysts contain some alkali hydroxide, it is not uncommon for the catalyst to be consumed by this reaction.

## 5.7. Reaction Vessels

Alkali isomerization of linoleyl soaps requires a containment vessel that is both tolerant of heat and caustic. When a low boiling point solvent such as ethanol or water is used, the vessel must also be capable of maintaining the reaction under pressure. There are a limited number of materials that will meet these criteria. Polytetrafluoroethylene and other fluoropolymers are capable of withstanding both the heat of the reaction and the caustic environment, but they cannot withstand pressure and are poor heat conductors (122). Fluoropolymer coated parts and nickel and nickel alloys such as Monel may be used in the construction of reaction vessels for production of CLA. The high cost of these materials rules out their use in construction of large batch reactors. Furthermore, none of these materials has sufficient strength for use in pressure reactors if a reaction in water or alcohol is planned. The preferred choice for reactor construction is nickel-plated steel, which has the desired strength, heat transfer, and chemical properties for conducting reactions in strong caustic solutions. A coated vessel of this design requires regular inspection, as a flaw in the coating could lead to vessel failure.

## 5.8. Microbial Production of CLA

Pariza and Yang (123) have recently described the microbial production of 9,11-c,t-CLA from linoleic acid using cultures of *Lactobacillus sp.* In their patented method, early stationary phase *Lactobacillus* cultures were incubated with linoleic acid dissolved in propylene glycol. A total CLA level of 7 mg/g cells was produced, which was over 96% 9,11-c,t-CLA. This type of conversion may lead to improved CLA products in the future.

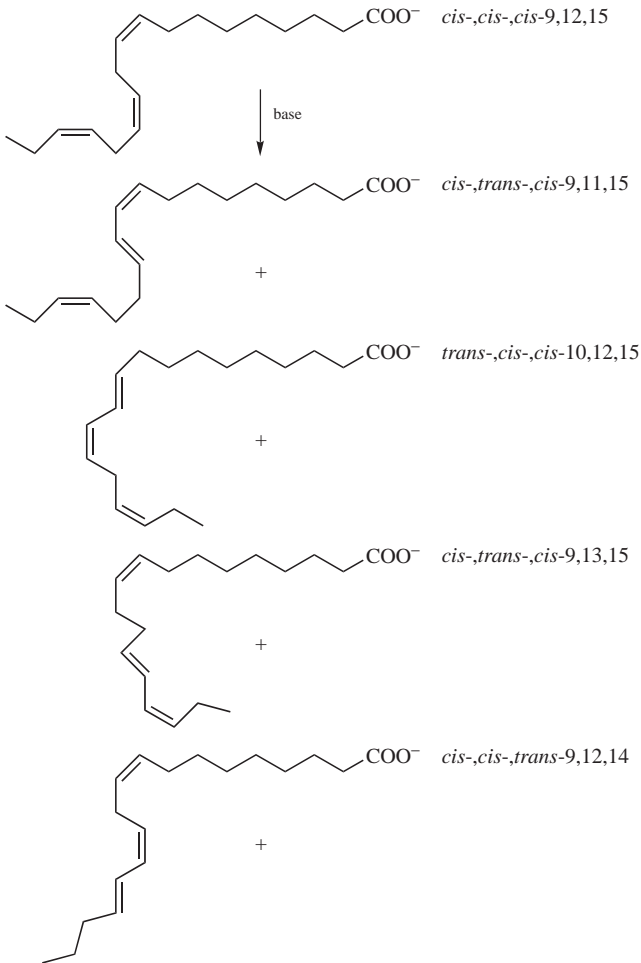
## 5.9. Synthesis of CLA by Dehydration of Ricinoleic Acid (12-Hydroxy-*cis*-9-Octadecadienoic Acid)

The most attractive method for production of pure 9,11-c,t-CLA is through the dehydration of ricinoleic acid. Synthesis from this relatively inexpensive starting material has proven elusive as it is difficult to control the formation of dehydration products (124). Synthesis of 9,11-c,t-CLA from ricinoleic acid has been reported (125), which, although an efficient reaction, uses expensive elimination reagents such as 1,8-diazobicyclo-(5,4,0)-undecene. For most applications, the high cost of the elimination reagent increases the production cost beyond the level at which commercial production of CLA is economically viable.

### 5.10. The Quality of Commercial CLA Products

The fate of other fatty acids and minor components during processing has not been investigated. The conditions used to conjugate linoleic acid have little or no effect on either monounsaturated or saturated fatty acids, however, any polyunsaturated fatty acids may be conjugated. The products of the reaction of alkali catalysts on these fatty acids are more complex than that discussed for linoleic acid (Reaction 4) and will not be discussed except to note that these reactions may produce undesirable products.

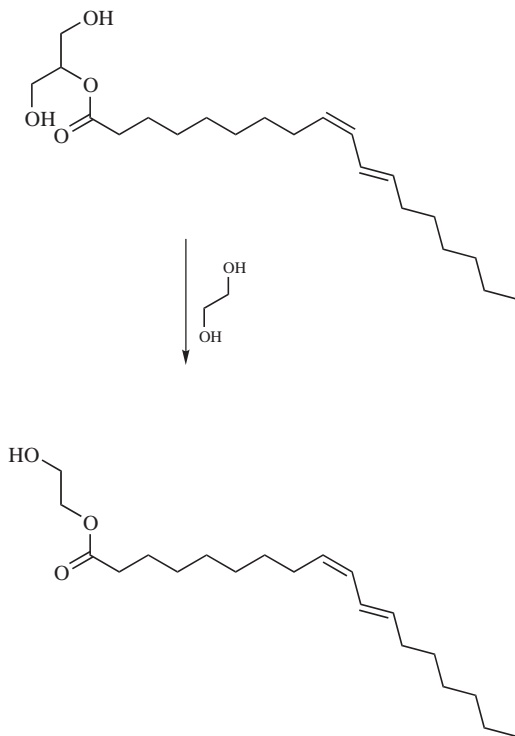
Reaction 4: Isomerization of *cis*-,*cis*-,*cis*-9,12,15-octadecatrienoyl soap to isomers.



From our observations, glycerol does not form undesirable compounds under the conditions of alkali isomerization of linoleic acid. However, we have found that a number of commercial fatty acids and CLA preparations contain appreciable levels

of monoacylglycerols (MAGs). MAGs themselves are not toxic, but it is possible that toxic compounds (such as ethylene glycol) may be incorporated into the final product through alcoholysis of the MAG by the low volatility alcohols used as a reaction medium (Reaction 5).

Reaction 5: Alcoholysis of MAG with ethylene glycol.



NMR and liquid chromatographic analysis of CLA samples from all sources indicated that, although CLA was the predominant compound, CLA esters and other unknown compounds may also have been formed.

The minor components of vegetable oil, such as tocopherol, sterol, or squalene, are stable to heat a strong alkali. However, tocopherol and other components present in vegetable oil readily react with oxygen in the presence of metals. Tocopherol stabilizes vegetable oils against oxidation and tocopherol loss through processing or high metal content may lead to a decreased shelf-life of the CLA product. Three commercial CLA products were analyzed for metal content using ICP. The metal contents are given in Table 4.

The CLA samples tested were free of potentially toxic metals at the levels tested, with the exception of a trace level of barium in product C. Products A and B had very low total metal contents, whereas product C had appreciable levels of calcium. The soaps probably derived from a CaCl<sub>2</sub> wash in a late stage of processing.

**TABLE 4. Metal Contents of Three Commercial CLA Products (ppm).**

Sample	Na	K	Fe	Cr	B	P	Ca	Ba	Mg	Li	Total
A	3	3	1	0	5	2	5	0	1	0	20
B	2	28	0	0	3	8	1	0	1	0	43
C	4	6	11	8	3	3	917	1	2	1	956

\*Not detected Si, Pb, Cu, Sn, Al, Ni, Ag, Ti, Zn, Mo, V, Sb, Be.

Product C also contained iron and chromium suggesting the use of a stainless-steel reactor for processing. Even these small levels of metals may contribute to rapid oxidation of product C, and it probably has a shortened shelf-life when compared with the other products.

Three commercial samples of CLA were subjected to a series of tests, which are reported in Table 5. Viscosity was measured using ASTM test D445. It was presumed that increased viscosity might be related to increased oxidation if it had occurred. Large differences in viscosity were not found. Color of the three samples is reported, but it is only a subjective statement regarding the apparent quality of the three materials. Included in the color analysis is the absorbance maximum observed for CLA dissolved in hexane (1:1000 CLA:hexane) using a Cary UV/Vis spectrometer. The UV spectra indicated that conjugated dienes were the major source of absorption of the oil samples.

Proton and carbon 13 NMR spectra were obtained from the three commercial samples. A series of unknown spectral components were observed in product A at 3.68 ppm, 3.98 ppm, 4.08 ppm, 4.15 ppm, 5.05 ppm, and 5.17 ppm. Comparison of the unknown peaks with the spectra of MAGs indicated that most of the observed peaks correlated with those present in the spectra of MAGs. An exact assignment of the spectra was not attempted as many forms of MAGs are possible. Observation of the spectral region of the olefinic protons revealed that the three samples were

**TABLE 5. Summary of Analyses of Three Commercial CLA Products.**

Sample	Viscosity cSt (40 °C)	Color	<sup>1</sup> H-NMR (400 MHz)	Size Exclusion	RP-LC	GC-FID	Isomer Mix
A	28.9	Clear $\lambda = 231.5$ A = 0.720	Acylglycerols	Acylglycerols	Polar contamination	Contamination	Good 95%
B	27.9	Light yellow $\lambda = 231.5$ A = 0.667	No acylglycerols	No acylglycerols	Polar contamination	OK	Poor 70%
C	29.7	Brown $\lambda = 231.5$ A = 0.676	No acylglycerols	No acylglycerols	Non-Polar contamination	OK	Good 90%



predominantly *cis*-, *trans*- or *trans*-, *cis*-fatty acids (126). We have found that a convenient measure of the CLA content of an oil can be obtained by comparing the integrated values of the protons at 6.0 ppm and 6.3 ppm with the integrated value of alpha methylene group (adjacent to the carbonyl) at 2.3 ppm. The olefinic protons were chosen as they do not overlap with oleic acid olefinic protons or olefinic protons from conjugated linoleic acid with *trans*-, *trans*-configurations. The ratio of *cis*-, *trans*-olefin to alpha methylene protons is a useful measure of CLA purity.

Carbon-13 olefinic carbons were observed at 400 MHz. The carbon-13 spectrum clearly demonstrates the formation of positional CLA isomers. As pure standards were not available, it was not possible to unequivocally assign the spectra of 8,10- or 11,13- CLA, but it is clear that these isomers are predominantly *cis*-, *trans*- or *trans*-, *cis*-fatty compounds.

Size exclusion chromatography was performed with a Waters GPC-Styragel<sup>TM</sup> HR 0.5 column (7.8 × 300 mm) at 35 °C using tetrahydrofuran as a solvent flowing at 1.0 mL/min and detecting compounds with both UV absorbance and evaporative light scattering detection (ELSD, 40 °C, 4.2 L/min gas flow). The goal of this analysis was to observe polymerization of the CLA products or the occurrence of acylglycerols. Under these conditions, 18-carbon fatty acids had a retention time of 6.6 minutes and MAGs had a retention time of 5.9 minutes. All three samples had two peaks observed by ELSD, one at the position expected for 18-carbon fatty acids and the other as expected for their respective MAGs. Product A had the largest peak at the position expected for MAGs. Observations made using UV absorbance at 233 nm reflected the observations made with the ELSD.

Reversed phase liquid chromatography was performed on a 150 mm × 3.0 mm Waters Symmetry<sup>TM</sup> C-8 column at 30 °C and a flow rate of 1.0 mL/min. The solvent phase was acetonitrile:tetrahydrofuran:0.1% aqueous phosphoric acid (50.4:21.6:28v/v/v). Under these operating conditions, most of the UV absorbance occurred as a peak at 3.83 minutes for all samples. Chromatograms of all samples had some small peaks (presumably the more polar compounds) eluting prior to the major peak. Product C presented a small but significant UV absorbing peak that eluted after the peak at 3.83 minutes.

## 5.11. Rapid Analytical Methods

Industrial CLA syntheses must be controlled to both maximize the content of preferred isomers such as 9,11-*cis*-, *trans*-octadecadienoic acid and minimize the formation of undesirable isomers. For the analysis to be useful, the results of the analysis should be available online or as quickly as possible. The authors are not aware of any existing online tests for the quality of CLA preparations, but several methods may have promise for use offline.

The potential offline analytical methods include UV, FTIR, proton NMR, carbon-13 NMR, gas chromatography, and capillary electrophoresis. With the exception of capillary electrophoresis and UV absorbance, none of these methods can

effectively analyze soap solutions and, therefore, for most analytical methods, neutralization of soaps would be necessary.

A reaction medium that contains 40% soaps by weight can usually be dissolved in ethanol. We have found that 100 mg of reaction mixture will totally dissolve in 10 mL of 95% ethanol when glycerol, water, or ethylene glycol are the reaction solvent and alkali hydroxides are used as the catalyst. It is then possible to dilute the reaction mixture solution 1000-fold to determine the UV absorbance at 231.5 nm. When there is no other interfering UV absorbance, this method is an excellent indication of the total conjugated double bonds. This method is also sensitive to the presence of conjugated linolenic acids derived from linolenic acid, which is indicated by a UV absorbance at 268 nm. None of the samples observed show three conjugated double bonds.

To obtain more detailed information regarding the composition of fatty acids requires additional analytical methods, some of which require extensive and time-consuming sample preparation. For these methods, we have found that it is possible to rapidly prepare a free fatty acid fraction. Alkali soaps from most reaction mixtures are readily dissolved in a mixture of hexane and ethanol (1:1v/v) or ethanol alone. When the soaps are neutralized by the addition of hydrochloric acid and water, a two-phase system is evolved. The fatty acids remain in the nonpolar phase while the polar solvent used in the reaction medium dissolves in the water. The solution of dissolved fatty acids can be directly injected onto a GC column specifically designed for separation of free fatty acids, such as the DB-FFAP column, or a suitable nonpolar GC column, such as the HP-5 column. Analysis by chromatography without derivitization affords the potential for rapid analytical feedback. We have found that a 30 m, DB-FFAP column (0.32 mm id, 0.25  $\mu$  film thickness) gave baseline resolution of most fatty acids without derivitization (program 50°C for 1 min, 50–200°C @ 25°C/min, 200–220°C @ 2°C/min, hold 20 min, He carrier 3 ml/min). The nonpolar HP-5 column (0.32 ID, 0.25 film) gave poorer resolution of underivatized fatty acids with some tailing (program 50°C for 1 min, 50–150°C @ 25°C/min, 150–290°C @ 10°C/min, hold 6 min, He carrier 2 ml/min). Both columns also partially separated isomers of CLA. It was possible to observe the formation of the all *trans*-isomers, but detailed analysis of positional isomers was not possible without additional effort.

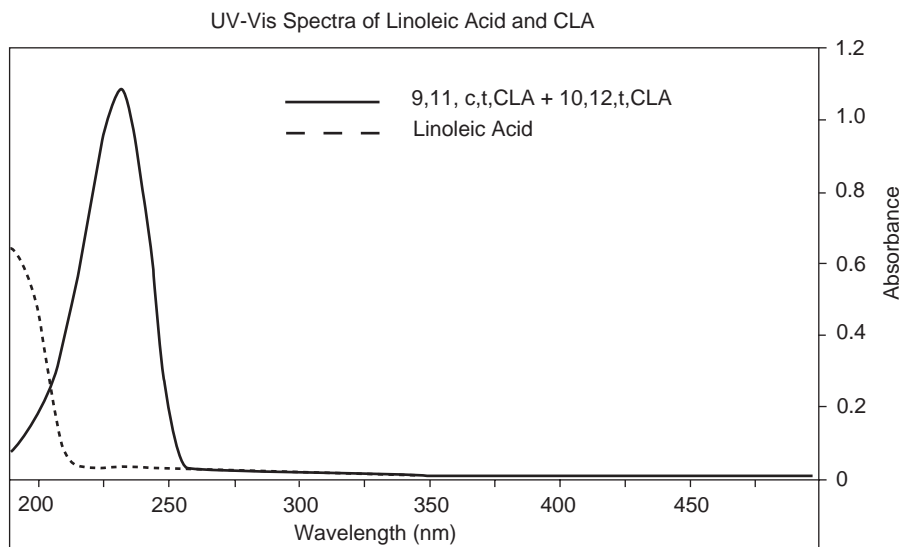
## 6. ANALYSIS OF CONJUGATED LINOLEIC ACIDS

An analyst needs to recognize three major variables before the selection of a suitable method of analysis of CLA. CLA preparations can differ in degree of conjugation, position of double bonds, and configuration of double bonds. The most basic variable is the degree of conjugation of the double bonds. Not all of the isolated double bonds may become conjugated either in biological or chemical conversions. The second issue to be addressed is the position of the double bonds in conjugated linoleic acids. Initially, in linoleic acid, the double bonds begin at the ninth and twelfth carbon atoms. After alkali isomerization, the predominant positional isomers

are 9,11 and 10,12 dienes. However, minor amounts of other positional isomers are also reported (127). In biological samples, the number of positional isomers is more varied with 16 isomers being separated by silver-ion chromatography (128). The final consideration is the geometric isomers present in CLA. Whereas the double bonds in linoleic acid are *cis*-, *cis*-, the isomerized product contains a predominance of *cis*-, *trans*- and *trans*-, *cis*-isomers plus lesser amounts of *cis*-, *cis*- and *trans*-, *trans*-isomers. Thus, the task for the analyst is to decide what information is required and then select a method or methods that will provide that information.

## 6.1. Ultraviolet Spectroscopy

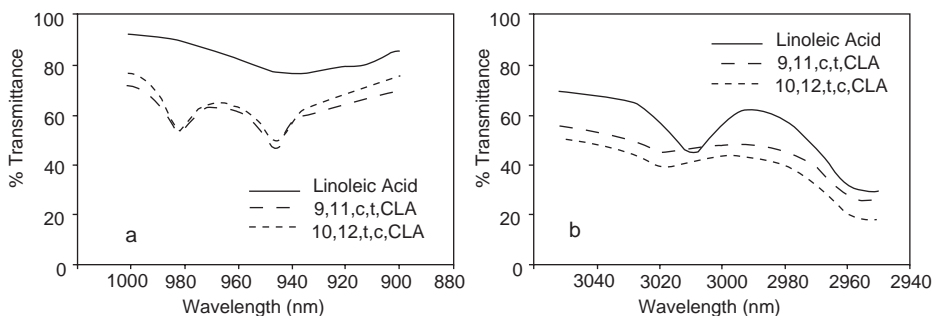
The early observation of an increase in the ultraviolet absorption (UV) near 233 nm was recognition that the composition of milkfat was not consistent throughout the year (129). This increase in absorption was not initially attributed to the increase in conjugated dienes present in milk fatty acids; however, it is now accepted that this is the case. There are subtle differences in the absorption maxima for the geometric isomers. Czauderna (130) reported a maximum of 231.9 nm for the *trans*-, *trans*-isomer, 234.3 nm for the *cis*-, *trans*- (or *trans*-, *cis*-) isomer and 235.4 nm for the *cis*-, *cis*-isomer. These small differences would not likely be discernable in isomer mixtures typically found in either biologically or chemically produced CLA. The UV measurement provides no information on the position of the double bonds. The advantage of using UV is the comparatively low cost of the spectrophotometer and the fact that it can be performed on either intact acylglycerols, fatty acids, or esters. In Figure 1, the UV spectra of linoleic acid and a commercial CLA product are shown.



**Figure 1.** The ultraviolet spectra of linoleic acid and conjugated linoleic acid (CLA).

## 6.2. Infrared Spectroscopy

Infrared spectroscopy (IR) initially used double beam optics, photocells, and alkali halide sample cells. These features at times tended to reduce the sensitivity of the instrument and to decrease the signal to noise ratio. Newer instruments have improved the signal to noise ratio by using Fourier Transform (FT) methodology and modern electronics. The application of infrared spectroscopy to the problem of identification of geometric isomers was first reported about 50 years ago. It was recognized that *cis*-, *trans*- (or *trans*-, *cis*-) dienes had a characteristic doublet at  $948\text{ cm}^{-1}$  and  $982\text{ cm}^{-1}$  (131). The corresponding *trans*-, *trans*-diene had a strong absorption band at  $988\text{ cm}^{-1}$ . Using FTIR and a direct deposit interface, Mossaba (132) reported the *cis*-, *trans*-doublet to occur at  $949\text{ cm}^{-1}$  and  $988\text{ cm}^{-1}$  and the *trans*-, *trans*-singlet at  $993\text{ cm}^{-1}$  for 4,4-dimethyloxazoline (DMOX) derivatives of CLA. An example of the spectra of linoleic acid and two CLA positional isomers obtained with a FTIR using a synthetic sample disc are shown in Figure 2A. In addition to these absorptions, there are other characteristic frequencies attributed to carbon-hydrogen stretching. In one report of 4,4-dimethyloxazoline derivatives (DMOX) of CLA isomers, it was possible to attribute unique differences among geometric isomers (133). The *cis*-, *trans*-isomers had characteristic bands at  $3020\text{ cm}^{-1}$  and  $3002\text{ cm}^{-1}$  (see Figure 2B); the *cis*-, *cis*-isomers had bands at  $3007\text{ cm}^{-1}$  and  $3005\text{ cm}^{-1}$ ; and the *trans*-, *trans*-isomer had a single band at  $3017\text{ cm}^{-1}$ . The use of these bands to determine geometric composition may be complicated by the much more intense absorption bands from other carbon-hydrogen bands present in the same region of the spectrum. Although it is theoretically possible to obtain a quantitative measurement of the amount of a substance present by IR, it is not often done. In the case of CLA, the bands that needed to be measured are minor bands compared with other bands, making the determination imprecise and difficult.



**Figure 2.** Infrared spectra of linoleic acid and 2 *cis*-, *trans*-, isomers of conjugated linoleic acid (A) 1000 to 900 nm of the =C-H vibration band and, (B) 3050 to 2950 nm of the C-H stretching bands.

### 6.3. Thin Layer Chromatography

Thin layer chromatography (TLC) has proven very useful in the separation of a vast array of chemicals both synthetic and naturally occurring. When it comes to separation of closely related diene isomers, it is a difficult task with unmodified adsorbents such as silica gel. Silver nitrate-modified thin layer chromatography ( $\text{Ag}^+$ -TLC) has been used to separate CLA isomers. Some *cis*-, *trans*-isomers of CLA have been separated as their methyl esters using hexane/diethyl ether, benzene, or toluene (102). The initial solvent mixture caused the *cis*-, *trans*-isomers to migrate faster than the *cis*-monoenes. Either of the later solvents resulted in the *cis*-, *trans*-isomer migrating between the *cis*- and *trans*-monoenes. Ackman (134) reported that the *cis*-, *trans*-isomer had a relative retention ( $R_f$ ) of 0.61, whereas the *trans*-, *trans*-isomer had a  $R_f$  of 0.65. Although the difference in the  $R_f$  values is not great, it is possible to separate the isomers to either collect larger quantities or investigate possible metabolites.

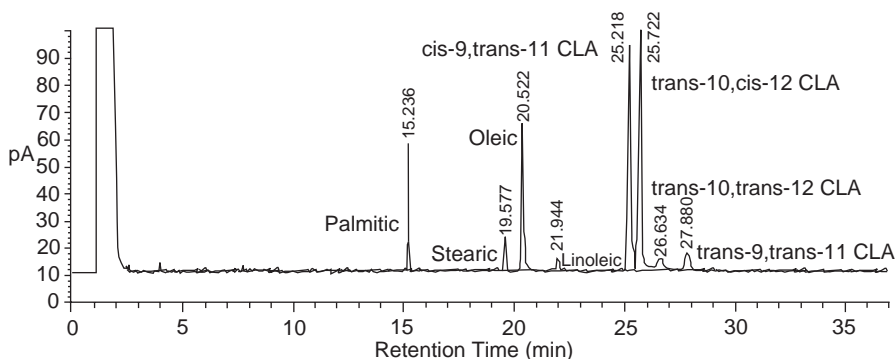
### 6.4. Gas Chromatography

Gas chromatography (GC), initially using packed columns and later using capillary columns, provides another method for the analysis of fatty acids and esters or other derivatives. As binding of the fatty acids to the column was problematic, fatty acid methyl esters (FAME) were traditionally used. The method of forming the methyl esters can be divided into three procedures: acid-catalyzed, base-catalyzed, and diazomethane alkylation (135). No one method is suitable for all situations and all suffer some deficiency.

Acid-catalyzed procedures typically are either boron trifluoride/methanol ( $\text{BF}_3/\text{CH}_3\text{OH}$ ) or hydrochloric acid/methanol ( $\text{HCl}/\text{CH}_3\text{OH}$ ). Many analysts use sulfuric acid instead of hydrochloric acid. Acid-catalyzed procedures are used for free fatty acids, phospholipids, or triacylglycerols, often at elevated temperatures. Although the procedures are relatively efficient at production of methyl esters, there will be some isomerization of some *cis*-, *trans*-isomers to the *trans-trans*-isomers (136). This isomerization can be reduced by using lower temperatures, for instance  $60^\circ\text{C}$ , for  $\text{HCl}/\text{CH}_3\text{OH}$  or room temperature for  $\text{BF}_3/\text{CH}_3\text{OH}$  (137). However, under these milder conditions, some phospholipids may not be esterified (137). In addition, methoxy adducts may be formed and hydroxy fatty acids may produce artifacts.

A base, such as sodium methoxide ( $\text{NaOCH}_3$ ), is useful in esterifying lipids as found in acylglycerols, sterol esters, and phospholipids (138). Free fatty acid and *N*-acyl lipids in sphingolipids will not be methylated. The  $\text{NaOCH}_3$  method does not apparently change the *cis*-, *trans*-isomer composition or form methoxy artifacts. However, some artifacts that could interfere with shorter chain fatty acids were observed.

Diazomethane is effective in esterifying free acids to their corresponding methyl esters under mild conditions and is fast. It will not produce methyl esters from acylglycerols, cholesterol esters, or phospholipids. Many researchers are concerned about the potential hazard of the diazomethane, its precursors, and the actual



**Figure 3.** Gas chromatogram of free fatty acids on a 30-m DBFFAP column (0.32 mm id, 0.25- $\mu$ m film thickness) using a temperature gradient of 50°C for 1 min; 50°C–150°C @ 25°C/min, 150°C–220°C @ 3°C/min; hold at 220°C for 3 min; He carrier gas @ 2 mL/min.

preparation of the reagent. Trimethylsilyl diazomethane is commercially available and can be used as a source of diazomethane. However, some artifacts (trimethylsilyl CLA esters) and other trimethylsilyl impurities may interfere with analysis (139). As there are limitations to each method of making methyl esters, the analyst will need to select reagents and conditions that are most appropriate for the substrate. For intact acylglycerols, sterol esters, and phospholipids, the NaOCH<sub>3</sub> method would be appropriate. If only free fatty acids are present, the diazomethane method would be appropriate, particularly if double bond isomerization is of concern.

The earliest of GC analyses were performed on columns packed with a solid support coated with a nonvolatile liquid phase. Packed columns are not frequently used today as they have been replaced by capillary columns where the liquid phase is immobilized on the internal surface of the capillary. As there are numerous liquid phases available, it is now possible to obtain commercial columns that will separate not only the methyl esters but also the underivatized fatty acids. This advancement obviates the need for derivatization and the associated problems. A typical chromatogram of free fatty acids is displayed in Figure 3. Individual isomers of CLA are now available to aid in the identification of isomers in the chromatogram. Gas chromatography can provide quantitative information on the degree of conjugation, positional, and geometric isomer distribution when suitable standards are available.

## 6.5. High-Performance Liquid Chromatography

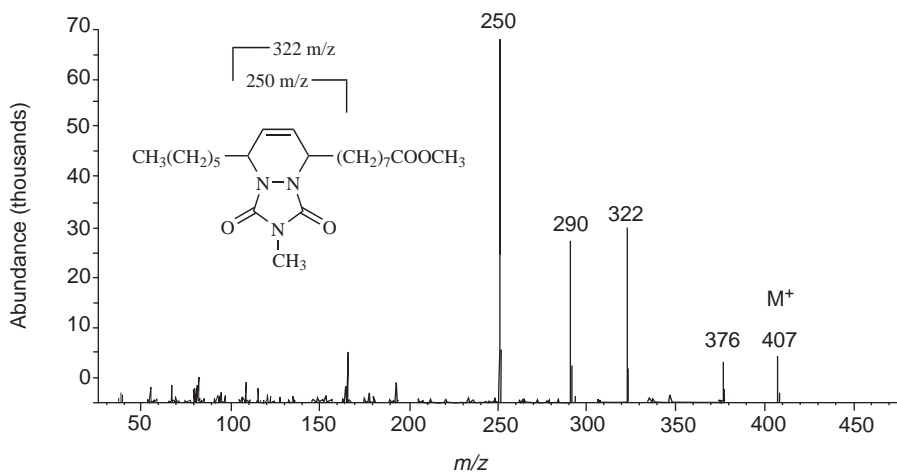
High-performance liquid chromatography (or less common, high-pressure liquid chromatography, HPLC) is a preferred method of analysis for many compounds because it does not require the high temperatures used in gas chromatography. Separations in HPLC can be based on either a size exclusion or on an adsorption principle. The size exclusion mode is useful for separating fatty acids from

acylglycerols and has been applied to CLA analysis. Reaney et al. (120) have used a Waters GPC-StyragelHR<sup>TM</sup> column for the purpose of observing possible polymerization of CLA or the presence of acylglycerols. In three commercial samples of CLA, the 18-carbon fatty acids could be separated from the MAGs. Both UV detection at 233 nm and evaporative light scattering detection (ELSD) were used. There was no reported separation of individual fatty acids or CLA isomers. Use of a reverse phase column (Waters C-8 Symmetry<sup>TM</sup> column) for separation of commercial CLA resulted in a major peak being observed that would correspond to the free fatty acids (120). As with the size exclusion column, there was no separation of either isomeric conjugated fatty acids or acids of different chain length. A preferred technique is silver ion-modified high-performance liquid chromatography (Ag<sup>+</sup>-HPLC). Using this modification, several groups have reported successful separation of both positional and geometric isomers of CLA (14, 140).

## 6.6. Mass Spectrometry

Mass spectrometry (MS) is a method to determine the mass of either an intact ionized molecule or an ionized fragment. When MS is combined with the separation power of gas or liquid chromatography, much valuable information can be obtained for structural determination. Although GC-MS or HPLC-MS may seem to be an ideal tool for CLA analysis, it suffers a major problem as originally practiced. In order to obtain a mass spectrum, it is necessary to produce an ionized species. With CLA, as well as other molecules, some ionization conditions cause the double bonds to migrate to new positions. If the double bonds migrate, it is only possible to obtain information on chain length and number of double bonds. To allow for less harsh ionization conditions, it is possible to make derivatives at either the carboxylic acid (remote site) or at the site of the conjugated double bonds (on site). The derivatives have been selected to allow for easier ionization. In the case of the on-site derivatives, the position of the diene is fixed at one location. At the carboxylic acid site pyrrolidide, picolinyl ester and 4,4-dimethyloxazoline (DMOX) derivatives are used. The fragmentation of the remote site derivative basically produces ions that are 14 mass units lighter than the previous ion for each methylene (CH<sub>2</sub>) group. When a double bond is encountered, the decrease observed is 12 mass units. A review on this subject has been compiled (141).

Two different approaches have been reported for on-site or double bond site derivatization of fatty acids or other conjugated dienes. One method involves the complete hydroxylation of the double bonds with osmium tetroxide (health caution) followed by trimethylsilylation (142). The second method is based on the well known Diels-Alder cyclo addition. MTAD (4-methyl-1,2,4-triazoline-3,5-dione) has been shown to be a useful reagent for adduct formulation with FAME conjugated dienes. Fragmentation patterns of adducts are usually dominated by cleavage fragments that include the ring formed during the cyclo addition plus either of the residual carbon chains. In Figure 4 of the MTAD derivative of methyl *cis*-, *trans*-9,11-octadecadienoate, these fragments occur at 322 m/z and 250 m/z, indicating



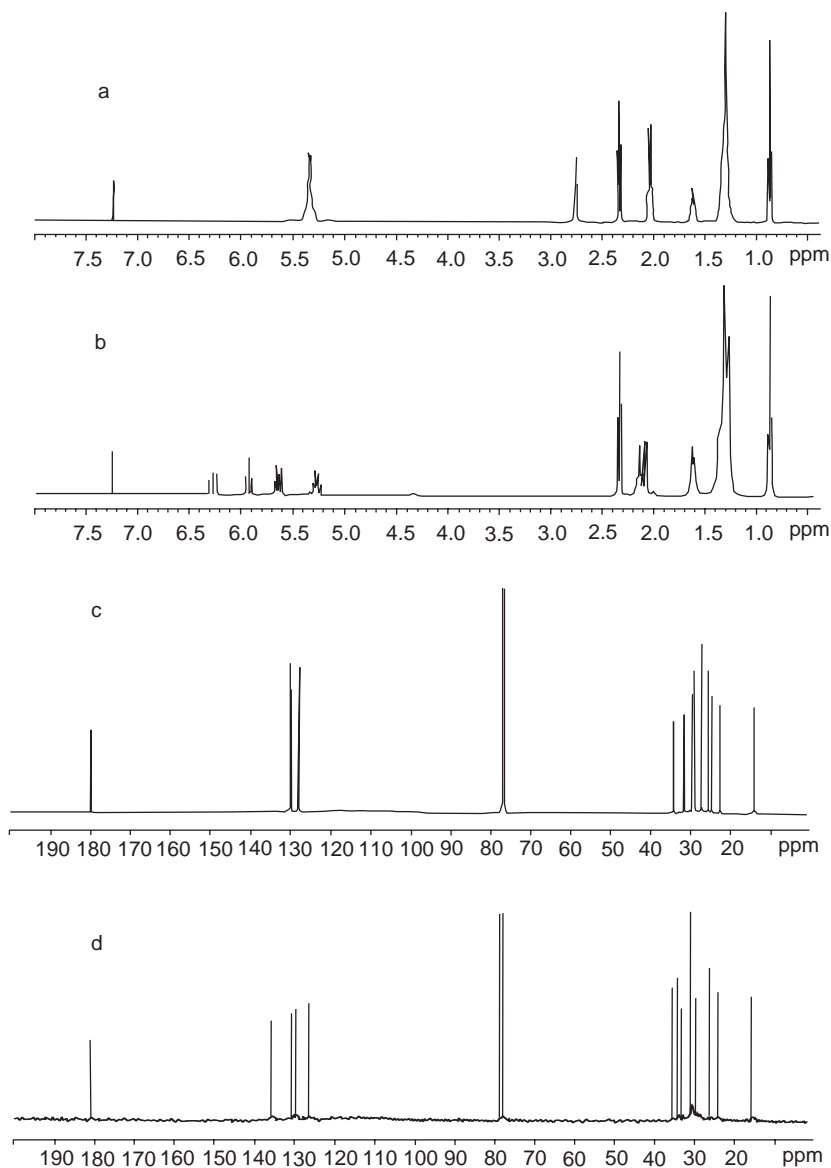
**Figure 4.** The mass spectrum of the adduct of methyl *cis*-, *trans*-9,11-octadecadienoate and 4-methyl-1,2,4-triazoline-3,5-dione (MTAD). Reproduced by permission of AOCS Press (143).

that the starting FAME was a 9,11 diene (143). Methyl *cis*-, *trans*-9,11-octadecadienoate and methyl *trans*-, *trans*-9,11-octadecadienoate readily formed adducts with similar mass spectra but with different retention times when analyzed by GC-MS (143). Methyl *cis*-, *cis*-9,11-octadecadienoate reacted more slowly to produce two products with similar fragmentation patterns but with different retention times. Unfortunately, it was demonstrated that the *cis*-, *cis*- and *cis*-, *trans*-isomers produced the same adduct, limiting usefulness when studying geometric isomers of CLA products.

## 6.7. Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) is a powerful technique for CLA analysis (144, 145). NMR spectroscopy can provide information on the environments of both the proton (<sup>1</sup>H) and the carbon-13 (<sup>13</sup>C) atoms in CLA and also provide correlation between the two atoms. In one of the most basic analyses, it is possible to observe the disappearance of the signals as a result of the isolated olefinic protons (5.35 ppm, Figure 5A) and the isolated methylene group (2.75 ppm) linoleic acid as well as the appearance of new signals associated with the conjugated diene (5.2–6.5 ppm, Figure 5B). Reaney et al. (120) reported that a convenient measurement of CLA content can be obtained by comparing the integrated areas of the olefinic protons at 6.0 ppm and 6.3 ppm with the area of the methylene protons adjacent to the carbonyl (2.3 ppm). The NMR <sup>13</sup>C spectra for both linoleic and *cis*-, *trans*-9,11-octadecadienoic acids are presented in Figure 5C and 5D, respectively. The complete assignment for this and other isomers of CLA has been reported (146, 147). A notable feature for CLA is the appearance of four well separated signals between 129–135 ppm (Figure 5D) compared with the two pairs of





**Figure 5.** (A) The  $^1\text{H}$  spectrum of linoleic acid; (B) the  $^1\text{H}$  spectrum of *cis*-, *trans*-9,11-octadecadienoic acid; (C) the  $^{13}\text{C}$  spectrum of linoleic acid; and, (D) the  $^{13}\text{C}$  spectrum of *cis*-, *trans*-9,11-octadecadienoic acid.

narrowly separated doublets observed in linoleic acid spectrum (Figure 5C). Detailed  $^1\text{H}$  and  $^{13}\text{C}$  and NMR spectroscopy have the potential to provide information on both positional and geometric isomers of CLA and can provide semiquantitative information on CLA concentration.

## 7. CONCLUSIONS

The research on CLA in growing animals is consistently showing effect on modulation of body mass and fat, however, the effect in humans is not consistent. More research is needed to delineate the effect of CLA and isomers on body composition in humans. Major research emphasis, at present, is focused on the effects of CLA and its isomers on body composition and carcinogenesis. Other areas that are attracting attention include the effects of CLA and isomers on cardiovascular, metabolic, and immune functions and the strategies to increase the content of CLA isomers in meat and dairy products.

At the same time, research is still needed to improve the commercial production of CLA. Although the content of desirable isomers in commercial CLA products has improved, there is still a demand for highly enriched or pure 9,11-*cis*-, *trans*-octadecadienoic acid products. The kinetic control of CLA synthesis will allow the development of CLA products that are virtually free of isomers other than 9,11-*ct* and 10,12-*tc*. Kinetic control of reactions requires exceedingly rapid analytical techniques that can be applied inexpensively and online or virtually online.

## REFERENCES

1. W. J. Dann and T. Moore, *Biochem. J.*, **27**, 1166–1169 (1933).
2. R. Kapoor, N. D. Westcott, M. J. T. Reaney, and S. Jones, *Inform*, **14**, 482–484 (2003).
3. M. J. T. Reaney, N. D. Westcott, R. Kapoor, and S. Jones, *Inform*, **13**, 802–807 (2002).
4. W. J. Dann, T. Moore, R. L. Booth, J. Golding, and S. K. Kon, *Biochem. J.*, **29**, 138–146 (1935).
5. R. Reiser, *Arch Biochem. Biophys.*, **32**, 113–120 (1951).
6. P. W. Parodi, *J. Dairy Sci.*, **60**, 1550–1553 (1977).
7. E. S. Miller and G. O. Burr, *Proc. Soc. Exp. Biol. Med.*, **36**, 726–729 (1937).
8. E. Aaes-Jorgensen, *J. Nutr.*, **66**, 465–483 (1958).
9. R. Reiser, *Proc. Soc. Exp. Biol. Med.*, **74**, 666–669 (1950).
10. R. H. Barnes, E. S. Miller, and G. O. Burr, *Proc. Soc. Exp. Biol. Med.*, **45**, 233–240 (1941).
11. M. A. Belury and A. Kempa-Steczko, *Lipids*, **32**, 199–204 (1997).
12. C. Ip, C. Jiang, H. J. Thompson, and J. A. Scimeca, *Carcinogenesis*, **18**, 755–759 (1997).
13. J. L. Sebedio, E. Angioni, J. M. Chardigny, S. Gregoire, P. Juaneda, and O. Berdeaux, *Lipids*, **36**, 575–582 (2001).
14. J. K. Kramer, N. Sehat, M. E. Dugan, M. M. Mossoba, M. P. Yurawecz et al., *Lipids*, **33**, 549–558 (1998).
15. J. L. Sebedio, P. Juaneda, G. Dobson, I. Ramilison, J. C. Martin et al., *Biochim. Biophys. Acta*, **1345**, 5–10 (1997).
16. J. L. Sebedio, S. Gnaedig, and J. M. Chardigny, *Curr. Opin. Clin. Nutr. Metab. Care.*, **2**, 499–506 (1999).

17. J. L. Sebedio, P. Juaneda, S. Gregoire, J. M. Chardigny, J. C. Martin, and C. Ginies, *Lipids*, **34**, 1319–1325 (1999).
18. L. Bretilion, J. M. Chardigny, S. Gregoire, O. Berdeaux, and J. L. Sebedio, *Lipids*, **34**, 965–969 (1999).
19. J. L. Sebedio, E. Angioni, J. M. Chardigny, S. Gregoire, P. Juaneda, and O. Berdeaux, *Lipids*, **36**, 575–582 (2001).
20. P. Urquhart, S. M. Parkin, J. S. Rogers, J. A. Bosley, and A. Nicolaou, *Biochim. Biophys. Acta*, **1580**, 150–160 (2002).
21. R. T. Holman, *Proc. Soc. Exp. Biol. Med.*, **76**, 100–102 (1951).
22. A. Akahoshi, Y. Goto, K. Murao, T. Miyazaki, M. Yamasaki et al., *Biosci. Biotechnol. Biochem.*, **66**, 916–920 (2002).
23. E. Ostrowska, D. Suster, M. Muralitharan, R. F. Cross, B. J. Leury et al., *Br. J. Nutr.*, **89**, 219–229 (2003).
24. M. Yamasaki, A. Ikeda, M. Oji, Y. Tanaka, A. Hirao et al., *Nutrition*, **19**, 30–35 (2003).
25. H. Blankson, J. A. Stakkestad, H. Fagertun, E. Thom, J. Wadstein, and O. Gudmundsen, *J. Nutr.*, **130**, 2943–2948 (2000).
26. U. Riserus, L. Berglund, and B. Vessby, *Int. J. Obes. Relat. Metab. Disord.*, **25**, 1129–1135 (2001).
27. E. Thom, J. Wadstein, and O. Gudmundsen, *J. Int. Med. Res.*, **29**, 392–396 (2001).
28. M. A. Belury, A. Mahon, and S. Banni, *J. Nutr.*, **133**, 257S–260S (2003).
29. K. L. Zambell, N. L. Keim, M. D. Van Loan, B. Gale, P. Benito et al., *Lipids*, **35**, 777–782 (2000).
30. M. M. Kamphuis, M. P. Lejeune, W. H. Saris, and M. S. Westerterp-Plantenga, *Int. J. Obes. Relat. Metab. Disord.*, **27**, 840–847 (2003).
31. Y. Park, K. J. Albright, W. Liu, J. M. Storkson, M. E. Cook, and M. W. Pariza, *Lipids*, **32**, 853–858 (1997).
32. J. M. Brown and M. K. McIntosh, *J. Nutr.*, **133**, 3041–3046 (2003).
33. E. A. De Deckere, J. M. van Amelsvoort, G. P. McNeill, and P. Jones, *Br. J. Nutr.*, **82**, 309–317 (1999).
34. Y. Park, J. M. Storkson, K. J. Albright, W. Liu, and M. W. Pariza, *Lipids*, **34**, 235–241 (1999).
35. K. Kang and M. W. Pariza, *Biochem. Biophys. Res. Commun.*, **287**, 377–382 (2001).
36. K. Nagao, Y. M. Wang, N. Inoue, S. Y. Han, Y. Buang et al., *Nutrition*, **19**, 652–656 (2003).
37. K. Kang, W. Liu, K. J. Albright, Y. Park, and M. W. Pariza, *Biochem. Biophys. Res. Commun.*, **303**, 795–799 (2003).
38. J. M. Brown, M. S. Boysen, S. S. Jensen, R. F. Morrison, J. Storkson et al., *J. Lipid. Res.*, **44**, 1287–1300 (2003).
39. N. Tsuboyama-Kasaoka, M. Takahashi, K. Tanemura, H. J. Kim, T. Tange et al., *Diabetes*, **49**, 1534–1542 (2000).
40. N. Tsuboyama-Kasaoka, H. Miyazaki, S. Kasaoka, and O. Ezaki, *J. Nutr.*, **133**, 1793–1799 (2003).
41. U. Riserus, P. Arner, K. Brismar, and B. Vessby, *Diabetes Care*, **25**, 1516–1521 (2002).
42. M. W. Pariza and W. A. Hargraves, *Carcinogenesis*, **6**, 591–593 (1985).

43. Y. L. Ha, N. K. Grimm, and M. W. Pariza, *Carcinogenesis*, **8**, 1881–1887 (1987).
44. Y. L. Ha, J. Storkson, and M. W. Pariza, *Cancer Res.*, **50**, 1097–1101 (1990).
45. T. D. Shultz, B. P. Chew, and W. R. Seaman, *Anticancer Res.*, **12**, 2143–2145 (1992).
46. C. Ip, M. Singh, H. J. Thompson, and J. A. Scimeca, *Cancer Res.*, **54**, 1212–1215 (1994).
47. F. Lavillonniere, V. Chajes, J. C. Martin, J. L. Sebedio, C. Lhuillery, and P. Bougnoux, *Nutr. Cancer*, **45**, 190–194 (2003).
48. A. Cesano, S. Visonneau, J. A. Scimeca, D. Kritchevsky, and D. Santoli, *Anticancer Res.*, **18**, 1429–1434 (1998).
49. J. L. Cheng, M. Futakuchi, K. Ogawa, T. Iwata, M. Kasai et al., *Cancer Lett.*, **196**, 161–168 (2003).
50. H. S. Park, J. H. Ryu, Y. L. Ha, and J. H. Park, *Br. J. Nutr.*, **86**, 549–555 (2001).
51. V. R. Durgam and G. Fernandes, *Cancer Lett.*, **116**, 121–130 (1997).
52. C. J. Kavanaugh, K. L. Liu, and M. A. Belury, *Nutr. Cancer*, **33**, 132–138 (1999).
53. H. J. Cho, W. K. Kim, E. J. Kim, K. C. Jung, S. Park et al., *Amer. J. Physiol. Gastrointest. Liver Physiol.*, **284**, G996–1005 (2003).
54. A. Miller, C. Stanton, and R. Devery, *Anticancer Res.*, **22**, 3879–3887 (2002).
55. P. A. Masso-Welch, D. Zangani, C. Ip, M. M. Vaughan, S. F. Shoemaker et al., *J. Nutr.*, **134**, 299–307 (2004).
56. C. Ip, Y. Dong, H. J. Thompson, D. E. Bauman, and M. M. Ip, *Nutr. Cancer*, **39**, 233–238 (2001).
57. C. Ip, J. A. Scimeca, and H. Thompson, *Nutr. Cancer*, **24**, 241–247 (1995).
58. A. Aro, S. Mannisto, I. Salminen, M. L. Ovaskainen, V. Kataja, and M. Uusitupa, *Nutr. Cancer*, **38**, 151–157 (2000).
59. K. L. Houseknecht, J. P. Vanden Heuvel, S. Y. Moya-Camarena, C. P. Portocarrero, L. W. Peck et al., *Biochem. Biophys. Res. Commun.*, **244**, 678–682 (1998).
60. J. W. Ryder, C. P. Portocarrero, X. M. Song, L. Cui, M. Yu et al., *Diabetes*, **50**, 1149–1157 (2001).
61. L. Clement, H. Poirier, I. Niot, V. Bocher, M. Guerre-Millo et al., *J. Lipid Res.*, **43**, 1400–1409 (2002).
62. K. M. Hargrave, M. J. Azain, S. D. Kachman, and J. L. Miner, *Obes. Res.*, **11**, 1104–1115 (2003).
63. K. Nagao, N. Inoue, Y. M. Wang, and T. Yanagita, *Biochem. Biophys. Res. Commun.*, **310**, 562–566 (2003).
64. E. Ostrowska, R. F. Cross, M. Muralitharan, D. E. Bauman, and F. R. Dunshea, *Br. J. Nutr.*, **88**, 625–634 (2002).
65. E. J. Noone, H. M. Roche, A. P. Nugent, and M. J. Gibney, *Br. J. Nutr.*, **88**, 243–251 (2002).
66. K. N. Lee, D. Kritchevsky, and M. W. Pariza, *Atherosclerosis*, **108**, 19–25 (1994).
67. D. Kritchevsky, S. A. Tepper, S. Wright, P. Tso, and S. K. Czarnecki, *J. Amer. Coll. Nutr.*, **19**, 472S–477S (2000).
68. R. J. Nicolosi, E. J. Rogers, D. Kritchevsky, J. A. Scimeca, and P. J. Huth, *Artery*, **22**, 266–277 (1997).
69. S. Toomey, H. Roche, D. Fitzgerald, and O. Belton, *Biochem. Soc. Trans.*, **31**, 1075–1079 (2003).

70. V. C. Gavino, G. Gavino, M. J. Leblanc, and B. Tuchweber, *J. Nutr.*, **130**, 27–29 (2000).
71. S. Yu-Poth, D. Yin, G. Zhao, P. M. Kris-Etherton, and T. D. Etherton, *J. Nutr.*, **134**, 68–71 (2004).
72. M. W. Pariza and K. N. Lee (assigned to Wisconsin Alumni Research Foundation, (Madison, Wisconsin), U.S. Patent 5,837,733, 1998).
73. A. Truitt, G. McNeill, and J. Y. Vanderhoek, *Biochim. Biophys. Acta*, **1438**, 239–246 (1999).
74. K. Nagao, N. Inoue, Y. M. Wang, J. Hirata, Y. Shimada et al., *Biochem. Biophys. Res. Commun.*, **306**, 134–138 (2003).
75. P. Benito, G. J. Nelson, D. S. Kelley, G. Bartolini, P. C. Schmidt, and V. Simon, *Lipids*, **36**, 229–236 (2001).
76. M. E. Cook, B. Ellen, J. L. Stahl, F. M. Graziano, M. Frank, and M. W. Pariza (assigned to Wisconsin Alumni Research Foundation, Madison, Wisconsin), U.S. Patent 5,585,400, 1996.
77. M. E. Cook, M. W. Pariza, X. Yang, and D. De Voney (assigned to Wisconsin Alumni Research Foundation, (Madison, Wisconsin), U.S. Patent 5,674,901, 1997).
78. M. G. Hayek, S. N. Han, D. Wu, B. A. Watkins, M. Meydani et al., *J. Nutr.*, **129**, 32–38 (1999).
79. L. D. Whigham, E. B. Cook, J. L. Stahl, R. Saban, D. E. Bjorling et al., *Amer. J. Physiol. Regul. Integr. Comp. Physiol.*, **280**, R908–R912 (2001).
80. M. W. Wong, B. P. Chew, T. S. Wong, H. L. Hosick, T. D. Boylston, and T. D. Shultz, *Anticancer Res.*, **17**, 987–993 (1997).
81. M. E. Cook, S. Kim, M. W. Pariza, and D. De Voney (assigned to Wisconsin Alumni Research Foundation, Madison, Wisconsin), U.S. Patent 5,914,346, 1999.
82. Y. Yu, P. H. Correll, and J. P. Vanden Heuvel, *Biochim. Biophys. Acta*, **1581**, 89–99 (2002).
83. J. Bassaganya-Riera, R. Hontecillas, D. R. Zimmerman, and M. J. Wannemuehler, *Vaccine*, **20**, 1435–1444 (2002).
84. J. Bassaganya-Riera, R. M. Pogramichniy, S. C. Jobgen, P. G. Halbur, K. J. Yoon et al., *J. Nutr.*, **133**, 3204–3214 (2003).
85. V. Bontempo, D. Sciannimanico, G. Pastorelli, R. Rossi, F. Rosi, and C. Corino, *J. Nutr.*, **134**, 817–824 (2004).
86. D. S. Kelley, P. C. Taylor, I. L. Rudolph, P. Benito, G. J. Nelson et al., *Lipids*, **35**, 1065–1071 (2000).
87. D. S. Kelley, V. A. Simon, P. C. Taylor, I. L. Rudolph, P. Benito et al., *Lipids*, **36**, 669–674 (2001).
88. W. L. Cheng, C. K. Lii, H. W. Chen, T. H. Lin, and K. L. Liu, *J. Agric. Food Chem.*, **52**, 71–78 (2004).
89. M. E. Cook, M. W. Pariza, K. N. Lee, and B. C. Wentworth (assigned to Wisconsin Alumni Research Foundation, Madison, Wisconsin), U.S. Patent 5,504,114, 1996.
90. R. H. Stock and J. D. Compton (assigned to LifeRight Foods LLC/N, Princeton, New Jersey), U.S. Patent 6,316,041, 2001.
91. M. E. Cook, A. Rahim, and M. W. Pariza (assigned to Wisconsin Alumni Research Foundation, Madison, Wisconsin), U.S. Patent 6,113,973, 2000.

92. M. L. Kelly, J. R. Berry, D. A. Dwyer, J. M. Griinari, P. Y. Chouinard et al., *J. Nutr.*, **128**, 881–885 (1998).
93. K. Nurmela and M. Griinari (assigned to Valio Oy, Helsinki, Finland), U.S. Patent 6,635,271, 2003.
94. A. T. Ward, K. M. Wittenberg, H. M. Froebe, R. Przybylski, and L. Malcolmsen, *J. Dairy Sci.*, **86**, 1742–1750 (2003).
95. T. R. Dhiman, G. R. Anand, L. D. Satter, and M. W. Pariza, *J. Dairy Sci.*, **82**, 2146–2156 (1999).
96. T. R. Dhiman, E. D. Helmink, D. J. McMahon, R. L. Fife, and M. W. Pariza, *J. Dairy Sci.*, **82**, 412–419 (1999).
97. T. R. Dhiman, L. D. Satter, M. W. Pariza, M. P. Galli, K. Albright, and M. X. Tolosa, *J. Dairy Sci.*, **83**, 1016–1027 (2000).
98. A. T. Ward, K. M. Wittenberg, and R. Przybylski, *J. Dairy Sci.*, **85**, 1191–1196 (2002).
99. L. A. Whitlock, D. J. Schingoethe, A. R. Hippen, K. F. Kalscheur, R. J. Baer et al., *J. Dairy Sci.*, **85**, 234–243 (2002).
100. J. J. Loor and J. H. Herbein, *J. Nutr.*, **128**, 2411–2419 (1998).
101. S. T. Franklin, K. R. Martin, R. J. Baer, D. J. Schingoethe, and A. R. Hippen, *J. Nutr.*, **129**, 2048–2054 (1999).
102. W. W. Christie, G. Dobson, and F. D. Gunstone, *Lipids*, **32**, 1231 (1997).
103. T. L. Mounts and H. L. Dutton, *Lipids*, **5**, 997–1005 (1970).
104. F. B. Padley, F. D. Gunstone, and F. B. Harwood, in F. D. Gunstone, H. Hashimoto, and F. B. Padley, eds., *The Lipid Handbook*, 2nd ed., Chapman and Hall, London, 1994, pp. 47–224.
105. R. W. Johnson and R. W. Daniels, in K. Othmer, ed., *Encyclopedia of Chemical Technology*, 4th ed, John Wiley and Sons, New York, 1996, pp. 168–177.
106. “Specifications and characteristics of Emery oleochemicals,” Technical Bulletin A-473, Henkel Corporation, Emery Group, Cincinnati, Ohio, 1991.
107. E. Fritz and R. W. Johnson, *Fatty Acids in Industry*, Marcel Dekker, New York, 1988.
108. F. D. Gunstone, M. Kates, and J. L. Harwood, in F. D. Gunstone, J. L. Harwood, and F. B. Padley, *The Lipid Handbook*, 2nd ed., Chapman and Hall, London, 1994, pp. 47–224.
109. C. Keulemans, Eur. Patent, 1990.
110. M. T. Cleary, S. Kulprathpanja, and R. W. Neuzil (assigned to UOP Inc., Des Plaines, Illinois), U.S. Patent 4,524,029, 1985.
111. M. T. Cleary and S. Kulprathpanja (assigned to UOP Inc., Des Plaines, Illinois), U.S. Patent 4,353,838, 1982.
112. H. G. Kirshner (assigned to Colgate-Palmolive Peet Company, Jersey City, New Jersey), U.S. Patent 2,389,260, 1945.
113. G. O. Burr, U.S. Patent 2,242,230, 1941.
114. J. D. Cawley, U.S. Patent 2,343,644, 1944.
115. A. Struve (assigned to Henkel Kommanditgesellschaft Auf Aktien, Dusseldorf-Holthausen, Denmark), U.S. Patent 4,381,264 1983.
116. T. F. Bradley, U.S. Patent 2,350,583, 1944.
117. K. E. Krajca (assigned to Sylvachem Corporation, Jacksonville, Florida), U.S. Patent 4,164,505, 1979.

118. T. F. Bradley and D. Richardson, *Indust. Engin. Chem.*, **34**, 237–242 (1942).
119. P. L. Nichols, R. W. Riemenschneider, and S. F. Herb, *J. Amer. Chem. Soc.*, **27**, 329–336 (1950).
120. M. J. T. Reaney, M. M. Liu, and N. D. Westcott, in M. P. Yurawecz, M. M. Mossoba, J. K. Kramer, M. W. Pariza, and G. J. Nelson, eds., *Advances in Conjugated Linoleic Acid Research*, AOCS Press, Champaign, Illinois, 1999, pp. 39–54.
121. A. Saebø, in J. L. Sebedio, W. W. Christie, and R. Adolf, eds., *Advances in Conjugated Linoleic Acid Research*. [5], AOCS Press, Champaign, Illinois, 2003, pp. 71–81.
122. T. A. Richardson, *Industrial Plastics: Theory and Application*, South West Publishing Co., Cincinnati, Ohio, 1993.
123. M. W. Pariza and Y. M. Yang (assigned to Wisconsin Alumni Research Foundation, Madison Madison, Wisconsin), U.S. Patent 5,856,149, 1999.
124. D. H. Solomon, *The Chemistry of Natural Film Formers*, 2nd ed., Robert E. Krieger Publishing Co., Huntington, New York, 1977, pp. 33–74.
125. D. A. Zabolotskij, P. M. Demin, and G. I. Mylagkoya, Russian Patent 2021,252, 1994.
126. M. S. F. Lie Ken Jie, M. K. Pasha, and M. S. Alam, *Lipids*, **32**, 1041–1044 (1977).
127. N. Sehat, M. P. Yurawecz, J. A. Roach, M. M. Mossoba, J. K. Kramer, and Y. Ku, *Lipids*, **33**, 217–221 (1998).
128. N. Sehat, R. Rickert, M. M. Mossoba, J. K. Kramer, M. P. Yurawecz et al., *Lipids*, **34**, 407–413 (1999).
129. R. L. Booth, S. K. Kon, W. J. Dann, and T. Moore, *Biochem. J.*, **29**, 133–137 (1935).
130. M. Czauderna and J. Kowalczyk, *J. Chromatogr. B. Biomed. Sci. Appl.*, **760**, 165–178 (2001).
131. J. E. Jackson, R. F. Paschke, W. Tolberg, H. M. Boyd, and D. H. Wheeler, *J. Amer. Oil Chem. Soc.*, **29**, 229–234 (1952).
132. M. M. Mossoba, M. P. Yurawecz, J. K. Kramer, K. Eulitz, J. Fritsche et al., in M. P. Yurawecz, M. M. Mossoba, J. K. Kramer, M. Pariza, and G. J. Nelson, eds., *Advances in Conjugated Linoleic Acid Research*, AOCS Press, Champaign, Illinois, 1999, pp. 141–151.
133. J. Fritsche, M. M. Mossoba, M. P. Yurawecz, J. A. G. Roach, N. Sehat, and H. Steinhart, *Z. Lebensm. Unters. Forsch. A* **205**, 415–418 (1997).
134. R. G. Ackman, C. A. Eaton, J. C. Sipos, and N. F. Crew, *J. Can. Inst. Food Sci. Technol.*, **14**, 103–107 (1981).
135. M. P. Yurawecz, J. K. Kramer, and Y. Ku, in M. P. Yurawecz, M. M. Mossoba, J. K. Kramer, M. Pariza, and G. J. Nelson, eds., *Advances in Conjugated Linoleic Acid Research*, AOCS Press, Champaign, Illinois, 1999, pp. 64–82.
136. E. Ostrowska, F. R. Dunshea, M. Muralitharan, and R. F. Cross, *Lipids*, **35**, 1147–1153 (2000).
137. S. A. Werner, L. O. Luedecke, and T. D. Shultz, *J. Agric. Food Chem.*, **40**, 1817–1821 (1992).
138. J. K. Kramer, V. Fellner, M. E. Dugan, F. D. Sauer, M. M. Mossoba, and M. P. Yurawecz, *Lipids*, **32**, 1219–1228 (1997).
139. Y. Park, K. J. Albright, Z. Y. Cai, and M. W. Pariza, *J. Agric. Food Chem.*, **49**, 1158–1164 (2001).

140. K. Eulitz, M. P. Yurawecz, N. Sehat, J. Fritsche, J. A. Roach et al., *Lipids*, **34**, 873–877 (1999).
141. V. Spitzer, in M. P. Yurawecz, M. M. Mossoba, M. W. Pariza, and G. J. Nelson, eds., *Advances in Conjugated Linoleic Acid Research*, AOCS Press, Champaign, Illinois, 1999, pp. 110–125.
142. G. Janssen and G. Paramentier, *Biomed. Mass Spectrom.*, **5**, 439–443 (1978).
143. M. J. T. Reaney, L. Y. Dong, and W. G. Taylor, *J. Amer. Oil Chem. Soc.*, **78**, 1083–1086 (2001).
144. M. S. F. Lie Ken Jie, M. K. Pasha, and M. S. Alam, in M. P. Yurawecz, M. M. Mossoba, J. K. Kramer, M. W. Pariza, and G. J. Nelson, eds., *Advances in Conjugated Linoleic Acid Research*, AOCS Press, Champaign, Illinois, 1999, pp. 152–163.
145. A. L. Davis, G. P. McNeill, and D. C. Caswell, in M. P. Yurawecz, M. M. Mossoba, J. K. Kramer, M. W. Pariza, and G. J. Nelson, eds., *Advances in Conjugated Linoleic Acid Research*, vol. 1, AOCS Press, Champaign, Illinois, 1999, pp. 164–179.
146. A. L. Davis, G. P. McNeill, and D. C. Caswell, *Chem. Phys. Lipids*, **97**, 155–165 (1999).
147. M. S. Jie, M. K. Pasha, and M. S. Alam, *Lipids*, **32**, 1041–1044 (1997).
148. B. A. Brice, S. F. Swain, S. F. Herb, P. L. Nicholas, Jr., and R. W. Reimenschneider, *J. Amer. Oil Chem. Soc.*, **29**, 279–287 (1949).
149. J. Ritz and J. Reese (assigned to Hoechst Aktiengesellschaft, Frankfurt, Germany), U.S. Patent 3,984,444, 1976.



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## *Diacylglycerols*

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### **1. INTRODUCTION**

Diacylglycerols (DAG) are natural components of various edible oils (1, 2). Typically, the level of DAG in edible oils is below 5% of total oil; however, several edible oils have levels above 5% (Table 1). Also, DAG have been used as emulsifiers for use in food systems, particularly baked goods, and are approved as such (3). Human consumption of DAG has been estimated at 3 g per day.

The traditional method of producing DAG is interesterification of triacylglycerols (TAG) with glycerol in the presence of a chemical catalyst at elevated temperatures (4). Preferred catalysts are alkali such as sodium/potassium hydroxide, sodium methoxide, or potassium acetate. Formation of monoacylglycerols (MAG) and DAG can be controlled to a certain degree by the molar ratio of glycerol-to-TAG in the initial reaction mixture. Nonetheless, the resulting DAG is part of a mixture of glyceridic components that present difficulties in obtaining a high-purity DAG fraction using standard industrial separation processes (i.e., chromatography, distillation, or winterization) caused by similar chemical and physical properties of these components.

DAG oil is prepared through the process of enzymatic esterification. Starting with a blend of soybean and canola oils, fatty acids are prepared then mixed

**TABLE 1. Glyceride Content of Various Edible Oils.<sup>1</sup>**

Oil	% of Total Oil			
	TAG	DAG	MAG	Others
Cottonseed	87.0	9.5	0.2	3.3
Palm	93.1	5.8	0.0	1.1
Olive	93.3	5.5	0.2	2.3
Corn	95.8	2.8	0.0	1.4
Safflower	96.0	2.1	0.0	1.9
Lard	97.9	1.3	0.0	0.8
Soybean	97.9	1.0	0.0	1.1
Rapeseed	96.8	0.8	0.1	2.3

<sup>1</sup>Data sourced from (1) and (2).

with glycerol. This mixture undergoes esterification using a 1,3-specific lipase from *Rhizomucor miehei*, which has been immobilized on a resin bed. After several finishing processes, including deodorization, the resulting edible oil is obtained. This oil is bland in flavor and light in color making it suitable for use as a bottled oil or oil ingredient for various food applications. For more information on DAG oil manufacturing, physical properties, and food application uses, a book containing chapters solely devoted to these issues of DAG oil has been published (5).

The nutritional characteristics of DAG oil ( $\geq 80\%$ ) have been compared with dietary TAG of similar fatty acid composition. In particular, the 1,3-DAG isoform appears to have distinct metabolic characteristics that can impact postprandial lipid metabolism and use of macronutrients for energy compared with TAG.

The rationale for anticipating metabolic differences is a result of the difference in metabolism of the digestion product of 1,3-DAG. The body digests DAG oil yielding monoacylglycerols (MAG) and free fatty acids (FFA) just as observed with TAG oil. As a result of the significant content of 1,3-DAG, 1-monoacylglycerol (1-MAG) is a major digestion product of DAG oil that is not observed in any significant amount upon TAG oil digestion. The small intestine typically reassembles digested monoacylglycerols and free fatty acids into TAG beginning with 2-MAG. Previous reports in the literature indicate that providing 1-MAG results in lower amounts of fat-rich particles appearing in serum following consumption. This difference in fat metabolism with DAG oil is apparent in fewer fat-rich particles appearing in the blood after a meal containing DAG oil. As a result, fatty acids not appearing as chylomicron triacylglycerols must be excreted in the feces or used by the gut or liver for energy or triacylglycerol storage.

The following review focuses on experimental data supporting different metabolic characteristics of 1,3-DAG or DAG oil containing 1,3-DAG (Table 2). Relevant areas of observed differences between 1,3-DAG/DAG oil and TAG/TAG oil metabolism include postprandial lipid metabolism and use of macronutrient fuels. Observations from animal and human experimental data are included.

**TABLE 2. General Differences in 1,3-DAG or DAG Oil Metabolism (vs. TAG).**

Topic	Animal	Human
1. Digestion products	1(3)-MAG vs. 2-MAG. <sup>s</sup>	—
2. Portal appearance of free fatty acids	Observed change. <sup>†</sup>	—
3. Lymph/serum appearance of chylomicron triglycerides	Decreased amount and rate. <sup>s</sup>	Decreased amount and rate. <sup>s</sup>
4. Oxidation and synthesis of fat	<i>Chronic</i> —Increased activities of enzymes associated with fat oxidation in liver, <sup>s</sup> increased expression of mRNA for genes associated with fat oxidation in small intestine, <sup>s</sup> decreased respiratory quotient. <sup>s</sup> Decreased activities of enzymes associated with fat synthesis in liver. <sup>s</sup>	<i>Chronic</i> —Decreased respiratory quotient over 36 hours. <sup>s</sup>
5. Energy expenditure	Enhanced energy expenditure. <sup>s</sup>	<i>Acute</i> —Enhanced <sup>s</sup> energy expenditure. <i>Chronic</i> —No change in energy expenditure observed.
6. Appetite suppression	No observations of appetite suppression.	Initial appetite suppression. <sup>s</sup>
7. Body weight and body fat	Decreased body weight and body fat. <sup>s</sup>	Greater losses of body weight and body fat. <sup>s</sup>

<sup>s</sup>Statistically significant experimental observations.

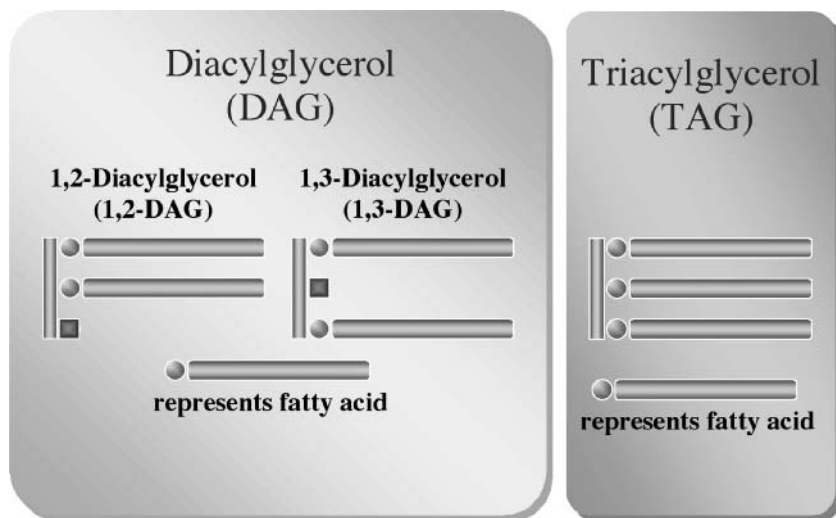
<sup>†</sup>Trend in experimental observations.

Differences caused by 1,3-DAG/DAG oil in the following discussion are made under the assumption of being compared with TAG/TAG oil control of similar fatty acid composition. DAG oil refers to an oil containing 80–90% DAG, 10–20% TAG, with the DAG portion containing 70% 1,3-DAG, 30% 1,2-DAG (Figure 1). TAG oil refers to a conventional edible oil.

## 2. COMPARISON OF DAG OIL VS. TAG OIL

### 2.1. Digestion Products

Digestion of 1,3- and 1,2-DAG (70%:30%, respectively, as diolein) results in the preferential formation of 1(3)-MAG and FFA in rats (6). 1(3)-MAG is 65% of the monoacylglycerol after 60 minutes of interaction with the small intestine. This observation has been demonstrated also in mice using labeled fatty acids incorporated into 1,3-DAG (7). After exposure of labeled 1,3-DAG to the small intestine in mice, the percentage of 1(3)-MAG from total lipid content increased to 14.2% compared with 1.5% ( $p < 0.001$ ), 2-MG decreased to 7.2% compared



% DAG in DAG oil:  $\geq 80$ , % DAG in TAG oil:  $\leq 20$ . Ratio 1,2-DAG : 1,3-DAG = 3:7. Typical fatty acid composition of DAG oil = ~45% linoleic acid, ~35% oleic acid, ~4% linolenic acid, ~4% saturated fat.

**Figure 1.** Glyceride composition, structure, and fatty acid profile of DAG oil. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

with 22.3% ( $p < 0.05$ ), and FFA increased to 59.1% compared with 22.3% ( $p < 0.001$ ). This change was reflected to some degree in 1(3) and 1,3 in mucosa of the small intestine ( $p < 0.001$ ). Although no corresponding data exists in humans with regard to discrete digestion products of DAG or DAG oil, fat digestion is not believed to be significantly different between rodents and humans.

## 2.2. Portal Appearance of FFA

A mix of 1,3- and 1,2-DAG (70%:30%, respectively, as diolein) was administered orally to rats followed by collection of blood from the portal vein at predetermined time periods. Oleic acid tended to be elevated from 0.5 hour to 1 hour after administration compared with triolein (8).

## 2.3. Lymph/Serum Appearance of Chylomicrons

Rats were infused continuously with emulsions containing diacylglycerol (66% 1,3-DAG, 33% 1,2-DAG) by stomach cannulation. DAG significantly retarded the rate of chylomicron TG formation over a 5-hour period with the difference between 2–3 hours being significant (6). Using labeled fatty acids, the rate of appearance of label from DAG in chylomicron triglycerides from lymph of rats was significantly

decreased during the initial 1 hour following administration by approximately 50% (17% in DAG oil vs. 31%) (9).

Two human studies have focused on serum levels of triglycerides after consumption of test emulsions. Using single doses of 10 g, 20 g, or 44 g of DAG oil, significant differences ( $p < 0.05$ ) were observed in the serum triglycerides for all doses at 6 hours after consumption (10). Additionally, the 44-g dose demonstrated a significant reduction ( $-23\%$ ,  $p < 0.05$ ) in the rate of postprandial triglycerides, whereas the 20-g dose showed a significant reduction in serum triglycerides at 4 hours ( $\sim 50\%$ ,  $p < 0.05$ ). A decrease in VLDL level was also observed at 4 hours during the administration of 20-g dose ( $p < 0.05$ ). Using a single dose of 30 g/m<sup>2</sup> of body surface area ( $\sim 55$  g), significant differences ( $p < 0.05$ ) were observed in the level of change in postprandial serum TG at 2 hours, 3 hours, and 8 hours after consumption of test emulsions (11). The suppression of the increase in serum TG after DAG oil consumption was approximately 50% at hours two and three. A significant decrease in VLDL was reported subsequently for this study at a recent scientific meeting (12).

## 2.4. Oxidation and Synthesis of Fat

Significantly higher activities ( $p < 0.05$ ) of enzymes involved in fat (or beta) oxidation were observed in liver homogenates of rats fed diets containing varying levels of DAG oil for 14 days (13). Correspondingly, a significant increase ( $p < 0.05$ ) in the oxidation rate of added palmitate was observed. At the same time, significantly lower activities ( $p < 0.05$ ) involved in fatty acid synthesis (including fatty acid synthase) were observed. In mice, mRNA for genes associated with beta-oxidation and lipid metabolism as well as small intestinal beta-oxidation were increased significantly after 10 days on DAG oil-containing diets (14). Significantly greater beta-oxidation ( $+139\%$ ,  $p < 0.001$ ) was observed by incubating labeled fatty acid in homogenates of small intestine. Increased oxygen consumption indicating increased energy expenditure, increased portal vein FFA, increased beta-oxidation in the liver and small intestine, and increased mRNA expression of acyl Co-A oxidase (ACO) and uncoupling protein-2 (UCP-2) in the small intestine after 1,3-DAG or DAG oil consumption has been shown in animal models (7, 8, 13, 14). Enzymes involved in fatty acid oxidation in the liver [carnitine palmitoyltransferase, acyl-CoA dehydrogenase, acyl-CoA oxidase (ACO), enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, and d3/d2-enoyl-CoA isomerase] showed increased activity whereas enzymes related to fatty acid synthesis correspondingly showed decreased activity after 14 days of DAG oil consumption (13). Lower hepatic triacylglycerol content and lower serum cholesterol were observed along with these differences in liver enzyme activities. Different fat-digestion products in the gut corresponded with a tendency to use a greater amount of oxygen after DAG oil consumption in animals (8). Using an animal model for diet-induced obesity, significantly lower body weight during the lifespan of mice (C57BL/6J) was observed during ad libitum DAG oil consumption in place of conventional oil (7, 14). In mice fed DAG oil, ACO mRNA levels in the liver were

increased also significantly, consistent with increased ACO activity, compared with a conventional triacylglycerol oil (14). Enhanced beta-oxidation in the small intestine has been reported in mice fed DAG oil compared with triacylglycerol oil (7). Increased beta-oxidation in the small intestine caused by DAG oil consumption was associated with increased expression of genes involved in beta-oxidation and lipid metabolism, including ACO, medium-chain acyl-CoA dehydrogenase (MCAD), liver-fatty acid binding protein (L-FABP), fatty acid transporter (FAT), and UCP-2. These changes in beta-oxidation and mRNA expression are not apparently consistent with regard to tissue specificity. In mice, these changes occurred solely in the small intestine, whereas in rats, these changes occurred solely in the liver.

Indirect calorimetry provides the ability to measure the relative contribution of macronutrients toward energy use. The measurements of expired carbon dioxide and consumed oxygen are used to calculate respiratory quotient (RQ). An RQ of 1.0 indicates use of carbohydrate solely, an RQ of 0.7 indicates use of fat solely, whereas an RQ of 0.85 indicates mixed use of macronutrients. Data in rats demonstrates a significant increase in using fat as an energy substrate following DAG oil infusion (gastric) as observed by a decreased respiratory quotient value between 3–5 hours post-infusion (15).

In humans, a recently published study reported the influence of DAG oil versus conventional oil on energy expenditure, energy substrate use, and subjective appetite ratings during a 36-hour stay in a metabolic chamber (16). Using healthy women ( $n = 12$ ), each subject consumed a defined, eucaloric diet for 3 days prior to each chamber. During the chamber stay, DAG oil or conventional oil with a similar fatty acid composition provided 40% of the fat consumed, which was as part of a defined (55% en from carbohydrate, 15% en from protein, 40% en from fat) eucaloric diet. For data analysis, differences over the entire 36-hour experimental period were evaluated. A significant decrease in respiratory quotient ( $-0.006$ ,  $p < 0.05$ ) and a significant increase in fat oxidation ( $+4.9$  g on day 1,  $p < 0.004$ ;  $+4$  g on day 2,  $p < 0.05$ ) were observed following consumption of a eucaloric diet containing 12% of total energy from DAG (16% energy from DAG oil) over 36 hours compared with TAG oil (Table 3).

**TABLE 3. Summary of Substrate Utilization for Energy and Fat Oxidation Data after DAG Oil Consumption.<sup>1</sup>**

Parameter	DAG Oil	TAG Oil
RQ		
Total, 36 h (8 A.M. to 8 P.M.)	0.849*	0.855
Day 1 (8 A.M. to 8 A.M.)	0.846*	0.853
Day 2 (8 P.M. to 8 P.M.)	0.851*	0.857
Fat oxidation <sup>2</sup> (g/d)		
Day 1	60.7**	55.8
Day 2	64.6*	60.6

<sup>1</sup>Adapted from (14).

<sup>2</sup>Estimated from Figure 2 of (14).

\* $p < 0.05$  vs. TAG oil.

\*\* $p = 0.004$  vs. TAG oil.

Serum levels of ketones, as measured by beta-hydroxybutyrate, and free fatty acids tended to be greater following DAG oil consumption with serum ketone levels being significantly greater on day 2 (at first draw) after DAG oil consumption ( $p < 0.05$  vs. TAG oil using post-hoc analysis). Serum ketone levels after DAG oil consumption remained substantially below the level of serum ketones observed in ketogenic diets.

## 2.5. Energy Expenditure

Following ten days of DAG oil consumption, increased mRNA expression of UCP-2, a protein reported to be involved in thermogenesis, was observed in mouse small intestine (7). In an earlier rat study, oxygen consumption was observed to increase, but no RQ or EE was determined (8). Additionally, energy expenditure over 22 hours was significantly increased in rats on DAG oil diet after an acclimation to DAG oil for 6 days (17). A pronounced difference was observed during the 4 hours after DAG ingestion during the nocturnal cycle.

In humans, the area of energy expenditure continues to be investigated. A significant increase in energy expenditure ( $p < 0.05$ ) has been measured over the course of 6 hours after the consumption of 20-g DAG oil (18). In the human study involving a metabolic chamber, no change was observed in either overall energy expenditure or individual components of energy expenditure (sleeping metabolic rate, diet-induced thermogenesis, and activity-based energy expenditure) over the 36-hour experimental period (16).

## 2.6. Appetite Suppression

Subjective data suggested significant responses toward appetite suppression as estimated by decreased feelings of hunger ( $p < 0.01$ ) and appetite ( $p < 0.05$ ), estimate of prospective food intake ( $p < 0.01$ ), and desire to eat ( $p < 0.05$ ) over the final 12 hours during the 36-hour study (16). Significant differences were not observed after day 1; however, these significant differences caused by DAG oil consumption in cumulative day 2 ratings for hunger, appetite, estimate of prospective food intake, and desire to eat were observed. Decreased cumulative scores inferred that subjects felt less hunger, had less appetite, indicated lower estimate of prospective food intake, and had less desire to eat after DAG oil consumption compared with TAG oil. Similar tendencies have been observed after consumption of medium-chain triacylglycerols with regard to fuel substrate shifts and potential effects on appetite suppression (19–21).

## 2.7. Body Weight and Body Fat

In a long-term study using mice prone to diet-induced obesity, mice consuming a DAG-oil diet for five months gained significantly less weight ( $p < 0.01$ ) and had significantly reduced-fat pad weight ( $p < 0.01$ ) (14). Levels of mRNA from liver were greater for genes controlling enzymes used for beta-oxidation. This significant

**TABLE 4. Change in Body Weight Caused by DAG Oil versus TAG Oil Consumption.<sup>1</sup>**

	% Change in body weight from baseline <sup>2</sup>				
	1 month	2 months	4 months	6 months	Overall Trend
DAG oil	-2.0	-2.4	-3.3	-3.4	$p < 0.025$ vs. TAG
TAG oil	0.8	-1.5	-2.6	-2.4	

<sup>1</sup>Adapted from (22).<sup>2</sup>Estimated from Figure 1.

change in body weight ( $p < 0.05$ ) and fat pad weight (epididymal,  $p < 0.05$  and perirenal,  $p < 0.01$ ) was observed also in a subsequent study conducted for 8 months (7). Using the same mouse model, 18:3(n-3)-rich DAG oil diets reduced body weight significantly as well as certain regional areas of visceral fat (22). Neither of these studies showed decreases in food intake as a result of consuming DAG oil-containing diets.

Two well-controlled studies have been conducted in humans examining the impact of DAG oil on body weight and body fat. In subjects consuming approximately 5% total calories from DAG oil for 16 weeks, significantly greater reductions in body weight ( $p < 0.01$ ) and body fat area ( $p < 0.05$ ) were observed (23). In a larger study, overweight subjects consuming 15% of total energy from DAG oil for 6 months as part of a mildly hypocaloric diet ( $-500$ – $800$  kcal/d) observed a greater extent of body weight ( $p < 0.025$ ) and body fat loss ( $p < 0.037$ ) (24). In a larger study using overweight individuals consuming 15% of total energy from test oils for 6 months as part of a diet with mild caloric restriction ( $-500$ – $800$  kcal/d), subjects consuming DAG oil demonstrated a greater extent of body weight ( $p < 0.025$ ) and body fat loss ( $p < 0.037$ ) over the period of dietary intervention when compared with subjects consuming a conventional triacylglycerol oil (24) (Table 4). As importantly, DAG oil functioned as the oil ingredient for food items that included mayonnaise, crackers, muffins, and instant soups. No apparent decrease in subject compliance caused by product quality or flavor has been reported.

### 3. SUMMARY

From digestion to body weight, differences are observed caused by pure 1,3-DAG, mixed DAG (70:30 ratio for 1,3: 1,2) or DAG oil compared with TAG or TAG oil. These differences demonstrate that 1,3-DAG follows a metabolic route resulting in changes in fatty acid metabolism. Fatty acids appear to a greater extent in portal circulation and chylomicron triacylglycerols are formed to a smaller extent after DAG oil consumption. In supporting experiments, beta-oxidation is increased by the small intestine and liver from animal experiments whereas fatty acid synthesis is inhibited in the liver after extended consumption of dietary DAG oil diets. Animal experiments also demonstrate changes in respiratory quotient indicating a shift



toward greater fat use. Animal experiments demonstrate a smaller accumulation of body weight and body fat over extended periods of consumption.

In humans, DAG oil consumption decreases serum triglycerides (postprandial and fasting) and enables greater degrees of body fat and body weight loss compared with conventional oil when used as a dietary aid as part of a healthy diet or caloric management plan. Studies in Japanese individuals and overweight adult Americans show similar apparent changes in body weight and body fat with greater differences in both measures occurring in groups consuming DAG oil.

Relatively mild losses in body fat and body weight loss have occurred following consumption of DAG oil over a period of several months with an approximate difference in daily caloric expenditure or intake between 50–100 calories. The difference in greater weight loss over six months observed between DAG oil and conventional oil could be attributed to a daily caloric deficit of approximately 48 calories (24). Although the difference in weight loss over 4 months observed between DAG oil and conventional oil could be attributed to a daily caloric deficit of approximately 90 calories (23). Correspondingly, the data from the metabolic chamber study did not indicate a significant change in daily energy expenditure after DAG oil consumption compared with TAG oil in humans (16). Detecting an energy expenditure difference of 50–100 calories over a 24–36-hour period would be difficult in a metabolic chamber setting under the best of conditions. These investigators also indicate a significant increase in fat oxidation of approximately 4–5 g on a daily basis, which corresponds to a shift in using approximately 35–45 calories from fat rather than other fuel sources. Apparently, this shift in substrate use may affect overall energy intake (i.e., appetite) to a greater degree than overall energy expenditure. Interestingly, individuals subjected to acute overfeeding of 1-MAG did not show any differences with regard to energy intake and appetite regulation compared with TAG (25, 26), whereas jejunal infusions of free fatty acids have shown the ability to reduce food intake and body weight in rats (27). These studies may indicate that the combination of 1-MAG and FFA, potentially more so the FFA, caused by the digestion of 1,3-DAG results in observed differences in energy expenditure and appetite regulation.

From a mechanistic viewpoint, fatty acid oxidation as a metabolic control for energy intake appears to be an important relationship (28–30). Products of fatty acid oxidation have been implicated in playing a role in control of food intake. Historically, ketones have received considerable attention, whereas glycerol and free fatty acids have received less attention. Using ketogenic diets (defined as severe CHO restriction) for weight loss has been long espoused with an underlying assumption that elevated serum ketone levels provide a certain degree of appetite suppression. This effect of appetite suppression by elevated serum ketones remains to be conclusively documented.

Effects of compounds that inhibit enzymes of fat metabolism have been reported in recent literature. Changes in fatty acid oxidation have been inversely correlated to changes in food intake using rodent models as well as human subjects. After administration of compounds that directly inhibit fatty acid oxidation or inhibit fatty acid synthesis, significant increases or decreases in food intake, respectively,

as well as changes in body weight over extended durations have been observed (31–36). Etomoxir, a carnitine palmitoyl transferase I inhibitor, causes decreased mitochondrial oxidation of long-chain fatty acids and increases insulin resistance in mice as a result of intramyocellular lipid accumulation over prolonged administration (37). Limited studies with acute administration of etomoxir in human subjects show mixed evidence regarding an increase in food intake (38, 39). C75, a fatty acid synthase inhibitor, results in decreased fatty acid synthesis and decreased food intake and body weight in mice through its proposed modulation of hypothalamic neuronal activity and neuropeptide Y levels (40, 41). Studies in human subjects administered C75 have yet to appear in the scientific literature.

Use of fatty acids for energy during the postprandial period after DAG oil consumption has been reported to occur in the liver or small intestine via beta-oxidation based on rodent studies (13, 14, 42). Regulation of hepatic fat oxidation is believed to be under the control of hepatic glucose metabolism, rather than dietary fat intake, which implies that the status of glycogen stores and carbohydrate metabolism may be more important parameters in controlling total energy balance and fat mass than fat intake (43, 44). This mechanism suggests that increased postprandial fatty acid oxidation may potentially lead to smaller glycogen stores, which have been observed to correlate with lower body fat stores and decreased effort to replenish the loss of body fat.

## REFERENCES

1. A. A. Abdel-Nabey, A. A. Y. Shehata, M. H. Ragab, and J. B. Rossell, *Riv. Ital. Sostanze Grasse*, **69**, 443–447 (1992).
2. R. P. D'Alonzo, W. J. Kozarek, and R. L. Wade, *J. Amer. Oil Chem. Soc.*, **59**, 292–295 (1982).
3. Code of Federal Regulations, Title 21 184.1505, U.S. Government Printing Office, Washington, D.C., 2001.
4. N. O. V. Sonntag, *J. Amer. Oil Chem. Soc.*, **59**, 795A–802A (1982).
5. Y. Katsuragi, T. Yasukawa, N. Matsuo, B. D. Flickinger, I. Tokimitsu, and M. G. Matlock, *Diacylglycerol Oil*, AOCS Press, Champaign, Illinois, 2004.
6. M. Murata, K. Hara, and T. Ide, *Biosci. Biotech. Biochem.*, **58**, 1416–1419 (1994).
7. T. Murase, M. Aoki, T. Wakisaka, T. Hase, and I. Tokimitsu, *J. Lipid Res.*, **43**, 1312–1319 (2002).
8. H. Watanabe, K. Onizawa, and H. Taguchi et al., *J. Japan Oil Chem. Soc.*, **46**, 301–308 (1997).
9. T. Yanagita, *Digestion and Absorption of Diacylglycerol*, 5th International Congress on Essential Fatty Acids and Eicosanoids, Diacylglycerol and Human Health Session, Taipei, Taiwan, 2002.
10. H. Taguchi, H. Watanabe, and K. Onizawa et al., *J. Amer. Coll. Nutr.*, **19**, 789–796 (2000).
11. N. Tada, H. Watanabe, N. Matsuo, I. Tokimitsu, and M. Okazaki, *Clin. Chem. Acta*, **311**, 109–117 (2001).

12. N. Tada, *Effect of Oral Diacylglycerol Loading on Postprandial Hyperlipidemia in Men, 5th International Congress on Essential Fatty Acids and Eicosanoids*, Diacylglycerol and Human Health Session, Taipei, Taiwan, 2002.
13. M. Murata, T. Ide, and K. Hara, *Br. J. Nutr.*, **77**, 107–121 (1997).
14. T. Murase, T. Mizuno, T. Omachi et al., *J. Lipid Res.*, **42**, 372–378 (2001).
15. S. Kimura, *Studies on Energy Metabolism of Diacylglycerol Using Expiration Analysis*, The Japanese Society of Nutrition and Food Science, 2002.
16. M. M. Kamphuis, D. J. Mela, and M. S. Westerterp-Plantenga, *Amer. J. Clin. Nutr.*, **77**, 1133–1139 (2003).
17. H. Watanabe, Effect of dietary DAG on energy expenditure in rats, in press.
18. K. Mori, Effects of diacylglycerol diet on energy expenditure, in press.
19. V. Van Wymelbeke, J. Louis-Sylvestre, and M. Fantino, *Amer. J. Clin. Nutr.*, **74**, 620–630 (2001).
20. M. Krotkiewski, *Int. J. Obes. Relat. Metab. Disord.*, **25**, 1393–1400 (2001).
21. R. J. Stubbs and C. G. Harbron, *Int. J. Obes. Relat. Metab. Disord.*, **20**, 435–444 (1996).
22. T. Hase, T. Mizuno, and K. Onizawa et al., *J. Oleo. Sci.*, **50**, 701–710 (2001).
23. T. Nagao, H. Watanabe, and N. Goto et al., *J. Nutr.*, **130**, 792–797 (2000).
24. K. C. Maki, M. H. Davidson, and R. Tsushima et al., *Amer. J. Clin. Nutr.*, **76**, 1230–1236 (2002).
25. A. M. Johnstone, L. M. Ryan, C. A. Reid, and R. J. Stubbs, *Eur. J. Clin. Nutr.*, **52**, 603–609 (1998).
26. A. M. Johnstone, L. M. Ryan, C. A. Reid, and R. J. Stubbs, *Eur. J. Clin. Nutr.*, **52**, 610–618 (1998).
27. J. E. Cox, W. J. Tyler, and A. Randich et al., *Amer. J. Physiol. Regul. Integr. Comp. Physiol.*, **278**, R604–R610 (2000).
28. E. Scharrer, *Nutrition*, **15**, 704–714 (1999).
29. M. I. Friedman, *Amer. J. Clin. Nutr.*, **67**, 513S–518S (1998).
30. A. Tremblay, *Int. J. Obes. Relat. Metab. Disord.*, **16**, 953–957 (1992).
31. E. Del Prete, T. A. Lutz, J. Althaus, and E. Scharrer, *Physiol. Behav.*, **63**, 751–754 (1998).
32. M. I. Friedman, R. B. Harris, H. Ji, I. Ramirez, and M. G. Tordoff, *Amer. J. Physiol.*, **276**, R1046–R1053 (1999).
33. N. E. Rawson, P. M. Ulrich, and M. I. Friedman, *Amer. J. Physiol.*, **271**, R144–R148 (1996).
34. J. N. Thupari, L. E. Landree, G. V. Ronnett, and F. P. Kuhajda, *Proc. Natl. Acad. Sci. USA*, **99**, 9498–9502 (2002).
35. T. M. Loftus, D. E. Jaworsky, and G. L. Frehywot et al., *Science*, **288**, 2379–2381 (2000).
36. J. Triscari and A. C. Sullivan, *Int. J. Obes.*, **8** Suppl 1, 227–239 (1984).
37. R. L. Dobbins, L. S. Szczepaniak, B. Bentley, V. Esser, J. Myhill, and J. D. McGarry, *Diabetes*, **50**, 123–130 (2001).
38. V. B. Hinderling, P. Schrauwen, W. Langhans, M. S. Westerterp-Plantenga, *Amer. J. Clin. Nutr.*, **76**, 141–147 (2002).
39. A. Kahler, M. Zimmermann, and W. Langhans, *Nutrition*, **15**, 819–828 (1999).

40. T. Shimokawa, M. V. Kumar, and M. D. Lane, *Proc. Natl. Acad. Sci. USA*, **99**, 66–71 (2002).
41. S. Gao and M. D. Lane, *Proc. Natl. Acad. Sci. USA*, **100**, 5628–5633 (2003).
42. T. Murase, M. Aoki, T. Wakisaka, T. Hase, and I. Tokimitsu, *J. Lipid Res.*, **43**, 1312–1319 (2002).
43. J. P. Flatt, *Amer. J. Clin. Nutr.*, **61**, 952S–959S (1995).
44. J. P. Flatt, *Int. J. Obes. Relat. Metab. Disord.*, **20** Suppl 2, S1–S11 (1996).

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## *Citrus Oils and Essences*

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### **1. INTRODUCTION**

Citrus fruits are among the most popular fruits nowadays and have a very long history of production and use. However, within the past century, industrial technologies began to develop in order to convert citrus fruits into commercial products (1). Each year, millions of tons of citrus fruits are delivered to factories for processing and juice production. Historically, the oldest citrus product is the oil. In ancient Sicily, where early Italian citrus industry had just been introduced, lemons were primarily grown for production of lemon oil, and juice was treated as a waste product until its later use for citric acid recovery. The early use of lemon and orange oils was mainly in perfumery and pharmaceuticals (1). With rapid development of science and technology, more areas of use of citrus oils were found, for which more detailed information on chemical composition and properties were required. The modern perfume and flavor industries have benefited from further research on citrus peel oil and essence. Besides, the yield of citrus seed oil has increased since citrus seeds were discovered as a new source of edible oil.

Citrus has proven to be a very good option for the oil and essence production. The genus citrus, according to Tanaka's system, has been divided into eight groups, Papeda, Limonellus, Citraphorum, Cephalocitrus, Aurantium, Osmocitrus, Acrumen, and Pseudofortunella, with a number of species within each group and a larger

number of fine-quality hybrids as well (2). In this chapter, the chemical composition, properties, and uses of citrus oils and essences are provided.

## 2. OIL EXTRACTION

### 2.1. Extraction of Citrus Peel Oil

Citrus peel oils of very complex composition are contained in oval, balloon-shaped oil sacs, or vesicles, located in the outer rind, or flavedo, of the fruit (3). The oil is usually extracted by mechanical separation or hydrodistillation. The five main types of citrus from which peel oils are recovered are orange, grapefruit, tangerine, lemon, and lime (4). Mechanical separation, known as cold-pressing of peel oils, does not use heat in order to avoid loss of volatile components. Swisher and Swisher (1) described three general commercial methods that are widely used in citrus industry to extract crude oils from fruit peels:

- (1) Oil recovery from peel after juice extraction
- (2) Simultaneous extraction of juice and oil emulsion from whole fruit
- (3) Recovery of oil from the peel flavedo after removal from the whole fruit by abrasion or shaving

Citrus peel oils for small-scale use may be obtained by hand-pressing. Fruits are sliced, and mesocarp and albedo layers are peeled from the flavedo before hand-pressing. Peel oils are collected in brine solution on ice, and oil extract is centrifuged at 4°C. Afterwards, the supernatant is dehydrated with anhydrous sodium sulfate and filtered (5). The total final oil extract is about 1% of the flavedo by weight (6).

Citrus peel oils other than cold-pressed oils have a lower price in the marketplace and are known as distilled oil, which is recovered from peels by steam distillation. This oil possesses an odor and flavor that is generally inferior to that of the cold-pressed oil (1). The final oil extract is a liquid with its color varying depending on the species of the fruit. For instance, bergamot oil is green to greenish-yellow, grapefruit oil is greenish-yellow, lemon oil is pale yellow to pale greenish-yellow, lime oil is colorless to pale yellow, mandarin oil is greenish-yellow to reddish-orange, bitter orange oil is pale yellow to yellowish-brown, and sweet orange oil is yellow to reddish-yellow (7). In addition, different citrus peel oils have different physicochemical properties. Table 1 shows the comparison of physicochemical properties of two grapefruit species.

### 2.2. Extraction of Citrus Seed Oils

Citrus seeds are regarded as a new source of edible oil, especially in some developing countries where nutritionists and food chemists have been searching for

**TABLE 1. Average Values for Physical and Chemical Properties of Cold-pressed Red and White Grapefruit Peel Oils.**

	White Grapefruit Oil	Red Grapefruit Oil
Specific gravity, 25°C/25°C	0.8534	0.8522
Refractive index, $n^{20D}$	1.4759	1.4766
Refractive index, 10% distillate, $n^{20D}$	1.4718	1.4718
Optical rotation, $\alpha[25D]$	+92.67	+91.07
Optical rotation, 10% distillate, $\alpha[25D]$	+97.04	+96.84
Evaporation residue, %	6.32	7.12
Aldehyde content, %	1.56	1.38

From Wolford et al., 1971 (8).

economical sources of material with oil of high industrial potential to alleviate the shortage of oil (9). Citrus seeds contain about 36% oil, which can be recovered from seeds by crushing and solvent extraction.

After juice extraction, seeds are separated from the waste product by a paddle-type finisher. They are crushed after washing and drying, and the oil is extracted. In some cases, a commercial solvent extraction of press-cake, using *n*-hexane or petroleum ether, is used to improve oil recovery (1). Unrefined citrus seed oil is pale to light yellow in color and may possess a bland or almond-like aroma (1). The physicochemical characteristics of citrus seed oils, namely the refractive index, specific gravity, melting point, color, and viscosity, vary slightly from each other (Table 2) (9). Citrus seed oils can be used for cooking after refining. However, crude oils are used for preparation of detergents and soaps.

**TABLE 2. Physicochemical Characteristics of Different Citrus Seed Oils.**

Property	Citron	Orange	Mandarin	Mixed
Refractive index (25°C)	1.4681 ± 0.001	1.4684 ± 0.002	1.4672 ± 0.001	1.4682 ± 0.001
Density (25°C) [g/cm <sup>3</sup> ]	0.884 ± 0.01	0.914 ± 0.06	0.962 ± 0.09	0.910 ± 0.08
Melting point [°C]	7	7	7	7
Viscosity [10 <sup>-1</sup> Pa·s]	0.05	0.07	0.08	0.07
Color	2.6R	3.1R	7.4R/0.2B	4.0R
Acid value [mg KOH/g oil]	0.953 ± 0.08	0.673 ± 0.09	1.120 ± 0.09	0.762 ± 0.06
Saponification value [mg KOH/g oil]	189.5 ± 1.41	190.2 ± 1.87	187.2 ± 1.73	188.3 ± 1.76
Ester value [mg KOH/g oil]	188.5 ± 1.40	189.5 ± 1.72	186.8 ± 1.70	187.5 ± 1.41
Peroxide value [meq O <sub>2</sub> /kg oil]	5.95 ± 0.48	6.37 ± 0.51	5.90 ± 0.62	5.98 ± 0.57
Iodine value (Hanus) [mg I <sub>2</sub> /g oil]	96.23 ± 1.34	102.57 ± 1.57	91.54 ± 1.09	99.25 ± 1.07

From El-Adawy et al., 1999 (9).

## 2.3. Extraction of Citrus Essences

Citrus essences are distilled aqueous solutions of the more volatile components from the corresponding citrus juices, as defined by Shaw (10). Commercially, they are added to concentrated citrus juices to impart fresh fruit flavor that may be lost during the concentration process. Essence may be collected from fresh juice either by partial distillation prior to juice evaporation or by condensation of volatiles from the early stages of evaporation (11). Two phases, namely, aqueous essence and essence oil, are obtained during recovery.

## 3. CHEMICAL COMPOSITION

### 3.1. Chemical Composition of Citrus Peel Oils

Different methods have been adopted to analyze the chemical composition of citrus oils as well as their odor activities, including gas chromatography-mass spectrometry (GC-MS) (12, 13), gas chromatography-olfactometry (GCO) (6), nuclear magnetic resonance (NMR) (14), near infrared (NIR) (15), high-performance liquid chromatography (HPLC) (16), thin layer chromatography (TLC) (9), and other chromatographic procedures. Improvements in the available techniques has given rise to more precise and detailed data for qualitative and quantitative determination of citrus oils. Accordingly, 55 volatile components in lemon oil, 62 in lime oil (14), 79 in mandarin oil (17), 88 in tangerine oil (18), and some more components in hybrid citrus oils have been identified (5).

Citrus peel oils are characterized by complex mixtures containing mostly terpenes as well as oxygen-containing compounds (19). Volatile components account for more than 90% of the oil mass, whereas nonvolatile residues are present in very small amounts. For most citrus fruits, peel oils consist almost exclusively in the form of monoterpenes, sesquiterpenes, and other aliphatic hydrocarbons. The hydrocarbon contents in Vietnamese pummelo, orange, tangerine, and lime, respectively, exceeded 98.7%, 97.6%, 98.6%, and 95.4% (20). Among these, monoterpenes were dominant. Limonene, a monoterpene often used as a functional index of ripeness, was the major component of all citrus peel oils, followed by  $\beta$ -pinene in lemon oil (21),  $\gamma$ -terpinene in tangerine and lime oils (14), myrcene in sweet orange oil, and *p*-cymene in mandarin oil (19). The compounds  $\alpha$ -pinene, sabinene,  $\beta$ -phellandrene, and terpenolene were present at lower levels. A few sesquiterpenes were also found in very small amounts but made appreciable contributions to flavor and odor; these included *trans*- $\beta$ -farnesene, *trans*- $\alpha$ -bergamotene, *trans*-caryophyllene, germacrene A to D, and  $\beta$ -bisobolene (14, 22).

Although terpene hydrocarbons, especially monoterpenes, are the most abundant constituents of citrus peels oil, they serve only as a flavor carrier and contribute little to flavor on their own (23). These terpenoid hydrocarbons are usually removed by deterpenation in order to increase the concentration of flavor and fragrance compounds. Furthermore, unsaturated hydrocarbons (terpenes) are unstable to heat and



light, and may oxidize rapidly to produce undesirable off-flavor compounds that adversely affect the desirable aroma of products (24). Therefore, concentrated and deterpenated oils have become popular in the citrus oil market.

Oxygenated compounds, mainly oxygenated terpenes, rather than terpene hydrocarbons, have been found to be responsible for the characteristic odor and flavor of citrus fruits, although they occur in relatively small amounts. When the hydrocarbon fraction is removed from the oil, the oxygenated fraction becomes more odorous due to a higher concentration (25). Characterized by quantitative abundance in aldehydes and a relatively wide variety of alcohols, oxygenated fraction includes aldehydes, alcohols, ketones, esters, oxides, acids, and trace amounts of fugenol methyl ether (5). Geranial and neral are the major aldehydes, both of which account for the fresh floral and citrus-like character of lemon and lime oils (21, 26). Citronellal has a green-citrusy odor rather than a sweet and fruity odor (5). In addition, many simple aliphatic aldehydes, such as octanal, decanal, and dodecanal, impart a characteristic aroma to citrus peel oils (17, 26). Among alcohols, monoterpene alcohols such as linalool, followed by octanol and  $\alpha$ -terpineol, are most predominant (5). Nerol and geraniol are also found in high levels. Among these, linalool and octanol are regarded as the most odor-active compounds in such citrus as Hyuganatsu (*Citrus tamurana*) (27). Ketones, esters, oxides, and acids are less represented, but make appreciable contributions to flavor. Nootkatone is an important flavor compound of grapefruit oils (28); neryl acetate, geranyl acetate, and bornyl acetate have been used as sweeteners, and linalool oxide provides a powerful sweet odor (5). Flavor dilution (FD) factor is employed to express the odor potency or intensity of volatile components in citrus peel oils (Table 3). FD factor is defined as the ratio of concentration of a compound in the initial concentration to that in the most diluted concentration in which the odor could be detected by GCO (29).

Compositions of volatiles in different orange oils are shown in Tables 4 and 5. It is evident that most of the constituents belong to the terpene family and may be arranged into two groups, terpene hydrocarbons (terpenes and sesquiterpenes) and oxygenated terpene products (21). Aside from the volatile components, there are small amounts (2–15%) of nonvolatile residues in citrus peel oils that possess antioxidative property; these include coumarins, psoralens, and polymethoxylated flavones (30–34).

### 3.2. Chemical Composition of Citrus Seed Oils

Generally, lipids in citrus seed oil may be classified as nonpolar, nonionic polar, and ionic polar. Nonpolar lipids are mainly composed of triacylglycerols; nonionic polar lipids refer to sugar-containing lipids, including glycosylacylglycerols, sterol glucosides, and sphingosine-containing lipids. Meanwhile, ionic polar lipids contain phospho, sulfo, amino, or carboxyl reactive groups (35).

Analyzed by thin layer chromatography, crude citrus seed oils are reported to have eight classes of chemical constituents: triacylglycerols, free fatty acids, diacylglycerols, monoacylglycerols, sterols, phospholipids, alcohols, and hydrocarbons

**TABLE 3. Potent Odorants (FD  $\geq$  2) Identified in the Peel Oil of Clementines (*Citrus Reticulata* Blanco cv. Clementine)**

No.	Odorant	Odor Quality	FD Factor
1	$\alpha$ -pinene	pinetree-like	1024
2	(Z)-hex-3-enal	grassy	128
3	myrcene	geranium leaf-like	1024
4	limonene	mint-, citrus-like	512
5	octanal	citrus-like, fresh	1024
6	oct-1-en-3-one	mushroom-like	2
7	(Z)-octa-1,5-dien-3-one	geranium-leaf-like	2
8	nonanal	green, citrus-like	16
9	(Z)-non-6-enal	green, fresh, cucumber	64
10	citronellal	citrus-like, fresh	64
11	decanal	green, fresh	256
12	(E)-non-2-enal	fatty, green	128
13	(Z)-dec-4-enal	green, musty	8
14	linalol	flowery	16384
15	octan-1-ol	citrus-like, soapy	8
16	unknown	waxy	16
17	undecanal	citrus-like, soapy	4
18	unknown	metallic	16
19	(E,Z)-nona-2,4-dienal	deep-fried	8
20	unknown	flowery, citrus-like	16
21	(E,E)-nona-2,4-dienal	deep-fried	512
22	dodecanal	green coriander	16
23	carvone	mint-like	256
24	(E)-undec-2-enal	green coriander	16
25	unknown	soapy	8
26	unknown	flowery,soapy	64
27	unknown	flowery	64
28	(E,E)-deca-2,4-dienal	deep-fried	4096
29	(E)-dodec-2-enal	green coriander	128
30	unknown	fatty, cucumber-like	64
31	(E)-tridec-2-enal	green, metallic	8
32	unknown	fruity, pinetree-like	8
33	tr-4,5-epoxy-(E)-dec-2-enal	metallic	128
34	unknown	metallic	256
35	unknown	fruity	8
36	unknown	fruity	8
37	sotolone	spicy	16
38	3a, 4, 5, 7a-tetrahydro-3,6-dimethylbenzofuran-2(3H)-one	sweet, cocos	4096
39	$\beta$ -sinensal	metallic, green, sweaty	64
40	unknown	metallic	16
41	$\alpha$ -sinensal	metallic, green, sweaty	64
42	vanillin	vanilla-like	16

<sup>a</sup>FD factor (Flavor dilution factor): The ratio of concentration of a compound in the initial concentration to that in the most diluted concentration in which the odor could be detected by GCC.

From Buettner et al., 2003 (29).

TABLE 4. Major Components of Cold-pressed Navel Orange Oil.

Compound	Cold-Pressed Oil (%)	Compound	Cold-Pressed Oil (%)
<b>monoterpenes</b>	97.52	<b>alcohols</b>	0.58
limonene	94.74	linalool	0.4
$\beta$ -myrcene	1.66	<i>n</i> -octanol	0.12
$\alpha$ -pinene	0.46	$\alpha$ -terpineol	0.06
sabinene	0.46	<b>oxidized limonene</b>	0.33
carene-3	0.09	limonene oxide (cis)	0.08
$\beta$ -pinene	0.03	carvone	0.08
$\alpha$ -phellandrene	0.03	limonene oxide (trans)	0.05
ocimene	0.03	carveol (cis)	0.05
terpinolene	0.02	limonene oxide (trans)	0.05
$\alpha$ -thujene	<0.01	dehydro carveol (iso)	0.01
$\alpha$ -terpene	<0.01	<b>esters</b>	0.02
<i>p</i> -cymene	<0.01	neryl acetate	0.01
$\gamma$ -terpinene	<0.01	geranyl acetate	0.01
<b>aliphatic aldehydes</b>	0.75	<b>sesquiterpenes</b>	0.13
decanal	0.38	valencene	0.03
<i>n</i> -octanal	0.22	$\beta$ -farnesene	0.02
dodecanal	0.11	$\alpha$ -farnesene	0.02
<i>n</i> -nonanal	0.04	$\delta$ -cadinene	0.02
<b>terpen aldehydes</b>	0.23	$\alpha$ -copaene	0.01
perillaldehyde	0.06	$\beta$ -cubebene	0.01
citronellal	0.05	$\beta$ -caryophyllene	0.01
$\alpha$ -sinensal	0.04	germacrene-D	0.01
geranial	0.03		
$\beta$ -sinensal	0.03		
neral	0.02		

From Shen et al., 2002 (23).

(Table 6). Triacylglycerols are the major oil class in all citrus seed oils, followed by free fatty acids and then diacylglycerols. The presence of partial acylglycerols and free fatty acids is due to partial enzymatic hydrolysis of reserve triacylglycerols during seed storage (9).

The composition of citrus seed oil varies with species and storage conditions. For instance, Habib et al. (36) reported that mandarin seed oil is very high in its triacylglycerol content whereas citron oil has a large amount of free fatty acids. With respect to compositional patterns, lime seed oil is similar, in its degree of unsaturation, to soybean oil, and orange oil is similar to cottonseed oil. In general, citrus seed oil has a larger amount of volatile fatty acids than other edible oils (36).

Crude citrus seed oils need to be refined before use as edible oils. Only triacylglycerols, diacylglycerols, and polar lipids remain after degumming, refining, bleaching, and deodorization. However, trace amounts of phosphatides (lecithin) and plant sterols may also remain in the oil (37).

Citrus seed oil, as a potential edible oil, serves as a good source for essential fatty acids. More than 60 fatty acids have been found in various citrus seed oils, among which unsaturated fatty acids are present in a high amount (>65%)

**TABLE 5. Composition of Oils from Five Different Orange Species.**

Component	% Total Area				
	Bergamot	Bitter Orange	Mandarin	Sweet Orange	Tangerine
limonene	36.54	93.42	68.8	96.1	88.15
$\beta$ -pinene	8.63	0.91	2.15	0.52	0.86
<i>p</i> -cymene	7.24	1.66	16.06	0.06	2.75
$\gamma$ -terpinene	2.2	—	5.85	—	3.11
$\alpha$ -pinene	1.47	0.94	2.87	0.69	1.27
myrcene	0.95	2.05	1.66	1.77	1.78
$\alpha$ -bergamotene	0.39	—	—	—	—
$\alpha$ -thujene	0.36	—	0.9	—	0.18
$\beta$ -caryophyllene	0.29	0.05	0.03	0.03	0.01
terpinolene	0.27	—	0.51	0.01	0.08
linalool	8.6	0.1	0.06	0.18	0.57
neral	0.07	0.04	—	0.05	—
geranial	0.05	0.02	0.02	0.03	0.02
octanal	—	0.03	—	0.06	0.04
linalyl acetate	31.73	0.16	—	—	—
geranyl acetate	0.52	0.11	—	—	—
sum of 16 compounds	99.3	99.5	98.9	99.5	98.8
% hydrocarbons	58.73	99.13	99.22	99.29	98.68
% oxygenated compounds	41.27	0.87	0.78	0.71	1.32

From Veriotti et al., 2002 (19).

(35, 38). Linoleic (>30%), oleic (>18%), and linolenic (2–12%) acids are the most predominant unsaturated fatty acids present (9). Lemon, lime, and citron oils contain the highest amount of linolenic acid. In addition, very small amounts of myristoleic acid (C14:1) in polar lipids fraction, myristoleic (C14:1) and palmitoleic (C16:1) acids in diacylglycerols fraction, and myristoleic (C14:1) and eicosaenoic (C20:1) acids in triacylglycerols fraction of citrus seed oil were also identified (39).

Saturated fatty acids were less abundant than their unsaturated counterparts in citrus seed oils and consisted mainly of palmitic (C16:0) and stearic (C18:0) acids

**TABLE 6. Composition of Different Citrus Seed Oils (%).**

Oil Class	Citron	Orange	Mandarin	Mixed
Triacylglycerols	66.8	65.4	68.4	68.0
Free fatty acids	14.5	13.4	11.7	12.8
Diacylglycerols	12.1	12.0	10.5	11.3
Monoacylglycerols	2.49	1.97	2.97	2.51
Sterols	2.18	3.52	3.27	3.14
Phospholipids	1.96	3.66	2.64	2.23
Hydrocarbons	Tr.	Tr.	Tr.	Tr.
Alcohols	Tr.	Tr.	Tr.	Tr.

\*Tr. = Traces.

From El-Adawy et al., 1999 (9).

**TABLE 7. Fatty Acids Composition of Different Citrus Seed Oils.**

Fatty Acid [%]	Citron	Orange	Mandarin	Mixed
Lauric (C12:0)	0.39	0.36	0.65	0.37
Myristic (C14:0)	0.43	0.44	0.61	0.46
Palmitic (C16:0)	29.52	24.73	28.12	28.54
Stearic (C18:0)	4.32	5.27	4.34	4.37
Oleic (C18:1)	22.25	26.00	24.89	24.53
Linoleic (C18:2)	33.21	38.44	38.26	38.25
Linolenic (C18:3)	9.56	4.58	2.58	3.11
Arachidic (C20:0)	0.32	0.18	0.55	0.37
Total saturates	34.98	30.98	34.27	34.11
Total unsaturates	65.02	69.02	65.73	65.89
Total essential fatty acids	42.77	43.02	40.84	41.36

From El-Adawy et al., 1999 (9).

at 22–31% and 1–6%, respectively. Arachidic (C20:0), lauric (C12:0) and myristic (C14:0) acids were found in trace amounts compared with other fatty acids (9, 35, 40).

Table 7 shows the fatty acid composition of different citrus seed oils. The ratio of unsaturated to saturated fatty acids is approximately 2:1 (9), although this ratio was reported to be in the range of 3–5:1 by Nagy (35). Generally, different varieties, cultivar, location, storage condition, and harvesting time of citrus fruit may lead to this variation. Table 8 shows the content of the six major fatty acids in different citrus seed oils; these are linoleic (C18:2), palmitic (C16:0), oleic (C18:1), linolenic (C18:3), stearic (C18:0), and palmitoleic (C16:1) acids.

### 3.3. Chemical Composition of Citrus Essence

Distillation of citrus juices yields two volatile fractions, namely, aqueous essences and essence oils that are separated from each other by condensation of the distillate (7). Aqueous essence, the bottom layer of the condensate is comprised of organic acids, alcohols, aldehydes, esters, hydrocarbons, ketones, hydrogen sulfide, and oxides (10). Considering many components found in both cold-pressed peel oil and aqueous essence, essence oil has a flavor similar to that of the combined peel oil and aqueous essence (10). However, essence oil usually contains a larger amount of

**TABLE 8. Major Fatty Acids in Citrus Seed Oils (%).**

Seed Type	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic
Oranges	26–31	0.1	3–5	24–28	35–37	2–4
Grapefruit	26–36	0.1–0.3	1–4	18–25	32–41	3–6
Mandarins	22–30	0.1–1.0	2–5	20–25	37–45	3–5
Lemons	18–24	0.1–0.3	2–4	26–34	31–38	6–12
Limes	24–29	0.1–0.5	3–5	20–22	37–40	6–11

From, Nagy, 1977 (35).

aliphatic ethyl esters (e.g., ethyl butyrate in orange essence oil) compared with the peel oil (41). Thus, its aroma resembles that of the corresponding juice more than that of the peel oil (7). In general, citrus flavor results from a complex mixture of components in the appropriate proportions, as described by Monshonas et al. (42).

## 4. STORAGE OF CITRUS OILS

### 4.1. Changes of Composition During Storage

Citrus peel oils have been used widely in beverages, cosmetics, pharmaceuticals, and perfumery industry, whereas seed oils are used in cooking and for treatment of leather and textile. The quality, freshness, and uniqueness of citrus oils are major considerations pertaining to their value and applications (43). However, large amounts of volatile components, as well as unsaturated compounds, render the oils unstable and prone to change with time and storage conditions.

Most of the qualitative changes of citrus peel oil during storage occur due to evaporation, oxidation, polymerization, rearrangement, and cyclization of some labile constituents in the presence of heat, light, oxygen, moisture, and catalysts resulting in the loss of volatile components and formation of off-flavor artifacts (43, 44). This major deterioration in citrus peel oil occurs when stored at 20°C, with a notable decrease in monoterpenes and an increase in oxygenated compounds (33). Monoterpenes may undergo oxidation and, hence, formation of oxygenated terpenes, rearrangement and cyclization into sesquiterpenes, polymerization into artifacts, and evaporation that causes loss of these components as well. As reported by Njoroge (33), the total percentage of monoterpenes decreased progressively from 98.0 to 66.4 in Daidai (*Citrus aurantium*) peel oil after 12 months of storage at 20°C. The prominently decreased components were  $\beta$ -myrcene,  $\gamma$ -terpinene, and limonene. However, the concentration of *p*-cymene was increased, indicating loss of freshness of the oils, which might be explained by dehydrogenation and rearrangement of  $\alpha$ - and  $\gamma$ -terpinene, and hydrogenation and double-bond rearrangement of limonene as well (43, 45).

The content of oxygenated compounds increased considerably during storage (43). Monoterpene alcohols, as oxidized products of monoterpenes, showed the most significant increase. In contrast, linalool (both *cis* and *trans*) was found to decrease at 20°C after 12 months of storage (44, 45). Esters, such as linalyl and geranyl acetates, and most oxides also increased significantly. Besides, artifacts were formed in citrus peel oil upon prolonged storage at higher temperatures in the forms of alcohol, carbonyl compound, ester, epoxide, and hydrocarbon (33). Sesquiterpene alcohols were the dominant artefacts formed, as found in Yuzu (*Citrus junos Tanaka*) peel oil, with spathulenol being the main constituent. Spathulenol could be produced directly from bicyclogermacrene on air oxidation, or through isomerization of bicyclogermacrene to aromadendrene and *allo*-aromadendrene, and the subsequent oxidation of these isomeric tricyclic sesquiterpenes (43). The relative compositional change of Yuzu is shown in Table 9.

TABLE 9. Relative Compositional Changes in Yuzu (*C. junos Tanaka*) Cold-pressed Oil During a 12-Day Storage in 20 C.

Compound	Relative Concentration (%)					
	Fresh Oil	1	3	6	9	12
<b>monoterpene hydrocarbons</b>						
$\alpha$ -pinene	1.84	1.95	1.94	1.61	1.45	0.66
$\beta$ -pinene	0.69	0.74	0.74	0.75	0.59	0.3
sabinene	0.24	0.24	0.25	0.19	0.17	0.08
myrcene	2.14	2.02	1.84	1.36	1.05	0.56
$\alpha$ -terpinene	0.15	0.12	0.12	0.04	0.02	Tr.
limonene	78.13	77.06	76.54	66.7	51.87	31.49
$\gamma$ -terpinene	9.32	10.07	9.62	8.01	2.23	1.47
<i>p</i> -cymene	0.4	0.61	0.68	4.65	5.19	3.05
terpinolene	0.39	0.41	0.4	0.5	0.09	0.05
$\alpha$ - <i>p</i> -dimethylstyrene	0.04	0.04	0.04	0.08	0.16	0.06
+ <i>p</i> -mentha-1,4,8-triene	—	—	—	—	—	—
total	93.34	93.26	92.17	83.89	62.82	37.72
<b>monoterpene alcohols</b>						
linalool	1.79	1.95	1.97	5.43	7.08	3.67
$\alpha$ -terpineol	0.1	0.13	0.13	0.49	1.24	1.36
(Z)-carveol	0.07	0.06	0.06	0.24	0.36	0.63
nerol	—	—	—	0.07	0.33	0.59
geraniol	—	—	—	0.07	0.32	0.45
perillyl alcohol	—	—	—	0.05	0.71	1.22
thymol	0.12	0.12	0.11	0.56	2.13	4.2
<i>p</i> -mentha-1,8-dien-10-ol	—	Tr.	0.01	0.05	0.97	1.04
total	2.08	2.26	2.27	6.72	10.81	9.86
<b>oxides and epoxides</b>						
(Z)-limonene oxide	Tr.	Tr.	0.01	0.07	0.15	0.07
(E)-limonene oxide	0.01	0.03	0.03	0.03	0.09	0.06
(Z)-caryophyllene epoxide	—	0.01	0.02	0.09	0.24	0.53
(E)-caryophyllene epoxide	—	0.01	0.01	0.03	0.16	0.48
total	0.01	0.05	0.07	0.22	0.64	1.14
<b>sesquiterpene hydrocarbons</b>						
$\delta$ -elemene	0.09	0.08	0.06	0.09	0.16	0.23
$\alpha$ -copane	0.01	0.03	0.03	0.03	0.15	0.15
$\beta$ -elemene	0.04	0.04	0.04	0.13	0.18	0.16
caryophyllene	0.17	0.16	0.17	0.17	0.2	0.26
aroma dendrene	0.01	0.01	0.01	0.01	0.01	0.02
$\gamma$ -elemene	0.02	0.02	0.01	0.05	0.05	0.3
allo-aroma dendrene	0.01	0.01	0.01	0.05	0.05	0.1
(E)- $\beta$ -farnesene	0.45	0.6	0.63	2.13	2.78	2.51
$\alpha$ -humulene	0.04	0.03	0.04	0.06	0.19	0.2
$\delta$ -muurolene	—	Tr.	Tr.	Tr.	0.05	0.06
germacrene D	0.2	0.19	0.17	0.19	0.16	0.16
$\alpha$ -muurolene	0.04	0.04	0.03	0.04	0.22	0.13
bicycloger-macrene	0.99	0.82	0.52	0.31	0.14	0.03
$\delta$ - $\gamma$ -cadinene	0.04	0.04	0.04	0.07	0.15	0.32
sesquiphell-andrene	0.03	0.03	0.04	0.18	0.21	0.63
total	2.14	2.1	1.8	3.51	4.7	5.26

TABLE 9. (Continued)

Compound	Relative Concentration (%)					
	Fresh Oil	1	3	6	9	12
<b>sesquiterpene alcohols</b>						
globulol	0.01	0.01	0.01	0.02	0.1	0.35
elemol	—	—	Tr.	0.03	0.07	0.16
viridiflorol	—	—	—	Tr.	0.02	0.35
spathulenol	—	0.03	0.26	2.64	14.05	28.25
$\alpha$ -cadinol	—	Tr.	0.02	0.13	0.24	1.88
T-cadinol	—	—	—	Tr.	0.21	0.52
$\beta$ -eudesmol	—	Tr.	0.02	0.18	0.47	1.91
$\alpha$ -eudesmol	—	—	—	—	Tr.	0.11
(Z,E)-farnesol	—	—	—	Tr.	Tr.	2.55
total	0.01	0.04	0.31	3	15.16	36.08
total natural volatiles (%)	97.58	97.66	96.29	94.24	78.62	53.26
total artifact volatiles (%)	—	0.05	0.34	6.32	17.79	40.04

From Njoroge et al., 1996 (43).

Tr. = Traces.

These compositional changes usually negatively influence the odor and flavor of citrus peel oils by generating off-flavor products. It has been shown that nonvolatile residues of citrus peel oil contain some compounds that exhibit antioxidative activities, among which permethoxylated flavones, dehydroabiatic acid (46), coumarins, and psoralens have been identified (33). In this respect, cold-pressed citrus peel oil is more stable than distilled oil and essence oil, in which most of the natural antioxidants present are left behind when the oil is distilled (1).

Citrus seed oil, another byproduct in citrus industry, is required to have high stability for cooking purposes. Citrus seed oil is subject to oxidative changes because of the presence of a high percentage of unsaturated fatty acids. The oil is readily oxidized in the presence of air, generating hydroperoxides, alcohols, aldehydes, ketones, hydrocarbons, and carboxylic acids as the primary, secondary, and tertiary oxidized products, respectively.

## 4.2. Storage Conditions

One of the most important reactions involved in chemical changes in citrus oils is certainly the oxidation reaction, due to the high content of terpenes in citrus peel oil and unsaturated fatty acids in citrus seed oil (3). Usually, quality deterioration in oils may occur under autoxidation, photo-oxidation, lipoxygenase-assisted oxidation, or thermal oxidation, all of which should be controlled in order to protect the oils from deterioration and off-flavor development (47). To maintain the original quality of citrus oils, undue exposure to air and contact with metals such as iron and copper, which act as pro-oxidants, should be avoided during processing and subsequent storage (1). In some cases, citrus oils are stored refrigerated (0–5°C) under an



inert gas (33). Meanwhile, the addition of antioxidants may help retard or control oxidation of citrus oils. Tocopherols are present in citrus seed oil with  $\alpha$ -tocopherol predominating (1). They effectively protect the oil from oxidation, with phospholipids remaining in the oil preventing their degradation (1, 47). Some phenolic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ), are also used in limited dosage to inhibit the oxidation of edible oils such as citrus seed oil (47). In addition, partial hydrogenation provides satisfactory shelf life to some citrus seed oils, in which the high content of linolenic acid may pose stability problems (1). However, hydrogenation is not considered desirable as it leads to the loss of essential fatty acids and may also affect the flavor quality of the product.

Unlike citrus seed oils, citrus peel oils are themselves very good antioxidants capable of inhibiting free radical-mediated reactions (48). As reported by Song et al. (49), abundant tocopherols were found in citrus peel oils, yet there was little correlation between tocopherol content and antioxidative activity in the oils, suggesting that the composition of terpenes present might be a major determinant of the antioxidative status of citrus peel oils. The compounds  $\beta$ -pinene, myrcene,  $\alpha$ -terpinene, and  $\gamma$ -terpinene were identified to have higher or similar antioxidative activities compared with that of  $\delta$ -tocopherol. Encapsulation is a technique frequently used in the storage of citrus peel oils and essences, which isolates the oils from the atmospheric oxygen, moisture, temperature, and light, and hence minimizes the oxidation of oil and reduces the release of volatile flavor compounds (50). Citrus peel oil may be spray-dried and encapsulated in a double emulsion (50). A water activity of 0.628 was found to result in good resistance to oxidation without the occurrence of caking or stickiness during the storage of spray-dried encapsulated citrus peel oil (51).

## 5. APPLICATIONS OF CITRUS OILS AND ESSENCES

The applications of citrus oils are versatile and in many domains. As a result of their freshness, lightness, and fine fruity aroma, citrus peel oils and essences are widely used in the food and beverage industries as well as in some nonfood applications (1).

The applications of cold-pressed peel oils in food and beverage are mainly in the soft drinks, sherbet, confectionery, bakery, and household extracts (1). In addition, they can act as reducing agents of peroxidase activity in leafy vegetables and antioxidants for edible oils, such as olive oil, to improve their sensory properties (52, 53). Moreover, they are effective inhibitors for the formation of N-nitrosodimethylamine (NDMA), a known carcinogen, that may occur during production and storage of food (54). Citrus peel oils are also added as flavoring agents to pharmaceutical and drugs as well as herbal medicines in order to mask their unpleasant tastes (14). Distilled peel oils, different from cold-pressed oils, have found their place in the perfumery and cosmetic industries as well as in the manufacturing of soap and paper (1).

Citrus peel oils may also be used for their antioxidative, antitumor, and radical-scavenging activities. The radical-scavenging ability of citrus peel oil may help prevent free radical-induced and various chronic diseases (48, 55, 56). Monoterpenes from volatile components and polymethoxylated flavones from nonvolatile residues have been reported to be effective inhibitors of tumor cell growth, implicating that citrus peel oils may be good cancer preventive food additives (57–59). Furthermore, citrus peel oils are useful to alleviate pain from burnt skin (60). Demonstrating anxiolytic and sedative effect, they could also be used in primary medical care against insomnia, anxiety, and epilepsy (61).

The insecticidal property and antimicrobial activity of citrus peel oils have been reported. The oil can repel moth, mosquito, cockroach, domestica, and housefly (62–66). It also inhibits the growth of microbes such as fungi and salmonellae, with monoterpenes being the major compounds that account for pathogen fungi inhibition (46, 67, 68).

Unlike citrus peel oil, citrus seed oil is mainly used after refining as edible oil and a source of essential fatty acids. Refined citrus seed oil is widely used in margarines, shortenings, salad dressings, and salad and cooking oils. Meanwhile, crude citrus seed oils are useful in the preparation of fatty acid derivatives, soap and detergent, and for the treatment of leather and textile (1).

## 6. CHALLENGES

Despite increasing applications of citrus oils, certain challenges related to potential health-damaging properties and contamination of citrus oils should not be ignored.

Citrus peel oil, such as bergamot oil, has been reported to show potential health hazards. Bergamot oil has a pleasant refreshing scent, and had been used widely in cosmetics until it was restricted in most countries a few years ago because of certain adverse effects, primarily phototoxicity and Berloque dermatitis (69). More recently, there seems to be increasing application of bergamot oil in aromatherapy. However, as reported by Kaddu et al. (69), bergamot oil possesses potential phototoxic and photomutagenic properties, indicating that special attention should be paid in using of psoralen-containing aromatherapy oils such as bergamot oil.

Aside from potential adverse health effects, contamination is another problem that should be considered in production and application of citrus oils. Citrus oils may be contaminated with plastic materials employed in production process or storage. Chloroparaffins, phthalate esters, and phosphorylated plasticizers are the major contaminants extracted from plastic components by citrus oils as the oil/water emulsions pass through various production phases (70). Phthalate esters have a wide range of activities and may be hepatotoxic, carcinogenic, and possibly damaging to the gastrointestinal tract (71). Chloroparaffins, another class of plasticizers, are toxic by ingestion and subcutaneous route and are classified as being potentially carcinogenic to humans (72). It is suggested that contamination of citrus peel oils by plasticizers does not depend on the nature of the oil, but probably

correlated with the procedures used during production cycle or storage of the oils (70, 72).

Another contamination of citrus peel oils comes from chlorine-treated water used in the oil recovery process and sanitizers used in postharvest handling and process equipment cleaning, which serve as a potential source of hypochlorous acid (HOCl) (73). HOCl can react with a variety of terpenes similar to d-limonene in structure, including limonene,  $\alpha$ -pinene, and  $\alpha$ -terpineol, resulting in the formation of terpene chlorohydrins. The contamination of terpene chlorohydrins could be reduced through reduction of the chlorine levels in the treatment water (74).

Pesticide residues may contaminate citrus peel oils as well. Cultivation of citrus crops commonly involves the use of chemicals such as fertilizers and pesticides. Regulations are increasingly stricter in terms of residual levels of pesticides because of the application of citrus oils in food, pharmaceutical, and cosmetic industries (75). Citrus peel oils, extracted from citrus peels, contain a higher concentration of pesticide residues than the fruits, due to the direct contact of the peels with pesticides. Organophosphorus and organochlorine pesticide residues in citrus peel oils have shown a steady decrease in recent years (76).

## 7. CONCLUSIONS

There is considerable interest in the chemical composition and properties of citrus oils and essences as well as the role they play in food and nonfood industries. Citrus peel oils and essences possess a pleasant aroma, with oxygenated compounds being the major constituents that account for their characteristic odor. Terpenes, the most abundant components in cold-pressed citrus peel oil, are removed in concentrated oil production, usually by use of adsorbant and supercritical carbon dioxide, to increase the concentration of oxygenated compounds and to enhance the qualification of the oil. Meanwhile, citrus seed oils are composed largely of triacylglycerols and are rich in oleic and linoleic acids.

Citrus oils and essences are unstable to heat, light, oxygen, and metals. Thus, they should be stored under appropriate conditions in order to avoid compositional changes that lead to their quality deterioration. Citrus oils and essences are widely used in food and nonfood industries. Applications in the health-related areas are forthcoming.

## REFERENCES

1. H. E. Swisher and L. H. Swisher, "Specialty citrus products," in *Citrus Science and Technology*, vol. 2, S. Nagy, P. E. Shaw, and M. K. Veldhuis, ed., The AVI Publishing Company, Westport, Connecticut, 1977, pp. 290–345.
2. K. Ogawa, A. Kawasaki, T. Yoshida, H. Nesumi, M. Nakano, Y. Ikoma, and M. Yano, *J. Agric. Food Chem.*, **48**, 1763–1769 (2000).
3. M. Usai, G. Arras, and F. Fronteddu, *J. Agric. Food Chem.*, **40**, 271–275 (1992).

4. P. E. Shaw, "Essential Oils," in *Citrus Science and Technology*, vol. 1, S. Nagy, P. E. Shaw, and M. K. Veldhuis, eds., The AVI Publishing Company, Westport, Connecticut, 1977, pp. 427–462.
5. H-S. Choi and M. Sawamura, *J. Agric. Food Chem.*, **48**, 4868–4873 (2000).
6. H-S. Choi, *J. Agric. Food Chem.*, **51**, 2687–2693 (2003).
7. K. Bauer, D. Garbe, and H. Surburg, "Natural raw materials in the flavor and fragrance industry," in *Common Fragrance and Flavor Materials*, K. Bauer, D. Garbe, and H. Surburg, eds., VCH Publishers, New York, 1990, pp. 146–147.
8. R. W. Wolford, J. W. Kesterson, and J. A. Attaway, *J. Agric. Food Chem.*, **19**, 1097–1105 (1971).
9. T. A. El-Adawy, E. H. Rahma, A. A. El-Bedawy, and A. M. Gafar, *Nahrung-Food*, **43**, 385–391 (1999).
10. P. E. Shaw, "Aqueous Essences," in *Citrus Science and Technology*, vol. 1, S. Nagy, P. E. Shaw, and M. K. Veldhuis, eds., The AVI Publishing Company, Westport, Connecticut, 1977, pp. 463–478.
11. R. W. Wolford, M. H. Dougherty, and D. R. Petrus, *Int. Fruchtsaftunion. Ber. Wiss. Tech. Komm.*, **9**, 151–169 (1969).
12. H. S. Song, M. Sawamura, T. Ito, A. Ido, and H. Ukeda, *Flavor Fragr. J.*, **15**, 323–328 (2000).
13. L. Mondello, A. Casilli, P. Q. Tranchida, L. Cicero, P. Dugo, and G. Dugo, *J. Agric. Food Chem.*, **51**, 5602–5606 (2003).
14. M-L. Lota, D. de R. Serra, F. Tomi, C. Jacquemond, and J. Casanova, *J. Agric. Food Chem.*, **50**, 796–805 (2002).
15. B. Steuer, H. Schulz, and E. Lager, *Food Chem.*, **72**, 113–117 (2001).
16. F. Buiarelli, G. P. Cartoni, F. Coccioli, and T. Leone, *J. Chromatogr. A.*, **730**(1-2), 9–16 (1996).
17. A. Verzera, A. Trozzi, A. Cotroneo, D. Lorenzo, and E. Dellacassa, *J. Agric. Food Chem.*, **48**, 2903–2909 (2000).
18. W. Feger, H. Brandauer, and H. Ziegler, *J. Essential Oil Res.*, **15**, 143–147 (2003).
19. T. Veriotti and R. Sacks, *Anal. Chem.*, **74**, 5635–5640 (2002).
20. N. T. M. Tu, L. X. Thanh, A. Une, H. Ukeda, and M. Sawamura, *Flavor Fragrance J.*, **17**, 169–174 (2002).
21. S. A. Vekiari, E. E. Protopadakis, P. Papadopoulou, D. Papanicolaou, C. Panou, and M. Vamvakias, *J. Agric. Food Chem.*, **50**, 147–153 (2001).
22. W. Feger, H. Brandauer, and H. Ziegler, *J. Essential Oil Res.*, **13**, 274–277 (2001).
23. Z. Shen, V. Mishra, B. Imison, M. Palmer, and R. Fairclough, *J. Agric. Food Chem.*, **50**, 154–160 (2002).
24. M. Sato, M. Boto, and T. Hirose, *Ind. Eng. Chem. Res.*, **34**, 3941–3946 (1995).
25. N. T. M. Tu, Y. Onishi, H-S. Choi, Y. Kondo, S. M. Bassore, H. Ukeda, and M. Sawamura, *J. Agric. Food Chem.*, **50**, 2908–2913 (2002).
26. M. G. Chisholm, M. A. Wilson, and G. M. Gaskey, *Flavor Fragrance J.*, **18**, 106–115 (2003).
27. H-S. Choi, Y. Kondo, and M. Sawamura, *J. Agric. Food Chem.*, **49**, 2404–2408 (2001).
28. D. Sun and P. D. Petracek, *J. Agric. Food Chem.*, **47**, 2067–2069 (1999).

29. A. Buettner, M. Montse, A. Fischer, J. Guasch, and P. Schieberle, *Eur. Food Res. Technol.*, **216**, 11–14 (2003).
30. D. Chouchi, and D. Barth, *J. Chromatogr. A.*, **672**(1-2), 177–183 (1994).
31. P. Dugo, L. Mondello, G. Dugo, D. M. Heaton, K. D. Bartle, A. A. Clifford, and P. Myers, *J. Agric. Food Chem.*, **44**, 3900–3905 (1996).
32. P. Stremple, *HRC-J. High Resolution Chromatogr.*, **21**, 587–591 (1998).
33. S. M. Njoroge, H. Ukeda, and M. Sawamura, *J. Agric. Food Chem.*, **51**, 4029–4035 (2003).
34. V. Andrea, N. Nedia, A. Andrea, and R. M. Teresa, *J. Agric. Food Chem.*, **51**, 4978–4983 (2003).
35. S. Nagy, “Lipids: Identification, Distribution, and Importance,” in *Citrus Science and Technology*, vol. 1, S. Nagy, P. E. Shaw, and M. K. Veldhuis, eds., The AVI Publishing Company, Westport, Connecticut, 1977, pp. 266–301.
36. M. A. Habib, M. A. Hammam, A. A. Sakr, and Y. A. Ashoush, *J. Amer. Oil Chem. Soc.*, **63**, 1192–1197 (1986).
37. J. L. Weihrauch and B. B. Teter, “Fruit and Vegetable By-product as Sources of Oil,” in *Technological Advances in Improved and Alternative Sources of Lipids*, B. S. Kamel and Y. Kakuda, eds., Blackie Academic & Professional, London, 1994, pp. 178–180.
38. K. Ajewole and A. Adeyeye, *Food Chem.*, **47**, 77–78 (1993).
39. M. Saidani, W. Dhifi, and B. Marzouk, *J. Food Lipids.*, **11**, 242–250 (2004).
40. A. Sattar, S. Mahmud, and S. A. Khan, *Pakistan J. Sci. Indust. Res.*, **31**, 743–744 (1988).
41. A. J. Hognadottir and R. L. Rouseff, *J. Chromatogr. A.*, **998**, 201–211 (2003).
42. M. G. Moshonas and P. E. Shaw, *J. Agric. Food Chem.*, **31**, 334–336 (1983).
43. S. M. Njoroge, H. Ukeda, and M. Sawamura, *J. Agric. Food Chem.*, **44**, 550–556 (1996).
44. H-S. Choi and M. Sawamura, *Biosci. Biotechnol. Biochem.*, **66**, 439–443 (2002).
45. P. Schieberle and W. Grosch, *Lebensm. Wissenschaft und Technologie*, **21**, 158–162 (1988).
46. I. Vargas, I. Sanz, P. Moya, and E. Prima-Yufer, *J. Food Prot.*, **62**, 929–932 (1999).
47. F. Shahidi, *Nahrung-Food.*, **44**, 158–163 (2000).
48. H-S. Choi, H. S. Song, H. Ukeda, and M. Sawamura, *J. Agric. Food Chem.*, **48**, 4156–4161 (2000).
49. H. S. Song, H. Ukeda, and M. Sawamura, *Food Sci. Technol. Res.*, **7**, 50–56 (2001).
50. A. Edris and B. Bergnstahl, *Nahrung-Food*, **45**, 133–137 (2001).
51. C. I. Beristain, E. Azuara, and E. J. V. Carter, *J. Food Sci.*, **67**, 206–211 (2002).
52. M. Charai, M. Faid, and A. Chaouch, *J. Essential Oil Res.*, **11**, 517–521 (1999).
53. A. G. Ponce, C. E. del Valle, and S. I. Roura, *Lebensmittel. Wissenschaft. und Technologie.*, **37**, 199–204 (2004).
54. M. Sawamura, S. H. Sun, K. Ozaki, J. Ishikawa, and H. Ukeda, *J. Agric. Food Chem.*, **47**, 4868–4872 (1999).
55. V. Calabrese, S. D. Randazzo, C. Catalano, and V. Rizza, *Drugs. Exp. Clin. Res.*, **25**, 219–225 (1999).
56. V. Calabrese, G. Scapagnini, S. D. Randazzo, G. Randazzo, C. Catalano, G. Geraci, and P. Morganti, *Drugs. Exp. Clin. Res.*, **25**, 281–287 (1999).
57. J. Chen, A. M. Montanari, and W. W. Widmer, *J. Agric. Food Chem.*, **45**, 364–368 (1997).

58. P. L. Crowell, *J. Nutr.*, **129**, 775–778 (1999).
59. Y. Takahashi, N. Inaba, S. Kuwahara, W. Kuki, K. Yamane, and A. Murakami, *J. Agric. Food Chem.*, **50**, 3193–3196 (2002).
60. T. P. Brown, P. C. Hill, and F. B. Bailie, *J. Int. Soc. Burn Injuries*, **25**, 678 (1999).
61. M. I. Carvalho-Freitas and M. Costa, *Biol. Pharm. Bull.*, **25**, 1629–1633 (2002).
62. H. C. Su, R. D. Speirs, and P. G. Mahany, *J. Econ. Entomol.*, **65**, 1438–1441 (1972).
63. E. A. Omer, A. A. Youssef, E. N. Abo-Zeid, and A. M. Sharaby, *Egyptian J. Horticul.*, **24**, 207–218 (1997).
64. T. Bruno, *Fitoterapia*, **71**, S29–S37 (2000).
65. A. A. Shalaby, K. A. Allam, A. A. Mostafa, and S. M. Fahmy, *J. Egypt. Soc. Parasitol.*, **28**, 595–606 (1998).
66. F. C. Ezeonu, G. I. Chidume, and S. C. Udedi, *Bioresour. Technol.*, **76**, 273–274 (2001).
67. D. R. Caccioni, M. Guizzardi, A. Renda, and G. Ruberto, *Int. J. Food Microbiol.*, **43**(1-2), 73–79 (1998).
68. M. E. Parish, D. Baum, R. Kryger, R. Goodrich, and R. Baum, *J. Food Protec.*, **66**, 1704–1707 (2003).
69. S. Kaddu, H. Kerl, and P. Wolf, *J. Amer. Acad. Dermatol.*, **45**, 458–461 (2001).
70. G. D. Bella, M. Saitta, S. Locurto, F. Salvo, G. Licandro, and G. Dugo, *J. Agric. Food Chem.*, **49**, 3705–3708 (2001).
71. G. D. Bella, M. Saitta, M. Pellegrino, F. Salvo, and G. Dugo, *J. Agric. Food Chem.*, **47**, 1009–1012 (1999).
72. G. D. Bella, M. Saitta, S. Locurto, V. L. Turco, A. Visco, and G. Dugo, *J. Agric. Food Chem.*, **48**, 4460–4462 (2000).
73. E. R. Weiss, J. Pika, and R. J. Braddock, *J. Agric. Food Chem.*, **51**, 2277–2282 (2003).
74. E. R. Weiss, R. J. Braddock, R. M. Goodrich, J. F. Gregory, and J. Pika, *J. Food Sci.*, **68**, 2146–2149 (2003).
75. S. Barrek, O. Paisse, M. Florence, and G. Loustalot, *Anal. Bioanal. Chem.*, **376**, 157–161 (2003).
76. M. Saitta, G. D. Bella, F. Salvo, S. Locurto, and G. Dugo, *J. Agric. Food Chem.*, **48**, 797–801 (2000).

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## *Gamma Linolenic Acid Oils*

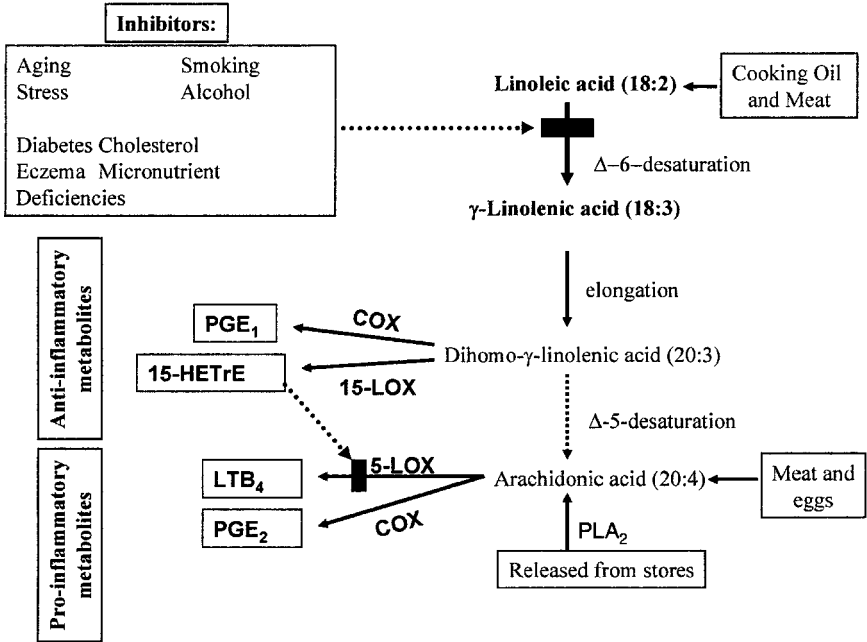
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### **1. INTRODUCTION**

Essential fatty acids (EFAs) can be defined by classic definition, which defines EFAs as the fatty acids that are required for proper functioning of cells, but the body cannot synthesize them and, therefore, must be supplied by diet. According to this definition, there are only two EFAs: linolenic acid (LA, C18:2, n-6) and alpha-linolenic acid (ALA, C18:3, n-3). The functional definition of EFAs includes the fatty acids that can correct the symptoms produced by elimination of all EFAs from the diet. According to this definition, LA, gamma linolenic acid (GLA, C18:3, n-6), and arachidonic acid (AA, C20:4, n-6) are EFAs of n-6 family (1, 2).

Gamma linolenic acid (*cis*-6, *cis*-9, *cis*-12-octadecatrienoic acid) is an 18-carbon polyunsaturated fatty acid containing three double bonds. It is produced in the body from desaturation of LA by the reaction catalyzed by enzyme delta-6-desaturase (D-6-D) (Figure 1). GLA is rapidly elongated to DGLA by elongase enzyme. Cats do not have this enzyme; hence, they cannot synthesize GLA and subsequent metabolites from LA (3). Therefore, cats must eat a meat-based diet to obtain longer chain metabolites of LA (DGLA, AA). DGLA can be acetylated and incorporated into membrane phospholipids. A small amount can be converted into AA, and this



**Figure 1.** Metabolic pathway for linoleic acid. COX, Cyclooxygenase, LOX, Lipoxygenase, PGE<sub>1</sub>, Prostaglandin E<sub>1</sub>, PGE<sub>2</sub>, Prostaglandin E<sub>2</sub>, PGL<sub>2</sub>, Prostaglandin I<sub>2</sub>, TXA<sub>2</sub>, Thromboxane A<sub>2</sub>, 15-HETE, 15-hydroxy eicosatrienoic acid, LTB<sub>4</sub>, Leukotriene B<sub>4</sub>.

reaction is catalyzed by delta-5-desaturase enzyme. Different animal species and different tissues differ in their capacity to convert DGLA to AA. Rat metabolizes DGLA to AA in significant amounts, whereas humans and other species have limited capacity to form AA from DGLA.

The reaction catalyzed by delta-6-desaturase enzyme is the slowest reaction in the metabolic pathway of LA and is considered as a rate-limiting step (4, 5). Activity of this enzyme further decreases with age and in people suffering from various diseases, including arthritis, diabetes, hypertension, eczema, psoriasis, and so on. Lifestyle factors like stress, smoking, excessive consumption of alcohol, linoleic acid (6), saturated and *trans*-fatty acids and nutritional deficiencies of Vitamin B6, zinc (7), and magnesium inhibit this desaturase. As a result of limitations in *in vivo* production of GLA, supplementation with preformed GLA is becoming important. This has led to interest in development and commercialization of the sources of GLA.

## 2. SOURCES OF GLA

GLA is present in small amounts in many plants belonging to the families Aceraceae, Boraginaceae, Cannabinaceae, Liliaceae, Onagraceae, Ranunculaceae,



Saxifragaceae, and Scrophulariaceae. Kleiman et al. (8) investigated 29 species of family Boraginaceae for the presence of GLA and tetraenoic (stearidonic acid, SDA) fatty acid. They observed 0–27% GLA, 0–56% ALA, and 0–17% SDA in seed oils from different plants in Boraginaceae. Janick et al. (9) identified 32 plants in which the content of GLA in seed oil can be more than 5% weight/weight (w/w) of total fatty acids (Table 1). The important crops that have been commercialized as sources of GLA-rich oils are discussed below.

**TABLE 1. Selected Plant Species High in Gamma-Linolenic Acid.**

Family Genus and Species	Oil Content of Seed (%)	GLA Content (%) of Oil	of Seed
<b>Boraginaceae</b>			
<i>Adelocaryum coelestinum</i>	22.0	12.0	2.7
<i>Alkanna orientalis</i>	23.0	12.0	2.8
<i>Anchusa azurea</i>	21.0	13.0	2.7
<i>Anchusa capensis</i>	29.0	10.0	2.9
<i>Anchusa hybrida</i>	20.0	13.0	2.6
<i>Borago officinalis</i>	28–38	17–25	5.0–8.4
<i>Brunnera orientalis</i>	27.0	15.0	4.2
<i>Cerintho minor</i>	10.0	10.0	1.0
<i>Cynoglossum amabile</i>	23.0	11.0	2.5
<i>Cynoglossum lanceolatum</i>	25.0	13.0	3.3
<i>Echium rubrum</i>	15.0	14.0	2.1
<i>Echium vulgare</i>	22.0	11.0	2.4
<i>Gastrocatyle hispida</i>	28.0	16.0	4.5
<i>Lithospermum arvense</i>	17.0	14.0	2.4
<i>Lithospermum purpureocaeruleum</i>	14.0	18.0	2.5
<i>Moltkia aurea</i>	10.0	10.0	1.0
<i>Moltkia coerulea</i>	10.0	11.0	1.1
<i>Nonea macrosperma</i>	39.0	13.0	5.1
<i>Onosma sericeum</i>	20.0	13.0	2.6
<i>Onosmodium molle</i>	17.0	20.0	3.4
<i>Onosmodium occidentale</i>	17.0	18.0	3.1
<i>Paracarum caelestinum</i>	21.0	12.0	2.5
<i>Pectocarpa platycarpa</i>	15.0	15.0	2.3
<i>Symphaticum officinale</i>	21.0	27.0	5.6
<b>Cannabaceae</b>			
<i>Cannabis sativa</i>	38.0	3–6	1.1–2.3
<b>Onagraceae</b>			
<i>Oenothera biennis</i>	17–25	7–10	1.2–2.5
<i>Oenothera grandifolia</i>	4.0	9.3	0.3
<b>Saxifragaceae</b>			
<i>Ribes alpinum</i>	19.0	9.0	1.7
<i>Ribes nigrum</i>	30.0	15–19	4.6–5.8
<i>Ribes rubrum</i>	25.0	4–6	1.0–1.5
<i>Ribes uva-crispa</i>	18.0	10–12	1.8–2.2
<b>Scrophulariaceae</b>			
<i>Scrophularia marilandica</i>	38.0	10.0	3.6

## 2.1. Borage (*Borago Officinalis* L.)

Borage is also known as star flower because of the shape of its flowers. Borage is the only member of the Boraginaceae family that is being grown commercially for its seeds at present. It is an annual herb native to Europe, Asia Minor, and North America. The morphology of this plant has been reviewed in many publications (9–11). It is an erect, hispid plant that can grow up to 100 cm in height. It has indeterminate vegetative growth habits that pose a significant challenge in commercial cultivation and harvesting of this plant. The plant has simple alternate leaves that are obovate, ovate, or oblong with an obtuse apex and create margin. The upper surface of leaves is dark-to-medium green, whereas the lower surface is light green. The stem is cylindrical, hollow, succulent, and occasionally susceptible to lodging. Stems, leaves, and calyx are covered with white, stiff, unicellular trichomes that can cause contact dermatitis to susceptible people. Flowers are bright blue, violet, pink, or white and are star shaped, hence, the other name for borage—star flower. Ovary is four lobed, and as the flower matures, it develops into 3–4 ovoid or oblong seeds, also known as nutlets. As they mature, they change color from green to brown to black and abscise rapidly. Flowering continues over a long period of time, and at any time, the plant bears the flowers and seeds in different stages of maturity. These factors pose a significant challenge in commercial production and harvesting.

Borage is mainly grown in the United Kingdom, Holland, Canada, New Zealand, and Poland, and it is estimated that about 95% of the world crop of borage is grown in these five countries. Borage is still not a major crop as its cultivation is very labor intensive. Most of the production of borage is under contract with oil producers with buy-back arrangement for seeds, and the production data regarding tonnage and acreage is not reported in any of the commercial publications. The acreage for borage grew consistently between 1980 and 1999 from a few hundred acres to about 7000 ha. There was an excellent harvest in 1999 in Canada, and the demand flattened leading to an over supply. This situation led to very limited production between 1999 and 2002 (unpublished information supplied by Bioriginal Food & Science Corp.). In 2002, the cultivation of borage increased again, but the severe drought in many of the borage-growing areas reduced the yield (unpublished information, Bioriginal Food & Science Corp.). Foliage is not used commercially any more, although in the folklore, it was used by early Europeans as a salad or vegetable and as a decoction for the treatment of various disease conditions.

As a result of seed drop and the continuous and long period of flowering and seed maturation, the seed yield is variable and a significant amount of potential crop is lost. The yields vary between 100 and 300 kg/ha in Canada and New Zealand and 300 and 500 kg/ha in the United Kingdom. The difference between Canada and Europe is probably caused by climatic conditions as borage prefers cool, moist conditions.

A limited amount of work has been done on optimization of cultivation, harvesting conditions, and plant variety development for borage and is reviewed by Clough (12) and Janik et al. (9) Because of the semiperennial nature of the crop and the seed drop, swathing and combining is the preferred harvest method. Simpson

(13) concluded from his studies that substantially higher yields could be obtained by swathng borage than by desiccating it with diquat or glyphosate. El Hafid et al. (14) studied the effect of seeding dates and nitrogen fertilization effects on borage in Alberta, Canada. In this case, early planting resulted in significantly higher seed yield and harvest index. Nitrogen fertility levels had no significant effect on the seed yield. The major objectives of borage variety development are to improve yield through development of varieties that are seed retentive on maturation and mature evenly. Other traits of interest include high oil content and high GLA content and morphological and yield stability. There is significant variability between the wild and cultivated borage accessions in terms of the amount of GLA and other fatty acid components as a percentage of oil indicating the possibility of developing lines with better quality parameters with additional breeding efforts (15). Hoffman La Roche has registered two varieties of borage (Tyreman and Spruce), but neither of these varieties are seed retentive. Bioriginal Food and Science Corp. has registered varieties of borage that have better seed retention compared with traditional borage.

From studies done on the *in vitro* propagation and somatic embryology (16–18) in borage, it appears that it is possible to produce GLA from cotyledonary somatic embryos. However, the authors observed that the system needs to be adapted to liquid media to facilitate large-scale production.

**2.1.1. Chemistry** The average weight of seed is between 16.1 and 24.5 mg (18). The oil content varies between 28% and 37% (w/w) and consists chiefly of linoleic (C18:2, 34–38%), GLA (C18:3, n-6; 16–27%), and oleic (C18:1, 14–18%) acids. The variation in GLA content is a result of geographical location, length of light period during growing season, average temperature, and diurnal temperature variations. There is no relationship between content of LA and GLA, although an inverse relationship is reported for content of 18:1 and GLA.

The average fatty acid composition of several seed lots over years, as provided by Bioriginal Food & Science Corp (unpublished data) and published information, is reported in Table 2. It has been observed that geographical location only affects the content of GLA and has no effect on the oil content of seeds, whereas plant density has no effect on the content of GLA (19). Oil also contains minor components that are characteristic of all vegetable oils and include sterols, tocopherols, and pigments. Borage oil contains about 650 ppm of  $\gamma$ - and 50 ppm of  $\delta$ -tocopherols (20). The content of unsaponifiable fraction is dependent on the method of extraction of oil and the degree of refining and varies between 0.8% and 1.5%. The relative proportion of different sterols in borage oil is independent of the method of refining. The major proportion of sterols in borage oil belongs to 4-desmethylsterols, although small amounts of 4-monomethyl and 4,4-dimethylsterols are also present. Campesterol and sitosterol constitute more than 50% of 4-desmethylsterols (21). Major sterols belonging to 4-monomethylsterol family include gramisterol, obtusifoliol, and citrostadienol (21).

Like other members of the Boraginaceae family, the Borage plant also contains pyrrolizidine alkaloids. Seven pyrrolizidine alkaloids have been identified so far in

**TABLE 2. Average Composition of Commercial Borage Crops.**

Crop Year	2002	1999	1998	1997	1996
Sample size	17	87	71	53	43
Moisture (%)	10.39	10.54	8.90	9.80	8.80
Oil (%)	31.16	31.40	30.60	30.70	32.40
Free Fatty acid	1.23	1.34	1.70	2.40	1.50
Fatty Acid Profile					
C16:0	9.9	10.6	10.2	10.3	10.4
C 16:1	0.3	0.3	0.2	0.2	0.2
C18:0	4.1	4.3	3.3	3.5	3.6
C18:1	17.7	17.6	14.8	14.7	14.3
C18:2	36.7	35.7	37.9	37.6	37.3
C18:3 (n-3)	0.3	0.2	0.2	0.2	0.2
C18:3 (n-6)	22.4	21.6	24.6	24.1	24.9
C18:4	0.2	0.2	0.2	0.1	0.2
C20:0	0.3	0.3	0.2	0.1	0.2
C20:1	4.1	4.1	3.9	3.9	3.9
C20:2	0.2	0.2	0.2	0.2	0.2
C22:0	0.2	0.2	0.1	0.1	0.1
C22:1	2.6	2.7	2.6	2.5	2.5
C24:1	1.7	1.7	1.5	1.5	1.5

borage leaves, flowers, and seeds. Thesinine, a saturated pyrrolizidine alkaloid, is the major alkaloid, and the six unsaturated pyrrolizidine alkaloids identified so far include amabiline, supinine, lycopsamine, interemedine, acetyllycopsamine, and acetylintermediate and are minor constituents. The total alkaloid content of the plant was reported to be less than 0.001%, whereas mature seeds yield about 0.03% crude alkaloids (22). Herrman et al. (23) reported the presence of thesesinine as a glycoside (Thesinine-4'-O- $\beta$ -D-glucoside) in seeds. As borage oil is an item of commerce for its content of GLA, there is a concern about the content of pyrrolizidine alkaloids in the oil. Published research to date could not detect the presence of pyrrolizidine alkaloids in borage oil. Dodson and Stermitz (22) used a method with a detection limit of 5 ppm, whereas Parvais and Stricht (24) employed a method with a detection limit of 0.1 ppm. These authors reported the absence of pyrrolizidine alkaloids in the oil samples tested. Mierendroff et al. (25) developed a method of detection of pyrrolizidine alkaloids at a limit of 4 ppb. Employing Mierendroff's method at an independent testing lab in Germany, Bioriginal tested several lots of borage oil over several years and could never detect any traces of pyrrolizidine alkaloids at such a low detection limit. The German Health Authority has limited the intake of unsaturated pyrrolizidine alkaloids to 1  $\mu$ g per day. Based on the above results, and assuming that borage oil contains PAs at a level of 4 ppb, one may have to consume more than 250 capsules (1000 mg each) every day to get a total of 1- $\mu$ g pyrrolizidine alkaloids. Based on this analysis, there is no likelihood of toxicity from pyrrolizidine alkaloids from borage oil ingestion.

## 2.2. Evening Primrose (*Oenothera biennis* L.)

Evening primrose of commerce consists of three species of family Onagraceae: *Oenothera biennis*, *O. lamarckiana*, and *O. parviflora*. It is native to North America and is now commonly found in many temperate zones around the world. Evening primrose thrives in open areas, especially in dunes and sandy soil. Evening primrose is grown commercially for its seed oil. China has become the major supplier of evening primrose seed and oil to world markets because it has the lowest cost of production and is estimated to supply about 90% of world supply of oil. Until 1985, it was mainly collected from wild growing plants; however, increase in demand justified the commercial production. In the wild, seeds exhibit physiological dormancy, whereby they may cause problems in cultivation as the plant density depends on the conditions after seeds are sown into the ground, of which the farmer has no control. Seeds may lay dormant for a long period of time (up to several years in the wild). Seeds can be forced by cold stratification. Germination is equal in light and dark. Plant also flowers over a period of 2–3 months, with 2–4 flowers at any time, and seed maturation takes place in pods that split when ripe and shatters seeds on the ground. New varieties of evening primrose have been developed over the last two decades that germinate within 3 weeks under test conditions and retain pods intact on the plant. Seeds are small, angular, and dark brown, and 1000 seeds may weigh between 0.2 and 0.6 g and each pod may contain up to 180 seeds. Each plant can have up to 250 pods.

Some of the species of evening primrose plant, including *O. biennis* and *O. lamarckiana*, are very unique and are challenging for their unique genetics. They do not obey Mendelian genetics and have been a subject of study for over 100 years. These species differ from common plants in that, during meiosis, the chromosomes do not pair up but form a circle by joining end to end. This prevents reshuffling of genes through translocation. The plants are self-pollinating, but inbreeding does not occur because of the presence of a set of lethal genes.

**2.2.1. Chemistry** Evening primrose plant is cultivated for its seeds that contain about 16% protein and 23–28% fat. The oil is rich in linoleic acid (70–75%) and gamma-linolenic acid (8–14%). The average fatty acid content of commercial evening primrose oils is given in Table 3. The varieties containing up to 14% GLA have been developed (26) but not yet commercialized on a large scale, as the majority of evening primrose oil in trade contains between 9% and 10.5% GLA. The oil contains about 98% triacylglycerols, small amounts of other lipids (free fatty acids, diacylglycerols), and 1–2% unsaponifiable matter consisting chiefly of sterols. Sterols contain about 90%  $\beta$ -sitosterol, 5% citrastadienol, 1.5% gramisterol, and 1% obtusifoliol. It also contains traces of  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherols (27). Evening primrose also contains traces of phenolic compounds consisting of catechin, (-)-epicatechin, and gallic acid (28).

**TABLE 3. Average Fatty Acid Profile of Oils of Evening Primrose, Black Currant, and Hemp.**

Fatty Acid	Evening Primrose Oil	Black Currant Oil	Hemp Oil
C16:0	5.98	7.10	5.60
C16:1	0.05	0.10	0.10
C18:0	1.84	1.50	2.60
C18:1	7.20	10.90	11.50
C18:2	73.87	45.20	56.60
C18:3 (n-3)	0.28	13.20	18.50
C18:3 (n-6)	9.74	16.90	1.60
C18:4	0.07	3.30	0.50
C20:0	0.30	0.10	0.90
C20:1	0.19	0.80	0.60
C22:0	0.10	0.10	0.30
C22:1		0.20	
C24:0	0.04		0.10

### 2.3. Black Currant

Black currant (*Ribes nigrum*) belongs to the family Saxifragaceae. The seeds are byproducts of the fruit processing industry that mainly uses black currant juice for jam, jellies, and cordial. In the early 1900s, cultivation of any *Ribes* species in the United States was prohibited by a federal ban, as they are the alternative host of white pine blister rust, a problem for all five-needle pines and the lumber industry. The ban was rescinded in 1966, but several states continue to ban the cultivation of *Ribes* species. Limited commercial acreage, the short harvest season (from mid-June until mid-July), and limited access to fruit for commercial processing has prohibited widespread distribution in North America. For oil extraction, seeds and pomace left as residue from food processing industry is used to extract the oil. Black currant oil contains about 13–17% GLA, 10–14% ALA, and 2–4% SA. The average composition of black currant oil is given in Table 3.

### 2.4. Hemp

Hemp (*Cannabis sativa* L., family Cannabinaceae) is a fast-growing annual herbaceous plant that is well suited to the temperate climates. The largest supply of the world hemp production comes from China and Eastern Europe. Hemp is mainly used for fiber, although there is a long history of food use. As a source of GLA, it has recently become popular. The hemp contains psychoactive substances (cannabinoids), because of which its trade was restricted. Fiber hemp that is traditionally grown for fiber contains less than 0.3% delta-9 tetrahydrocannabinol (THC) and is classified as low THC hemp. However, no THC is present in the seed or seed oil. Seed contains 30–35% oil, of which 2.5% to 3.6% is gamma-linolenic acid and 15–20% alpha-linolenic acid (29). The average composition of the oil is given in Table 3.

When grown as a seed crop, a considerably lower seeding rate of 15–30 kg/ha is used as compared with a fiber crop. As hemp is a dioecious crop with male and female plants, for adequate seed set, approximately 3–5% of the plants should be male to ensure pollination (30). A few popular French monoecious varieties are also available in the market. Seed yields can vary greatly depending on the variety used and growing conditions but averages around 500 kg/ha and can be as high as 1200 kg/ha. In the northern climates, soil temperature should be above 10°C when planting. Although, the once growing vigorously hemp is a good competitor and will suppress most weeds, early establishment is a must to reduce weed pressure. Pesticides are generally considered not necessary to grow hemp. Crop length is between 3 and 4 months, depending on variety. Breeding and development work on this crop has been limited, and the movement of certified seed is highly regulated and expensive. Traditional breeding has concentrated on developing varieties with superior fiber yield. Thus, there is potential to improve seed yields domestically through breeding programs.

## 2.5. Echium

Echium has not been commercialized to any significant level because of regulatory requirements for registration. The genus *Echium* contains about 30 species distributed across Europe, the Mediterranean region, Madeira, the Canaries, and the Azores. The plants grow in the wild and are cultivated in home gardens as flowering plants. *Echium plantagineum* L. has been discovered to contain significant amounts of GLA, ALA, and stearidonic acid in seed lipids. *Echium plantagineum* is also known by the common names of Purple Vipers Bugloss, Paterson's Curse, and Salvation Jane. Agricultural production of this species is largely limited to Eastern and parts of Western Europe at present. Trials with *Echium* production in Canada have had reasonable success.

*Echium plantagineum* is an erect biennial 20–60 cm high, softly hairy, with one or many flowering stems. The basal leaves are ovate with prominent lateral veins and soft appressed setae. The cauline leaves are oblong to lanceolate, the uppermost being more or less cordate at the base. Inflorescence is usually branched. Calyx is 7–10 mm at anthesis, and up to 15 mm in fruit. Corolla is 18–30 mm long, infundibuliform is blue becoming pink through purple, and is hairy on veins and margins only. Two stamens are exerted from corolla tube, the remaining stamens are included or only slightly exerted, and the stigmas are distinctly bifid.

As a crop, echium is a spring-sown annual with crop duration of 3 to 3.5 months. It requires warm, sunny conditions for quick establishment, which helps in terms of early weed competition. The best oil quality is maintained when crop-growing temperatures are around 25°C. Well-worked medium-textured soils are preferable with adequate moisture. Seed bed should be prepared to a firm, fine, moist tilth, and the seed should be planted 1–2 cm deep at the seeding rate of 3–5 kg/ha. Weed control is an issue, and as a minor crop, no herbicides are currently registered for this crop. Pre-emergence weed control might be necessary in weedy fields to reduce the weed pressure during establishment. No significant problems have been reported on this

crop yet. Yields are highly variable, however, and usually average 300 kg/ha. Very little breeding work has been done to date on this crop, and no varietal information is available at this time.

*Echium plantagineum* occurs over significant areas of farmland in Australia (31). The young growth is eaten readily by livestock. The plant is considered a weed in good pastures, whereas on the poor land, it is considered a reserve fodder (32). The level of pyrrolizidine alkaloids is normally between 0.1% and 0.3% of the dry weight of the whole plant, but levels as high as 0.9% have been reported (33). Field evidence strongly indicates that horses, pigs, and, to a lesser extent, sheep are all affected. They are mainly cultivated as ornamental flowers. However, John K. Kings and Sons, Ltd. and Croda started a program on commercial cultivation of *E. platigenium* because of the presence of GLA and stearidonic acid (SA) in the seeds (unpublished information).

Echium oil is mainly composed of  $\alpha$ -linolenic (30–33%), linoleic (14–18%),  $\gamma$ -linolenic (10–13%), stearidonic (13–15%), oleic (14–17%), and palmitic (6–7%) acids. Like other vegetable oils, echium oil contains between 0.6% and 1.8% unsaponifiable matter. The oil samples analyzed by Bioriginal Food & Science Corp. showed an average content of 0.91% total unsaponifiable matter, campesterol was 15.71%, beta-sitosterol was 12.53%, stigmasterol was 0.55%, and others were 33.52%. Tocopherols constituted 8.37% of total unsaponifiable matter and consisted of alpha- (0.53%), gamma- (6.92%), and delta-(0.92%) tocopherols. The fatty acid profile of echium oil is given in Table 3.

Echium seeds, like other members of family boraginaceae, contain pyrrolizidine alkaloids. The seeds contain echimidine as a major alkaloid and many minor alkaloids, including retronecine, lycopsamine, 7-acetyllycopsamine, and their derivatives.

### 3. EXTRACTION OF OIL

Of the above discussed sources of GLA, the commercial oils are mainly produced from borage and evening primrose. Black currant oil is limited as a result of availability of seeds for oil production, whereas hemp is still subject to trade restrictions in many countries because of the potential tetrahydrocannabinoid (THC) content. Echium is a new crop and has not been commercialized to any significant extent so far. All of these seeds are processed following general methods of oil extraction common in the vegetable oil industry as discussed here.

Borage, hemp, and black currant seeds have oil content in the range of 26–40% (on 8% moisture basis); they are best suited to mechanical pressing followed by solvent extraction. In North America, mechanically expressed borage oil is a major item of commerce, constituting about 80–90% of total sales of borage oil, whereas in Europe, solvent-extracted oil is the major item of commerce. Mechanically expressed oil is sold to a limited extent in the European markets, although it is gaining popularity. Evening primrose seeds are small and hard and contain 18–20% oil. Therefore, they are difficult to expeller press. Black currant seeds, being a byproduct



of the food industry, are usually associated with pomace and must be separated. The separation of pomace and seed can be done either by washing with alcohol or special cleaning processes. Washing with alcohol is not very common because of cost and environmental regulations.

### 3.1. Seed Cleaning

Seed cleaning is essential to protect the quality of the oil because seeds, as obtained from the farm, are contaminated with weed seeds, other grain seeds, and extraneous matter. These contaminating weed seeds may impart undesirable flavors and may negatively affect the stability of the oil. Seeds can be cleaned on the farm, but commercial seed-cleaning plants are used by most oil producers. The seed cleaning process involves aspiration of dust and lighter materials, followed by two-stage screening to remove larger and smaller sized particles. Cleaning is done to reduce the extraneous matter to less than 1.0%.

### 3.2. Expeller Pressing

The clean seed is stored in silos from where it is either conveyed to the screw-press or to a cooker, where they are conditioned by heating to about 50–90°C. The conditioning helps by improving the oil yield and inactivates the enzymes (lipase) that can affect the quality of the oil. Preheated seeds are conveyed to a continuous screw-press where they are crushed between a stationary cage (barrel) and rotating screw as they move forward. The pressure built up during their forward move causes oil to be released. The screw-press is similar to those used for other seeds, including canola and soybeans. The major manufacturers of these screw-presses include Anderson International, French Oil Mills, Krupp, and DeSmet. There are many smaller manufacturers of screw-presses whose equipment is better suited for small output plants common for GLA-containing oils. The oil is pumped to storage tanks and contains between 4% and 8% fines coming from the seed. The oil is clarified either by decantation or filtration. The press-cake so obtained contains between 12% and 18% oil and is either used as animal feed or subjected to solvent extraction to recover the remaining oil.

### 3.3. Solvent Extraction

The majority of evening primrose, black currant, and some borage seeds are extracted using this method. Prior to solvent extraction, the seed must be crushed or flaked to rupture the cell walls, enabling better extraction efficiency at a lower energy cost. The combination of expeller pressing and solvent extraction is a common practice for GLA-rich oils. The press-cake obtained from expeller press may be extruded or used as is. Flaking is commonly done for evening primrose seeds using a smooth roller mill. Food-grade hexane is the solvent of choice, although some work has been done replacing hexane with alcohol. Alcohol contains about 5% water. During the desolventization process, alcohol is removed first, leaving

water in the oil. Removal of water from the oil adds to the energy cost and adds additional steps in the processing, further complicating the process. The majority of the GLA oil producers are small and low-volume entities. They often use a batch process employing either percolation or immersion. Continuous process is also used but poses processing challenges in that the plant has to be optimized to produce different oils, as processing one type of seed will not be able to sustain the plant because of low-volume requirements. In either process, the cake or flaked seeds first come in contact with a solvent rich in oil (miscella) followed by an oil-poor solvent and the last stage, with a pure solvent.

### **3.4. Supercritical Extraction**

Supercritical extraction using carbon dioxide under high pressure is also becoming popular for GLA-rich oils; however, it is not being used to any major extent because of the high cost of the plant and the oil obtained by this technology. Some work is being done on optimization with respect to operating conditions (34). Major emphasis is on the flow rate of carbon dioxide, pressure, and temperature to optimize the yield. The particle size of the seed pieces and the moisture content also play a role in extraction efficiency. It is reported that the lower the moisture content, the better the yield. The supercritical extraction usually results in an oil with similar fatty acid composition when compared with solvent extracted oil, but the oil is low in sterol content and may be more prone to oxidation. The biggest advantage of supercritical extraction is that it eliminates the need for further processing of oil such as distillation for desolventization, degumming, and so on.

### **3.5. Desolventization**

The oil-solvent mixture and the meal is stripped of the solvent to recover solvent-free oil and meal. The solvent-enriched meal is conveyed to vertical desolventizer where heat and vacuum facilitate removal of solvent vapors. Desolventizer contains trays with sweeping arms to agitate the meal for improved efficiency. Some plants purge the cake with steam to remove the solvent, whereas others use hot air, although application of vacuum is most common. The solvent oil miscella are stripped of solvent in a three-stage evaporator. The hexane is reused for the extraction of oil.

### **3.6. Further Processing**

The desolventized as well as expeller-pressed oil is further processed to reduce/remove the pigments and phosphatides (gums). Crude oils may contain 1–2% phosphatides, which are removed by the degumming process. The degumming process is similar to that employed in the vegetable oil industry and uses water, citric acid, phosphoric acid or a combination of acid, and water. After the oil is contacted with these agents, the phosphatides settle as sludge and are removed by either filtration or centrifugation.

The water degumming process involves addition of about 2% water to the oil, intensive mixing under vacuum at 80°C for 10–30 min, and filtration/centrifugation. Water degumming removes most of the hydratable phosphatides, leaving behind between 50 and 200 mg/kg of phosphorous depending on the extraction conditions employed. Acid degumming using a combination of citric acid or phosphoric acid with water also removes nonhydratable phosphatides. In this process, oil is heated to 60–80°C, and 0.1–0.4% citric acid or phosphoric acid is added with intense mixing for 1–5 min. To this mixture, 2% water is added and mixing is continued for 30–60 min. After contact with water, the oil is clarified of the precipitated gums by centrifugation or filtration using clays.

Degummed oil is further purified by physical or chemical refining. Alkali refining is rarely used in oil for the health food/dietary supplement industry, although it may be used for oil for cosmetic/pharmaceutical applications. In processing of oil for the health food/dietary supplement industry, water degumming and bleaching processes may be combined when the oil is heated with water and citric or phosphoric acid with activated bleaching clay to 80°C in a vacuum reactor. The mixture is intensely agitated under vacuum for 30–60 min. During this time, the gums are precipitated and are adsorbed onto the bleaching clay along with pigments and chloroplasts. The oil mixture is cooled and filtered to remove gums, and pigments. The resulting bleached oil has a lighter color and a phosphorous content of less than 50 ppm. As these oils also contain wax esters and other compounds that may settle with time at room temperature and are collectively called waxes, they are subjected to the winterization process where they are chilled to 4°C and filtered to remove the waxes. The oil for the dietary supplement industry is not winterized. Finally, the oil may be subjected to steam stripping (deodorization). In this process, the oil is steam distilled to remove free fatty acids and other volatile impurities. This is the last process in the refining of oils, and the oil is then packed in drums or totes under nitrogen atmosphere. The oil must be stored in a cool dry place, tightly packed in the container under nitrogen atmosphere to protect against oxidation.

### 3.7. Quality Control

The GLA-containing oils are used for nutritional and health-promoting or disease-preventive actions. They must be of high quality and free from contaminants. The quality of oil is dependent on many factors, including seed quality and purity, herbicide and pesticide residues in seed, processing and storage conditions for seed and oil, and so on. Improper storage and drying of seeds can raise the free fatty acid levels in seed that can result in off flavors in the oil. Being a polyunsaturated fatty acid, GLA is prone to oxidation. The oxidation process for GLA-rich oils involves addition of an oxygen atom at the double bond in unsaturated fatty acids leading to formation of hydroperoxides. These hydroperoxides are unstable and decompose to form aldehydes and ketones. These oxidation products not only impart off flavors to the oil, making it unacceptable organoleptically, but also may have adverse health effects. The quality of the oil is tested by checking for peroxide value, an indicator of primary oxidation product. Oil with a peroxide value of less than

**TABLE 4. General Specifications of GLA-Rich Oils.**

Parameter	Units	Value		
Peroxide Value	meq/kg	<5		
Anisidine Value		<15		
Acid Value (unrefined oils)	mg KOH/g	<4		
Acid Value (refined oils)	mg KOH/g	<0.7		
Unsaponifiable matter	%	<2		
Pesticides/Herbicides	mg/kg	<0.05		
Solvent residues	ppm	<1.0		
Color (Lovibond 1 inch)		<3 red		
Heavy metals	ppm	<10		
Lead	ppm	<0.1		
Mercury	ppm	<0.1		
Cadmium	ppm	<0.1		
Arsenic	ppm	<0.1		

Major Fatty Acid (% of total fatty acids)	Evening		
	Primrose Oil	Borage Oil	Black Currant Oil
Oleic acid (C18:1)	6–9%	14–22%	9–15%
Linoleic acid (C18:2)	70–77%	32–38%	40–50%
Gamma Linolenic acid (C18:3, n-6)	8–12%	18–25%	15–19%
Alpha Linolenic acid (C18:3, n-3)	0.1–1.0%	0.1–2.0%	12–15%
Stearidonic acid (C18:4)	0.1–0.3%	0.1–0.3%	2–5%

10 milliequivalent of KOH/kg oil is considered good for consumption. Peroxide value alone is not a good indicator of oxidative stability of oil as it measures the primary oxidation products, which degrade to secondary oxidation product, including aldehydes and ketones. These secondary oxidation products can be measured by several methods, including conjugated dienes, anisidine value, and so on. In addition to oxidative stability indices, the oils are tested for fatty acid profile to ensure the quality and purity of the oil. The presence of free fatty acids is tested by acid value. The free fatty acid content of the oil should be as low as possible. These oils are also tested for heavy metal contamination. The total content of heavy metal should be less than 10 ppm. The oils should be free of any herbicide or pesticide residues and the solvent used in extraction of oil. The quality parameters for these oils are listed in Table 4.

#### 4. METABOLISM OF GLA

When GLA-rich oils are taken orally, GLA is rapidly absorbed. It first appears in serum phospholipids, and with continuous administration, it is distributed in other phospholipid fractions. Part of absorbed GLA is oxidized, and the rest is taken up by various tissues/cells and is rapidly elongated to dihomogammalinolenic acid (DGLA) (Figure 1). The oxidation rate of GLA was found to be 28% of that for

LA (35). DGLA can be acetylated and incorporated into membrane phospholipids, or it can be desaturated to AA by delta-5-desaturase. DGLA competes with AA for cyclooxygenase (COX) and lipoxygenase (LOX) enzymes. DGLA produced prostaglandins of series 1 (PGE<sub>1</sub>) and thromboxane A<sub>1</sub> (TxA<sub>1</sub>) by the action of COX. These products of COX action exert anti-inflammatory, vasodilatory, and anti-aggregatory actions. DGLA produces 15-hydroxyeicosatrienoic acid (15-HETrE) by the action of 15-lipoxygenase. 15-HETrE is a strong inhibitor of 5-lipoxygenase, whereby it inhibits production of leukotriene B<sub>4</sub> (LTB<sub>4</sub>) from inflammatory cells, including neutrophils (36).

Hassam et al. (37) were the first to study the absorption and metabolism of GLA using <sup>14</sup>C-labeled GLA in rats. They observed accumulation of labeled DGLA and AA in brain and liver after 22 hours of administration, suggesting that GLA is rapidly metabolized to DGLA and AA. Leyton et al. (35) reported that DGLA is preferentially incorporated in liver phosphoacylglycerols, mainly in choline and inositol phosphoacylglycerols. Feeding a GLA-rich diet to rats caused accumulation of DGLA in milk (38) and a rise in DGLA and AA in aorta and platelets (39) and in immune cells, including macrophages, kupfer cells, and endothelial cells (40, 41). Barre et al. (42) observed a rise in GLA and DGLA with no change in AA levels in different platelet phospholipid fractions in human volunteers following daily administration of 5.23-g GLA from borage oil for 42 days. There was a differential distribution of DGLA in various phospholipid fractions with phosphatidylcholine had maximal (67.6%) followed by phosphatidylethanolamine (16.7%), phosphatidylserine (12.9%), and phosphatidylinositol (2.6%). There was no change in sphingomyelin. In all phospholipid fractions, the ratio of DGLA/AA decreased significantly. In a later study, they observed a rise in GLA and DGLA levels in phosphatidylcholine fraction of plasma HDL and cholesteryl esters. AA levels increased only in phosphatidylcholine fraction of HDL (43). In these studies, the dose of GLA employed is much higher than used in any of the clinical trials. The difference in the observed rise in platelet AA levels after feeding of GLA sources in above studies is caused by species difference. Rat platelets have delta-5-desaturase enzyme required for conversion of DGLA to AA, whereas human platelets lack this enzyme and obtain preformed AA from the circulation.

In one study on six healthy volunteers, time of administration of GLA-rich oil was found to affect the peak serum levels of GLA (44). Administration of evening primrose oil (equivalent to 240-mg GLA) in the evening caused a rapid peak in serum levels (2.7 ± 1.2 hours) compared with administration in the morning (4.4 ± 1.9 hours). There was a small but insignificant increase in serum DGLA and AA levels. In this study, the second dose of evening primrose oil was given 12 hours after the morning dose. This might have contributed to the observed rapid rise in peak serum GLA levels, or this could reflect faster absorption of GLA in the evening. A rapid rise in plasma triacylglycerols level after the second meal has been observed. Manku et al. (45) studied the effect of feeding evening primrose oil (containing GLA) for a period of 10 days to 12 weeks on plasma fatty acid levels. In this study, they collected the blood samples of 392 individuals who were part of 20 different studies. In all of these studies, DGLA levels in plasma phospholipids were

increased significantly. In 17 of these studies, there was a small but significant rise in phospholipid AA levels, whereas in 3 studies, there was no rise, and in 2 of these studies, there was a fall in AA levels. In these 3 studies, EPO was administered for 10 days only. In all of the studies, the ratio of AA/DGLA fell, suggesting a greater rise in DGLA levels. These results indicate that, in humans, DGLA is slowly desaturated to AA. Feeding borage oil for 7 weeks to normotensive (WKY) and spontaneously hypertensive (SHR) rats resulted in an increase in GLA and DGLA levels in plasma, liver, aorta, and renal artery in both strains of rats, although AA was increased only in plasma and liver (46). These observations indicate that there is a tissue-specific rise in AA after administration of GLA.

From the above discussion, it is clear that GLA is rapidly absorbed and elongated to DGLA. DGLA levels increase in most of the tissues after GLA administration, but the levels of AA rise to a smaller extent mainly in the liver. The capacity of other tissues to desaturate DGLA is limited and depends on the species. Chilton et al. (47) studied the effect of in-vivo administration of GLA and in vitro incubation of human neutrophils with GLA on metabolism of GLA. They observed that in vivo administration of GLA to humans caused an increase in DGLA in the neutrophils and no GLA was detected. Incubation of neutrophils with GLA resulted in a rise in the DGLA concentration of neutrophils. Stimulation of these neutrophils with ionophore A23187 caused a release of AA and DGLA from neutrophil phospholipids. DGLA was metabolized to 15-HETRe that inhibited  $LTB_4$  production with an  $IC_{50}$  of 5  $\mu$ M.

#### 4.1. Effect of Triacylglycerol Structure on Bioavailability of GLA

Major sources of GLA include borage oil, evening primrose oil, and fungal oils. GLA is mainly distributed at *sn*-2 position in triacylglycerols in borage oil, at *sn*-3 position in black currant and evening primrose oils, and at *sn*-1 and *sn*-3 positions in fungal oils (48). Evening primrose oil was reported to provide higher levels of GLA compared with borage oil in rats, although the latter oil contains a higher amount of GLA/g. This was a surprising finding and attributed to positional differences for GLA in the triacylglycerol structure and the inability of gastric and pancreatic lipases to hydrolyze fatty acids at *sn*-2 position. The fatty acid in the *sn*-2 position of triacylglycerols is preferentially absorbed as the 2-monoacylglycerol and serves as the template for re-esterification by intestinal cells to reform triacylglycerols. The *sn*-2 fatty acids are also preferentially preserved as components of chylomicrons and very-low-density lipoprotein particles for ultimate incorporation in tissue membranes. Subsequently, Raederstroff and Moser (49) repeated the studies in rats and failed to reproduce similar results. They observed that the levels of GLA and DGLA in liver, aortic, and erythrocyte phospholipids reflected the amount of GLA present in the source oil. This indicated that different oils were well absorbed and that the amount of GLA absorbed was dose dependent, and the source of GLA did not matter. To further resolve this matter, Chung et al. (50) studied the efficacy of borage oil, evening primrose oil, or a combination of borage oil with

safflower oil to match the GLA content to evening primrose oil in reversing the epidermal hyperproliferation induced by essential fatty acid deficient diets. In this study, they observed that GLA-rich diets reversed epidermal hyperproliferation caused by essential fatty acid deficiency and the potency order was borage oil greater than borage-safflower oil combination, greater than evening primrose oil. Finally, two diets had similar amounts of GLA, but they differed in the structural location of GLA on triacylglycerol molecule. There were higher levels of DGLA in epidermal phospholipids and ceramides on the borage oil or the borage oil and safflower oil diet than from the evening primrose oil diet. They proposed that borage oil, being richest in GLA at *sn*-2 position, is more bioavailable; hence, borage oil was more potent. Higher bioavailability of GLA at *sn*-2 position is also supported by data from other laboratories studying the effect of triacylglycerol structure on fat digestion and absorption. During digestion, gastric and pancreatic lipases hydrolyze fatty acids at *sn*-1 and *sn*-3 position forming free fatty acids and *sn*-2 monoacylglycerols. The absorption of free fatty acids is reduced in the presence of divalent ions (calcium and magnesium) because of soap formation, whereas *sn*-2 monoacylglycerols are favorably absorbed. The differences in the results obtained by these two groups [Chung et al. (50) and Raederstroff and Moser (49)] could be caused by species differences, or by a difference in study design, as Chung et al. (50) performed the studies in the essential-fatty-acid-deficient guinea pigs, or by tissue differences.

## 5. CARDIOVASCULAR EFFECTS

Cardiovascular disease is a major cause of mortality and morbidity in industrialized countries. Several risk factors have been linked to incidence of cardiovascular disease and include hypertension, lipid abnormalities (high plasma cholesterol and triacylglycerol levels), atherosclerosis, obesity, diabetes, smoking, stress, heredity, and diet. Dietary GLA affects many of these parameters and is discussed below.

### 5.1. Effect on Blood Pressure

Arterial blood pressure is regulated by the interaction of cardiac output and peripheral vascular resistance. Several factors can influence these interactions, and they can include renin-angiotensin system, local metabolic factors, stress hormones, and so on. Interventions that interfere with these modulators can affect the blood pressure regulation. In 1975, Rose et al. (51) observed a biphasic response of intravenously administered DGLA on systemic arterial pressure in dogs that was characterized by an initial fall in blood pressure followed by a sustained fall and an increase in myocardial contractility. Only the sustained fall in blood pressure was blocked by cyclooxygenase inhibition, whereas the early fall in blood pressure and positive inotropic effects were not affected, suggesting that DGLA causes a blood pressure-lowering effect directly and through PGE<sub>1</sub> pathways. In 1982, evening primrose oil was shown to inhibit the blood pressure-increasing activity of

intravenously administered renin and angiotensin II in rats given evening primrose oil for 3 months (52). This observation suggested that GLA-rich oils may reduce the blood pressure by interfering with the renin-angiotensin system in the body. GLA-inhibited isolation (psychological) stress-induced rise in blood pressure in rats when administered at a dose of 0.018 or 0.04 mg/hour via an osmotic pump (53). In the unstressed rats, there was no effect of GLA on blood pressure. No effect on heart rate, heart weight, or adrenal weight was observed in any animal. Mills et al. (54) repeated the experiments on humans to observe if GLA has similar actions on stress reactivity and performance. They selected 30 normotensive male university students for the study and divided into various groups. One group ( $n = 10$ ) received olive oil capsules for 28 days, and another group received borage oil capsules ( $n = 10$ ) providing 1.3-g GLA per day. These volunteers were given Stroop color word conflict test before commencement of supplement therapy and after 28 days of supplementation. Borage oil supplementation significantly reduced the stress-induced rise in systolic blood pressure and heart rate and did not affect diastolic blood pressure or plasma norepinephrine levels. Borage oil treatment increased the skin temperature and the performance as compared by number of correct responses. These data confirm the observations obtained earlier in rats and indicate increased tissue perfusion by borage oil treatment. Leng et al. (55) also observed a blood pressure-lowering effect in patients with peripheral arterial diseases. In their study, they used a combination of GLA with EPA, so the probable contribution of EPA to blood pressure-lowering effect cannot be entirely ruled out.

The exact mechanism of blood pressure-lowering effect is not very clear, and GLA-rich oils appear to act via several mechanisms. Borage (56) and evening primrose oils (57) were shown to reduce *in vivo* pressor responses to angiotensin-II and norepinephrine without affecting *in vitro* contractile response of aorta to potassium chloride and serotonin in rats. These observations suggest that GLA may be interfering with agonist-receptor interactions without affecting the contractility of vascular smooth muscles. Subsequent studies in spontaneously hypertensive rats demonstrated the blood pressure-lowering effect of borage oil (58) without affecting the pressor response to angiotensin and norepinephrine, suggesting the role of other mechanisms. These findings suggest that there may be a species difference in responsiveness to angiotensin II and norepinephrine, although the blood pressure-lowering effect was similar in magnitude. GLA was shown to prevent development of hypertension in SHR rats (59), which could have been mediated via the cyclooxygenase pathway as an increase in aortic levels of 6-keto  $\text{PGF}_{1\alpha}$  was observed. In hypertensive rats, GLA was shown to significantly reduce the ratio of plasma aldosterone to renin that was caused by a insignificant decrease in plasma aldosterone levels and a small increase in plasma renin activity (60). There was no effect of borage oil treatment on plasma cortisol levels compared with rats fed control diet free of GLA. Borage oil treatments also reduced angiotensin receptor number and affinity in SHR rats, suggesting a reduction in the responsiveness of adrenal glomerulosa cells to angiotensin and interference with the renin-angiotensin-aldosterone axis might contribute to the hypotensive effects. These studies cannot alienate the exact mechanism by which borage oil interferes with angiotensin receptors.



Mills et al. (61) studied the effects of dietary borage oil on baroreflexes in normotensive, healthy males. These males were subjected to lower body negative pressure of  $-10$  and  $-40$  mm Hg. A negative pressure of  $-10$  mm Hg unloads cardiopulmonary baroreceptors, whereas the negative pressure of  $-40$  mm Hg unloads both cardiopulmonary and arterial baroreceptors. They observed that borage oil treatment augmented the baroreflex response to  $-40$  mm Hg without affecting the response to  $-10$ -mm Hg negative pressure, suggesting that GLA may be affecting only high-pressure arterial baroreflex responses. This could be mediated either by altering the sensitivity of baroreceptor stimulus-response relationship or by shifting the operating point of the reflex to a much steeper point on the baroreceptor stimulus-response relationship curve. In human hypertension, baroreceptor responses are decreased, which may be contributing to structural changes in hypertensive patients.

## 5.2. Platelet Function and Plasma Lipids

Increased levels of plasma triacylglycerols and cholesterol and platelet dysfunction (increased aggregation) are independent risk factors for cardiovascular disease. The effects of GLA on blood lipids and platelet function are controversial. Chaintreuil et al. observed a fall in serum triacylglycerols and cholesterol levels in insulin-dependent diabetic patients administered 2 g/day GLA, but not with 500 mg daily dose for 6 weeks (62, 63). In hypertriglyceridemic patients, GLA had no effect on plasma triacylglycerol levels or platelet function, although there was an increase in GLA and DGLA levels in plasma and platelet phospholipids (64). Viikari et al. (65) also failed to observe the lipid-lowering effect of evening primrose oil in hyperlipidemic subjects in an open study. They continued administration of evening primrose oil for 3 months but changed the dose every month from 2.4 ml (first month) to 7.2 ml (third month). They observed a rise in GLA levels in serum cholesteryl esters, phospholipids, and triacylglycerols. The differences in the results of above studies could be attributed to dose differences. Guivernau et al. (66) fed GLA at a dose of 240 mg/day for 12 weeks to 12 hypertriacylglycerolmic patients and 12 rats. They observed a significant decrease in plasma triacylglycerols, total cholesterol, and LDL cholesterol and an increase in HDL-cholesterol. Reactivity of platelets to low doses of adenosine diphosphate and epinephrine was significantly reduced. A reduction in plasma thromboxane  $B_2$  levels was also observed in humans. In rats, a rise in plasma 6-keto-PGF $_{1\alpha}$  levels was observed, suggesting an increase in PGE $_1$  production by GLA administration. Changes in eicosanoids may contribute to the observed effects of GLA on platelet aggregation as thromboxane  $B_2$  is a potent platelet aggregator. GLA is rapidly metabolized to DGLA, and DGLA has been shown to inhibit platelet aggregation in *in vitro* (67) and *in vivo* studies (68, 69).

Ishikawa et al. (70), in a double-blind, cross-over trial in hypercholesterolemic patients, demonstrated that GLA lowered low-density lipoprotein cholesterol and apolipoprotein B in plasma and increased HDLC levels without affecting the levels of total cholesterol. Jantti et al. (71) observed a decrease in plasma

apolipoprotein B concentrations in rheumatoid arthritis patients given evening primrose oil at a dose of 20 ml (about 1.8 g GLA) per day for 12 weeks. In this trial, no effect on plasma triacylglycerols or total or high-density lipoprotein cholesterol was observed. Horrobin and Manku (72) found that evening primrose oil exerted cholesterol-lowering effects in people with plasma cholesterol levels above 5 mmol/l but had no effect in people having plasma cholesterol levels lower than 5 mmol/l. Fukushima et al. (73) fed conventional diets enriched with 10% borage oil, palm oil, perilla oil, evening primrose oil or mixed oils, and 0.5% cholesterol for 15-week to 8-week-old rats. GLA-rich diets lowered plasma total cholesterol and the sum of LDL, IDL, and VLDL cholesterol. Cholesterol-lowering effects of a GLA-rich diet could be mediated by changes in membrane lipid composition affecting absorption of cholesterol. This observation is confirmed by Koba et al. (74) in Cacao cells. When these cells were incubated with GLA, the absorption of cholesterol from the growth medium was attenuated and the cell membranes were enriched with GLA, DGLA, and AA.

### 5.3. Atherosclerosis

Atherosclerosis is the most common cause of morbidity and mortality in patients with cardiovascular diseases. The exact cause of atherosclerosis is not clear. Atherosclerosis is a culmination of several events, including vascular dysfunction, which may be caused by an injury to vasculature, recruitment of inflammatory cells including monocytes and neutrophils, activation of macrophages, vascular smooth muscle cell proliferation, deposition of lipids, and synthesis of extracellular matrix. Oxidized low-density lipoprotein cholesterol plays a role in initiation of atherogenesis. It stimulates monocytes with the resultant formation of foam cells. These cells release mediators that stimulate expression of adhesion molecules like cadherin, vcams, and so on. Macrophages, on stimulation, release eicosanoids and cytokines that may stimulate proliferation of vascular smooth muscle cells. Proliferation of vascular smooth muscle cells appears to be a central event in atherogenesis. Essential fatty acids are substrate for the production of eicosanoids, and the membrane composition of inflammatory cells reflects dietary intake of various fatty acids. It appears that dietary manipulation of the composition of cell membranes is the easiest target to control atherogenesis. Renaud et al. (75) demonstrated that dietary polyunsaturated fatty acids, including GLA, reduced severity of atherosclerotic lesion in rabbits compared with saturated-fatty-acid-rich diets. In Japanese quail, dietary primrose oil was shown to inhibit atherogenesis (76). Fan et al. (77) observed inhibitory action of dietary evening primrose oil either alone and in combination with fish oil on aortic smooth muscle cell proliferative action of peritoneal macrophages from mice. The inhibitory action appeared to be mediated through cyclooxygenase pathway as indomethacin (cyclooxygenase inhibitor)-inhibited PGE<sub>1</sub> release and antiproliferative actions. Addition of 5-lipoxygenase inhibitor to the culture medium had no effect on antiproliferative or DNA synthesis inhibitory actions of primrose oil. In vitro incubation of endothelial cells with PUFAs, including GLA, AA, ALA, EPA, or DHA, stimulated the oxidation of LDL and

metabolism of oxidized LDL by macrophages (78). These interventions also increased the release of superoxide anions by endothelial cells. These observations suggest pro-atherosclerotic actions of PUFAs in humans. In apolipoprotein E knockout mice, evening primrose oil inhibited aortic smooth muscle cell proliferation and reduced the aortic vessel wall medial layer thickness and the size of atherosclerotic lesion (79). This study confirms the beneficial effects of GLA in lowering cardiovascular risks by inhibiting atherosclerotic plaque development.

#### 5.4. Cardiac Arrhythmia

Several studies have demonstrated that LA (present in vegetable oils) exert antiarrhythmic activity in several models, including ischemic-reperfusion injury and catechol-induced arrhythmias. Li et al. (80) observed that PGE<sub>1</sub> and PGI<sub>2</sub> exert antiarrhythmic activity in cultured, spontaneously beating neonatal rat cardiac myocytes, while PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2 $\alpha$</sub> , and TXA<sub>2</sub> exert proarrhythmic activity. Charnock et al. (81) studied the effects of evening primrose oil and black currant oil on ventricular fibrillation in rats induced by ischemia. They compared the effects of these two oils to sunflower oil (a source of LA) and sheep fat (saturated fat). They observed that, compared with the saturated fat group, all other dietary treatments significantly reduced the number of premature ventricular beats, however, there was no difference between the three PUFA groups. The effect on duration of ventricular fibrillation was dependent on diet with saturated fat showing the longest duration that was significantly reduced by the other three oils and the potency order of these three oils was sunflower oil < evening primrose oil < black currant oil. Evening primrose oil contains similar amounts of LA but additional amounts of GLA, suggesting GLA might have been playing an additional protective role. As black currant oil contains twice the amount of GLA and additional ALA, it is difficult to assess from this study if additional protection provided by black currant oil was caused by high amounts of GLA or synergistic action of GLA and ALA.

### 6. CANCER

Cancer is a collective term that defines a group of conditions caused by excessive growth of cells in any organ/tissue. It can occur in any part of the body. It is a complex phenomenon, the etiology of which is not very well understood. Risk of cancer increases with age, and about 77% of cancers are diagnosed in people after 55 years of age. Risk factors for cancer include lifestyle factors (diet, tobacco, excessive alcohol use, and physical inactivity), radiations, chemicals, infections, heredity (inherited mutations), immune conditions, obesity, and hormones. Heredity increases the predisposition to cancer but in itself is not responsible for initiation of cancer and requires interaction with other factors. About 5–10% of total cancers are hereditary because of inheritance of mutated gene. According to the American Cancer Society (<http://www.cancer.org/downloads/PRO/12>), about 1.37 million new cases of cancer are expected to be diagnosed in 2004. This estimate excludes

basal and squamous cell carcinoma of skin and carcinoma in situ of any site except urinary bladder. About 563,700 people are expected to die from cancer in the United States in 2004. Of these deaths, 170,000 deaths will be related to tobacco, and a similar number of deaths will be caused by nutrition, obesity, physical inactivity, and other lifestyle factors.

Basic treatment for cancer includes chemotherapy, radiations, and surgery. Strategies for prevention include modification of lifestyle factors and dietary interventions. The role of dietary fat in cancer is controversial. Many prospective studies found an increase in cancer risk (82–84), whereas others reported no association between fat intake and cancer (85–87).

GLA has been studied in several studies for its effects on various cancer cell lines in vitro. It has been observed to exert cytotoxic activities against several tumor cell lines in vitro and tumor implants in experimental animal models. There are limited studies on the effect of GLA on tumors in humans. In cell lines, the effect of GLA appears to depend on the cell line, dose, and incubation time. In a study by Dippenaar et al. (88), GLA caused significant (up to 70%) growth inhibitory effects on mouse BL6 melanoma cells in vitro at a dose of 20  $\mu\text{g/ml}$ . At this dose, GLA did not affect the growth of normal bovine kidney epithelial MDBK cells, suggesting that GLA acts as an anticancer agent and inhibits the growth of cancer cells without affecting the normal cells. Human hepatoma cell lines differ in sensitivity to GLA as they require continuous presence of GLA in culture media for 4 days to observe growth inhibitory effects (88); withdrawal of GLA from the growth media after 5-day treatment suppressed the growth for 5 more days (89). This observation suggests that cancer cells may lack delta-6-desaturase and, hence, cannot make GLA and, subsequently, DGLA. Cancer cells incorporate GLA and DGLA in their cell membranes and DGLA may be acting via a cyclooxygenase pathway in inhibiting cancer cell growth as PGE<sub>1</sub> stimulates cyclic-AMP formation and induces cell death in cancer cell lines (90). In 1985, Begin et al. (91) confirmed that GLA has growth inhibitory actions against human prostate, breast, and lung cancer cells with no effect on normal cells.

Experiments were conducted to study the effects of GLA treatment on carcinogen-induced cancers in animals. Lee and Sugano (92) failed to observe any tumor inhibitory action of evening primrose oil in pathogen-free female Sprague Dawley rats in whom the tumor was induced by intragastric administration of 10 mg of 7,12-dimethylbenz(a)anthracene (DMBA) one week after animals were on experimental diets containing 5% evening primrose oil, sunflower oil, or palm oil. In another study, 50-day old female rats (Sprague Dawley) were given either 5 mg or 10 mg 7,12-dimethylbenz(a)anthracene (DMBA) intragastrically to induce mammary tumors (93). On 14-(5 mg DMBA rats) or 21-(10 mg DMBA rats) day post-DMBA administration, rats were divided into two groups and were fed a high-fat diet containing either 20% evening primrose oil or 20% corn oil (93). The group of rats on the evening primrose oil diet had significantly lower number of rats bearing tumors, and malignant tumors. Linoleic acid content of the primrose oil diet was higher than that of corn oil diet and linoleic acid has been linked to promote mammary tumorigenesis in rats and mice. The two diets differ in GLA only, which

suggests that GLA may be responsible for the tumor-inhibiting effects of the evening primrose oil diet. The different results of the above two studies (92, 93) can be due to differences in the dose of GLA given to rats. The other difference could be in the immune status of the rats, as Lee and Sugano conducted their studies on pathogen-free rats. Gonzalez et al. (94) performed a case controlled study in 4 regions of Spain investigating the association of dietary factors and risk of gastric cancer. Zaragova is an area in Spain where people eat borage leaves and stem, usually cooked by boiling in water. After adjusting for intake of fruits and vegetables and caloric intake, a strong negative association was observed between risk of gastric cancer and borage intake. The negative association showed a strong dose-response effect, when the population was subdivided into quartiles. On analysis, they found that boiled borage leaves contained about 4.4% GLA, while boiled stems contained 14.6% GLA. This is the first study on association between dietary borage consumption and risk of gastric cancer. As very few populations are habitual borage eaters, it is difficult to repeat the studies and also this study cannot definitely link GLA as an anticancer agent in borage leaves and stems.

To confirm if the cytotoxic effects of GLA are, in fact, mediated by prostaglandin pathway, Botha et al. (95) cultured human breast carcinoma cell line NUB1 with 50  $\mu$ l GLA or DGLA and studied the effects on prostaglandin production and cell growth. They observed that GLA had inhibitory actions on NUB1 cell growth that were accompanied by an increase in production of prostaglandin E and F. On the other hand, DGLA caused a significantly higher increase in the level of these prostaglandins but had no effect on cell growth, indicating GLA exerts cancer-cell growth-inhibitory actions by some other mechanisms. Kenny et al. (96) co-administered 2.8 g GLA with 20 mg tamoxifen to 38 breast cancer patients. The control group consisted of 47 breast cancer patients on 20 mg tamoxifen only. They observed that GLA acted synergistically with tamoxifen in reducing the expression of estrogen receptors in tumor cells and enhanced the efficacy of tamoxifen. GLA + tamoxifen group of patients showed early response to therapy and had significantly better quality of life by 6 weeks on therapy. GLA treatment was well tolerated with 42% of patients reporting no side effect and a general feeling of well being, 34% of patients reported alterations in the bowel habits with a tendency towards loose stool (many elderly patients found this beneficial). In early responders, the GLA group had a much higher reduction in expression of estrogen receptors (ER) than tamoxifen alone. The GLA group also had downregulation of expression of bcl-2 gene at 6 weeks, compared with no effect or transient increase in bcl-2 protein in the tamoxifen group. As bcl-2 plays a role in prevention of apoptotic cell death, this observation suggests that, by reducing the expression of antiapoptotic protein, GLA stimulates apoptotic cell death in cancer cells, which may have contributed to faster response at 6 weeks.

GLA has been shown in experimental model of cancer to inhibit metastasis of cancer. Urokinase concentration is increased in malignant cancer cells, and it is reported to play a role in invasiveness and metastasis of cancer. du Toit et al. (97) studied the effect of GLA on urokinase activity. They observed that GLA is a competitive inhibitor of urokinase activity with a  $K_i$  value of 120  $\mu$ M. In a

subsequent study, they observed that GLA inhibited production of urokinase activity in human prostate tumor (DU-145) cells (98). These observations suggest that GLA, by inhibiting urokinase activity, may be playing a role in preventing metastasis of cancers. Jiang et al. (99) studied the effect of GLA on motility and invasiveness of three colon cancer cell lines (HT115, HT29, and HRT18) induced by hepatocyte growth factor. They observed GLA and its lithium salt reduced metastasis and invasiveness of all the cancer cell lines by upregulating expression of E-cadherin and inhibiting attachment of cancer cells to fibronectin without affecting fibronectin receptors. Dissociation of tumor cells from the main tumor is the first requirement for metastasis. By increasing the expression of E-cadherin, GLA increases the adhesiveness of tumor cells, so the incidence of metastasis is reduced. In subsequent studies, they further demonstrated reduced metastasis and increased adhesion of tumor cells that are E-cadherin negative (HT115 and MDA-MB 231) suggesting that other mechanisms play a role in reducing the invasiveness of cancer cells. They reported increased formation of desmosomes by increasing the expression of desmoglein. As desmosomes play a role in cell–cell adhesion, this observation indicates a role of GLA in preventing metastasis by increasing the adhesiveness of tumor cells so they fail to dissociate and, hence, metastasize. At the same time, GLA inhibits cell-matrix interaction and the exact mechanism is not clear. Integrins play a major role in cell-matrix interactions. GLA has been shown to inhibit this interaction at several stages by inhibiting focal adhesion kinase activation and paxilin activation. Both of these molecules are activated by tyrosine phosphorylation, which is inhibited by GLA in tumor cells. GLA also upregulates expression of metastasis suppressor nm-23 gene (100). A reduction in the level of nm-23 gene expression has been reported in various cancers, including colorectal, breast, liver, ovarian, and bladder cancers. These studies indicate that GLA may act on different targets at the gene level to reduce metastasis and invasiveness of cancers. Jiang et al. (101) demonstrated that GLA may be acting through activation of peroxisome proliferator activated receptor-gamma (PPAR- $\gamma$ ) through increased phosphorylation of these receptors. On phosphorylation, these receptors are translocated to the nuclear membranes and regulate the expression of various genes. They demonstrated that removal of PPAR- $\gamma$  with antisense oligos abolished the effect of GLA on expression of adhesion molecules and tumor-suppressor genes.

## 6.1. Prostate Cancer

GLA has been shown to inhibit 5 $\alpha$ -reductase activity in androgen-sensitive (LNCaP) and androgen-insensitive (PC3) human-prostate cancer-cell lines (102). This observation may suggest that GLA could be acting as an anticancer agent against androgen-dependent prostate and skin cancers.

## 6.2. Glioma

Patient suffering from malignant cerebral glioma are treated aggressively with radiation, chemotherapy, and surgery, although surgery is the first option combined with

the other two treatments. The median survival time after aggressive treatment is about one year (103, 104). Naidu et al. (105) treated six patients suffering from histochemically confirmed malignant glioma with GLA. Of these patients, four patients received 1 mg GLA daily for 10 days, whereas the other two patients were treated only on alternate days. Treatment started 10 days after surgery; all these patients demonstrated marked necrosis of tumor immediately after the therapy. Of these six patients, three were alive after two years, whereas two were lost to follow-up and one died. No side effect of therapy was observed during or after treatment. During subsequent follow-up, authors did not observe any increase in size of residual tumor or recurrence of tumor. Based on the results of this study, authors extended the treatment to 15 more patients and found increased survival by one and one-half to two years. This study also confirmed necrosis of tumor cells and safety of GLA. They also injected GLA to normal dogs intracerebrally and found no cytotoxic effects (106). These studies demonstrated that GLA injected directly into tumor mass may potentially be useful treatment for malignant glioma.

### 6.3. Liver Cancer

Merve et al. (107) conducted a double-blind placebo-controlled trial of evening primrose oil in patients suffering from primary liver cancer, a fatal disease. The patients were randomly assigned to the GLA or placebo group. The GLA group patients received 36 capsules per day supplying 18 g evening primrose oil containing 1.44 g GLA. The control group received the same amount of olive oil. They observed a mean survival time of 58 days in the treatment group compared with 42 days in the placebo group, although the difference was not statistically significant. Gamma glutamyl transaminase enzyme activity was decreased in seven patients in the treatment group compared with two patients in the placebo group. This difference was statistically significant, suggesting that evening primrose oil may have some effect on tumor. In this study, patients had up to 3-kg tumor weight, suggesting an advanced stage of cancer. Probably, the dose of GLA was not sufficient to obtain a statistically significant effect on survival time. A major finding was that the quality of life was better for the evening primrose oil group as indicated by the patients self assessment. Falconer et al. (108) studied the effect of lithium salt of GLA on pancreatic cancer in 18 patients who had unresectable pancreatic cancer and had undergone either surgical bypass or had pancreas endoscopically stented. These patients were administered GLA intravenously for 10 days and then were switched to oral GLA therapy. During the infusion period, the dose of GLA was gradually increased for the first five days and then continued at maximal tolerated dose for a subsequent 5 days. Patients received a mean dose of 5.7g lithium GLA for the last 5 days and mean oral dose of 3 g afterwards. They observed a median survival of 8 months and 4 patients were still alive compared with normal life expectancy of 3–6 months for these patients. GLA treatment increased T-cell function and reduced TNF production. In this report, the study design was not well defined; therefore, it was difficult to assess if the protocol had any beneficial effect on patient survival, though the treatment was reported to be well tolerated.

## 6.4. Mechanism of Anticancer Effects of GLA

The exact mechanism of anticancer effects of GLA is not very clear and may depend on the cancer type. Many cancer cells have been shown to lack phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activity and delta-6-desaturase activity. PLA<sub>2</sub> is essential in releasing free fatty acids from the membrane phospholipids. Released free fatty acids, like DGLA, AA, EPA, etc, act as a substrate for cyclooxygenase and lipoxygenase enzyme to produce prostaglandins and leukotrienes. Delta-6-desaturase is essential for the conversion of dietary LA to GLA. Therefore, administration of GLA can bypass these metabolic steps and show anticancer effects. GLA, being a polyunsaturated fatty acid, can increase lipid peroxidation in the cancer cells. Free radicals have been implicated in cytotoxic actions of several anticancer drugs. It is possible that GLA may be showing its anticancer effects through oxidative mechanisms (109). Leaver et al. (110) analyzed the effect of GLA and AA on free radical production and cell death by necrosis and apoptosis in 30 human glioma types. The brain samples were obtained from patients undergoing surgery. Patients had grade 1 to grade 4 tumors. They observed that tumor cells in general produced less free radicals than normal cells and amongst the tumor cells, total free radical production was higher for advanced tumors (Grade 4). GLA and AA, both increased the production of free radicals in normal and tumor cells; however, tumor cells responded with a much higher increase in the production of free radicals and GLA was more potent than AA in increasing the free radical production in glioma cells. In this study, the necrotic cells produced less free radicals than nonnecrotic tumor cells and they showed a lower degree of rise in free radical production when incubated with GLA or AA. As necrotic cells are rich in phagocytic cells, this observation suggests that GLA or AA increase the production of free radicals in tumor cells mainly and the phagocytic cells are not the major source of free radical in gliomas incubated with GLA or AA. This observation also indicates that GLA is free from toxic effects on healthy cells in contrast to cancer therapeutics probably because it does not promote formation of free radicals from phagocytic cells that may release free radicals at several sites and damage healthy cells.

GLA could show its cancer-cell growth-inhibitory action by inhibiting cell proliferation or by increasing apoptotic cell death. de Kock et al. (111) demonstrated that GLA acts differently on human osteogenic sarcoma cells (MG-63 cells) and human epithelial cervix carcinoma cells (HeLa cells). In MG-63 cells, GLA-induced inhibition of mitosis was associated with abnormal metaphase cell spindle formation and inhibition of protein synthesis in G<sub>1</sub> and S-phase. HeLa cells respond differently, showing increased hypercondensation of chromosome, suggesting increased apoptotic cell death that was associated with increased protein synthesis for all of the G<sub>1</sub> proteins and selective S-phase proteins. In a subsequent study on HeLa cells, they further demonstrated that GLA inhibits MAP-kinase pathway and c-Jun expression. As c-jun is the transcription factor involved in cell proliferation and is activated by MAP-kinases, GLA is interfering with nuclear processes in inducing apoptosis in HeLa cells (112). Jiang et al. (113) observed a decrease in phosphorylation of p27<sup>kip1</sup> and p57<sup>kip2</sup> that are inhibitors of cyclin-dependent kinases



and play a role in progression of mitotic growth (progression from G1 to S phase). Decreased phosphorylation resulted in increased binding of these proteins to cyclin-dependent kinases including CDK4, cyclin E, and CDC2. Seegars et al. (114) studied the involvement of p53 protein in apoptotic cell death induction by GLA and AA in skin fibroblasts and lymphoblast cells containing wild type and mutant p53. They confirmed the earlier observations that normal cells are not affected by GLA to any appreciable extent. They also observed that AA was more toxic to normal cells than GLA, as GLA at much higher doses induced apoptosis in normal cells. Transformed cells were more susceptible to apoptotic cell death induction by GLA. The p53 does not appear to play a role in apoptosis induction by GLA as transformed cells containing wild type and mutant p53 responded to apoptosis induction by GLA.

## 7. IMMUNE FUNCTION AND AUTOIMMUNE DISEASES

Immune function is a very complex function that involves interplay of several cell types and humoral and cellular factors. Immune cells, including lymphocytes, polymorphonuclear leukocytes, monocytes, splenocytes, kuppfer cells, etc, have a high content of polyunsaturated fatty acids in their membrane phospholipids. The composition of PUFAs in membrane phospholipids can be altered by dietary interventions. GLA is taken up by inflammatory cells and is rapidly elongated to DGLA. In some species, it can be desaturated to AA but, in human immune cells, it is not desaturated probably because of, very limited to no delta-5-desaturase in immune cells. By the action of enzyme phospholipase A<sub>2</sub>, free DGLA is released from the membrane phospholipids and competes with AA for cyclooxygenases and lipoxygenases. DGLA produces PGE<sub>1</sub> and thromboxane A<sub>1</sub> (TxA<sub>1</sub>). The actions of PGE<sub>1</sub> have been reviewed in detail by Horrobin (115). It mainly exerts anti-inflammatory and vasodilatory properties. DGLA produces 15-hydroxyeicosatrienoic acid (15-HETrE) by the action of 15-lipoxygenase. This metabolite of DGLA is a strong inhibitor of 5-lipoxygenase whereby it inhibits production of leukotriene B<sub>4</sub> (LTB<sub>4</sub>) from neutrophils (116). LTB<sub>4</sub> has a diverse array of inflammatory actions: It is a very potent chemotactic factor that attracts neutrophils at the site of inflammation, increases adherence of leukocytes to endothelial cells, enhances migration of T-lymphocytes in vitro stimulates release of interferon gamma and IL-2 production by T cells, and promotes the biosynthesis of IL-1 from monocytes. Thus, dietary administration of GLA-rich oils has a potential in modulating immune function. Several in vitro and in vivo studies have investigated the effect of GLA on immune functions.

Ziboh and Fletcher (117) observed a dose-dependent inhibition of calcium ionophore stimulated release of LTB<sub>4</sub> by human neutrophils obtained from healthy human volunteers fed either 0.48 or 1.5 g GLA per day for 6 weeks from borage oil. A linear relationship between rise in polymorphonuclear neutrophil (PMN) phospholipid DGLA and inhibition of LTB<sub>4</sub> production was not observed. Kaku et al. (118) observed inhibitory effects of dietary GLA on LTB<sub>4</sub> production by

rat peritoneal exudates cells. They also reported stimulation of immunoglobulin production from mesenteric lymph node leukocytes by GLA. This action may suggest that GLA may strengthen gut immune responses and may prevent allergic reactions.

Santoli and Zurier (119) studied the effect DGLA, AA, and EPA on mitogen-induced production of interleukin 2 (IL-2) by human peripheral blood mononuclear cells (PBMCs). They observed inhibition of IL-2 production by AA or DGLA in a dose-dependent manner. EPA showed inhibitory action in some donors only. Indomethacin, a cyclooxygenase inhibitor, caused an increase in IL-2 release and suppressed PGE release from PBMCs. It inhibited PGE release from fatty-acid-incubated PBMCs but did not attenuate IL-2 inhibitory action of fatty acids, suggesting that the suppressive effect of AA and DGLA on IL-2 release is not mediated through prostaglandin pathway. The inhibition of IL-2 release could be mediated by the effect of GLA and DGLA on early response genes, as both these fatty acids have been shown to reduce a rise in *c-fos* and a fall in *c-myc* oncogenes in T cells (120). DeMarco et al. (121) observed a reduction in IL-2-dependent proliferation of T-lymphocytes isolated from synovial tissue and synovial fluid from arthritic patients. Rotondo et al. (122) studied the effect of GLA, DGLA, AA, and EPA on IL-1-induced proliferation of thymic lymphocytes and observed that GLA was less potent than DGLA in inhibiting the IL-1-induced proliferation of lymphocytes. The actions of these fatty acids were not mediated through the prostaglandin pathway, as cyclooxygenase inhibitors had no effect on the actions of these fatty acids, which might exert a direct effect on lymphocytes. Rothman et al. (123) observed stimulation of production of IL-1 $\beta$  in human peripheral mononuclear cells by DGLA. Incubation with LPS further stimulated the production of IL-1 $\beta$ . Intracellular IL-1 $\beta$  was entirely pro-IL-1 $\beta$ . Incubation with DGLA also stimulated release of pro-IL-1 $\beta$  and small amounts of mature IL-1 $\beta$ . LPS failed to stimulate the further release of IL-1 $\beta$  from PBMC. This could be due to the maturation of monocytes to macrophages during 16 hours of incubation. Mature macrophages are reported to release decreased amounts of IL-1 $\beta$  in response to LPS stimulation. The observations of Rothman et al. (123) are contrary to expectations, as GLA and DGLA exert anti-inflammatory actions, one would expect a decrease in production and a release of IL-1 $\beta$ . It could be caused by experimental design as they incubated the cells for 24 hours followed by incubation with LPS for 16 hours. DeLuca et al. (124) stimulated the PBMCs for 30 minutes followed by stimulation with LPS for 16 hours. They observed a dose-dependent decrease in LPS-induced release of IL-1 $\beta$  and TNF $\alpha$  by GLA and DGLA. EPA also inhibited mediator release but required twice the amount. They observed a similar reduction in the release of these mediators when 2.4 g GLA was administered to human volunteers as a single dose.

A recent study by Furse et al. (125) demonstrated that LPS-stimulated IL-1 $\beta$  release is further increased by IL-1, and this process is known as auto-induction. GLA inhibits IL-1 $\beta$  release from LPS-stimulated monocytes mainly by inhibiting the auto-induction process. This information may suggest that GLA may be inhibiting excessive release of IL-1 $\beta$  to prevent inflammation but may not interfere with basal release of IL-1 $\beta$ , which plays a role in host defense.

GLA and DGLA inhibit protein kinase C (PKC) activity in PMA-stimulated T-lymphocytes. However, only GLA inhibited basal PKC activity. Both fatty acids stimulated translocation of PKC from cytosol to membrane (126). GLA and DGLA inhibited anti-CD3 monoclonal antibody induced early and late rise in intracellular calcium in T cells and also inhibited a rise in inositol-1,4,5-triphosphate (IP<sub>3</sub>) production (127). Stimulation of T cells resulted in the formation of IP<sub>3</sub> and diacyl glycerol (DAG). IP<sub>3</sub> stimulates the early rise in intracellular calcium by releasing the calcium ions from intracellular stores (SR). DAG was found to stimulate the formation of PKC, which phosphorylates several proteins in the cells and plays a role in late rise in intracellular calcium. GLA and DGLA promote translocation of PKC to cellular membranes, whereby they may be inhibiting phosphatidylinositol turnover. These studies provide a strong support to the hypothesis that GLA and DGLA interfere with signal transduction pathways and exert antiproliferative actions on T cells, and these actions may mediate immune modulating and anti-inflammatory actions of these fatty acids.

Wu et al. (128) studied the effect of supplementation of black currant oil on immune function in healthy elderly volunteers. They isolated the mononuclear lymphocytes pre- and post-supplementation and studied the release of IL-1 $\beta$ , IL-2, and PGE<sub>2</sub>, and proliferation of lymphocytes in response to mitogens, including concanavalin A and phytohemagglutinin A (PHA). No effect of black currant oil administration was observed on lymphocyte proliferation in response to concanavalin A, but it increased in response to PHA. There was no effect on the release of IL-1 $\beta$  and IL-2, while PGE<sub>2</sub> release was significantly decreased. Black currant oil supplementation also increased delayed type hypersensitivity (DTH) response as shown by the increase in the total diameter of induration at 24 hours and response to specific antigens (tetanus toxoid and *T. mentagrophides*). DTH response is depressed in aged populations and may contribute to increased mortality and morbidity. In this study, volunteers consumed 675-mg GLA and 653-mg ALA per day for 2 months. Therefore, it was not possible to ascribe the results to GLA only. Nerad et al. (129) demonstrated that administration of 2 g GLA for 12 weeks from borage oil to healthy volunteers caused an increase in total score of indurations, suggesting that the increase in induration observed by Wu et al. (128) may be contributed by GLA content of black currant oil. Immune enhancing activity observed in these studies could be contributed by a reduction in PGE<sub>2</sub>, as it is well known inhibitor of lymphocyte proliferation and T-cell function. Zurier et al. (130) observed *in vitro* suppression of T-lymphocytes proliferation by GLA, DGLA, AA, and EPA. Of all fatty acids examined, GLA and DGLA were more potent than AA and EPA. In this study, preincubation of lymphocytes with fatty acids was required, but the continuous presence of GLA or DGLA was not needed for inhibition of proliferation suggesting that these fatty acids are incorporated in the membrane phospholipids of the cells and exert inhibitory actions on proliferation. Addition of these fatty acids along or after the addition of a stimulant has no effect on T-cell proliferation indicating that they interact at earlier stages of signal transduction, which leads to inhibition of proliferation. Thus, the fatty acids tested may reduce the stimulant-induced rise in cytosolic calcium that is required for proliferation.

## 7.1. Rheumatoid Arthritis

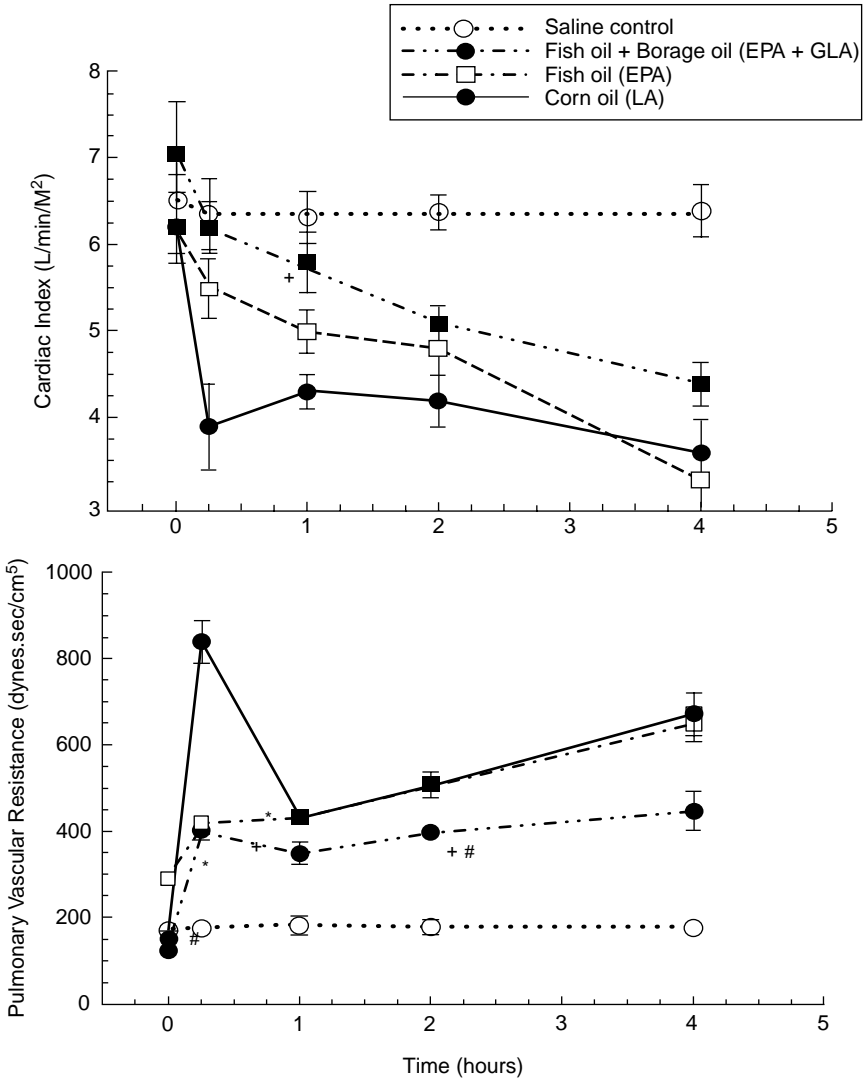
Rheumatoid arthritis is an autoimmune disease characterized by inflammation of the joints and cartilage destruction. Several studies discussed above have demonstrated anti-inflammatory potential of GLA-containing oils. These studies suggest that GLA-rich oils can be used to treat inflammatory conditions.

Tate et al. (131) demonstrated in rats that a GLA-rich diet can reduce the inflammation induced by injection of monosodium urate. GLA inhibited polymorphonuclear leukocyte recruitment, crystal phagocytosis, and lysosomal enzyme release. In a subsequent study, the authors demonstrated anti-inflammatory effects of GLA in Freund's adjuvant induced arthritis in rats (132). The anti-inflammatory effect of GLA was associated with inhibition of proliferation of pouch-lining cells and maintenance of architecture of these cells. Hansen et al. (133) administered 4 g of evening primrose oil supplying 360-mg GLA per day along with zinc, ascorbic acid, niacin, and pyridoxine to a group of 20 arthritis patients for 12 weeks. They did not observe any effect of treatment on several parameters of arthritis (number of tender and swollen joints, pain, erythrocyte sedimentation rate, and duration of morning stiffness). The failure of GLA to exert any beneficial effect could be caused by low dosage short duration of treatment. Belch et al. (134) studied the effect of 540-mg GLA or 450-mg GLA and 240-mg EPA per day on symptoms of arthritis and NSAID requirement. They continued the treatment for 12 months and observed that a significant number of patients had reduced requirements for NSAIDs at the end of 12 months. After 12 months, the treatment with GLA was stopped and three months after stopping the treatment, all the patients needed a full dose of NSAIDs, indicating that GLA or EPA had NSAID sparing effects and were not disease-modifying agents. In an open label clinical study, 1.1-g GLA given for 12 weeks reduced inflammation in arthritic patients and also reduced release of PGE<sub>2</sub>, LTB<sub>4</sub>, and LTC<sub>4</sub> (135). Laventhal et al. (136), in a randomized, placebo-controlled trial, observed that 1.4-g GLA given as borage oil for 6 months resulted in significant reduction in swollen joint count and score, tender joint count and score, and platelet counts. They also observed a 33% reduction in duration of morning stiffness. The only side effects of GLA treatment were belching, flatulence, and soft stools. Zurier et al. (137) repeated the trial by increasing the dose to 2.8 g of GLA. In this trial, the patients were randomized to receive either 2.8-g GLA/day from borage oil or placebo for 6 months and, after 6 months, all the patients were switched to GLA arm. Patients on GLA group at the end of 6 months showed reductions in swollen joint count and score, morning stiffness, and tender joint count and score. At the end of 12 months, the patients who started with GLA from the beginning continued to show improvement in their symptoms. Patients who started GLA after 6 months on placebo also started to improve. None of the patients in the GLA group experienced deterioration of condition in first 6 months, but, at the end of 12 months, two patients (out of 21) reported deterioration in condition. Seven of these 21 patients required a reduction in the dosage of nonsteroidal anti-inflammatory drugs or prednisone. Three months after stopping the treatment with borage oil, most of the patients showed exacerbation of disease condition suggesting that borage oil must be continued for relief of symptoms.

Overall, research with GLA-containing oils has shown that GLA provides benefit in the reduction of morning stiffness by about 73 minutes and exerts a NSAID-sparing effect. However, the dosage of GLA required for the treatment of arthritis is not well established as, in various studies, from 340-mg to 2.8-g GLA per day has been used.

## 7.2. Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome (ARDS) is an acute, severe injury to the lungs. Patients with ARDS suffer from severe shortness of breath, requiring mechanical ventilation. It is associated with increased pulmonary capillary permeability, pulmonary edema, increased pulmonary vascular resistance, and progressive hypoxemia. ARDS can also lead to damage and failure of other organs. The exact cause of ARDS is not known but several factors can contribute, including chest trauma, sepsis, bacterial infections, and so on. At the cellular level, oxygen-free radicals, cytokines, and prostaglandins can play a role. Recent research is focused on dietary manipulations that help in reducing the inflammation and generation of pro-inflammatory mediators. Oxidative metabolism of AA results in the formation of pro-inflammatory mediators, including PGE<sub>2</sub>, TXB<sub>2</sub>, LTB<sub>4</sub>, etc. LTB<sub>4</sub> is a potent chemotactic factor and attracts neutrophils and exacerbates the damage to tissues. GLA and its metabolic product DGLA counter the effects of AA by forming anti-inflammatory mediators, such as PGE<sub>1</sub> and 15-HETrE, and reduce the formation of AA-derived inflammatory mediators. Kumar et al. (138) observed a significant reduction in plasma phospholipid levels of GLA, DGLA, ALA, and EPA in patients at risk of developing ARDS while patients with established ARDS additionally had lower amounts of AA. This study suggests that treatment with GLA and EPA is warranted in these patients. Gadek et al. (139) conducted a multicenter double-blind, placebo-controlled clinical trial on patients with ARDS. The treatment group (51 patients) was administered a mixture of borage oil, fish oil (providing 5.8 ± 0.3-g GLA, 6.9 ± 0.3-g EPA, and 2.9 ± 0.1-g DHA per day), and antioxidants via gastric or jejunal tube. They observed a significant reduction in the number of total cells and neutrophils in bronchoalveolar fluid by day 4 compared with the fluid obtained from the control group. This was associated with improved arterial oxygenation. Patients in the treatment group had a lesser requirement for ventilator support, supplemental oxygen, and lower number of days of stay in ICU compared with patients in the control group. Significantly fewer patients in the treatment group developed new organ failure, and there was about a 17% reduction in the total number of infections in the treatment group. As this study used a combination of EPA and GLA with antioxidants, it is difficult to differentiate the effects of GLA alone, although this study provides a strong support for using a combination of EPA and GLA. Murray et al. (140) studied the effect of fish oil alone or in combination with borage oil on cardiac function in pigs during acute lung injury induced by infusion of *E. coli* endotoxin. They observed that fish oil or a fish oil and borage oil combination attenuated lung injury induced depression of cardiac function. A combination of fish oil and borage oil acted synergistically compared with fish



**Figure 2.** Effect of various treatments on cardiac index (upper panel) and pulmonary vascular resistance (lower panel). Data adapted from (140).

oil alone in attenuating the cardiac depression (Figure 2, Upper panel). A fish oil and borage oil combination had lower pulmonary vascular resistance during the 4-hour experiment duration than either the control group or the group given fish oil alone (Figure 2, Lower panel). The other interesting finding of this study was that GLA in combination with EPA prevented the loss of platelets from circulation, whereas EPA alone did not exert this effect. This observation indicates that GLA decreased the aggregatory and adhesive properties of platelets in vivo. A significant

reduction in the amount of TXB<sub>2</sub> (EPA, and EPA + GLA groups) and 6-keto prostaglandin F<sub>1</sub> $\alpha$  in the alveolar fluid in EPA + GLA group was also observed, suggesting that the beneficial effects of the treatment may be mediated by a reduction in the formation of pro-inflammatory and vasoconstrictor metabolites of AA. In a subsequent study, the administration of EPA or EPA+GLA to pigs altered the composition of pulmonary surfactant by reducing the concentration of oleic acid and increasing the concentration of DGLA, EPA, and DHA. However, there was no effect on the pulmonary compliance or surfactant function. Mancuso et al. (141) observed that a combination of fish oil and borage oil attenuated endotoxin induced rise in pulmonary microvascular protein permeability in rats, which was associated with a decrease in LTB<sub>4</sub>, TxA<sub>2</sub>, and PGE<sub>2</sub> production by pulmonary alveolar macrophages. Additionally, this treatment also attenuated endotoxin induced early and late hypotension.

## 8. SKIN CONDITIONS

Skin is the largest organ of the body and provides a barrier function, whereby it protects the inner organs from environmental toxins and bacteria. It also plays an important role in temperature regulation, sensory perception, and excretion. It undergoes constant renewal. Burr and Burr (142) observed that rats on a fat-free diet developed dried, scaly skin suggesting the role of fats in normal physiology of skin. Subsequent studies have demonstrated that skin contains essential fatty acids and is metabolically an active organ. It has the capability to elongate the fatty acids but lacks the capacity to desaturate. This information suggests that dermal cells take up preformed long-chain metabolites of LA (GLA, DGLA, and AA) and ALA (EPA, DPA, and DHA).

During EFA deficiency, the levels of LA, DGLA, and AA are reduced in the skin and may contribute to dry and scaly appearance of the skin, with the increase in epidermal water loss. In studies with EFA-deficient rats, mice, and guinea pigs, it has been demonstrated that skin undergoes hyperproliferation (acanthosis, hypergranulosis, and hyperkeratosis) with increased DNA synthesis. LA levels were significantly decreased with an increase in mead acid (20:3 n-9, abnormal fatty acid characteristic of EFA deficiency). Supplementing diets with a large dose of safflower oil (rich in LA) or much smaller dose of evening primrose oil (rich in LA and GLA) reversed the signs of EFA deficiency on skin, whereas fish oil failed to reverse these symptoms (143). In this study, a rise in EPA, DPA, and DHA levels in skin phospholipids was observed, but the levels of LA did not increase. This study also confirmed, by labeled fatty acid incubation, that skin lacks delta-6 and delta-5 desaturase activities, indicating that skin cannot metabolize LA or ALA.

### 8.1. Atopic Dermatitis

Atopic dermatitis, or eczema, is a skin disorder characterized by dry, itchy, and hypersensitive skin. It is common in children but can occur at any time and age.

The exact cause of eczema is not well understood and it can be hereditary. Soon after the discovery of essential fatty acids, it was observed that infant patients suffering from atopic eczema had low levels of LA and AA (144) and responded well to supplemental lard containing LA and AA (144). Very high doses of LA (20–50 g) provided partial relief from symptoms of eczema but failed to raise the levels of metabolites of LA in the blood. Later on, it was confirmed that the plasma phospholipids of adults suffering from atopic dermatitis had higher concentrations of LA and a lower concentration of GLA, DGLA, and AA (145), suggesting that atopic patients may suffer from defective delta-6-desaturation. These patients also failed to show flushing response to topically applied niacin, suggesting that they have defects in prostaglandin pathways and fail to produce vasodilatory prostaglandins. Subsequent studies have shown lower levels of DGLA in breast milk of atopic mothers than in the normal mothers (146, 147). As the breast-fed infants get their nutrient requirements from breast milk, they do not receive sufficient quantities of DGLA and may be prone to dermatitis.

Based on these observations, it seems logical that dietary GLA or DGLA should help prevent/treat atopic dermatitis. Wright and Burton (148) conducted a double-blind, placebo-controlled clinical trial of evening primrose oil. They recruited 60 adults and 39 children suffering from moderate to severe atopic dermatitis in the study. Adult patient groups received 4, 8, or 12 capsules daily, whereas children were given 2 or 4 capsules daily providing 45 mg GLA per capsule, and the placebo was liquid paraffin. Treatment was continued for 12 weeks. All of the patients had moderate to severe eczema. They observed that the lower dose of GLA only provided relief from itch, while the other two groups of adult patients on higher doses of GLA showed better improvements in itch, scaling, and the general impression of severity as assessed by the physician and the patient. Children in this study did not perform as well as the adults, possibly caused by either insufficient dose of GLA or high placebo effects in children. Manku et al. (149) analyzed the blood samples of adult patients from the above study for plasma phospholipid fatty acids. They observed that LA levels were higher in the atopic patients, and the scatter of values for LA was also very high. Levels of DGLA and AA were lower in these patients. Treatment with 4 capsules per day did not affect blood GLA or DGLA or plasma PGE<sub>1</sub> levels, whereas 8 and 12 capsules per day caused a significant elevation in the levels of DGLA and PGE<sub>1</sub>. Schafer and Kragballe (150) observed that neutrophils and epidermis of atopic dermatitis patients have high levels of monounsaturated fatty acids (MUFAs), which correlated positively with the severity of disease, and lower ratios of n-6 PUFAs/MUFA. Feeding 6 g of evening primrose oil for 10 weeks increased the ratio of n-6 PUFA/MUFA and increased the levels of DGLA in neutrophils and epidermal phospholipids. They did not evaluate the effect of the treatment on dermatitis as the patients were allowed to use emollients; this study cannot shed any light on the efficacy of GLA in dermatitis. In another large multicenter study, 179 patients with eczema were treated with 4.0 g of evening primrose oil per day, and they demonstrated clinical improvements as evaluated by a dermatologist. Scarff and Lloyd (151) studied the effect of treatment with evening primrose oil in dogs suffering from dermatitis. In this study, the dogs



were on olive oil placebo for 3 weeks followed by either olive oil or evening primrose oil for 9 weeks. At the end of 9 weeks, the treatments were switched over without any wash out period. They observed a deterioration in condition during first 3 weeks on olive oil in all of the dogs; however, during the first treatment period, all dogs showed improvement that could be ascribed to placebo effect in the olive oil group. In the second treatment period, dogs on olive oil worsened, whereas those on evening primrose oil improved. They observed an interaction in the order of treatment with the evening primrose oil that could be caused by a change in treatment between active and placebo without any washout period. Fiocchi et al. (152) evaluated safety and efficacy in children suffering from atopic eczema. They treated these children (average age 11.4 months) with 3.0 g/day of GLA for 28 days. None of the children showed complete recovery, although gradual improvement in erythema, excoriations, and lichenification was reported. They also reported a significant reduction in itching and the use of antihistamines without observing any side effect because of the treatment. Borrek et al. (153) compared the effect of borage oil with corn oil in 24 subjects suffering from atopic eczema in a double-blind cross-over trial. The subjects were between 3 and 17 years old and received 360-mg GLA daily for 10–14 weeks. They did not observe any difference between the two groups, and the placebo treatment also showed improvements. In this study, 10 patients on borage oil treatment showed improvements but they did not differ from nonresponders in any of the characteristics (age, sex, symptom severity, etc.). As there was a large placebo effect, the effectiveness of borage oil may have been masked due to a small number of subjects in this study.

Eczematous skin also has high transepidermal water loss compared with normal skin. Hartop and Protty (154) observed that application of pure GLA triacylglycerol to rats with dry skin due to essential fatty acid deficiency reduced the transepidermal water loss. In essential fatty acid deficiency, there was a loss of LA, ALA, and their long-chain metabolites in plasma and other organs. However, the eczema differed from essential fatty acid deficiency in which there is usually an excess of LA with imbalance of longer chain metabolites. Tolleson and Frithz (155) studied the effects of topically applied borage oil on the transepidermal water loss from the skin of infants suffering from seborrheic dermatitis. They observed that topically applied borage oil relieved the symptoms of dermatitis within 3–4 weeks and also normalized the elevated transepidermal water loss. Topically applied borage oil also caused a rise in serum LA content, suggesting transdermal absorption of LA from borage oil. The site of application of borage oil was not important as borage oil in the napkin area of the infants also relieved the symptoms at other sites.

Henz et al. (156) evaluated the efficacy of borage oil in the treatment of atopic dermatitis in a double-blind, placebo-controlled, multicenter clinical trial. In this study, 160 patients with moderate eczema (Costa score between 20 and 36 points) were divided into two groups. The active group received 3.0 g borage oil (690 mg GLA) daily for 24 weeks and the placebo group received migliol, an oil containing no GLA. Patients were allowed to use a steroid cream during the trial. Some patients did not follow the guidelines and violated the conditions of protocol and included poor compliance (less than 70% of dose consumed; 7 patients on placebo,

6 on borage oil), excessive use of steroid cream (three times above median dose; 1 patient on placebo and 4 on borage oil), and less than 11 weeks of treatment (6 patients on each treatment), and patients with unstable disease (Costa score of less than 18 at week 2; 32 patients on placebo and 21 on borage). When all the patients, including those who did not follow the protocol, were included in the data analysis, no significant differences in Costa scores between the two groups was observed, although borage oil treatment improved erythema, vesiculation, crusting, excoriation, lichenification, and insomnia scores over placebo group. A marked reduction in serum IgE levels was observed, but the difference was statistically insignificant due to large intersubject variations. Borage oil treatment also increased plasma and erythrocyte levels of GLA and DGLA in the majority of patients. When the subgroup of patients who did not follow the protocol was excluded from the analysis, the borage oil treatment showed significant improvement on the reduction of steroid cream use. Borage oil was well tolerated with minor side effects (headache, nausea, vomiting, and diarrhea). The frequency of side effects was not different from that observed with the placebo treatment.

Takwale et al. (157) conducted a single-center, double-blind clinical trial to study the efficacy and tolerability of borage oil in the treatment of atopic eczema in children and adults. Adult patients were given 8 capsules of borage oil (supplying 920-mg GLA) and children were given 4 capsules (supplying 460 mg of GLA) daily for 12 weeks. Patients were allowed to continue using a steroid ointment for symptomatic relief. The efficacy of the treatment was evaluated from the change in total symptom score measured with the six-area, six-sign, atopic dermatitis (SASSAD) score as the primary endpoint. Secondary end points included symptom score assessment on visual analogue scales, topical corticosteroid requirement, and global assessment of response by participants. This study failed to observe any effect of borage oil treatment on eczema, although the treatment was safe, well tolerated, and was free from major side effects. This study suffers from several major limitations. They recruited 151 patients, of which 11 were lost at week two of the 12-week study. An additional 16 participants withdrew during the trial, leaving only 124 subjects who completed the trial. However, they analyzed the data for 140 patients, including those who did not complete the protocol. Good clinical trial demands inclusion of data only from those patients that follow the protocol. Noncompliance with the treatment protocol is the single most important reason for failure of treatment in dermatological practice and was evident in the study by Henz et al. (158). They used two different placebo treatments: Liquid paraffin for adults and olive oil for children. Liquid paraffin is an inert material for its effect on atopic dermatitis, whereas olive oil is not as inert because it can modify the cellular fatty acid profile. It has been reported to increase tissue levels of DGLA (159, 160). By increasing tissue levels of DGLA, olive oil may increase the dermal levels of lipooxygenase and cyclooxygenase metabolites of DGLA that are reported to exert anti-inflammatory actions (161, 162). Therefore, olive oil may show some beneficial effects because of the above-mentioned biochemical pathways, and hence, may not be a true placebo and dampen the effect of treatment. Therefore, separate analysis in adults and children was highly desirable to avoid the potential variations in

outcome induced by different placebo. The scoring system (SASSAD) used in this study as a primary outcome parameter is reported to have a very high interobserver variation (7–30, median 15.5, out of a possible score of 108) (163).

## 8.2. Other Skin Conditions

Radiation-induced damage: Skin is sensitive to radiations. Hopewell et al. (164) studied the effect of GLA or GLA and EPA combinations on radiation-induced skin damage in pigs. They treated female pigs for 4 weeks prior to and 10–16 weeks after irradiation of skin. Control pigs were treated with a placebo oil devoid of GLA and EPA. The pigs were irradiated with a single or fractionated (20 F/28 days) dose of  $\beta$ -rays. They observed that prior administration of GLA or GLA + EPA had no protective effect, while given before and after irradiation, both of these interventions reduced the development of early (bright red erythema or moist desquamation) and late (dusky/mauve erythema and dermal necrosis) reactions of radiations. This observation suggests that GLA alone or in combination with EPA may help improve the efficacy of radiation treatment by reducing the side effects.

## 9. DIABETES

Diabetes is a metabolic syndrome resulting in disturbed glucose homeostasis. This can be mediated because of decreased production of insulin (because of damage to insulin-producing pancreatic  $\beta$ -cells), as in type 1 diabetes, or increase in tissue resistance to the action of insulin (type 2 diabetes). According to the American Diabetes Association, approximately 17 million people in the United States, or 6.2% of the population, have diabetes. Although an estimated 11.1 million have been diagnosed, unfortunately, 5.9 million people (or one-third) are unaware that they have the disease. It affects people of all ages and races, although it is more common in African Americans, Latinos, Native Americans, Asian Americans, and Pacific Islanders. Incidence of diabetes increases with age in all populations. Diabetics are also at high risk of other complications, including cardiovascular (atherosclerosis, heart attack, stroke, peripheral vascular disease), neurological (neuropathies), renal failure, skin diseases (dry, itchy skin, skin infections, dermopathy), slow wound healing, retinopathy, and impotence. A combination of neuropathy and vascular disease in diabetics leads to more amputations. The mechanism of complications of diabetes is not well understood. High blood-glucose levels may be contributing to various complications, although several pathways, including increased oxidative stress, modification of proteins by glycosylation, reduction in production of vasodilator mediators including nitric oxide, prostacyclin, altered cytokine production, and so on, may be involved. Reduced tissue perfusion and resulting cellular damage contribute to several pathophysiological situations. The management of diabetes involves controlling the blood-glucose levels by diet, exercise, or drugs.

Several studies have confirmed that diabetes inhibits the activity of delta-6-desaturase (165–167), which is the first enzyme in the metabolism of LA and ALA. As a result of inhibition of this enzyme, diabetics have a lower content of DGLA and AA in various tissues (168). This may lead to imbalance in different eicosanoids in diabetics and may contribute to various complications common in diabetics. Based on these observations, it was hypothesized that GLA may help correct many of the complications of diabetes. To test this hypothesis, many studies were conducted in animals and humans. Some of these studies are discussed here.

Diabetic patients and animals show reduced nerve conduction velocity. Julu (169) from the University College of London performed a detailed study investigating the effect of GLA alone or in combination with EPA on nerve conduction velocity. During diabetes, nerve conduction velocity was reduced. Rats made diabetic by injection of streptozotocin (a pancreatic toxin) suffered about a 20% decline in motor nerve-fiber conduction velocity. Supplementation with GLA alone significantly attenuated the diabetes-induced deficit in nerve conduction velocity. Combined treatment with GLA and EPA completely prevented diabetes-induced reduction in motor nerve conduction velocity. In this study, they observed no effect of GLA or EPA on diabetes-induced weight loss, increase in blood-glucose, and glycosylated hemoglobin levels. In the subsequent study, Julu and Mutamba (170) studied the comparative effect of GLA and insulin for 3 or 5 days of treatment after induction of diabetes with streptozotocin in rats. They again observed a reduction in conduction velocity in myelinated sensory and motor nerve fibers. Unmyelinated sensory fiber also showed a trend for reduction in conduction velocity, but because of high intersubject variability, the fall could not reach statistical significance. Treatment with insulin for 3 days partially corrected the deficit in sensory-nerve conduction velocity, whereas motor-nerve conduction velocity was brought back to normal levels. Treatment for 5 days with insulin returned the motor-nerve conduction velocity to normal levels. GLA treatment for 3 days overcorrected the sensory-nerve conduction velocity, whereas the motor-nerve conduction velocity was brought back to normal levels. Treatment with GLA for 5 days brought sensory-nerve conduction velocity to normal levels. The overcorrection in sensory-nerve conduction velocity by three-day treatment with GLA cannot be explained. GLA treatment had no effect on blood-glucose levels or diabetes-induced weight loss, whereas insulin corrected both of these parameters. Cameron et al. (171) studied the effect of GLA alone or in combination with fish oil on diabetes-induced reduction in nerve conduction velocity and resistance to conduction block and found that diabetes increased the resistance of nerves to hypoxic conduction block and reduced the nerve conduction velocity. Treatment with GLA prevented these changes, whereas a combination with fish oil was less effective. Their results on conduction velocity are different from those reported by Julu and Mutamba (170) who observed better efficacy of GLA and fish oil combination. This difference is difficult to explain except for the sex differences in the rats in the two studies. Julu and Mutamba (170) used female rats, whereas Cameron's (171) group used male rats. The role of female sex hormones in differential observation of two groups cannot be discounted as the polyol pathway is differentially affected in

males and females. This pathway may be mediating a greater role in conduction velocity reduction in males than in females because of reduction of perfusion of vasa nervosum. In addition, Cameron et al. (171) observed that GLA treatment reduced the resistance to ischemic conduction block that was increased by diabetes. This may be caused by improved perfusion of vasa nervosum. During diabetes, the perfusion to vasa nervosum is reduced, which may cause ischemic preconditioning of nerves leading to increased resistance to subsequent ischemic conduction block. They also demonstrated increased capillary density in the sciatic nerve of GLA-treated diabetic rats leading to improved perfusion. The fatigue index of skeletal muscle was increased in diabetes, which increase was attenuated significantly by GLA treatment. This was also associated with increased capillary vascularization of muscle. In the subsequent study, Cameron and Cotter (172) observed that streptozotocin-induced diabetes reduced endoneural blood flow and oxygen tension that may be causing the hypoxic injury to the nerve cells leading to reduced nerve conduction and neuropathy. Treatment with evening primrose oil prevented the decrease in endoneural blood flow that was primarily caused by increased perfusion through capillaries leading to normal endoneural oxygen tension. However, in non-diabetic rats, evening primrose oil treatment increased the bulk flow (flow through major arteries, arteriols, veins, and arterio-venous shunts) without affecting capillary blood flow. The chronic increased blood flow through major vessels may be a stimulus to increased angiogenesis observed in an earlier study. Treatment with vasodilator drugs similarly caused an increase in blood flow and stimulated angiogenesis. After confirming the beneficial effects of GLA treatment in amelioration of diabetic nerve-conduction deficit, Cameron and Cotter (173) studied the effect of GLA and antioxidant treatment on nerve conduction velocity in diabetes. They selected a dose of GLA that would only correct the nerve conduction velocity reduction by about 20%. When the diabetic rats were given 20 mg/kg/day of GLA alone or in combination with an antioxidant (BM15.0639), the combination had a synergistic effect in improving the velocity. This synergistic effect on nerve conduction velocity was mediated by synergistic improvement in sciatic nerve capillary endoneural blood flow by the combined treatment. Several later studies confirmed the beneficial effects of antioxidants and GLA given in combination or as conjugates. In these studies, investigators studied the effects of GLA conjugates with ascorbic acid or  $\alpha$ -lipoic acid or in combination with various antioxidants, including Vitamin E, ascorbic acid,  $\alpha$ -lipoic acid, n-acetylcystein, butylated hydroxytoluene, and so on. These observations confirm previously reported observations that diabetic complications may be mediated by a combination of increased oxidative stress and abnormalities in essential fatty acid metabolism.

Jamal and Carmichael (174) conducted a double-blind, placebo-controlled trial of evening primrose oil in patients with type 1 and 2 diabetes with established neuropathy for at least 6 months. The patients were given 380 mg of GLA per day for 6 months and were evaluated before and after the treatment for neurological symptoms (pain, parasthesia, numbness, weakness, and abnormal sensation to heat and cold) as well as nerve conduction. At the end of the 6-month treatment, a significant improvement in 9 out of 12 variables was observed on GLA treatment. Treatment

with GLA also increased plasma phospholipid content of GLA, DGLA, and AA that are reduced in diabetes. The GLA treatment had no effect on glycosylated hemoglobin levels. These observations indicate that GLA-induced improvements may be mediated by improved perfusion of nerves rather than correction in metabolic derangements. By improving the tissue perfusion, treatment may have prevented hypoxic insult and related injury to the nerves, whereby improving the nerve conduction and reducing the associated pain and numbness. Keen et al. (175) conducted a double-blind, placebo-controlled, multicenter clinical trial of GLA in 111 diabetic patients with mild neuropathy. They studied the effect of treatment over 1 year on 13 symptoms, including motor-nerve conduction velocity, muscle strength, hot and cold thresholds, sensation, and tendon reflexes. GLA treatment at a dose of 480 mg per day was demonstrated to render benefits on functions, and the effects were more pronounced in well-controlled diabetics than in poorly controlled subjects. Keen et al. (175) used a higher dose of GLA than Jamal (174), and the study was of longer duration.

Diabetes is also known to impair immune response in humans (176) and animals, which may contribute to slow wound healing in diabetics. In streptozocin-induced diabetes in rats, a number of circulating T and B lymphocytes decreased with no effect on the number of circulating monocytes and neutrophils. In a study by Oon et al. (177), it was demonstrated that feeding evening primrose oil prevents a diabetes-induced fall in lymphocyte number that may have been mediated by increased production of PGE<sub>1</sub> in this group.

## 10. PREMENSTRUAL SYNDROME (PMS)

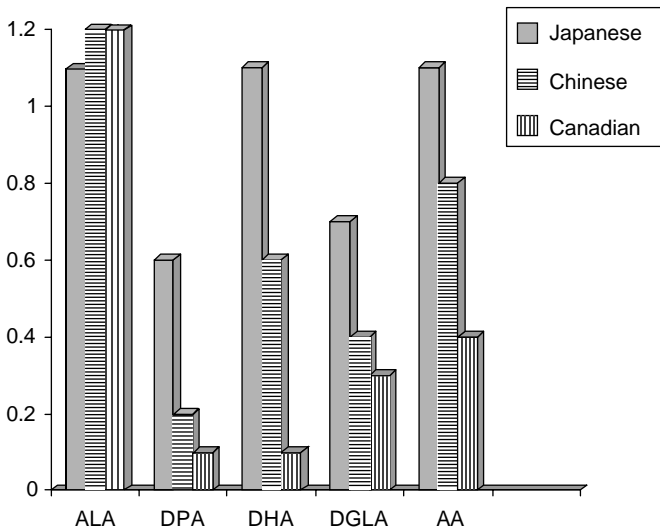
Premenstrual syndrome (PMS) is a recurrent cyclic disorder associated with the cyclic hormonal rhythms of the menstrual cycle. A large number of symptoms have been associated with PMS that are divided into physical, behavioral, and emotional symptoms. PMS may be associated with dysmenorrhea and other menstrual irregularities. Physical symptoms include bloating, abdominal and back cramps and discomfort, change in appetite, weight gain, breast tenderness and pain, and headache. Behavioral changes include anxiety, depression, lethargy, hypersomnia or insomnia, moodiness, irritability, anger, and social withdrawal. These symptoms vary in intensity from mild to severe and affect up to 90% of women some time in their child-bearing age. About 40% of women in industrialized countries suffer from mild to moderate symptoms of PMS, whereas about 10% of North American women suffer from moderate to severe symptoms affecting their daily life activities (178).

The objective of treatment is to prevent the symptoms of PMS. Several therapeutic agents are available to control the symptoms of PMS, but none of the agents are effective in controlling more than a few symptoms. In a study on 42 women suffering from PMS, a deficiency of long-chain metabolites of LA was reported with above-normal LA levels in plasma phospholipids, suggesting inhibition of delta-6-desaturase enzyme (179). As prostaglandins play an important role in regulation

of reproductive function, it was suggested that an imbalance between different prostaglandins may contribute to the symptoms of PMS. GLA, being the only natural source of DGLA that can be supplemented, may benefit these women by improving the ratio of prostaglandins of series 1 and 2. Puolakka et al. (180) performed a placebo-controlled trial of evening primrose oil in 30 women suffering from severe PMS. They observed that evening primrose oil treatment reduced the symptoms of PMS, although it was more effective in reducing depression. Treatment with evening primrose oil also reduced the production of thromboxane B<sub>2</sub> by the platelets during clotting. The effectiveness of GLA oils in treatment of PMS is not equivocally proven. Some studies reported no effect of evening primrose oil treatment over placebo (181).

### 11. INFANT NUTRITION AND DEVELOPMENT

The role of GLA in infant nutrition and development as such is not very clear. The body weight of infants at birth was positively associated with the proportions of AA and DGLA in plasma triacylglycerols and choline phosphoacylglycerols in premature infants (182) and infants born at full term (182, 183). The positive association of DGLA with birth weight is more consistent than that observed for AA or DHA. This information suggests that DGLA is playing an important role in fetal development. The exact role, however, is not clear. Careful analysis of breast milk composition from women of different geographical areas revealed that women who had a higher amount of DHA in their breast milk lipids also had a higher amounts of DGLA (Figure 3) (184). The role of DHA is well established in infant development,



**Figure 3.** Breast milk fatty acid composition from Japanese, Chinese, and Canadian women. Data adapted from (184).

while the role of GLA and DGLA is not clear. This observation suggests that DGLA may play an important role in infant development. Earlier discussion in this chapter revealed that GLA and DGLA may be playing a role in atopy in infants as the breast milk of mothers of atopic children had lower GLA and DGLA levels in breast milk. Recently popularized, the Barker hypothesis (185) (the fetal origins hypothesis) states that, during fetal development, the environmental exposures set the limit to metabolic capacity. When this capacity is exceeded later in life, it sets as an overt disease. According to this theory, the effect of nutrient deficiency may be more marked than that during later life and may cause predisposition to many chronic diseases, such as heart disease, diabetes, metabolic syndrome, etc. (186, 187). In support of this hypothesis, it was observed that children who had higher levels of GLA in umbilical cord plasma phospholipids at birth did not develop insulin resistance at the age of 7 years, while those with lower levels of DGLA had increased insulin resistance, body fat, insulin, proinsulin, and leptin concentrations (188). The exact mechanism of how DGLA in infancy and fetal development can affect the health conditions in later life is not clear. It may be possible that GLA, as a ligand for peroxisome proliferator activated receptors (PPARs), affects the transcriptional regulation of glucose and lipid homeostasis.

## 12. DRUG/NUTRIENT INTERACTIONS

GLA modulates the second messengers at the cellular levels. Many drugs act by interfering with these second messengers. This suggests that GLA may interact with drugs to affect their actions. These actions could include alterations in the therapeutic potential or side effect profiles. Cyclosporin is a potent immunosuppressant drug that is commonly used in the prevention of graft rejection in transplant recipients and in the treatment of severe psoriasis. The major side effects of cyclosporin include hypertension and renal toxicity (189, 190). In borderline hypertensive rats, GLA was shown to inhibit hypertensive and glomerular filtration-rate-reducing actions of cyclosporin (191). In Wistar rats, GLA was shown to attenuate nephrotoxic effects of cyclosporin (192). These actions could be mediated by increased production of PGE<sub>1</sub>, as demonstrated by the increased ratio of 6-keto-PGF<sub>1 $\alpha$</sub> /TXB<sub>2</sub>. GLA has been shown to enhance the sensitivity of 36B10 astrocytoma cells to radiation (193). The enhanced sensitivity could be mediated by increased free radical production as it was blocked by Vitamin E. GLA has been shown *in vitro* to enhance the cytotoxicity of paclitaxel to various breast cancer cell lines, including MDA-MB-231, MCF-7, SK Br3, and T47D (194). In this study, the authors observed the synergistic action of GLA when the cells were co-incubated with paclitaxel, whereas preincubation of cancer cell lines with GLA, followed by treatment with paclitaxel, resulted in only additive effects. These actions of GLA were only partly inhibited by Vitamin E, suggesting that increased oxidative stress may partly be contributing to the cytotoxic actions of GLA against breast cancer cell lines. Thus, GLA was found to be most potent in enhancing cytotoxic actions of paclitaxel followed by ALA, EPA, DHA, and OA, whereas LA had no effect.



Similar results were obtained for vinorelbine and GLA in breast cancer cells (MDA-MB-231, T47D, and SK-Br3) (195).

Recently, it was shown that GLA acts synergistically with tamoxifen in enhancing the antitumor activity (196). This study was conducted in nude mice implanted with estrogen receptor positive breast cancer cells (MCF-7 BIM). GLA acted synergistically with tamoxifen in inhibiting tumor growth and expression of estrogen receptors.

Ikushima et al. (197) studied the interaction of GLA on cytotoxicity of various anticancer drugs in human neuroblastoma cell lines in culture. They observed that GLA enhanced absorption and cytotoxicity of vinca alkaloids (vincristine, vinblastin, and vindesine) 2–2.5-fold. This was associated with increased lipid peroxidation of cancer cells. In the same cell lines, GLA inhibited the cytotoxic action of platinating agents like cisplatin and carboplatin. This study suggests that GLA may react differently with various anticancer drugs. Liu and Tan (198) observed that GLA and DHA increase the absorption of doxorubicin into doxorubicin-sensitive and-resistant lymphoma cancer cells. The resistant cells became sensitive to doxorubicin toxicity, which was associated with increased superoxide dismutase activity with no effect on catalase activity and p-glycoprotein levels. This observation suggests that GLA has no effect on p-glycoprotein, which plays a role in multidrug resistance development. However, by increasing the levels of only superoxide dismutase and not catalase activity, GLA may stimulate the formation of hydrogen peroxide in the cancer cells, which may contribute to oxidative toxicity of doxorubicin. Hydrogen peroxide can form hydroxyl radicals, which are highly toxic to adjacent molecules.

Kaku et al. (199) studied the interaction of GLA with soy protein and casein in mediating immune response and LTB<sub>4</sub> production by rat peritoneal exudates cells. They observed that dietary borage oil reduced production of LTB<sub>4</sub> from the peritoneal exudates cells and the effect was stimulated by soy protein but not by casein. This study suggests that soy protein, but not casein, may stimulate anti-inflammatory action of GLA-rich oils.

### 13. SAFETY OF GLA-CONTAINING OILS

GLA-containing oils have been studied in several clinical trials on humans, in addition to laboratory animal studies. All of these studies have revealed that these oils are safe and are devoid of serious side effects. The more commonly observed side effects with these oils include gastric upsets (nausea, vomiting, diarrhea, belching), and headache. Evening primrose oil was associated with reducing sensitivity to seizure threshold in patients suffering from temporal lobe epilepsy (200). Although similar effects with borage or black currant oils have not been reported, it is advisable to observe caution when giving these oils to epileptic patients. Potential of hepatotoxicity from pyrrolizidine alkaloids in borage is nonexistent (as discussed in section 2.1.1) as the content of pyrrolizidine alkaloids in borage oil is less

than 4 ppb. At this level, one may have to consume more than 250 g of oil per day to expose to toxic levels of alkaloids.

## 14. CURRENT RESEARCH FOCUS

Current focus of research is on increasing the concentration of GLA in oils and to find new sources of GLA for commercial use. The strategies include genetic manipulations, variety development, and concentrations of existing GLA-rich oils like borage and evening primrose. GLA-containing oils can be concentrated to higher GLA levels by employing common techniques such as hydrolysis of oil to form free fatty acids followed by urea complexation to remove saturated and monounsaturated fatty acids (201). Employing this technique, the oil can be concentrated to 40–80% GLA (202, 203). The resultant oil contains GLA as a free fatty acid or can be converted to ethyl ester or triacylglycerol form by chemical/enzymatic esterification. The triacylglycerol form produced in this way contains about 50–70% triacylglycerols, 10–25% diacylglycerols, and 5–10% monoacylglycerols (Bioriginal Food and Science Corp). The enzymatic process involves the use of microbial lipases (from *Pseudomonas* sp.). Other areas of research include increasing the content of GLA in foods and alternative crops by genetic engineering. Cook et al. (204) inserted the delta-6-desaturase gene from borage into tomato. This strategy resulted in an increase in the content of GLA in tomato fruit along with a reduction in LA content. Although this variety has not been commercialized, there is a potential in optimizing the variety. Similar efforts have been made on other plants, including tobacco (205, 206) and canola (207). GLA levels in tobacco plants could be increased to about 14% of total fatty acids. At this level, it is not an economical source for production of GLA. Recently, Ross Labs has been successful in producing a transgenic variety of canola plant that contains up to 40% GLA in its seed oil (207). The transgenic canola oil containing GLA was compared with borage oil for its pharmacological actions and was found to be similar to borage oil, demonstrating that the transgenic canola oil could become an economically viable source of GLA.

Another area of current research is development of structured lipids where GLA is combined with a fatty acid of omega-3 family, preferably EPA or DHA, into one triacylglycerol molecule. Structured lipids can be produced by interesterifying a mixture of conventional fats and oils of interest using chemical or enzymatic methods. Chemical methods provide random distribution of different fatty acids on the glycerol backbone, whereas enzymatic reactions could be position specific, affording controlled production of triacylglycerols with desired configuration (208). Interesterification using the chemical method usually involves a reaction between two oils using metal alkoxide (sodium methoxide) as a catalyst. The unreacted fatty acids are removed by vacuum distillation. The alternative and more researched process involves acidolysis using lipases. In this process, either pure fatty acid is reacted with a triacylglycerol molecule or relatively rich fraction of fatty acid of interest is taken/prepared before acidolysis reaction.

The structured lipids have unique chemical, physical, or physiologic properties that are not observed by simply blending mixtures of the starting fats and oils (209). At present, the enzymatic process is under development but has not been widely commercialized so far due to the economy of the process. Laboratory research is in progress with the objective to develop a process that can be economically scaled up. The current emphasis is on optimization of lipases, reaction conditions including water activity of the reaction mixture, mole ratios of fatty acids to triacylglycerol, amount of enzyme, reaction temperature, and duration. The nonspecific lipases can be obtained from *Candida rugosa*, *Pseudomonas* sp, while 1, 3-position specific lipase are obtained from *Aspergillus niger*, *Mucor javanicus*, *Rhizomucor miehei*, *Rhizopus* sp., *Geotrichum candidum*, *Candida cylindracea*, *Candida parapolitytica*, *Rhizopus delemar*, etc. One can utilize either pure EPA or DHA as free acid or ethyl esters for incorporation into borage or evening primrose oil or GLA can be added to fish oils containing EPA and DHA. In these approaches, a structured lipid containing EPA, DHA, and GLA in one triacylglycerol molecule is produced. Spurvey et al. (210) studied the effect of reaction conditions on the incorporation of GLA into menhaden and seal blubber oils. They observed that the best conditions include a mole ratio of 3:1 for GLA to triacylglycerol, enzyme concentration of 500 units/g of oil, reaction temperature of 40°C, and time of 24 hours for incorporation of GLA into fish oils. They utilized concentrated GLA from borage oil for acidolytic reaction. The GLA of 91% concentration was prepared by chemical hydrolysis followed by urea complexation (201). This concentrate was reacted with fish oils using lipase from *Pseudomonas* sp (nonspecific enzyme) and *Mucor miehei* (sn 1,3-specific enzyme). Lipase from *Pseudomonas* sp. gave higher incorporation of GLA into fish oil. Using a similar approach, production of EPA-rich borage oil and evening primrose oil has been produced (208).

## REFERENCES

1. A. G. Hassam, J. P. Rivers, and M. A. Crawford, *J. Nutr.*, **107**(4), 519–524 (1977).
2. A. G. Hassam, J. P. Rivers, and M. A. Crawford, *Nutr. Metab.*, **21**(Suppl 1): 190–192 (1977).
3. J. P. Rivers, A. J. Sinclair, and M. A. Crawford, *Nature*, **258**(5531), 171–173 (1975).
4. H. Sprecher, *Prog. Lipid Res.*, **20**, 13–22 (1981).
5. R. R. Brenner, *Adv. Exp. Med. Biol.*, **83**, 85–101 (1977).
6. G. D. de, I. M. J. de Alaniz, and R. R. Brenner, *Lipids*, **19**(2), 91–95 (1984).
7. S. Ayala and R. R. Brenner, *Acta Physiol. Lat. Am.*, **33**(3), 193–204 (1983).
8. R. Kleiman, F. R. Earle, I. A. Wolff, and Q. Jones, *J. Am. Oil Chem. Soc.*, **41**(7), 459–460 (1964).
9. J. Janick, J. Simon, J. Quinn, and N. Beaubaire, *Herbs, Spices and Med. Plants*, **4**, 147–167 (1989).
10. K. R. Fell and J. M. Peck, *Planta Medica*, **16**, 29–42 (1968).

11. M. L. Fernald, *Grey's Manual of Botany*, 8th ed., D. Van Nostrand Co., New York, NY, 1970, 1198.
12. P. Clough, in *Structured and Modified Lipids*, F. D. Gunstone, ed., Marcell Dekker, Inc., New York, NY, 2000, pp. 75–117.
13. M. J. A. Simpson, *Ann. Appl. Biol.*, **123**, 105–108 (1993).
14. R. El Hafid, S. F. Blade, and Y. Hoyano, *Industrial Crops and Products*, **16**, 193–199 (2002).
15. A. De Haro, V. Dominguez, and M. del Rio, *J. Herbs, Spices and Med. Plants*, **9**(4), 297–304 (2002).
16. J. Janick, J. E. Simon, and A. Whipkey, *Hort. Sci.*, **22**, 493–495 (1987).
17. J. Quinn, J. E. Simon, and J. Janick, *J. Am. Soc. Hort. Sci.*, **114**(3), 511–515 (1989).
18. A. Whipkey, J. Simon, and J. Janick, *J. Am. Oil Chem. Soc.*, **65**(6), 979–984 (1988).
19. N. A. Beabaire, Master's Thesis, Purdue University, West Lafayette, Indiana, 1987.
20. A. Sensidoni, G. Bortolussi, C. Orlando, G. Lognay, P. Fantozzi, and M. Paquot, *Lebensm. Wiss. Technol.*, **28**(3), 343–346 (1995).
21. I. Wretnesjo and B. Karlberg, *J. Amer. Oil Chem. Soc.*, **79**(11), 1069–1074 (2002).
22. C. Dodson and F. R. Stermitz, *J. Nat. Prod.*, **49**(4), 727–728 (1986).
23. M. Herrmann, H. Joppe, and G. Schmaus, *Phytochemistry*, **60**(4), 399–402 (2002).
24. O. Parvais and V. Stricht, *J. Planar Chromat.*, **7**(1/2), 80–82 (1994).
25. H. J. Mierendorff, *Fett. Wissenschaft Technologie.*, **97**(1), 33–37 (1995).
26. H. W. Hulan, I. Hall, and D. M. Nash, *Crop. Res.*, **27**, 1–9 (1987).
27. W. W. Christie, *Industrial Crops and Products*, **10**, 73–83 (1999).
28. M. Wettasinghe, F. Shahidi, and R. Amarowicz, *J. Agric. Food Chem.*, **50**(5), 1267–1271 (2002).
29. B. D. Oomah, M. Busson, D. V. Godfrey, and J. C. G. Drover, *Food Chem.*, **76**(1), 33–43 (2002).
30. Hemp Oil Canada Inc. (2004) *Nutritional Composition*. Available: <http://hempoilcanada.com/compo.html>.
31. C. C. J. Culvenor, *Trends Pharmacol. Sci.*, **6**(1), 18–22 (1985).
32. C. C. J. Culvenor, *Aust. J. Chem.*, **9**, 512 (1956).
33. A. A. Seawright, M. P. Hegarty, L. F. James, and R. F. Keeler, in *Plant Toxicology—Proceedings of the Australia / USA Poisonous Plants Symposium Yeerongpilly, Brisbane, Australia*, 1985.
34. A. Molero Gomez and E. Martinez de la Ossa, *Chem. Eng. J.*, **88**(1–3), 103–109 (2002).
35. J. Leyton, P. J. Drury, and M. A. Crawford, *Lipids*, **22**(8), 553–558 (1987).
36. L. Chilton, M. E. Surette, D. D. Swan, A. N. Fonteh, M. M. Johnson, and F. H. Chilton, *J. Immunol.*, **156**(8), 2941–2947 (1996).
37. A. G. Hassam, A. J. Sinclair, and M. A. Crawford, *Lipids*, **10**(7), 417–420 (1975).
38. A. G. Hassam and M. A. Crawford, *Nutr. Metab.*, **20**(2), 112–116 (1976).
39. M. M. Engler, J. W. Karaian, and N. Salem, Jr., *Nutr. Res.*, **11**, 753–763 (1991).
40. J. D. Palombo, S. J. DeMichele, E. E. Lydon, T. J. Gregory, P. L. Banks, R. A. Forse, and B. R. Bistriani, *Am. J. Clin. Nutr.*, **63**(2), 208–219 (1996).

41. J. D. Palombo, S. J. DeMichele, E. Lydon, and B. R. Bistrrian, *J. Parenter. Enteral Nutr.*, **21**(3), 123–132 (1997).
42. D. E. Barre and B. J. Holub, *Lipids*, **27**(5), 315–320 (1992).
43. D. E. Barre and B. Holub, *J. Nutr. Res.*, **12**(10), 1181–1194 (1992).
44. J. Martens-Lobenhoffer and F. P. Meyer, *Int. J. Clin. Pharmacol. Ther.*, **36**(7), 363–366 (1998).
45. M. S. Manku, N. Morse-Fisher, and D. F. Horrobin, *Eur. J. Clin. Nutr.*, **42**(1), 55–60 (1988).
46. M. M. Engler and M. B. Engler, *Prostaglandins Leukot. Essent. Fatty Acids*, **59**(1), 11–15 (1998).
47. L. Chilton, M. E. Surette, D. D. Swan, A. N. Fonteh, M. M. Johnson, and F. H. Chilton, *J. Immunol.*, **156**(8), 2941–2947 (1996).
48. L. D. Lawson and B. Hughes, *Lipids*, **23**(4), 313–317 (1988).
49. D. Raederstorff and U. Moser, *Lipids*, **27**(12), 1018–1023 (1992).
50. S. Chung, S. Kong, K. Seong, and Y. Cho, *J. Nutr.*, **132**(10), 3090–3097 (2002).
51. J. C. Rose, M. Johnson, P. W. Ramwell, and P. A. Kot, *Proc. Soc. Exp. Biol. Med.*, **148**(4), 1252–1256 (1975).
52. B. A. Scholkens, D. Gehring, V. Schlotte, and U. Weithmann, *Prostaglandins Leukot. Med.*, **8**(3), 273–285 (1982).
53. D. E. Mills and R. Ward, *Proc. Soc. Exp. Biol. Med.*, **176**(1), 32–37 (1984).
54. D. E. Mills, K. M. Prkachin, K. Harvey, and R. Ward, *J. Hypertens.*, **3**(2), 111–116 (1989).
55. G. C. Leng, A. J. Lee, F. G. R. Fowkes, R. G. Jepson, and G. D. O. Low, *Clin. Nutr.*, **17**(6), 265–271 (1998).
56. M. M. Engler, M. B. Engler, S. Erickson, and S. M. Paul, *J. Hypertens.*, **10**(10), 1197–1204 (1992).
57. B. A. Scholkens, D. Gehring, V. Schlotte, and U. Weithmann, *Prostaglandins Leukot. Med.*, **8**(3), 273–285 (1982).
58. M. M. Engler, M. B. Engler, S. K. Erickson, and S. M. Paul, *J. Hypertens.*, **10**(10), 1197–1204 (1992).
59. P. Hoffmann, H. U. Block, J. Beitz, C. Taube, W. Forster, P. Wortha, P. Singer, E. Naumann, and H. Heine, *Lipids*, **21**(12), 733–737 (1986).
60. M. M. Engler, M. Schambelan, M. B. Engler, D. L. Ball, and T. L. Goodfriend, *Proc. Soc. Exp. Biol. Med.*, **218**(3), 234–237 (1998).
61. D. E. Mills, M. Mah, R. P. Ward, B. L. Morris, and J. S. Floras, *Am. J. Physiol.*, **259**(6 Pt 2), R1164–R1171 (1990).
62. J. Chaintreuil, L. Monnier, C. Colette, P. Crastes de Paulet, A. Orsetti, D. Spielmann, F. Mendy, and A. Crastes de Paulet, *Hum. Nutr. Clin. Nutr.*, **38**(2), 121–130 (1984).
63. P. Singer, P. Hoffmann, J. Beitz, W. Forster, M. Wirth, and W. Godicke, *Prostaglandins Leukot. Med.*, **22**(2), 173–177 (1986).
64. M. Boberg, B. Vessby, and I. Selinus, *Acta. Med. Scand.*, **220**(2), 153–160 (1986).
65. J. Viikari and A. Lehtonen, *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **24**(12), 668–670 (1986).
66. M. Guivernau, N. Meza, P. Barja, and O. Roman, *Prostaglandins Leukot. Essent. Fatty Acids*, **51**(5), 311–316 (1994).

67. M. Lagarde, M. Burtin, M. Dechavanne, B. Sicard, and B. Coiffier, *Prostaglandins Med.*, **4**(3), 177–183 (1980).
68. P. B. A. Kernoff, A. L. Willis, K. J. Stone, J. A. Davies, and G. P. McNicol, *Br. Med. J.*, **2**(6100), 1441–1444 (1977).
69. J. C. Rose, M. Johnson, P. W. Ramwell, and P. A. Kot, *Proc. Soc. Exp. Biol. Med.*, **148**(4), 1252–1256 (1975).
70. T. Ishikawa, Y. Fujiyama, O. Igarashi, M. Morino, N. Tada, A. Kagami, T. Sakamoto, M. Nagano, and H. Nakamura, *Atherosclerosis*, **75**(2–3), 95–104 (1989).
71. J. Jantti, T. Nikkari, T. Solakivi, H. Vapaatalo, and H. Isomaki, *Ann. Rheum. Dis.*, **48**(2), 124–127 (1989).
72. D. F. Horrobin and M. S. Manku, *Lipids*, **18**(8), 558–562 (1983).
73. M. Fukushima, K. Shimada, E. Ohashi, H. Saitoh, K. Sonoyama, M. Sekikawa, and M. Nakano, *J. Nutr. Sci. Vitaminol. (Tokyo)*, **47**(3), 228–235 (2001).
74. K. Koba, J. W. Liu, L. T. Chuang, S. N. Anderson, T. Bowman, E. Bobik, Jr., M. Sugano, and Y. S. Huang, *Biosci. Biotechnol. Biochem.*, **64**(12), 2538–2542 (2000).
75. S. Renaud, L. Mcgregor, R. Morazain, C. Thevenon, C. Benoit, E. Dumont, and F. Mendy, *Atherosclerosis*, **45**(1), 43–51 (1982).
76. A. M. Sadi, T. Toda, H. Oku, and S. Hokama, *Exp. Anim.*, **45**(1), 55–62 (1996).
77. Y. Y. Fan, K. S. Ramos, and R. S. Chapkin, *Arterioscler. Thromb. Vasc. Biol.*, **15**(9), 1397–1403 (1995).
78. C. Maziere, F. Dantin, M. A. Conte, J. Degonville, D. Ali, F. Dubois, and J. C. Maziere, *Biochem. J.*, **336**(Pt 1) 57–62 (1998).
79. Y. Y. Fan, K. S. Ramos, and R. S. Chapkin, *J. Nutr.*, **131**(6), 1675–1681 (2001).
80. Y. Li, J. X. Kang, and A. Leaf, *Prostaglandins*, **54**(2), 511–530 (1997).
81. J. S. Charnock, P. L. McLennan, M. Y. Abeywardena, and W. F. Dryden, *Ann. Nutr. Metab.*, **29**(5), 306–318 (1985).
82. A. R. Kristal, J. H. Cohen, P. Qu, and J. L. Stanford, *Cancer Epidemiol. Biomarkers Prev.*, **11**(8), 719–725 (2002).
83. F. Levi, C. Pasche, F. Lucchini, and C. La Vecchia, *Ann. Oncol.*, **13**(3), 369–373 (2002).
84. A. J. Littman, S. A. Beresford, and E. White, *Cancer Causes Control*, **12**(8), 691–702 (2001).
85. C. Byrne, H. Rockett, and M. D. Holmes, *Cancer Epidemiol. Biomarkers Prev.*, **11**(3), 261–265 (2002).
86. R. Jarvinen, P. Knekt, T. Hakulinen, H. Rissanen, and M. Heliövaara, *Br. J. Cancer*, **85**(3), 357–361 (2001).
87. S. A. Smith-Warner, D. Spiegelman, H. O. Adami, W. L. Beeson, P. A. van den Brandt, A. R. Folsom, G. E. Fraser, J. L. Freudenheim, R. A. Goldbohm, S. Graham, L. H. Kushi, A. B. Miller, T. E. Rohan, F. E. Speizer, P. Toniolo, W. C. Willett, A. Wolk, A. Zeleniuch-Jacquotte, and D. J. Hunter, *Int. J. Cancer*, **92**(5), 767–774 (2001).
88. N. Dippenaar, J. Booyens, D. Fabbri, P. Engelbrecht, and I. E. Katzeff, *S. Afr. Med. J.*, **62**(19), 683–685 (1982).
89. J. Booyens, N. Dippenaar, D. Fabbri, P. Engelbrecht, C. C. Louwrens, and I. E. Katzeff, *S. Afr. Med. J.*, **65**(15), 607–612 (1984).
90. P. Coffino, H. R. Bourne, and G. M. Tomkins, *Am. J. Pathol.*, **81**(1), 199–204 (1975).

91. M. E. Begin, U. N. Das, G. Ells, and D. F. Horrobin, *Prostaglandins Leukot. Essent. Fatty Acids*, **19**(2), 177–186 (1985).
92. J. H. Lee and M. Sugano, *Nutr. Rep. Int.*, **34**(6), 1041–1049 (1986).
93. S. Abou El-Ela, K. W. Prasse, R. Carroll, and O. R. Bunce, *Lipids*, **22**(12), 1041–1044 (1987).
94. C. A. Gonzalez, J. M. Sanz, G. Marcos, S. Pita, E. Brullet, E. Saigi, A. Badia, A. Agudo, and E. Riboli, *Cancer Epidemiol. Biomarkers Prev.*, **2**(2), 157–158 (1993).
95. J. H. Botha, K. M. Robinson, N. Ramchurren, and R. J. Norman, *Prostaglandins Leukot. Essent. Fatty Acids*, **35**(2), 119–123 (1989).
96. F. S. Kenny, S. E. Pinder, I. O. Ellis, J. M. Gee, R. I. Nicholson, R. P. Bryce, and J. F. Robertson, *Int. J. Cancer*, **85**(5), 643–648 (2000).
97. P. J. du Toit, C. H. van Aswegen, and D. J. du Plessis, *Prostaglandins Leukot. Essent. Fatty Acids*, **51**(2), 121–124 (1994).
98. P. J. du Toit, C. H. van Aswegen, and D. J. du Plessis, *Prostaglandins Leukot. Essent. Fatty Acids*, **55**(3), 173–177 (1996).
99. W. G. Jiang, S. Hiscox, M. B. Hallett, C. Scott, D. F. Horrobin, and M. C. Puntis, *Br. J. Cancer*, **71**(4), 744–752 (1995).
100. W. G. Jiang, S. Hiscox, R. P. Bryce, D. F. Horrobin, and R. E. Mansel, *Br. J. Cancer*, **77**(5), 731–738 (1998).
101. W. G. Jiang, A. Redfern, R. P. Bryce, and R. E. Mansel, *Prostaglandins Leukot. Essent. Fatty Acids*, **62**(2), 119–127 (2000).
102. T. Liang and S. Liao, *Biochem. J.*, **285**(Pt 2), 557–562 (1992).
103. L. Kleinberg, S. A. Grossman, K. Carson, G. Lesser, A. O'Neill, J. Pearlman, P. Phillips, T. Herman, and M. Gerber, *J. Clin. Oncol.*, **20**(14), 3149–3155 (2002).
104. A. A. Brandes, U. Basso, and L. M. Pasetto, *Expert. Rev. Anticancer Ther.*, **1**(3), 357–370 (2001).
105. M. R. C. Naidu, U. N. Das, and A. Kishan, *Prostaglandins Leukot. Essent. Fatty Acids*, **45**, 181–184 (1992).
106. U. N. Das, W. S. K. Prasad, and D. R. Reddy, *Cancer Let.*, **94**(2), 147–155 (1995).
107. C. F. van der Merwe, J. Booyens, H. F. Joubert, and C. A. van der Merwe, *Prostaglandins Leukot. Essent. Fatty Acids*, **40**(3), 199–202 (1990).
108. J. S. Falconer, K. C. Fearon, J. A. Ross, and D. C. Carter, *World Rev. Nutr. Diet.*, **76**, 74–76 (1994).
109. P. Sangeetha, U. N. Das, R. Koratkar, G. Ramesh, M. Padma, and G. Sravan Kumar, *Cancer Let.*, **63**, 189–198 (1992).
110. H. A. Leaver, J. R. Williams, J. W. Ironside, E. P. Miller, A. Gregor, B. H. Su, R. J. Prescott, and I. R. Whittle, *Eur. J. Clin. Invest.*, **29**(3), 220–231 (1999).
111. M. de Kock, M. L. Lottering, and J. C. Seegers, *Prostaglandins Leukot. Essent. Fatty Acids*, **51**(2), 109–120 (1994).
112. M. de Kock, M. L. Lottering, C. J. Grobler, T. C. Viljoen, M. le Roux, J. C. Seegers, *Prostaglandins Leukot. Essent. Fatty Acids*, **55**(6), 403–411 (1996).
113. W. G. Jiang, R. P. Bryce, D. F. Horrobin, and R. E. Mansel, *Int. J. Oncol.*, **13**(3), 611–617 (1998).

114. J. C. Seegers, M. de Kock, M. L. Lottering, C. J. Grobler, D. H. van Papendorp, Y. Shou, R. Habberset, and B. E. Lehnert, *Prostaglandins Leukot. Essent. Fatty Acids*, **56**(4), 271–280 (1997).
115. D. F. Horrobin, *Wien. Klin. Wochenschr.*, **100**(14), 471–477 (1988).
116. L. Chilton, M. E. Surette, D. D. Swan, A. N. Fonteh, M. M. Johnson, and F. H. Chilton, *J. Immunol.*, **156**(8), 2941–2947 (1996).
117. V. A. Ziboh and M. P. Fletcher, *Am. J. Clin. Nutr.*, **55**(1), 39–45 (1992).
118. S. Kaku, K. Ohkura, S. Yunoki, M. Nonaka, H. Tachibana, M. Sugano, and K. Yamada, *Prostaglandins Leukot. Essent. Fatty Acids*, **65**(4), 205–210 (2001).
119. D. Santoli and R. B. Zurier, *J. Immunol.*, **143**(4), 1303–1309 (1989).
120. W. V. Williams, H. Rosenbaum, and R. B. Zurier, *Pathobiology*, **64**(1), 27–31 (1996).
121. D. M. DeMarco, D. Santoli, and R. B. Zurier, *J. Leukocyte Biol.*, **56**(5), 612–615 (1994).
122. D. Rotondo, C. R. Earl, K. J. Laing, and D. Kaimakamis, *Biochim. Biophys. Acta*, **1223**(2), 185–194 (1994).
123. D. Rothman, H. Allen, L. Herzog, A. Pilapil, C. M. Seiler, and R. B. Zurier, *Cytokine*, **9**(12), 1008–1012 (1997).
124. P. DeLuca, R. G. Rossetti, C. Alavian, P. Karim, and R. B. Zurier, *J. Investig. Med.*, **47**(5), 246–250 (1999).
125. R. K. Furse, R. G. Rossetti, and R. B. Zurier, *J. Immunol.*, **167**(1), 490–496 (2001).
126. R. G. Rossetti, C. M. Seiler, M. Laposata, and R. B. Zurier, *Clin. Immunol. Immunopathol.*, **76**(3), 220–224 (1995).
127. D. Vassilopoulos, R. B. Zurier, R. G. Rossetti, and G. C. Tsokos, *Clin. Immunol. Immunopathol.*, **83**(3), 237–244 (1997).
128. D. Wu, M. Meydani, L. Leka, Z. Nightingale, G. Handelman, and J. Blumberg, *Am. J. Clin. Nutr.*, **70**(4), 536–543 (1999).
129. J. L. Nerad, S. N. Meydani, and C. A. Dinarello, *Cytokine*, **3**, 513 (1991).
130. R. B. Zurier, R. G. Rossetti, C. M. Seiler, and M. Laposata, *Prostaglandins Leukot. Essent. Fatty Acids*, **60**(5–6), 371–375 (1999).
131. G. A. Tate, B. F. Mandell, R. A. Karmali, M. Laposata, D. G. Baker, H. R. Schumacher, Jr., and R. B. Zurier, *Arthritis Rheum.*, **31**(12), 1543–1551 (1988).
132. G. A. Tate and R. B. Zurier, *Agents Actions*, **43**(1–2), 35–38 (1994).
133. T. M. Hansen, A. Lerche, V. Kassis, I. Lorenzen, and J. Sondergaard, *Scand. J. Rheumatol.*, **12**(2), 85–88 (1983).
134. J. J. Belch, D. Ansell, R. Madhok, A. O'Dowd, and R. D. Sturrock, *Ann. Rheum. Dis.*, **47**(2), 96–104 (1988).
135. S. Pullman-Mooar, M. Laposata, D. Lem, R. T. Holman, L. J. Leventhal, D. DeMarco, and R. B. Zurier, *Arthritis Rheum.*, **33**(10), 1526–1533 (1990).
136. L. J. Leventhal, E. G. Boyce, and R. B. Zurier, *Ann. Intern. Med.*, **119**(9), 867–873 (1993).
137. R. B. Zurier, R. G. Rossetti, E. W. Jacobson, D. M. DeMarco, N. Y. Liu, J. E. Temming, B. M. White, and M. Laposata, *Arthritis Rheum.*, **39**(11), 1808–1817 (1996).
138. K. V. Kumar, S. M. Rao, R. Gayani, I. K. Mohan, and M. U. Naidu, *Clin. Chim. Acta*, **298**(1–2), 111–120 (2000).



139. J. E. Gadek, S. J. DeMichele, M. D. Karlstad, E. R. Pacht, M. Donahoe, T. E. Albertson, C. Van Hoozen, A. K. Wennberg, J. L. Nelson, and M. Noursalehi, *Crit. Care Med.*, **27**(8), 1409–1420 (1999).
140. M. M. Murray, M. Kumar, T. Gregory, P. Banks, H. D. Tazelaar, and S. J. DeMichele, *Am. J. Physiol.*, **269**(6 pt 2), H2090–H2099 (1995).
141. P. Mancuso, J. Whelan, S. J. DeMichele, C. C. Snider, J. A. Guszczka, K. J. Claycombe, G. T. Smith, T. J. Gregory, and M. D. Karlstad, *Crit. Care Med.*, **25**(3), 523–532 (1997).
142. G. O. Burr and M. M. Burr, *J. Biol. Chem.*, **82**(2), 345–367 (1929).
143. R. S. Chapkin, V. A. Ziboh, and J. L. McCullough, *J. Nutr.*, **117**(8), 1360–1370 (1987).
144. A. E. Hansen, *Proc. Soc. Exp. Biol. Med.*, **31**, 160–161 (1933).
145. M. S. Manku, D. F. Horrobin, N. Morse, V. Kyte, K. Jenkins, S. Wright, and J. L. Burton, *Prostaglandins Leukot. Med.*, **9**(6), 615–628 (1982).
146. S. Wright and C. Bolton, *Br. J. Nutr.*, **62**(3), 693–697 (1989).
147. L. Businco, M. Ioppi, N. L. Morse, R. Nisini, and S. Wright, *J. Allergy Clin. Immunol.*, **91**(6), 1134–1139 (1993).
148. S. Wright and J. L. Burton, *The Lancet*, **2**(8308), 1120–1122 (1982).
149. M. S. Manku, D. F. Horrobin, N. L. Morse, S. Wright, and J. L. Burton, *Br. J. Dermatol.*, **110**(6), 643–648 (1984).
150. L. Schafer and K. Kragballe, *Lipids*, **26**(7), 557–560 (1991).
151. D. H. Scarff and D. H. Lloyd, *Vet. Record*, **131**(5), 97–99 (1992).
152. A. Fiocchi, M. Sala, P. Signoroni, G. Banderali, C. Agostoni, and E. Riva, *J. Int. Med. Res.*, **22**(1), 24–32 (1994).
153. S. Borrek, A. Hildebrandt, and J. Forster, *Klin. Padiatr.*, **209**(3), 100–104 (1997).
154. P. J. Hartop and C. Prottey, *Br. J. Dermatol.*, **95**(3), 255–264 (1976).
155. A. Tolleson and A. Frithz, *Acta Derm. Venereol.*, **73**(1), 18–20 (1993).
156. B. M. Henz, S. Jablonska, P. C. van de Kerkhof, G. Stingl, M. Blaszczyk, P. G. Vandervalk, R. Veenhuizen, R. Muggli, and D. Raederstorff, *Br. J. Dermatol.*, **140**(4), 685–688 (1999).
157. A. Takwale, E. Tan, S. Agarwal, G. Barclay, I. Ahmed, K. Hotchkiss, J. R. Thompson, T. Chapman, and J. Berth-Jones, *Br. Med. J.*, **327**(7428), 1385 (2003).
158. B. M. Henz, S. Jablonska, P. C. van de Kerkhof, G. Stingl, M. Blaszczyk, P. G. Vandervalk, R. Veenhuizen, R. Muggli, and D. Raederstorff, *Br. J. Dermatol.*, **140**(4), 685–688 (1999).
159. M. D. Giron, F. J. Mataix, and M. D. Suarez, *Biochim. Biophys. Acta.*, **1045**, 69–73 (1990).
160. K. L. Campbell and G. P. Dorn, *Res. Vet. Sci.*, **53**, 172–178 (1992).
161. C. C. Miller and V. A. Ziboh, *Biochem. Biophys. Res. Commun.*, **154**(3), 967–974 (1988).
162. V. A. Ziboh, C. C. Miller, and Y. Cho, *Am. J. Clin. Nutr.*, **71**(1 Suppl), 361S–366S (2000).
163. C. R. Charman, A. J. Venn, and H. C. Williams, *Br. J. Dermatol.*, **146**(6), 1057–1060 (2002).
164. J. W. Hopewell, G. J. van den Aardweg, G. M. Morris, M. Rezvani, M. E. Robbins, G. A. Ross, E. M. Whitehouse, C. A. Scott, and D. F. Horrobin, *Int. J. Radiat. Oncol. Biol. Phys.*, **30**(5), 1119–1125 (1994).
165. S. Ayala and R. R. Brenner, *Acta Physiol. Lat. Am.*, **25**(5), 371–378 (1975).

166. J. P. Poisson, *Enzyme*, **34**(1), 1–14 (1985).
167. O. Mercuri, R. O. Peluffo, and R. R. Brenner, *Biochim. Biophys. Acta*, **116**(2), 409–411 (1966).
168. M. Arisaka, O. Arisaka, and Y. Yamashiro, *Prostaglandins Leukot. Essent. Fatty Acids*, **43**(3), 197–201 (1991).
169. P. O. Julu, *J. Diabet. Complications*, **2**(4), 185–188 (1988).
170. P. O. Julu and A. Mutamba, *J. Neurol. Sci.*, **106**(1), 56–59 (1991).
171. N. E. Cameron, M. A. Cotter, and S. Robertson, *Diabetes*, **40**(5), 532–539 (1991).
172. N. E. Cameron and M. A. Cotter, *Acta Diabetologica*, **31**(4), 220–225 (1994).
173. N. E. Cameron and M. A. Cotter, *Am. J. Physiol.*, **271**(3 Pt 1), E471–E476 (1996).
174. G. A. Jamal and H. Carmichael, *Diabetic Medicine*, **7**(4), 319–323 (1990).
175. H. Keen, J. Payan, J. Allawi, J. Walker, G. A. Jamal, A. I. Weir, L. M. Henderson, E. A. Bissessar, P. J. Watkins and M. Sampson, *Diabetes Care*, **16**(1), 8–15 (1993).
176. B. Singh, J. Lauzon, J. Venkatraman, A. B. Thomson, R. V. Rajotte, and M. T. Clandinin, *Diabetes Res.*, **8**(3), 129–134 (1988).
177. B. B. Oon, D. Muggleston, and A. Warley, *Exp. Physiol.*, **77**(1), 185–190 (1992).
178. D. N. Ugarriza, S. Klingner, and S. O'Brien, *Nurse Pract.*, **23**(9), 40–52 (1998).
179. M. G. Brush, S. J. Watson, D. F. Horrobin, and M. S. Manku, *Am. J. Obstet. Gynecol.*, **150**(4), 363–366 (1984).
180. J. Puolakka, L. Makarainen, L. Viinikka, and O. Ylikorkala, *J. Reprod. Med.*, **30**(3), 149–153 (1985).
181. S. K. Khoo, C. Munro, and D. Battistutta, *Med. J. Aust.*, **153**(4), 189–192 (1990).
182. A. A. Leaf, M. J. Leighfield, K. L. Costeloe, and M. A. Crawford, *Early Hum. Dev.*, **30**(3), 183–191 (1992).
183. P. Rump, R. P. Mensink, A. D. M. Kester, and G. Hornstra, *Am. J. Clin. Nutr.*, **73**(4), 797–806 (2001).
184. L. Wang, Y. Shimizu, S. Kaneko, S. Hanaka, T. Abe, H. Shimasaki, H. Hisaki, and H. Nakajima, *Pediatrics. Int.*, **42**(1), 14–20 (2000).
185. D. J. Barker, P. D. Winter, C. Osmond, B. Margetts, and S. J. Simmonds, *Lancet*, **2**(8663), 577–580 (1989).
186. D. W. Lamont, L. Parker, M. A. Cohen, M. White, S. M. Bennett, N. C. Unwin, A. W. Craft, and K. G. Alberti, *Public Health*, **112**(2), 85–93 (1998).
187. A. A. Jackson, *Adv. Exp. Med. Biol.*, **478**, 41–55 (2000).
188. P. Rump, C. Popp-Snijders, R. J. Heine, and G. Hornstra, *Diabetologia*, **45**(3), 349–355 (2002).
189. J. M. Rabkin, C. L. Corless, H. R. Rosen, and A. J. Olyaei, *Am. J. Surg.*, **183**(5), 595–599 (2002).
190. T. Markham, A. Watson, and S. Rogers, *Clin. Exp. Dermatol.*, **27**(2), 111–114 (2002).
191. D. E. Mills, M. Mah, R. P. Ward, B. Morris, and J. Floras, *Am. J. Physiol.*, **259**(6 Pt 2), R1164–R1171 (1990).
192. P. Morphake, J. Bariety, I. Darlametsos, and G. Tsipas, *Prostaglandins Leukot. Essent. Fatty Acids*, **50**(1), 29–35 (1993).
193. S. Vartak, M. E. Robbins, and A. A. Spector, *Lipids*, **32**(3), 283–292 (1997).

194. J. A. Menendez, M. D. Barbacid, S. Montero, E. Sevilla, E. Escrich, M. Solanas, H. Cortes-Funes, and R. Colomer, *Eur. J. Cancer*, **37**(3), 402–413 (2001).
195. J. A. Menendez, S. Ropero, M. M. del Barbacid, S. Montero, M. Solanas, E. Escrich, H. Cortes-Funes, and R. Colomer, *Breast Cancer Res. Treat.*, **72**(3), 203–219 (2002).
196. F. S. Kenny, J. M. Gee, R. I. Nicholson, I. O. Ellis, T. M. Morris, S. A. Watson, R. P. Bryce, and J. F. Robertson, *Int. J. Cancer*, **92**(3), 342–347 (2001).
197. S. Ikushima, F. Fujiwara, S. Todo, and S. Imashuku, *Anticancer Res.*, **10**(4), 1055–1059 (1990).
198. Q. Y. Liu and B. K. Tan, *Life Sci.*, **67**(10), 1207–1218 (2000).
199. S. Kaku, S. Yunoki, K. Ohkura, M. Sugano, M. Nonaka, H. Tachibana, and K. Yamada, *Biosci. Biotechnol. Biochem.*, **65**(2), 315–321 (2001).
200. K. S. Vaddadi, *Prostaglandins Med.*, **6**(4), 375–379 (1981).
201. S. P. Senanayake and Shahidi F, *J. Food Lipids*, **7**, 51–61 (2000).
202. J. C. Philips and Y. S. Huang, in *Gamma-linolenic acid: Metabolism and its roles in Nutrition and Medicine*, Y. S. Huang and D. E. Mills, eds. AOCS Press, Champaign, IL, 1996, pp. 1–13.
203. F. C. Huang, Y. H. Ju, and J. C. Chiang, *J. Am. Oil Chem. Soc.*, **76**(7), 833–837 (1999).
204. D. Cook, D. Grierson, C. Jones, A. Wallace, G. West, and G. Tucker, *Mol. Biotechnol.*, **21**(2), 123–128 (2002).
205. A. S. Reddy and T. L. Thomas, *Nat. Biotechnol.*, **14**(5), 639–642 (1996).
206. O. Sayanova, M. Smith, P. Lapinskas, and A. K. Stobart, *Proc. Natl. Acad. Sci. USA*, **94**(8), 4211–4216 (1997).
207. P. E. Wainwright, Y. S. Huang, S. J. DeMichele, H. Xing, J. W. Liu, L. T. Chuang, and J. Biederman, *Lipids*, **38**(2), 171–178 (2003).
208. S. P. Senanayake and F. Shahidi, *J. Agric. Food Chem.*, **50**(3), 477–483 (2002).
209. K. T. Mok, A. Maiz, K. Yamazaki, J. Sobrado, V. K. Babayan, L. L. Moldawer, B. R. Bistrrian, and G. L. Blackburn, *Metabolism*, **33**(10), 910–915 (1984).
210. S. A. Spurvey, S. P. Senanayake, and F. Shahidi, *J. Am. Oil Chem. Soc.*, **78**(11), 1105–1112 (2001).

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## *Oils from Microorganisms*

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### **1. GENERAL INTRODUCTION AND BACKGROUND INFORMATION**

As this chapter is the only one in this new edition that is solely devoted to the potential use of microorganisms as sources of oils and fats, we have felt it appropriate to preface our review by including a brief outline of the importance of microbial biotechnology in general so that the extent to which microorganisms can now be grown and the amounts of products that are produced can be appreciated by the general reader who may not be familiar with the field of microbial biotechnology.

#### **1.1. Microorganisms and Biotechnology**

Microorganisms range from bacteria, which are known as the prokaryotic microbes as they do not contain a defined nucleus, although they do, of course, contain DNA, to the eukaryotic microorganisms that do have a nucleus and range from the simple yeasts to fungi, which may show more complex structural variations and be capable of some limited differentiation. Some fungi can differentiate into macromolecular forms and give rise to, for example, the mushrooms and toadstools. There are also a range of microorganisms in the aquatic environments, and these will include both

prokaryotic and eukaryotic forms. Many of these will only grow in the presence of light (phototrophs), although a few will grow in the dark if some form of fixed carbon (e.g., sugar) is supplied (i.e., they will grow heterotrophically like other microorganisms). Thus, the term “microbe” applies to a very wide range of living cells, although all would be regarded, at least in the first instance, as being unicellular and, as such, could only be seen as individual cells when examined down a microscope.

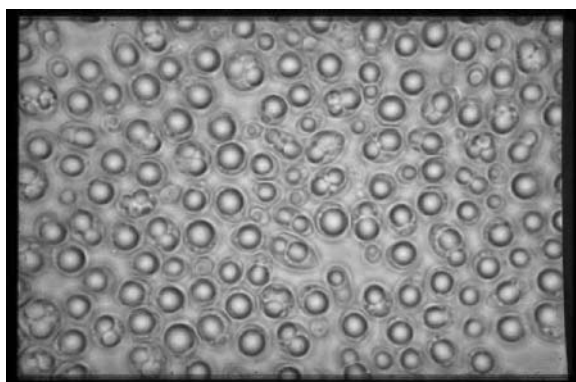
The advantages of microorganisms, which are used extensively in biotechnology systems, are as follows:

- The range of products that they can produce—from simple molecules like ethanol and citric acid, to complex proteins, carbohydrates, etc.
- They can be grown phenomenally fast: some bacteria can divide once every 10 minutes, although yeasts and fungi may take several hours to accomplish this doubling of cell numbers;
- They can be randomly mutated, by chemicals and other mutagens, so that products can be produced in vastly increased quantities. Penicillin, for example, when first produced, was at a few milligrams per liter; now the current mutated strains will produce up to 100-g penicillin/L.
- Microorganisms can also be modified by genetic manipulation where genes (that is, specific sections of DNA coding for the synthesis of particular proteins) taken from other living cells, i.e., other microorganisms, plants, or even animals cells, can be easily incorporated into a microorganism so that it will now produce not only its own proteins, but also the foreign protein.

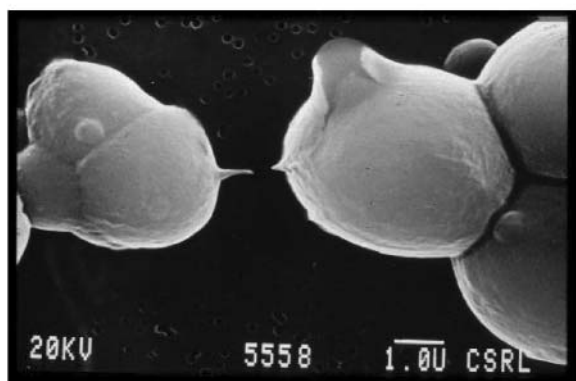
Microorganisms can therefore produce, at least in theory, any product that we currently can identify in any living cell. They will produce them faster, more safely (you do not need to spray them with pesticides, herbicides, etc.) than any other system. Moreover, the products can be produced to a guaranteed quality on a year-round basis as productions do not depend on the vagaries of the weather or climate. They are the ultimate controlled living system. This, then, is the world of microbial biotechnology (1). The scale of cultivation can be in small fermenters (about 1–5 m<sup>3</sup>) for production of very high-value, low-volume materials, such as some of the current healthcare products, up to fermenters of 100–500 m<sup>3</sup>, which can be used to produce bulk materials such as citric acid, ethanol, and even whole cells destined for animal or human food—the so-called single cell proteins.

## 1.2. Microbial Oils

Microorganisms, like every other living cell system, produce lipids. All cells are surrounded by membranes that require the synthesis of fatty acids (except in the case of the most ancient of bacteria—the Archaea—where long-chain, branched terpenoid structures are used), which are then attached to glycerol 3-phosphate giving rise to the phospholipids and triacylglycerols. This system of fatty acid and lipid



(a)



(b)

**Figure 1.** (a) Cells of an oleaginous yeast, *Cryptococcus curvatus* (formerly *Candida curvata*), in which the lipid droplets constituting about 70% of the cell weight of the cells can be clearly seen. (b) Electron micrograph of extracted oil from the same yeast shows a possible boundary layer around the oil droplets. The oil is virtually pure triacylglycerol. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

biosynthesis is then used, in some cases, for the overproduction of triacylglycerols that then serve as reserve storage materials within the microbial cell (see Figure 1). These are the so-called **oleaginous species**, and the oils are then not only useful to the microorganism to reuse during any subsequent period of starvation, but also may be considered as sources of these commodities. The oils are referred to as **single cell oils** (SCO), which is a euphemism similar to the term “single cell protein” used to indicate protein derived from microbial (single) cells. “Single cell oils” is now a generally accepted term and is used in preference to “microbial oil,” which may often create an undesirable impression with an otherwise unappreciative public.

Microorganisms have been considered as potential sources of oils since the early decades of the twentieth century when scientists, particularly in Germany, began to explore them as an alternative to plant oils, which were increasingly in short supply because of the advent of two world wars. Indeed, during the Second World War,

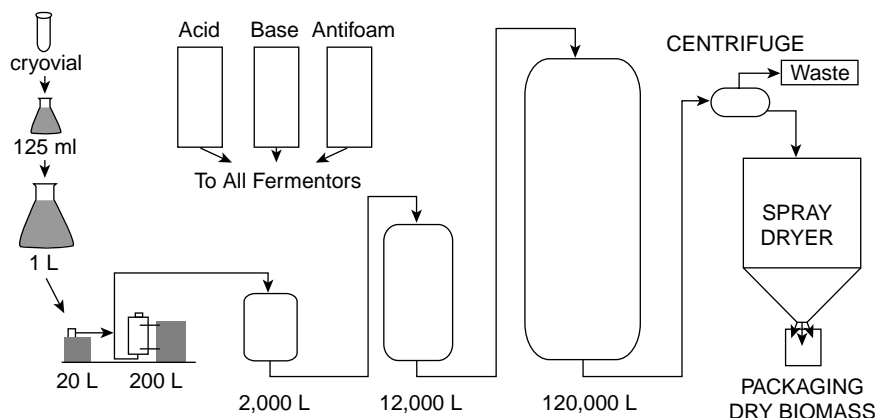
processes had been developed that produced small amounts of the oils and fats, although, because of the lack of large-scale fermentation technology, this never proceeded beyond a demonstration scale (2, 3). None of the microbial fat seems to have been used for human consumption, however. Nevertheless, there was considerable interest in microbial oils and fats and in exploring their potential as alternatives to plant seed oils, which were poorly developed at this time.

Work on understanding which were the best oil-producing strains of microorganism to use and how they had to be cultivated to induce the highest possible lipid contents was carried out during both war-time and peace-time in the first half of the twentieth century, so that by the late 1950s, a shrewd understanding of the range of oils that could be produced had been gained as well as knowing how these organisms had to be grown to produce the highest yields (2). However, the economic value of the microbial oils was always the Achilles heel to their commercialization. Advances in agriculture, particularly after the end of the Second World War in 1945, meant that all agricultural commodities, and not just oils and fats, became cheaper and more plentiful than ever before. As the microorganisms being considered for oil production had to be grown on sugar, which was an agricultural product, it was clear that it was nonsensical to grow one field of sugar cane (or sugar beet) for it to be converted at the ratio of 5 tons of sugar to 1 ton of oil in an expensive bioreactor (i.e., the fermenter) to produce oil that could be produced just as easily using the self-same field to grow a plant oilseed crop.

Thus, by the 1960s, there seemed to be no arguable case in favor of microbial oils being an economic proposition. And yet today, in the first decade of the twenty-first century, we have at least four microbial oils that are, or have been, in full-scale production, with the prospect that others may soon follow.

This change in attitude toward microbial oils has originated mainly, but not exclusively, by the appreciation of the need for specific polyunsaturated fatty acids (PUFAs) to be included in our diets, both for our infants and babies as well as ourselves in later life. Such polyunsaturated fatty acids, which have been known to exist in microorganisms for many years, are now in demand. They cannot be easily produced by plants and, in some cases, cannot be produced at all by plants. The sources of these PUFAs then are from animals and, in particular, fish. Fish, though, are a dwindling resource and the oils, which are mainly obtained from their livers or the bodies of fatty fish, can be contaminated with various pollutants, including organo-mercury compounds, dioxanes, and other materials that we would do best to avoid in our own diets. Thus, the commercial exploitation of microbial oils has originated by them being destined for human consumption and by them not being readily available from traditional sources, either plants or animals.

Coupled with the realization that microorganisms can be a unique source of certain desirable oils, has been the advent of large-scale fermentation technology throughout the latter half of the last century. This has culminated in the ability to design, build, and operate purpose-designed fermenters in excess of 1,000,000-L capacity for the production of microorganisms to provide a variety of products. A simple calculation tells us that a 1000-m<sup>3</sup> fermenter that produces 100-g cells/L in 4 days would yield a 100 tons of biomass with, perhaps, 40 tons of oil: an annual



**Figure 2.** A diagrammatic presentation of a fermentation system for the production of a single cell oil. The main fermenter is inoculated from a smaller vessel at about 10% (v/v). Additional nutrients, including glucose, may be added to the microbial culture within the fermenter during growth. The process operates in a batch mode so that when the cells reach their highest lipid contents (see Figure 3), the fermenter is emptied, the cells are removed from the broth, and then finally they are spray-dried. In this form, the oil within the cells is stable and can be extracted by solvents whenever, and wherever, is convenient. (From 50, with kind permission of the author, Dr. David J. Kyle, and the publishers.)

yield of over 3000 tons of oil. In agriculture, about 4 square miles (9 km<sup>2</sup>) of land would need to be sown with sunflowers or oilseed rape to give the same yield of oil. A typical large-scale process for the production of SCO is shown diagrammatically in Figure 2. This would be typical of the current fermentation processes being used for these types of products.

In this review, we aim to provide details of microbial oils that have been considered of commercial potential and to describe in more detail those microbial systems that are in commercial operation to provide key fatty acids for the expanding nutraceuticals industry.

### 1.3. Microbial Lipid Production Systems

Microorganisms that accumulate more than 20% of their biomass are known as the *oleaginous* species. This value, though, is arbitrary and has no precise numerical definition. There are probably far more nonoleaginous species than oleaginous ones; that is, most microorganisms will accumulate, even under the most propitious conditions, only a few percent of their biomass as lipid. The lipid that is produced in the oleaginous species is usually in the form of triacylglycerols (see Figure 1) and is an intracellular reserve supply of both carbon and energy (and perhaps water) to be used in times of nutrient starvation. The extent of lipid accumulation may range from the lower limit of about 20% up to 70% of the cell weight being extractable oil. The range of oil contents of a selected range of microorganisms is given in Table 1 together with a profile of the constituent fatty acids. More detailed lists



**TABLE 1. Lipid Contents and Fatty Acid Profiles of Selected Oleaginous Microorganisms (Compiled from Lists Given in (4)–(6), Which Should be Consulted, if Needed, for Further Information).**

Organism	Lipid Content (% w/w)	Major Fatty Acyl Residues (Relative % w/w)											Others (%)
		14:0	16:0	16:1 (n-7)	18:0	18:1 (n-9)	18:2 (n-6)	18:3 (n-3)	18:3 (n-6)	20:4 (n-6)	20:5 (n-3)	22:6 (n-3)	
<b>A. Yeasts</b>													
<i>Candida</i> sp. 107	42	Trace	44	5	8	31	9	1	—	—	—	—	—
<i>Cryptococcus albidus</i>	65	Trace	12	1	3	73	12	—	—	—	—	—	—
<i>C. curvatus</i> D <sup>1</sup>	58	Trace	32	—	15	44	8	—	—	—	—	—	—
<i>Waltomyces lipofe</i> <sup>2</sup>	64	Trace	37	4	7	48	3	—	—	—	—	—	—
<i>Lipomyces starkeyi</i>	63	Trace	34	6	5	51	3	—	—	—	—	—	—
<i>Rhodospiridium toruloides</i>	66	Trace	18	3	3	66	—	—	—	—	—	—	23:0 (3%) 24:0 (6%)
<i>Rhodotorula glutinis</i>	72	Trace	37	1	3	47	8	—	—	—	—	—	—
<i>Trichosporon beigellii</i>	45	Trace	12	—	22	50	12	—	—	—	—	—	—
<i>Yarrowia lipolytica</i>	36	Trace	11	6	1	28	51	1	—	—	—	—	—
<b>B. Fungi</b>													
Zygomycetes													
<i>Conidiobolus nanodes</i>	26	1	23	—	15	25	1	—	4	4	—	—	20:1 (13%) 22:1 (8%) 12:0 (40%)
<i>Entomophthora coronata</i>	43	31	9	—	2	14	2	—	1	—	—	—	—
<i>Cunninghamella japonica</i>	60	Trace	16	—	14	48	14	—	8	—	—	—	—
<i>Mortierella isabellina</i>	86	1	29	—	3	55	3	—	3	—	—	—	—
<i>Rhizopus arrhizus</i>	57	19	18	—	6	22	10	—	12	—	—	—	—
<i>Mucor alpine-peyron</i>	38	10	15	—	7	30	9	—	1	5	—	—	20:0 (8%) 20:3 (6%)
Ascomycetes													
<i>Aspergillus terreus</i>	57	2	23	—	Trace	14	40	21	—	—	—	—	—
<i>Fusarium oxysporum</i>	34	Trace	17	—	8	20	46	5	—	—	—	—	—
<i>Pellicularia praticolo</i>	39	Trace	8	—	2	11	72	2	—	—	—	—	—

Notes :

Hyphomycetes													
<i>Cladosporium herbarum</i>	49	Trace	31	—	12	35	18	1	—	—	—	—	—
Clavicipitaceae													
<i>Claviceps purpurea</i>	60	Trace	23	—	2	19	8	—	—	—	—	—	12-HO- 18:1 (42%)
<b>C. Microalgae and thraustochytrids<sup>3</sup></b>													
													Continued
Prokaryota													
<i>Spirulina maxima</i>	22	8	63	2	—	4	9	—	12	—	—	—	—
<i>Spirulina platensis</i>	25	1	26	5	—	23	10	—	21	—	—	—	—
Eukaryota													
<i>Chlorella minutissima</i>	28	12	13	21	—	1	2	—	—	3	45	—	—
<i>Chlorella vulgaris</i>	52	—	16	2	—	58	9	14	—	—	—	—	—
<i>Cryptocodium cohnii</i> <sup>3</sup>	50	16	16	1	—	21	1	—	—	—	—	40	—
<i>Isochrysis galbana</i>	23	12	10	11	—	3	2	—	—	<1	25	11	18:4 (11%)
<i>Monodus subterraneus</i>	20	—	19	10	—	5	2	—	<1	14	34	—	—
<i>Nannochloropsis oculata</i>	45	4	15	22	—	3	1	—	—	4	38	—	14:1 (13%)
<i>Phaeodactylum tricornutum</i>	24	—	10	21	—	1	4	—	1	1	33	4	—
<i>Porphyridium cruentum</i>	5	—	30	5	—	<1	5	—	1	16	38	—	—
Thraustochytrids													
<i>Schizochytrium</i> sp. <sup>3</sup>	40	17	32	8	—	5	—	—	—	—	1	28	22.5 (n-5) (8%)
<i>Thraustochytrium aureum</i> <sup>3</sup>	25	3	8	—	—	16	2	—	2	3	—	52	—

1. This yeast was initially known as *Candida curvata*, then was renamed *Apiotrichum curvatum*, and now is regarded as *Cryptococcus curvatus*.

2. Formerly known as *Lipomyces lipofer*.

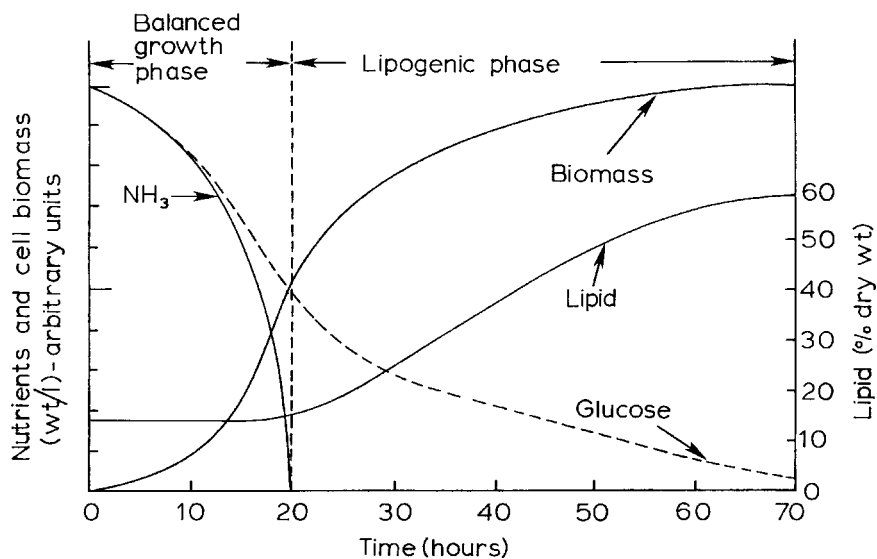
3. Microalgae cultivated phototrophically except for *C. cohnii*, *Schizochytridium* sp., and *T. aureum*, which are nonphotosynthetic and are grown heterotrophically.

are provided in other reviews (4-6) and in an earlier comprehensive monograph on microbial lipids (7).

As SCO are usually triacylglycerols that can account for over 90% of the total lipid in the microbial cell, this makes them potential substitutes for plant oils and animal fats, although clearly their economic potential will rest on their intrinsic value. It is to be emphasized, however, that an SCO will only compete with other commercial sources if it can be shown to be better in some respect or cheaper than the traditional source.

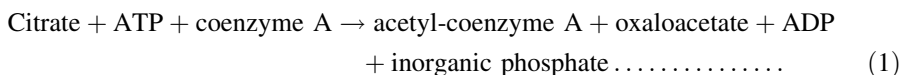
The biochemical procedure by which microorganisms accumulate lipid is now understood in some detail and has been recently described (8). Readers wishing more detailed information should consult this article. In brief, oleaginity, which is not shared by all microorganisms, depends on the presence of just a few key enzymes.

The process of lipid accumulation begins by the cells being grown in a culture medium in which the supply of a nutrient; usually it is nitrogen in the form of an ammonium salt, becoming exhausted (this is shown schematically in Figure 3). At the same time, there remains in the medium a surfeit of carbon, usually glucose or some other assimilatable carbon source. The cells, as a consequence of this nutrient exhaustion, can no longer grow and multiply, but they do continue to take up the sugar present in the medium. It is this surplus sugar that then becomes the source of carbon for lipid biosynthesis.

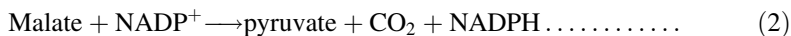


**Figure 3.** Stylistic presentation of the course of lipid accumulation by an oleaginous microorganism. The concentration of nitrogen ( $\text{NH}_3$ ) in the medium is adjusted so that it becomes exhausted after the first 24 hours growth; after this point, the cells enter the lipid accumulation phase in which the excess carbon (e.g., glucose) continues to be assimilated by the cells, and because there is no new cell synthesis because of the lack of nitrogen, the surplus carbon is converted into lipid, which functions as a reserve of carbon and energy for the cells.

Under the conditions of nitrogen-limited growth, the first requirement is for the cells to cease generating energy (i.e., ATP), which is no longer needed for the synthesis of new macromolecules, (e.g., proteins and nucleic acids) as the cells are unable to grow and divide because of the lack of nitrogen (required for protein biosynthesis). A key enzyme in the citric acid cycle (Krebs' cycle or tricarboxylic acid cycle), isocitrate dehydrogenase, becomes inactive immediately following the depletion of nitrogen. This leads to isocitrate not being metabolized, and consequently, both it and citric acid, with which it is in equilibrium, rapidly accumulate. This event occurs in the mitochondria of the cells, but the citrate is quickly transported out of this compartment into the cytoplasm of the oleaginous cell and is immediately cleaved by an enzyme known as ATP:citrate lyase (1):



This is a key reaction as it generates the C<sub>2</sub> building unit (acetyl-CoA) for fatty acid biosynthesis. Without this enzyme being present, there would be no abundant supply of the acetyl-CoA units and, indeed, many if not all of the nonoleaginous yeasts do not possess this enzyme. The oxaloacetate generated in this cleavage reaction is immediately converted to malate by malate dehydrogenase and then the malate, in turn, is converted to pyruvate by the action of malic enzyme (2).



This is the second key enzyme needed to produce high amounts of lipid as the reaction catalysed simultaneously produces the necessary reducing equivalent, NADPH, by which the growing long acyl chain, derived from acetyl-coenzyme A (see above), is reduced to the final long-chain fatty acid. Fatty acid biosynthesis, and consequently lipid accumulation, requires both a continuous supply of acetyl-CoA and reducing power (NADPH), and these are provided by the key reactions mentioned above.

How the oleaginous microorganism then produces variable amounts of lipid (see Table 1) lies with the activity of malic enzyme rather than in ATP:citrate lyase. Malic enzyme activity is controlled by the genetic makeup of the cell: in cells that accumulate considerable amounts of lipid (up to and even above 70%), the gene that controls the synthesis of the malic enzyme is kept switched on all the time, whereas in the low-lipid cells, the gene is switched off shortly after nitrogen exhaustion. When this happens, malic enzyme activity quickly disappears and, concomitantly, lipid accumulation ceases. Thus, it is now possible to explain not only the reason why some microorganisms are able to accumulate lipid and other cannot, but also why the amount of lipid accumulated can vary considerably within the lipid-accumulating organisms (8). This biochemical information is now leading to the identification of the key genetic elements that are involved in lipid biosynthesis. From this will then come the opportunity to genetically modify microorganisms

to give them increased quantities of lipid and, simultaneously, to produce more of the desired fatty acids.

## 2. COMMERCIAL MICROBIAL OILS

### 2.1. Initial Ventures Into Single Cell Oil Production

**2.1.1. Fungal Oils Rich in Gamma-Linolenic Acid** Although microbial lipids, SCO, were considered as possible commercial sources of oils and fats for almost all of the last century, no industrial production of a microbial oil took place simply because no economically attractive target had been identified. However, by the late 1970s, certain polyunsaturated fatty acids were being used medicinally as over-the-counter treatments for a variety of disorders, chief among which was the use of evening primrose oil (9) as a possible treatment for multiple sclerosis.

Evening primrose oil contains the relatively unusual fatty acid, gamma-linolenic acid, 18:3n-6. (GLA) (see Table 2). Claims for the efficacy of this oil in the treatment of many disorders, including atopic eczema (10, 11), which go back many centuries in a historical context, are then attributed to the presence of this fatty acid, which does not occur in most other plant seed oils. Other claims for the efficacy of GLA-rich oils include the treatment of premenstrual tension, which is the main selling point of this oil in the United Kingdom, as well as for various cancers (9, 12). These have added impetus to identifying a cheaper and perhaps more reliable source of GLA as the cultivation of evening primrose is not easy, with it being a biennial crop and which produces very tiny seeds requiring careful harvesting and processing (13). As it was known that GLA is also present in a large number of simple fungi, known as the lower fungi or Zygomycetes since the 1940s, a fungal route to its production was recognized as a feasible alternative to evening primrose

**TABLE 2. Fatty Acid Profiles of GLA-SCOs—Microbial Oils Rich in Gamma-Linolenic Acid (18:3 n-6)—in Comparison with Plant Sources of GLA [Adapted from (4) and 6)].**

Oil	Content (% w/w)	Relative% (w/w) of Major Fatty Acids								
		16:0	16:1	18:0	18:1	18:2 (n-6)	18:3 (n-6)	18:3 (n-3)	20:1	22:1
<i>Mucor circinelloides</i> <sup>1</sup>	25	22	1	6	40	11	18	—	—	—
<i>Mortierella isabellina</i> <sup>2</sup>	ND	27	1	6	44	12	8	—	0.4	—
<i>Mortierella ramanniana</i>	~40	24	—	5	51	10	10	—	—	—
<i>Mucor hiemalis</i>	30	25	1	10	32	12	15	—	—	—
Evening primrose	16	6	—	2	8	75	8	0.2	0.2	—
Borage	30	10	—	4	16	40	22	0.5	4.5	2.5
Blackcurrant	30	6	—	1	10	48	17	13	—	—

<sup>1</sup> Production organism used by J & E Sturge Ltd., Selby, N. Yorks., UK.

<sup>2</sup> Production organism used by Idemitsu Ltd., Japan.

ND—Not disclosed but believed to be 40% to 50%.

oil. Work was carried out in the authors' laboratory at the University of Hull beginning in 1976 by screening a large number of these fungi for their potential to produce an oil rich in this fatty acid. This led to *Mucor circinelloides* (formerly known as *Mucor javanicus*) being identified as the most productive organism (3): although this fungus was not the highest producer of GLA, there were three important and interdependent variables that had to be satisfied:

- The organism had to be able to grow quickly and to a high cell density. A working target was for an organism to achieve more than 50-g dry cell per liter of fermenter in a time not to exceed 4 days.
- The organism had to have more than 20% oil content; otherwise extraction would be difficult, and the costs of oil production would be increased. Equally, the extracted oil should be at least 90% as a triacylglycerol so that subsequent refinement and encapsulation would be relatively easy to accomplish.
- The content of GLA in the oil had to be considerably higher than that in evening primrose oil, which was only about 10%; a working target of 20% was therefore chosen.
- A fourth preference was for the chosen organism to be already recognized as being safe to use for food purposes: the so-called Generally Recognised As Safe (GRAS) category. However, this was not regarded as absolutely essential as the product would be an extracted, purified oil. It would then be the quality and safety of this that would be assessed rather than the safety of the whole organism. Nevertheless, one would not want to use a microorganism that had any association with any disease for very obvious reasons. Also, the chosen organism should not be a plant pathogen because of possible environmental risks when growing the organism on a large scale. (All organisms being used in large-scale cultivations have to be intrinsically safe to handle and even organisms that could cause allergic reactions in factory operatives are best avoided.)

The finally chosen production organism, *Mucor circinelloides*, satisfied all of the above criteria, including it being a GRAS-status organism as it has a historically long association with tempe, a well-known oriental food material. The profile of its fatty acids is given in Table 2 along with its lipid content.

In Japan, Idemitsu Co. Ltd. adopted a slightly different strategy to isolate GLA-producing organisms. They opted to go primarily for organisms producing high oil contents and, seemingly, hoped that the GLA also would be high in such organisms. In the event, although high oil-producing species were found, none produced more than 10% of the total fatty acids as GLA (see Table 2). For a reason not yet understood, but possibly related to the limited capacity of the cells to generate NADPH that is used both in fatty acid synthesis and in fatty acid desaturation (see Section 1.3), there is an inverse relationship between oil content and GLA formation. The higher the oil content of the cells, the lower is the GLA content (see Table 2). The Idemitsu oil from *Mortierella isabellina*, therefore, had only half the GLA content

of the U.K. organism, *Mucor circinelloides*, although the Japanese organism had twice the oil content of the U.K. organism. Commercially, however, the higher the GLA content of an oil, the greater its value, and an oil with 20% GLA is probably more than twice as valuable as an oil with only 10% GLA, so it is more cost-effective to produce more GLA at the expense of producing less oil.

The GLA-SCO was produced in the United Kingdom by John & E. Sturge, Selby, North Yorkshire, using *Mucor circinelloides*. It was first offered for sale in 1985 under the name of *Oil of Javanicus*, which took account of the oriental origins of the organism and its original name, *Mucor javanicus*. The oil was sold commercially throughout the United Kingdom between 1985 and 1990. Production was at about 10–15 tons of oil per year. The fermenters used were 220,000 L and were normally used for the production of citric acid using *Aspergillus niger*. The overall fermentation configuration was similar to that shown in Figure 1. To grow *M. circinelloides* for oil production, rather than *A. niger* for citric acid, all that had to be done was to reformulate the growth medium so that it now contained an insufficient supply of nitrogen and so that cell proliferation would cease after about 36–40 hours and lipid accumulation would then begin (see Figure 2).

The Oil of Javanicus was of high purity and passed all toxicity tests: It was superior in safety evaluations to conventional plant oils, which always contain very small residual amounts of pesticides, insecticides, and herbicides from the various sprays that are used on such crops. These levels, though, are always below the recommended threshold values laid down by regulatory authorities. Being cultivated in fermenters, *M. circinelloides* does not, of course, need to be sprayed with any chemical pesticide, herbicide, or fungicide.

The arrival of this novel oil on the market resulted in a sharp decline in the price that evening primrose oil was being offered for sale. Competition between it and Oil of Javanicus was much fiercer than had been anticipated. Although for all intents and purposes, the fungal oil was superior to the plant oil, in that it contained twice the GLA content of the evening primrose oil, nevertheless, and perhaps not surprisingly, there was a certain reluctance on behalf of the general public, who were buying these oils to switch to an oil of fungal origin. However, the marketing of the oil carefully eschewed specific mention of the word “microorganism” or “fungus,” but nevertheless there was a reluctance on the behalf of major purchasers, the health-food stores, over-the-counter medicine shops, and so on, to purchase the oil in spite of its technical superiority. Although interest in this GLA-rich oil was high, it quickly became apparent that it was being outcompeted by evening primrose oil in terms of price. Unfortunately, within the European Union, agricultural crops not designated as food crops could be financially subsidized from the Common Agricultural Policy. This meant that growers of evening primrose oil received cash benefits directly from the EU for growing this plant, which was designated as a non-food crop. At the same time, the fermentation process was being financially penalized by the sugar used as the feedstock had to be bought within the EU at EU prices and not at world prices, which were less than half this cost. Sugar within the EU has a tariff placed on it so that farmers in the EU can receive

adequate remuneration for growing this crop. Thus, the Oil of Javanicus was doubly disadvantaged by its commercial rival, evening subsidized primrose oil, which was subsidized by the EU, and at the same time the cost of production was increased by the artificially high price of the fermentation feedstock.

The final blow to the production of the fungal oil came with the introduction of borage oil (*Borago officinalis*) as a superior source of GLA. This new oil had a GLA content of 20–22% (see Table 2) and, although it was technically just as difficult to grow and process as evening primrose oil, it was considered superior and was, of course, cheaper, than both the evening primrose oil and the fungal oil. Again, growing this borage crop still enjoyed the financial benefits that had accrued from the EU Common Agriculture Policy for evening primrose cultivation.

In 1990, and against this background of increasing erosion of the profit for the microbial SCO, production of Oil of Javanicus ceased. The 6 years in which the oil had been in production, though, established a number of important points. First, microbial oils could be produced on the very largest of scales, up to 220 m<sup>3</sup> in this case. The oil was intrinsically safe, posed no safety problems, and passed all toxicity trials to which it was subjected. It was well accepted by all people who consumed it, and no adverse reactions to it were ever recorded. There were no particular difficulties in extracting the oil (a process using hexane was used), and the oil could be easily purified using conventional procedures of the oils and fats industry. The oil was also remarkably stable from oxidation, presumably because of the presence of endogenous natural antioxidants. Thus, the way was now open for other microbial oils to enter the market even though Oil of Javanicus was no longer in commercial production.

In Japan, the GLA-SCO from the Idemitsu Co. Ltd process using *Mort. isabellina* appears also to have ceased production, and GLA, when needed, is derived either from evening primrose oil or borage oil.

**2.1.2. Cocoa-Butter Equivalent Yeast Fat** The initial interest in producing high-value microbial oils in the late 1970s quickly led to serious consideration being given to the prospects of producing a facsimile oil to cocoa butter, i.e., cocoa butter equivalent fat or CBE (14–16). The price of cocoa butter, which is used extensively in chocolate manufacture, is very variable, but in the early 1980s, its price was exceeding \$8000 per ton. CBEs are traditionally made by palm oil fractionation to give an oil containing a high content (approximately 30–35%) of stearic acid (see Table 3) with equal proportions of oleic acid and palmitic acid (17) and command a price that is usually fixed at about 50% to 60% of the price of cocoa butter. CBEs can be added into cocoa butter at up to 5% in various countries, including the United Kingdom, without invalidating the claim for the product to be called “chocolate.” In other countries, such mixtures can only be used in confectionery chocolate.

The high price of cocoa butter at this time then made it an attractive target to emulate. A sufficient margin of profit was considered possible if a microbial oil could be produced that mimicked the fatty acid profile of cocoa butter and, most importantly, had the same melting profile as cocoa butter. This manifests itself



**TABLE 3. Fatty Acid Profiles of CBE-SCOs—Microbial Oils for use as a Cocoa Butter Equivalent—in Comparison with Cocoa Butter.**

	Relative % (w/w) of Major Fatty Acids						Ref
	16:0	18:0	18:1	18:2	18:3 (n-3)	24:0	
<i>Cryptococcus curvatus</i> WT <sup>a</sup>	30	15	45	5	0.5	2	27
<i>C. curvatus</i> NZ	18	24	48	3	1	2	28
<i>C. curvatus</i> R26-20	15	47	25	8	2	—	21, 23
<i>C. curvatus</i> R25-75	33	25	33	7	1	—	21, 23
<i>C. curvatus</i> F33.10	24	31	30	6	—	4	22, 23
<i>Trichosporon cutaneum</i> DRL-D221	30	13	47		7	—	24
<i>T. cutaneum</i> DRL-ole <sup>-</sup>	26	38	16	←14		—	24
Yeast isolate K7-2	26	25	38	6	1	1	29
Cocoa butter	23-30	32-37	30-37	2-4	—	—	—

WT<sup>a</sup> = wild type.

by the fat being solid at ambient temperature (up to about 25°C) but then melting completely at 30–32°C. As most microbial oils contain less than 10% of the total fatty acids as stearic acid (18:0), the task was then to increase the content of stearate and, simultaneously, to ensure that the resulting triacylglycerol had the correct fatty acid distribution (i.e., was a *sn*-1 palmitoyl, *sn*-2 oleoyl, *sn*-3 stearyl glycerol) so that the ensuing oil would meet the very stringent requirement for its inclusion in chocolate.

The most attractive production organisms appeared to be yeasts, which do not usually contain high amounts of 18:2 or other polyunsaturated fatty acids and therefore were immediately attractive for this reason. They also could be grown extremely rapidly and to high cell densities with high lipid contents. Attempts in the early 1980s had been made by several groups to increase the amount of stearic acid in a yeast by growing the yeast on stearic acid, or esters of stearic acid, as feedstocks (5). Not surprising, such yeasts then contained high amounts of this acid, but as no cheap source of the stearic acid existed at this time, this did not represent an economic route to an SCO-CBE product.

An alternative strategy was developed by Cadbury-Schweppes plc, the large U.K.-based, multinational chocolate and food company, to inhibit the conversion of stearic acid to oleic acid, which is mediated by fatty acid delta-9 desaturase, using sterculic acid, *cis*-9,10-methyleneoctadecenoic acid (18, 19). This inhibitor, which can be derived from sterculia and kapok oils (18), increased the stearic acid content of several oleaginous yeasts up to nearly 50% of the total fatty acids when they were grown on glucose. However, the selectivity of the inhibitor was such that the yeast still contained too much linoleic acid, and consequently, another inhibitor of the delta-12 desaturase (converting oleic acid to linoleic acid) was needed. This was *cis*-12,13-methyleneoctadecenoic acid, which had to be synthesized

chemically. In the presence of both inhibitors at about 100 mg/L, one yeast, *Rhodospiridium toruloides*, now produced a SCO-CBE product that was close to the required fatty acid profile (5, 19). The costs of the fatty acid desaturase inhibitors unfortunately proved to be too high for the process to be sustained, and there was also clear unease at using metabolic inhibitors that might find their way into the final product. Consequently, this interesting and novel approach was abandoned.

Nevertheless, the clear conclusion was reached from the work with sterculic acid that an SCO-CBE was possible if the activity of the delta-9 (and the delta-12) desaturase could be diminished. This was then the concept behind the subsequent mutation program that was developed by several groups but most notably by a group at the Free University of Amsterdam, The Netherlands. The yeast chosen for this mutational work was *Candida curvata* (also known as *Apitrichum curvatum* and now renamed *Cryptococcus curvatus*). Using conventional mutational procedures, various mutants were produced that had lost the ability to synthesize oleic acid and now needed this fatty acid to be included in the growth medium (20–23) (see also Table 3). Clearly the gene coding for the delta-9 desaturase had been affected by the mutations.

By judicious selection of the various mutants and adjustment of the mutational makeup, it was possible to select mutants that had a low activity of the delta-9 desaturase and no longer needed oleic acid to be added as a supplement to the growth medium. The composition of the fatty acids of two of these mutants, R26-20 and R25-75, are shown in Table 3. The best results were, obtained though, using a hybrid of two mutants, F33.10, which gave an almost ideal fatty acid profile for an SCO-CBE (see Table 3). Similar mutational programs were carried out by other research groups (24–26), although with no greater success than the Dutch group (23).

One factor that is a prerequisite for using mutated strains of micro-organisms is their long-term genetic stability and, in particular, their stability when grown in large-scale fermenters where they have to undergo many generations to reach the required cell densities. Also, it is important that the mutants should grow as rapidly as the original parent organism. During the growth of the mutants from small cultures (say, 200 mL) up to the final level at perhaps 100,000 L or higher (see Figure 3), there has to be complete genetic stability; otherwise the organism reverts to its original constitution and the desired product is no longer produced. For these reasons, the mutants of the yeast used for SCO-CBE production were not entirely dependable when used in large-scale growth trials and yields of the required CBE were below expectation. This is probably attributed to there being other mutational changes to the DNA, besides the alteration in the oleic acid desaturase gene, which then affect the long-term performance of the organism. Today, current molecular techniques would allow one to identify the key genes that needed to be changed and then these could be individually manipulated in such a way that the remainder of the DNA would not be affected. Thus, in principle, specifically, genetically modified yeasts could now be produced that would yield a very high-quality SCO-CBE even when grown in large-scale fermenters. However, in the 1980s, before this

current technology was available, further efforts to produce a yeast oil CBE focused on controlling the production of oleic acid using a restricted supply of oxygen to the cells.

As the conversion of stearic acid to oleic acid (and indeed all desaturase reactions) requires the active participation of  $O_2$  in the reaction, it was attractive to consider that the content of stearic acid in the yeast oil could be increased by restricting the supply of air to the growing cultures. This, in fact, was achieved by Davies et al. (28) using the yeast, *C. curvatus*. To achieve the necessary low levels of oxygen within the fermenter, a 500-L system had to be employed. With smaller fermenters, it was not feasible to restrict the oxygen supply sufficiently to cause the desired effects. The fatty acid profile of the yeast when grown under such conditions is shown in Table 3 and was considered to be a reasonable approximation to that needed for the oil to be considered as a suitable CBE (27).

Extensive large-scale cultivation of various yeasts, but principally *C. curvatus*, for the production of SCO-CBE was carried out by Davies et al. in New Zealand from the early 1980s until the 1990s. The concept behind this work was the use of cheese creamery waste, which in New Zealand—the location of Davies's laboratory—was a major waste resource. As whey contains 4% to 5%(w/v) lactose, this could be used as an appropriate substrate for yeast growth. The yeasts chosen by Davies (see 29), therefore, were all lactose-users, including *C. curvatus*, which had originally been isolated by Earl Hammond et al. from dairy waste-processing areas (30). By virtue of the extensive work carried out on this process, Davies et al. was able to calculate the likely costs of production of an SCO-CBE (27, 29). Davies, writing in 1992 (27), calculated that the manufacturing cost of producing 1 ton of refined yeast oil would be \$800–\$1000. This was based on a process that would use 200,000 m<sup>3</sup> of whey per year and that would be available at a single location in New Zealand. These costs, though, did not include plant depreciation, the interest payable on the capital investment needed, nor the manufacturing overheads. Collectively, these costs could then double the original estimate. At this time (late 2003), cocoa butter had slumped in world prices from its all-time high of \$8000/ton in the early 1980s to \$3500/ton. As a CBE could only command about two-thirds of this price, this meant that the yeast CBE would only be valued at \$2000–\$2500/ton. The margin of profit, therefore, was not great enough to warrant the large investment of capital that would be needed to establish the process. Consequently, after huge efforts by many groups around the world, it was generally agreed that this approach was not economically viable. As far as the present authors are aware, no further commercial interest has been shown in developing a yeast-based process for the production of a CBE. Even if the economics of cocoa butter production were to change in the next decade, there are now sufficiently good alternative systems, including the use of immobilized lipase technology, to produce CBE material more cost-effectively than the microbial route of manufacture (17, 31). It is worth pointing out, however, that the cost-calculations of Davies for the bioengineering aspects of SCO-CBE production could form the basis for future calculations of such processes aimed at the production of other microbial oils.

### 3. SCOS IN CURRENT (2003) PRODUCTION

As already discussed, the cost of SCO manufacture is high because of the high capital costs involved in the construction of large fermenters and associated machinery as well as in the costs of operation. If a microbial oil is to be exploited commercially, then the SCO must command a premium price (32, 33). In reality, this means that microbial sources for oils can only be a commercial reality if the SCO produced is (1) destined for human consumption and (2) not readily available from traditional sources, either plant or animal.

Such opportunities exist in terms of long-chain polyunsaturated fatty acid (LC-PUFA) rich oils that have well-defined and publicly recognized benefits for human health. Currently, >95% of the global SCO production are oils rich in two distinct fatty acids: arachidonic acid (ARA, 20:4 n-6) and docosahexanoic acid (DHA, 22:6 n-3). Both are being produced either directly by, or under contract, for Martek Biosciences Corporation, MD. The production of SCOs is no longer a small-scale operation. The blend of SCO produced by Martek as a neonate nutritional supplement (for inclusion in infant formula) has established itself worldwide in the past few years, to the extent that current production is measured in the "hundreds" of tons annually. An estimate has been made that for 2003, about 560 tons of SCOs will be produced, the majority of which will be SCOs rich in either ARA or DHA. For 2004, this amount is predicted to double and possibly double again in 2005. ARA and DHA are LC-PUFA, having a carbon chain of greater than 18 carbons and are not available from plant sources. Agricultural plants usually produce only 18 carbon (or shorter) fatty acids, and hence, the most unsaturated fatty acids they produce are 18:3 of the n-3 or n-6 type. Although some plants, though, do produce C20+ fatty acids, these are monounsaturated such as erucic acid (22:1, n-9) or nervonic acid (24:1, n-9) and do not possess the beneficial physiological effects of ARA and DHA. Animals (including fish) are potential sources of a multitude of polyunsaturated fatty acids with carbon chains as long as 22 and as many as six double bonds. However, these animal oils have potential problems of generally low LC-PUFA content and complex fatty acid profiles, as well as acceptability problems to various sectors of the community based on religion or lifestyle (34). Furthermore, there are increasing concerns about the presence of viruses and prions in materials of animal origin, and with fish, there are worries concerning their long-term availability as a cheap resource. More recently, reports of the possible accumulation of toxic pollutants, including heavy metals, from the marine environment into fish livers have added another dimension to the argument against the use of fish oils. Fish livers, together with whole fish bodies, are, of course, the current major source of LC-PUFAs.

SCOs have the advantage over these traditional sources for the provision of LC-PUFA for the very reason that they are produced by fermentation. The quality and supply of SCO can be closely controlled and guaranteed on a yearly basis (32, 33). Such guarantees are hard to provide for plant- or animal-derived oils because of environmental conditions and variations that are outside of the producers' control. Weather, diet (for animal oils), and environmental pollution (including the spraying

of almost all commercially grown crops with mixtures of herbicides, pesticides, and fungicidal chemicals) have a considerable impact on the supply and quality of oils from traditional sources. Also, the growth of micro-organisms is very rapid in comparison with agricultural crops and animals; usually fermentation times are 4 to 10 days. As a result, the supply of SCO can far more easily be matched to the market requirement, avoiding oversupply or shortage problems that can be an issue with traditional oils.

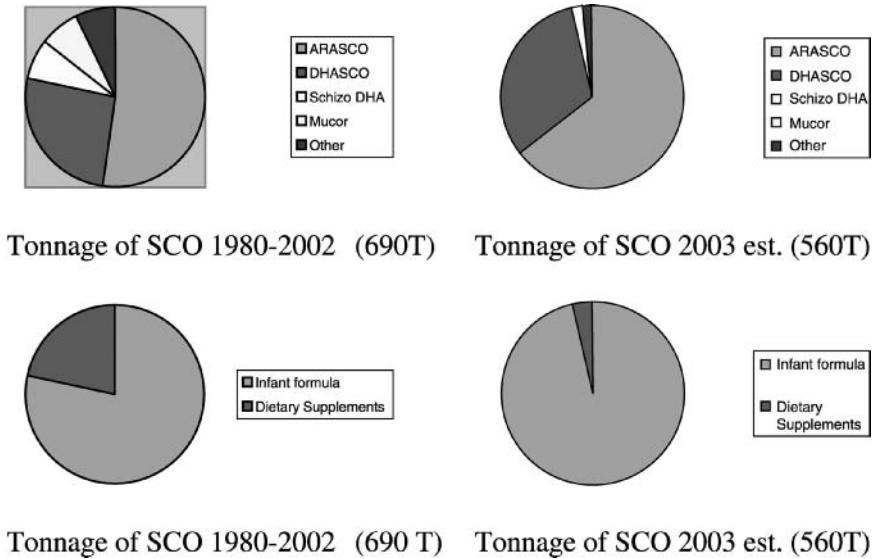
Initially, public reluctance to consume a microbe-derived oil in preference to those from plant or animal sources was a considerable problem, contributing to the decline of "Oil of Javanicus" (the first commercially produced SCO); see Section 2.1.1. However, a mixture of more astute marketing, "rebranding" of SCO as either "fermented oils," "designer oils," or even "vegetarian oils" when in competition with animal oils, and the improved public acceptance of "microbial" foods, as exemplified by the success of the mycoprotein (SCP) product, Quorn™, in the United Kingdom and Europe, has helped establish PUFA-SCOs as acceptable products.

### 3.1. Archidonic Acid

After the success (at least temporarily) of "Oil of Javanicus," the next fatty acid to be developed on a commercial basis was ARA. The early development occurred in the Far East, with two Japanese companies, Lion Corp. and Suntory. Both companies developed processes for producing arachidonic acid using fungal sources. It is interesting to note that the interest of Lion Corp. in an arachidonic acid-rich oil was not for nutritional applications but as the basis of cosmetic creams, another area that can withstand commodities with a premium price. This development resulted in patents being awarded to these two companies in the late 1980s covering the production of arachidonic acid from *Mortierella alpina* (35, 36).

ARA-rich SCO continues to be produced commercially in the Far East by Suntory and is produced in Europe by Dutch State Mines (DSM), formerly Gist-brocades. DSM produces ARASCO™ (an oil produced from the fungus *Mortierella alpina* and containing >40% w/w ARA) under contract for Martek Biosciences (USA) for inclusion in the neonate "Formulaid®" nutritional supplement, which is used in infant formulas and baby foods in 60 countries worldwide (including the United States since February 2002). Currently, infant formula is, by far, the most important market for SCO. Over 95% of global sales of SCO is made up by a single product, Martek's Formulaid® (Figure 4).

The astonishing success of this product is evidenced by the fact that although it was introduced into infant formula in the United States in February 2002 (and the formula containing this supplement commands a premium price), by the time of writing (September 2003), the market share of infant formula containing Formulaid® in the United States has reached 30%. The ARA-rich SCO produced by Suntory, which is similarly used in infant formula, is mainly for export as the Japanese market for such products is still being developed. However, the oil is available within Japan as a health supplement. In China, the Wuhan Alking Bioengineering Co.



**Figure 4.** The production and uses of commercial SCO over the past 20 years and comparison with current estimated production and use (Dr. David J. Kyle, personal communication). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

Ltd., located in Wuhan City, has been producing arachidonic acid using *Mort. alpina* since 2001 (see [www.alking.com.cn](http://www.alking.com.cn)). Production appears to be at the 50,000-L level although larger fermenters may be under commission. The oil is thought to be used in infant milk powder but also may be exported to unknown destinations.

As *Mort alpina*, unlike *Mucor circinelloides* (also known as *Mucor javanicus*), did not, prior to the production of ARA-rich SCO, have any definite historical association to any food product, extensive toxicological studies were performed to confirm the safety of ARASCO (37–40). This requirement for toxicological screening is a hurdle that must be overcome for any SCO that cannot claim GRAS status by association with a preexisting human food. Obtaining this toxicological data *ab initio* can be a costly and time-consuming process.

Many micro-organisms have been examined for the ability to produce substantial amounts of ARA, but the overwhelming consensus is that fungi of the genus *Mortierella* (and in particular the subgenus of *Mortierella Mortierella*) are the organisms of choice (41–43). Although a number of species of *Mortierella* have been suggested, and even perhaps developed, as production organisms (44), the commercial production of ARASCO™ is currently carried out using *Mortierella alpina*. The major culture collections ATCC (14 strains), IMI (4 strains), CBS (19 strains), and so on, contain multiple isolates of *Mortierella alpina*, which have been exhaustively examined for their commercial viability. The most productive strain will depend to a large degree on the process developed as strain selection, and process development must occur in parallel. The interdependence of the strain/process complicates the isolation/selection of new improved strains once a process is established.

The most productive strain in the “open access” culture collections appears to be ATCC 32222 (45, 46). However, the group of Shimizu working in collaboration with Suntory in Japan have isolated a very high ARA-producing environmental strain that has been designated *Mortierella alpina* 1S-4 (32, 36, 41). This strain has several unusual and advantageous characteristics (ease of sporulation amongst them). Much experimental data pertaining to this strain exist in the literature (32, 47–49). However, because of the proprietary nature of this strain, little of this published data has had the opportunity to be corroborated by other workers in the field.

Although relatively high-producing strains of *Mortierella alpina* are available “off the shelf” from culture collections, it is inevitable that commercial production will use proprietary “improved” strains. The processes used to obtain these strains involve a certain amount of “lore” because of the difficulties obtaining spores of the organism, which are a prerequisite for carrying out a successful mutation program. Also, as increased ARA/oil production within a mutant organism confers no easily recognizable trait in a mutant strain, considerable effort must be expended to screen many thousands of potential mutants to isolate one new strain that overproduces ARA.

**3.1.1. Commercial Production** The commercial production of ARA-rich SCO starts with the thawing of a cryovial of a certified stock culture of the production strain of *Mort. alpina*. The thawed cryovial is used to inoculate a series of shake-flask cultures and seed fermenters of increasing volume to maintain a 5–10% inoculum for each successive stage of the seed train. Final production scale is typically 50 to 200 m<sup>3</sup> (see Figure 2) (50).

Media used for ARA-rich SCO production vary depending on the process/strain used; however, they tend to be relatively simple complex media composed of a base of yeast extract and glucose (51), although the ion composition is known to be crucial for optimal productivity as well as the carbon to nitrogen ratio in the medium (48, 52, 53).

Unlike “classic” fungal fermentations, such as for the production of penicillin, citric acid, and gibberellic acid (1), the desired product in the case of ARA-rich SCO is an intracellular product and this leads to the ARA-rich SCO process having some distinctive features. Although in the other processes fungal biomass is, in effect, an unwanted byproduct and can be minimized, in the ARA-rich SCO process, a very high cell density must be achieved. Cell densities as high as 50–60 g/L dry weight have been reported (48), and this creates problems in terms of culture mixing and mass transfer as LC-PUFA biosynthesis is an aerobic process caused by the O<sub>2</sub>-requirement of the fatty acid desaturases.

In order to obtain maximal ARA-rich SCO productivity, the fungus must be cultivated in the correct morphological form. *Mort. alpina* can grow as either dispersed hyphal filaments or as pellets (of varying sizes; depending on conditions). Although “feather-like” hyphal filaments yield the optimal ARA production at low cell densities, because of the ready access of nutrients and ease of gas exchange between the hyphae and the medium (54), this morphology is not suitable for cultivation at high cell densities. Under intensive cultivation conditions, hyphal growth causes the

viscosity of the culture to increase and agitation of the culture to be difficult (48). Under these conditions, gas exchange and nutrients become limited, thereby deleteriously affecting ARASCO production. For large-scale industrial ARA-rich SCO production, the optimal morphology is pellets, which causes a decrease in the viscosity of the culture and promotes mixing. When pellets form, however, the culture becomes heterogeneous as the biomass in the interior of the pellet can experience nutrient limitation (49). Depending on the size of the pellets, the ARA-rich SCO production when the fungus changes from hyphal to pellets can be decreased by as much as 50% (45). Therefore, achieving the optimum morphology for ARA-rich SCO production is not a trivial matter. It appears small pellets < 2 mm in diameter are optimal to decrease culture viscosity, while maintaining adequate nutrient and oxygen transfer to the pellet interior (48, 55).

The ARA-rich SCO fermentation operates under nitrogen-limiting conditions to promote cell lipid accumulation. A glucose-fed batch system is employed to allow high levels of carbon to be used without the toxic effects of very high initial glucose concentrations. Once the biomass has accumulated a suitable amount of lipid and ARA (approximately 200 mg ARA/g dry weight), the fungal biomass must be removed from the culture medium, either by continuous centrifugation or filter pressing, and dried before the oil can be extracted and purified using techniques essentially identical to those used for vegetable oil production. The resulting oil is a pale yellow brilliant oil with a relatively bland flavor that is remarkably resistant to oxidation. The fatty acid profile of the commercial oil is given in Table 4 (55).

### 3.2. Docosaheptaenoic Acid

The most unsaturated fatty acid found in significant quantities in nature (some algal lipids contain trace amounts of fatty acids with as many as eight double bonds) is DHA. This fatty acid is known to be especially important in the neural development in animals; indeed, DHA alongside ARA are the predominant PUFA in neural tissue (56). A deficiency in DHA in has been linked to impaired brain and visual development in neonates (56).

Once the physiological role of DHA had been realized, this fatty acid became an obvious candidate for commercial microbial production as no "traditional" lipid source rich in this LC-PUFA alone was known. As mentioned above, plants generally do not synthesize fatty acids longer than 18 carbons and no plant makes any DHA. DHA is found in animal-derived oils, particularly fish oils; however, the use of fish oil as a DHA supplement is problematic. Fish, like all animals, possess fatty acid profiles that, to a large extent, reflect their dietary intake rather than metabolic ability to synthesize fatty acids. As a result, the fatty acid profiles of fish oil are complex and contain a host of other PUFA as well as DHA. The presence of another LC-PUFA, eicosapentaenoic acid (EPA, 20:5 n-3), is a particular problem associated with fish oil if the intended use is neonate nutrition. EPA, a precursor of important signal molecules (prostaglandins and eicosanoids), is thought to act antagonistically to the beneficial effects of ARA-rich SCO supplementation of infant formula and is associated with growth retardation in neonates (57).



**TABLE 4. Fatty Acid Profiles of Microbial Oils Rich in Arachidonic Acid and Docosahexaenoic Acid that are Produced Commercially (from 55).**

Oil	Fatty Acid Composition (Rel. % w/w)													
	12:0	14:0	16:0	16:1	18:0	18:1	18:2 (n-6)	18:3 (n-6)	18:3 (n-3)	20:3 (n-6)	20:4 (n-6)	22:5 (n-6)	22:6 (n-3)	24:0
ARASCO <sup>a</sup>	—	0.4	8	0	11	14	7	4	—	4	49	—	0	1
DHASCO <sup>b</sup>	4	20	18	2	0.4	15	0.6	—	—	—	—	—	39	—
Schizo-SCO <sup>c</sup>	—	13	29	12	1	1	2	—	3	1	—	12	25	—

<sup>a</sup> Production organism: *Mortierella alpina* (see 55).

<sup>b</sup> Production organism: *Cryptocodinium cohnii* (see 55).

<sup>c</sup> Production organism: *Schizochytrium* sp. (see 55).

NB: ARASCO and DHASCO are registered tradenames of Martek Corp. Inc.

Other problems associated with the administration of fish oil to the general population, and in particular to neonates, is the potential for the presence of environmental toxins in fish oils. As the fish from which the oils are derived are free living in the world's oceans, they are prone to contamination with pesticide residues and industrial wastes (including heavy metals) that are all too frequently released into the marine environment. Although the levels of these chemicals are usually low, they are of sufficient amounts to cause concern that the British Government commissioned a report that has highlighted the extent of contamination of fish stocks (58). In particular, the presence in fish oils of dioxins and polychlorinated biphenyls (PCBs) was noted and the report recommended that "oily fish" should not be eaten more than once a week. More recently, the specter of mercury in fish oils has led to the recommendation by the Food and Drug Administration (FDA) and the Environmental Protection Agency in America (59) that pregnant women should restrict their oily fish intake. Furthermore, a recent study has determined that the mercury content of fish oil diminishes the cardiovascular benefits of these oils (60). Any inclusion of the oils from such fish, mainly derived from liver, an organ that often concentrates ingested toxins in infant formula, even if the levels of contaminants is low and less than safety limits, must be seen as foolhardy. Alternative sources of DHA that would be outside the current supplies are clearly needed, and such sources may then be expected to command a premium price far in excess of that of fish oils.

**3.2.1. Microbial Sources of DHA** It has been recognized for over 30 years that certain marine microorganisms have the ability to synthesize DHA *de novo* and to accumulate significant amounts of this fatty acid in their cellular lipid in a relatively pure form (61). Indeed, it is these microbes at the base of the food chain that synthesize the DHA that eventually appears in fish oil (62). Two microalgal sources, in particular one dinoflagellate (*Cryptocodinium cohnii*) and one chytrid (*Schizochytrium*), have been found to synthesize sufficient quantities of DHA (in an oil devoid of EPA) to become commercially viable. Although both organisms produce a DHA-rich SCO, important differences exist in the fatty acid profiles of the resultant oils that have significant impact on their potential use. Although *C. cohnii* oil contains DHA as >99% of the PUFA (and >40% of the total fatty acids), the oil from *Schizochytrium* contains a significant amount of another PUFA docosapentaenoic acid (DPA n-6; 22:5n-6). The presence of DPA in the chytrid oil was initially a cause for sufficient concern that this oil was not considered suitable for neonate nutritional applications. However, the DPA appears to be completely benign and has, moreover, been recognized as a natural component of the phospholipids of human blood platelets (63, 64). Studies of DPA metabolism in rat hepatocytes (65) have further indicated that DPA is retroconverted to ARA if the ARA content of the diet is low, but when ARA is administered along with a mixture of DPA and DHA, then the DPA serves to maintain the DHA at a high circulating concentration. Thus, there may be positive benefits of including DPA in a dietary supplement of ARA + DHA, and this would occur by using the *Schizochytrium* oil along with the ARA-SCO.

Initially, the two organisms were developed as competing processes by two pioneering U.S. firms, Martek (using *C. cohnii* to produce DHASCO™, an oil containing >40% DHA) and OmegaTech (using *Schizochytrium* to produce Sea Gold™, now known as DHAGold™, an DHA-rich SCO also containing DPA n-6). In April 2002, Martek acquired OmegaTech to combine the expertise of the two companies. Currently, the DHA-rich SCO produced by Martek is almost exclusively DHASCO™ and is destined for infant formula as part of the SCO blend Formulaid®. The production of the *Schizochytrium* oil continues at a low level and is aimed more directly at food applications. Several other companies, Norferm in Norway and Celanese in Germany, have developed competing processes to some level at least, and indeed Celanese, through their subsidiary company Nutrinova, Frankfurt, Germany, have recently expressed their intent to launch a chytrid-derived DHA-rich oil, to be known as DHActive™. To date, however, no DHA-rich SCO other than Martek's DHASCO™ has reached a large-scale market. In Japan, Nagase-Suntory are thought to be producing small amounts of DHA-SCO to be used as a dietary supplement, although it is uncertain to what extent production is taking place.

Both *C. cohnii* and *Schizochytrium* were selected for both their capacity to accumulate large amounts of DHA containing oil (cells contain >30% oil containing >40% DHA) and ease of cultivation in conventional stirred tank fermenters. Although both *C. cohnii* and *Schizochytrium* are microalgae, they are heterotrophic and therefore do not need light as a prerequisite for growth. As a result, both can be grown in fermenters using similar technologies used for other microbial fermentations. One peculiarity of these organisms, however, is a consequence of their marine heritage: Both organisms require the growth medium to contain a substantial concentration of salt (NaCl) in order to grow. Seawater contains 18–19,000-ppm Cl<sup>-</sup>, which is not a problem in glass vessels, such as laboratory bench fermenters; however, it is a major problem at industrial scale where stainless steel vessels are the norm. The “standard” grade of stainless steel, S 30400 (more normally referred to as simply 304), which is relatively inexpensive and is used for a large percentage of applications (approximately 50% of all stainless steel used is 304) from household pans and cutlery to industrial plants. A total of 304 can withstand high Cl<sup>-</sup> conditions for short periods of time, assuming thorough washing in between, but is susceptible to crevice corrosion at above 200-ppm Cl<sup>-</sup> if contact is prolonged. The higher grade of stainless steel 316 is also used for industrial plants, although less often because of increased expense and can withstand Cl<sup>-</sup> concentration up to 1000 ppm, still insufficient for cultivation of microorganisms in media with salt concentrations similar to seawater. As a result, specific media have been developed (66), and strains of both organisms that are capable of growth in “low” salt conditions have been identified.

As with the ARASCO process, the organism is cultivated in sequentially larger vessels to ensure sufficient inocula size, to final production vessels up to 200 m<sup>3</sup> (see Figure 2). The development of the culture is followed to ensure optimal conditions, and once sufficient biomass and oil have accumulated, the biomass is harvested by centrifugation, spray dried, and then hexane extracted using technology essentially identical to that used for vegetable oils (50, 67). The resulting oil is a

transparent orange oil that is bland in taste and odor. Despite its high DHA content, DHA-rich SCO and in particular DHASCO™ is very stable, far more stable than fish oils (67–69), to the extent that microbial oils have to be relatively badly abused before they take on the taste/odor characteristics of even the most refined fish oils.

## 4. PROSPECTS FOR PRODUCTION OF OTHER PUFAS BY MICROORGANISMS

### 4.1. Eicosapentaenoic Acid (20:5, n-3)

The markets for ARA and DHA are now well established in Europe and the United States and are clearly being developed in Japan as well as in China. The next PUFA that appears likely to be produced is EPA, which could be used as a nutraceutical for over-the-counter sales or, more likely, as a possible pharmaceutical, as there have been numerous reports of its benefits for the treatment of various diseases and disorders. Such conditions as atherosclerosis, cancer, rheumatoid arthritis, psoriasis, bipolar disorder, schizophrenia, and Alzheimer's disease have all been said to have been improved or relieved by the oral administration of EPA, sometimes on its own, although sometimes with DHA (34, 70–72). EPA and DHA, of course, occur together in many fish oils, and when treatment with both these PUFAs is advocated, then the use of fish oils would seem to be the appropriate recommendation.

EPA has long been recognized as having anti-inflammatory properties and therefore has potential uses against autoimmune diseases such as arthritis. However, inflammation is now being recognized as a major factor in the progression of heart disease (73). Thus, the cardioprotective activity of EPA may be associated with its anti-inflammatory properties. There is also some evidence that growth of some mouse tumors can be decreased by oral administration of large doses of EPA (74–76). The EPA may work by affecting fatty acid uptake by the tumor cells and, in particular, by inhibiting linoleic acid uptake, which is converted into the 13-hydroxy derivative that is a positive promoter of tumor growth (77).

Opportunities for producing EPA, besides purifying it from fish oils, which will always leave some DHA behind, using micro-organisms are currently somewhat limited. *Mortierella alpina* strain 1S-4, which is used commercially to produce arachidonic acid (see above) can produce EPA if it is grown during the lipid accumulation stages at 12°C rather than at the normal 28°C (78, 79). Also, EPA can be produced in increased quantities if alpha-linolenic acid (18:3, n-3) is fed to this particular strain of *M. alpina* (80). Up to 42-mg EPA/g cells has been achieved. However, ARA is still present in all of these oils. Some species of the fungus, *Pythium*, has also been shown to produce EPA at up to 34-mg/g cells (81–83), but other PUFAs, including arachidonic acid, are also synthesized simultaneously, making production of an EPA-rich oil, devoid of ARA, somewhat difficult.

The best current sources of EPA would appear to be the photosynthetic microalgae, of which *Porphyridium cruentum*, *Isochrysis galbana*, *Nannochloropsis oculata*, and *Phaeodactylum tricornerutum* appear to be the prime candidates (84–87). All produce oils with EPA between 25% and 38% of the total fatty acids (see

Table 1), but in each case, there is always some ARA or DHA, or even both, that is present. The oil content of these algae is also not very encouraging as it is generally less than 20% of the cell mass. The oil is not high in triacylglycerols, which is the desirable form for it, but it is a variety of complex lipids involved in the photosynthetic apparatus of the cells.

Thus, there is as yet no equivalent oil to the DHASCO from *Cryptocodinium cohnii*, in which EPA is the sole PUFA that is present and moreover is present principally as its triacylglycerol. Some researchers, though, have advocated the use of genetically engineered microorganisms to produce EPA by taking appropriate genes from bacteria (a few of which can also produce EPA) and cloning them into more amenable species, but this is far from an easy task if one also needs to increase the total oil content of the species. There is considerable effort currently being placed into finding suitable micro-organisms for the production of EPA-rich oils, and it is not inconceivable that within the next 2 to 3 years, such organisms will be found, but at the present time, no microbial source of EPA is in production.

## 4.2. Other PUFAs

Prospects of producing a variety of other PUFAs using microorganisms exist, although whether any of these will reach commercial fruition will depend on the demand for such materials. In many cases, these unusual fatty acids can be produced using *Mortierella alpina*, which normally produces arachidonic acid (see above). However, Shimizu et al. have shown (32, 88) that it is possible to mutate this fungus so that the normal sequence of desaturation and elongation of the C18 PUFAs can be disrupted at various key points. Thus, mutants of this organism have been created, which can accumulate:

- **Stearidonic acid** (18:4, n-3) [here the elongation of gamma-linolenic acid (18:3, n-6) to 20:3, n-6, had been prevented by a mutation that knocked out the gene coding for this elongase enzyme activity; the GLA was now desaturated with an existing delta-15 desaturase]. The production of this PUFA has, however, also been demonstrated in transgenic crop plants by Monsanto, so the potential for this PUFA as the basis of a SCO is limited.
- **Dihomolinolenic acid** (DHGLA; 20:3, n-6) (where the final desaturation of this fatty acid to arachidonic acid by a delta-5 desaturase had been either deleted by mutation or blocked by the presence of a specific inhibitor of the desaturase).
- **Eicosatrienoic acid** (ETA; 20:3, n-9: Mead acid) (here a double mutant of the parent fungus was produced that lacked both delta-12 and delta-15 desaturase activities so that oleic acid, 18:1,n-9, could only be desaturated between its existing double bond and the carboxylate head group).
- **Eicosatetraenoic acid** (EteA; 20:4, n-3) [where the mutant that had accumulated DHGLA (see above) was now grown with linseed oil (as a source of alpha-linolenic acid, 18:3, n-6) instead of the normal glucose; this fatty acid was then channeled down the n-3 route of metabolism].

In all cases, the amounts of the various PUFA were relatively low, although for DHGLA, the amount reached 23% of the total fatty acids. Some of the above PUFAs also occur in small amounts in various marine microalgae, although no routes to optimize their commercial productions have been indicated.

The unusual PUFA, **docasopentaenoic acid** (22:5, n-6), which occurs at about 12% of the total fatty acids of the *Schizochytrium* sp. used for DHA production (see Table 4), is thought to be produced in Japan by Nagase-Suntory, possibly as a byproduct from the purification of the DHA-rich oil that this organism produces, although the exact means of production are not certain. The direct applications of DPA are uncertain, although as discussed in Section 3.2.1, there may be some benefits of including DPA along with both DHA and ARA as it may serve to maintain DHA at a higher circulating level (65).

## 5. THE FUTURE OF MICROBIAL OILS

The past two decades have seen the commercial productions of several single cell oils: oils rich in gamma-linolenic acid, arachidonic acid, and docosahexaenoic acid. Although gamma-linolenic acid rich-SCO was in production for only about 6 years in the United Kingdom during the 1980s and is no longer considered an economic reality, the production of DHA-and ARA-rich-SCOs has demonstrated the potential of this technology, in dramatic style. These SCOs are enjoying a period of enormous growth in demand, to the point where the market is supply- rather than demand-limited. The main global supplier of SCO, Martek Bioscience Corporation (>95% of the global market in SCO are "Martek-oils") is doubling its production capacity every year for at least the next two years (2004 and 2005). As a consequence, Martek has become a major fermentation company with millions of Liters of fermentation capacity solely dedicated to SCO production.

Against this background, it is more than likely that an SCO rich in eicosapentaenoic acid will also become commercially viable within the next few years as the demands for improved supplies of this fatty acid are now beginning to accelerate. Stearidonic acid could well be the next one after that, although in this particular case, this PUFA can be produced using selected species of plants known as *Echium*, and transgenic crop plants producing this fatty acid have been produced by Monsanto.

The microbial route to production will, however, always be expensive, and thus, only the most expensive of the PUFAs will justify being produced by this means. Fermentation technology is never going to be a cheap alternative to agriculture. The logical progression, therefore, is to see the next phase of PUFA production moving from micro-organisms toward the use of plants. This can, though, only take place by genetic manipulation as no plants are known that produce ARA, EPA, or DHA and it is these three PUFAs that are the major targets for production because of the potential size (both in volume and in price) of the markets for each of them.

To design a plant that will produce these oils, it is necessary to clone into the plant of choice (probably sunflower or rapeseed—canola) genes that will code for one or two elongating enzymes (to convert C18 fatty acids into C20 fatty acids

and then to elongate the C20 fatty acids into C22 fatty acids) and a number of desaturases that will then convert a diunsaturated fatty acid (i.e., 18:2, which is the major fatty acid of these plants) into, eventually, a hexa-unsaturated fatty acid. Thus, to produce DHA (via a "classic" fatty acid route), a minimum of six different genes will be needed and possibly more to ensure that these genes will function correctly.

The expression of the introduced genes must also be carefully controlled so that the proteins (i.e., enzymes that they are coding for) will be specifically targeted (both spatially and temporally) to ensure that the LC-PUFA are produced only in seed tissue and only during the oil accumulation phase. Although this targeting is not a major technical obstacle, the apparent innate inability of plants to metabolically process LC-PUFA could be a considerable challenge. Although the introduction of fatty acid desaturase genes (even those not usually found in plants) leads to an appreciable build-up in new unsaturated fatty acids, the introduction of an fatty acid elongase has not yet engendered a significant accumulation of C20 or C22 PUFAs (to the authors' knowledge). It is possible that this failure to produce C20 PUFA is not caused by a lack of elongase activity *per se*, but it is associated with the inability of plant acyltransferases to efficiently transport C20 fatty acids. The acyltransferases are enzymes involved in the "shuttling" of fatty acids into cellular membranes for their final desaturation and then incorporation into triacylglycerols (TAG). If this is the case, then several more genes (for LC-PUFA acyltransferases) may need to be introduced into plants to facilitate LC-PUFA accumulation, and this would add enormously to the complexity of producing a satisfactory GM plant for LC-PUFA production.

Some of the problems associated with the transgenic production of LC-PUFA in plants (although not the problems associated with fatty acid transport) may be solved by harnessing the newly discovered "PKS-like" route for LC-PUFA biosynthesis. This route, completely separate from "classic" fatty acid biosynthesis, is catalysed by a single enzyme complex, and not using fatty acid desaturases or discrete elongases appears to operate in certain marine prokaryotes and in *Schizochytrium* (89). The "PKS-like" enzymatic machinery is encoded by three or four open reading frames, decreasing the number of genes that would be required to be transformed into a potential plant host. However, the very large size of these genes could introduce problems of their own as it becomes increasingly more difficult to clone genes as their size increases.

A further, and as yet unresolved, problem develops as to how the necessary reducing power that is needed in each of the elongating and desaturating reactions will be generated. The plant of choice for genetic manipulation is already in metabolic balance: It produces what it needs, no more, no less. When an increased metabolic burden is then placed on a plant to produce products that require additional resources from the central metabolic pathways, it is not clear how these resources will be achieved. An increased demand for reducing power in one part of the plant means that metabolic economies will have to take place elsewhere. Plants do not have the capacity to increase their metabolic capacity at the whim of genetic engineers. They are governed by the availability of light and of CO<sub>2</sub>. Thus, how the plants that are being designed to produce high contents of PUFAs, particularly

DHA, will be able to achieve this is, at least to the present authors, far from clear. It is not beyond the bounds of possibility that such genetically modified plants may produce much less oil than the normal plant as the energy and reducing power needed (by the desaturase and elongase reactions) to produce the PUFAs must come from the same sources that are being used to synthesize the “normal” fatty acids. The situation in plants may parallel what was found with the production of GLA in *Mucor* spp. (see Section 2.2.1): You can either find strains that produce a lot of oil but have little GLA, or you can find strains that produce a lot of GLA but have little oil, but you cannot have both occurring simultaneously. It may then take additional genetic manipulations to correct this imbalance, but distortion of one metabolic pathway can only be achieved at the expense of another so this is not likely to be a trivial task.

Thus, although the GM plant route to PUFA formation is attractive, it is by no means going to be as simple a task as the molecular biologists would appear to consider. One can expect that it will take the labors of many people many years to achieve these objectives, but, given the high rewards that are at stake, it may seem to many people just a question of time before the genetic manipulators are successful. The question then that microbial oil producers have to decide is just how long they have before the GM plant people achieve their goals, for when that happens, it will be the end of SCO productions. They will simply be too expensive to compete. The pessimists would suggest no more than 10 years; the optimists might suggest 20 or even 30 years.

But there is one final question that no one can yet resolve. Will GM plants be accepted for the production of PUFAs? The public opinion of GM crops is, at present, very much against their use in Europe. A survey conducted in the United Kingdom during 2003 has indicated that over 80% of the population is opposed to the introduction and use of GM crops, with only about 7% of the population being willing to consume any such GM product (90). The view against the use of GM plants, which has no scientific basis but is wholly based on irrational fears, now appears to be spreading to the United States and the rest of the world. If there should be a moratorium against the use of GM crops in general, then it may be that the industrial companies who are currently funding much of this work will pull out these endeavors. If this should happen, no matter how regrettable this might be scientifically, then this would spell the end of GM plant PUFAs. If this should happen, the only way in which the demand for these desirable oils is going to be met will be by the microbial route. Single cell oils could then have a long and distinguished future ahead of them.

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## REFERENCES

1. C. Ratledge and B. Kristiansen, *Basic Biotechnology*, 2nd ed., Cambridge University Press, Cambridge, U.K., 2001.
2. M. Woodbine, *Progr. Indust. Microbiol.*, **1**, 179–245 (1959).
3. C. Ratledge, in D. J. Kyle and C. Ratledge, eds., *Industrial Applications of Single Cell Oils*, American Oil Chemists' Society, Champaign, Illinois, 1992, pp. 1–15.
4. C. Ratledge, in B. S. Kamel and Y. Kakuda, eds., *Technological Advances in Improved and Alternative Sources of Lipids*, Blackie, Glasgow, U.K., 1994, pp. 235–291.
5. C. Ratledge, in H. J. Rehm, R. Reed, A. Puhler, P. Stadler, H. Kleinkauf, and H. von Dohren, eds., *Biotechnology*, Vol. 7: *Products of Secondary Metabolism*, 2nd ed., VCH, Weinheim, Germany, 1997, pp. 133–197.
6. C. Ratledge, in F. D. Gunstone, ed., *Structured and Modified Lipids*, Marcel Dekker, New York, 2001, pp. 351–399.
7. C. Ratledge and S. J. Wilkinson, eds., *Microbial Lipids*, Vol. 1, Academic Press, London, U.K., 1989.
8. C. Ratledge and J. P. Wynn, *Adv. Appl. Microbiol.* **51**, 1–51 (2002).
9. Y. S. Huang and D. E. Mills, eds.,  *$\gamma$ -Linolenic Acid: Metabolism and its Roles in Nutrition and Medicine*, American Oil Chemists' Society, Champaign, Illinois, 1996.
10. D. F. Horrobin and P. F. Morse, *Lancet*, **345**, 260–261 (1995).
11. D. F. Horrobin, *Amer. J. Clin. Nutr.*, **71**, 367S–372S (2000).
12. Y. S. Huang, and A. Ziboh, eds.,  *$\gamma$ -Linolenic Acid: Recent Advances in Biotechnology & Clinical Applications*, American Oil Chemists' Society, Champaign, Illinois, 2001.
13. P. M. Clough, in F. D. Gunstone, ed., *Structured and Modified Lipids*, Marcel Dekker, New York, 2001, pp. 75–117.
14. R. J. Davies, Report of the Department of Scientific and Industrial Research, No. IPD/T50/2011 DSIR, New Zealand, 1983.
15. R. J. Davies, *Food Technol. New Zealand*, **June** 33–37 (1984).
16. R. J. Davies, in R. S. Moreton, ed., *Single Cell Oil*, Longman, U.K./John Wiley, New York, 1988, pp. 99–145.
17. K. W. Smith, in F. D. Gunstone, ed., *Structured and Modified Lipids*, Marcel Dekker, New York, 2001, pp. 401–422.
18. R. S. Moreton, *Appl. Microbiol. Biotechnol.*, **33**, 41–45 (1985).
19. R. S. Moreton, ed., *Single Cell Oil*, Longman, U.K./John Wiley, New York, 1988, pp. 1–32.
20. A. Ykema, E. C. Verbee, H. J. J. Nijkamp, and H. Smit, *Appl. Microbiol. Biotechnol.*, **32**, 76–84 (1989).
21. A. Ykema, E. C. Verbee, I. I. G. S. Verwoert, K. H. van der Linden, K. H. Nijkamp, H. J. J. Nijkamp, and H. Smit, *Appl. Microbiol. Biotechnol.*, **33**, 176–182 (1990).
22. I. I. G. S. Verwoert, A. Ykema, J. A. C. Valkenburg, E. C. Verbree, H. J. J. Nijkamp, and H. Smit, *Appl. Microbiol. Biotechnol.*, **32**, 327–333 (1989).
23. H. Smit, A. Ykema, E. C. Verbree, I. I. G. S. Verwoert, and M. M. Kater, in D. J. Kyle and C. Ratledge, eds., *Industrial Applications of Single Cell Oils*, American Oil Chemists' Society, Champaign, Illinois, 1992, pp. 185–195.

24. M. Beavan, A. Kligerman, R. Droniuk, C. Drouin, B. Goldenberg, A. Effio, P. Yu, B. Guiliany, and J. Fein, in D. J. Kyle and C. Ratledge, eds., *Industrial Applications of Single Cell Oils*, American Oil Chemists' Society, Champaign, Illinois, 1992, pp. 156–184.
25. M. Hassan, P. J. Blanc, A. Pareilleux, and G. Goma, *Biotechnol. Lett.*, **16**, 819–824 (1994).
26. M. Hassan, P. J. Blanc, A. Pareilleux, and G. Goma, *World J. Microbiol. Biotechnol.*, **10**, 534–537 (1994).
27. R. J. Davies, in D. J. Kyle and C. Ratledge, eds., *Industrial Applications of Single Cell Oils*, American Oil Chemists' Society, Champaign, Illinois, 1992, pp. 196–218.
28. R. J. Davies, J. E. Holdsworth, and S. L. Reader, *Appl. Microbiol. Biotechnol.*, **33**, 569–573 (1990) *Erratum*: **34**, 832–833 (1991).
29. R. J. Davies, and J. E. Holdsworth, *Adv. Appl. Lipid Res.* **1**, 119–159 (1992).
30. N. J. Moon, and E. G. Hammond, *J. Amer. Oil Chem. Soc.*, **55**, 683–688 (1978).
31. F. D. Gunstone, in F. D. Gunstone, ed., *Structured and Modified Lipids*, Marcel Dekker, New York, 2001, pp. 11–35.
32. M. Certik and S. Shimizu, *Appl. Microbiol. Biotechnol.*, **54**, 224–230 (2000).
33. C. Ratledge, *Trends Biotechnol.*, **11**, 278–284 (1993).
34. B. F. Haumann, *Inform.*, **9**, 1108–1116 (1998).
35. S. Kazuhiko et al., Japanese Patent 1038007, 1989.
36. Y. Shinmen, S. Shimizu, and Y. Hideaki, European Patent 0276541, 1988.
37. H. Streekstra, *J. Biotechnol.*, **56**, 153–165 (1997).
38. K. Boswell, E-K. Koskelo, L. Carl, S. Glaza, D. J. Hensen, K. D. Williams, and D. J. Kyle, *Food Chem. Toxicol.*, **34**, 585–593 (1996).
39. R. A. Hempenius, J. M. H. van Delft, M. Prinzen, and B. A. R. Lina, *Food Chem. Toxicol.*, **35**, 573–581 (1997).
40. R. A. Hempenius, B. A. R. Lina, and R. C. Haggit, *Food Chem. Toxicol.*, **38**, 127–139 (2000).
41. Y. Shinmen, S. Shimizu, K. Akimoto, H. Kawashima, and H. Yamada, *Appl. Microbiol. Biotechnol.*, **31**, 11–16 (1989).
42. S. Stredanska and J. Sajbidor, *Folia Microbiol.*, **37**, 357–358 (1997).
43. V. K. Eroshin, E. G. Dedyukhina, T. I. Chistyakova, V. P. Zhelifonova, C. P. Kurtzman, and R. J. Bothast, *World J. Microbiol. Biotechnol.*, **12**, 91–96 (1996).
44. T. Aki, Y. Nagahata, K. Ishihara, Y. Tanaka, T. Morinaga, K. Higashiyama, K. Akimoto, S. Fujikawa, S. Kawamoto, S. Shigeta, K. Ono, and O. Suzuki, *J. Amer. Oil Chem. Soc.*, **78**, 599–604 (2001).
45. A. Singh, and O. P. Ward, *Appl. Microbiol. Biotechnol.*, **48**, 1–5 (1997).
46. P. K. Bajpai, P. Bajpai, and O. P. Ward, *J. Indust. Microbiol.*, **8**, 179–186 (1991).
47. S. Shimizu, *Yukagaku*, **44**, 804–813 (1995).
48. K. Higashiyama, T. Yaguchi, K. Akimoto, S. Fujikawa, and S. Shimizu, *J. Amer. Oil Chem. Soc.*, **75**, 1815–1819 (1998).
49. K. Higashiyama, K. Murakami, H. Tsujimura, N. Matsumoto, and S. Fujikawa, *Biotechnol. Bioeng.*, **63**, 442–448 (1999).
50. D. J. Kyle, *Lipid Technol.*, **8**, 107–110 (1996).
51. H. Yamada, S. Shimizu, Y. Shinmen, H. Kawashima, and K. Akimoto, *J. Dispersion Sci. Technol.*, **10**, 561–579 (1989).

52. J. Sajbidor, D. Kozelouhova, and M. Certik, *Folia Microbiol.*, **37**, 404–406 (1992).
53. Y. Koike, H. J. Cai, K. Higashiyama, S. Fujikawa, and E. Y. Park, *J. Biosci. Bioeng.*, **91**, 382–389, (2001).
54. E. Y. Park, Y. Koike, K. Higashiyama, S. Fujikawa, and M. Okabe, *J. Biosci. Bioeng.*, **88**, 61–67, (1999).
55. L. M. Arterburn, K. D. Boswell, T. Lawlor, M. A. Cifone, H. Murle, and D. J. Kyle, *Food Chem. Toxicol.*, **38**, 971–976 (2000).
56. G. Hornstra, *Am. J. Clin. Nutr.*, **71**, 1262S–1269S (2000).
57. K. Boswell, E.-K. Koskelo, L. Carl, S. Glaza, D. J. Hensen, K. D. Williams, and D. J. Kyle, *Food Chem. Toxicol.*, **34**, 585–593 (1996).
58. BBC Online Network. (Sept. 20, 1999). Toxin warning on oily fish. *BBC News*. Available: <http://news.bbc.co.uk/1/hi/health/452548.sym>.
59. United States Environmental Protection Agency. Press Release. (Jan. 12, 2001). Available: <http://www.epa.gov/mercury/fishadv.pdf>.
60. J. Gómez-Aracena, J. D. Kark, R. A. Riemersma, J. M. Martín-Moreno, and F. J. Kok, *New Eng. J. Med.*, **347**, 1747–1754 (2002).
61. G. W. Harrington, D. H. Beach, J. E. Dunham, and G. G. Holtz, *J. Protozool.*, **17**, 213–219 (1970).
62. R. J. Henderson, *Lipid Technol.*, **11**, 5–10 (1999).
63. S. M. Innis and J. W. Hansen, *Am. J. Clin. Nutr.*, **64**, 159–167 (1996).
64. J. A. Conquer and B. J. Holub, *Lipids*, **32**, 341–345 (1997).
65. P. S. Y. Tam, R. Umeda-Sawada, T. Yaguchi, K. Akimoto, Y. Kiso, and O. Igarashi, *Lipids*, **35**, 71–75 (2000).
66. W. R. Barclay, U. S. Patent 5,340,742, 1994.
67. P. W. Behrens and D. J. Kyle, *J. Food Lipids*, **3**, 259–272 (1996).
68. W. Barclay, R. Abril, P. Abril, C. Weaver, and A. Ashford, *World Rev. Nutr. Diet*, **83**, 61–76 (1998).
69. C. C. Becker and D. J. Kyle, *Food Technol.*, **52**, 68–71 (1998).
70. C. A. Drevon, I. Baksaas, and H. L. Krokan, eds., *Omega-3 Fatty Acids: Metabolism and Biological Effects.*, Birkhauser Verlag, Basel, 1993.
71. A. P. Simopoulos, R. R. Kifer, R. E. Martin, and S. M. Barlaw, eds., *Health Effects of n-3 Polyunsaturated Fatty Acids.*, S. Karger AG, Basel, 1991.
72. D. J. Garcia, *Food Technol.*, **52**, 44–49 (1998).
73. G. J. Blake and P. M. Ridker, *J. Intern. Med.*, **252**, 283–294 (2002).
74. A. S. Whitehouse and M. J. Tisdale, *Biochem. Biophys. Res. Commun.*, **285**, 598–294 (2002).
75. M. J. Tisdale, *J. Nutr.*, **129**, 243S–246S (1999).
76. M. J. Tisdale, *Support Care Cancer*, **11**, 73–78 (2003).
77. L. A. Sauer, R. T. Dauchy, and D. E. Blask, *Cancer Res.*, **60**, 5289–5295 (2000).
78. S. Shimizu, Y. Shinmen, H. Kawashima, K. Akimoto, and H. Yamada, *Biochem. Biophys. Res. Commun.*, **150**, 335–341 (1988).
79. S. Shimizu, H. Kawashima, Y. Shinmen, K. Akimoto, and H. Yamada, *J. Amer. Oil Chem. Soc.*, **65**, 1455–1459 (1988).

80. S. Shimizu, H. Kawashima, K. Akimoto, Y. Shinmen, and H. Yamada, *J. Amer. Oil Chem. Soc.*, **66**, 341–347 (1989).
81. S. R. Gandhi, and J. D. Weete, *J. Gen. Microbiol.*, **137**, 1825–1830 (1991).
82. D. J. O'Brien, M. J. Kurantz, and R. Kwoczak, *Appl. Microbiol. Biotechnol.*, **40**, 211–214 (1993).
83. E. E. Stinson, R. Kwoczak, and M. J. Kurantz, *J. Ind. Microbiol.*, **8**, 171–178 (1991).
84. E. Molina Grima, J. A. Sanchez Perez, F. Garcia Camacho, J. M. Fernandez Sevilla, and F. G. Acien Fernandez, *Appl. Microbiol. Biotechnol.*, **41**, 23–27 (1994).
85. A. Seto, K. Kumasaka, M. Hosaka, E. Kojima, M. Kashiwakura, and T. Kato, in D. J. Kyle and C. Ratledge, eds., *Industrial Applications of Single Cell Oils*, American Oil Chemists' Society, Champaign, Illinois, 1992, pp. 219–234.
86. Z. Cohen, *J. Amer. Oil Chem. Soc.*, **67**, 916–920 (1990).
87. W. Yongmanitchai and O. P. Ward, *J. Amer. Oil Chem. Soc.*, **69**, 584–590 (1992).
88. M. Certik, E. Sakuradani, and S. Shimizu, *Trends Biotechnol.*, **16**, 500–505 (1998).
89. J. G. Metz et al., *Science*, **293**, 290–293 (2001).
90. (2003). Available: [www.gmpublicdebate.org/ut\\_09/ut\\_9\\_6.htm](http://www.gmpublicdebate.org/ut_09/ut_9_6.htm).

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## *Transgenic Oils*

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### **1. INTRODUCTION**

Most commodity seed oils consist of triacylglycerols containing varying percentages of palmitic, stearic, oleic, linoleic, and linolenic acids esterified to the glycerol backbone. These oils and the fatty acids derived from them are used primarily for food and feed; yet they have important industrial applications as well. They provide precursors for soaps and other surfactants, derivatives used in production of certain plastics and polyamides, applications in low volatile organic carbon (VOC) coatings, cosmetics, as well as lubricant and grease compounds. The usefulness of an oil for food use lies in caloric value and the presence of essential fatty acids, specifically, those fatty acids that are not produced by humans, linoleic acids, and  $\alpha$ -linolenic acids. In many cases, the usefulness of a seed oil to industry derives from a high proportion of a specific fatty acid in the oil; for example, the high linolenate content of linseed oil results in its good properties as a drying oil. This article will briefly cover progress in developing new oils, examine the state of the art, and describe industrial oilseed crops projected to be developed for commercial use.

Oils containing high levels of oleic acid are considered to be beneficial for human health consequences. Such oils are stable to oxidation compared with oils

containing polyunsaturated fatty acids (PUFAs) and contain low levels of saturated fatty acids. Olive oil (70% oleate) is generally considered to have a beneficial composition for oleate content. It should be noted that continued research is adding information for fatty acid requirements and benefits. Linoleic and  $\alpha$ -linolenic acids have been known for decades to be essential fatty acids. More recently, the need for eicosapentaenoic and docosahexaenoic acids as part of the diet, especially for the developing fetus, and even  $\gamma$ -linolenic, underlie the need for intake of oils and fats from multiple sources that can supply what seems to be an expanding list of nutritionally important fatty acids.

Although oils containing PUFAs can be converted to high monounsaturate content by partial hydrogenation, the process results in the production of *trans*-fatty acids. There is a negative perception of *trans*-fatty acids, which are thought to behave physiologically as saturated fatty acids. These acids are considered to increase arterial plaque formation and may contribute to the development of type II diabetes. Thus, a considerable research-and-development effort has been put into designing food oils with a high content of oleic acid. However, for commercial use, the market for food-grade oils is often driven by price, with quality traits providing premium value.

Most vegetable oil that is produced is consumed as food. These food oils also have important industrial uses. For example, approximately 15% of soybean oil is used for industrial products, including inks, plasticizers, coatings, and composite materials. Other commodity oils are useful industrially because they contain uncommon fatty acids. Castor oil is 90% ricinoleate (12-hydroxy-octadec-9-enoate), and the hydroxy group imparts dramatically different physical and chemical properties that make castor oil an important industrial feedstock. Rapeseed oil contains up to 60% erucate (docosa-13-enoate), which is used in several lubricant applications. Tung oil contains up to 80% eleostearate (octadeca-9c,11t,13t-trienoate), a conjugated fatty acid that makes tung oil a prized drying oil because it does not yellow during the drying process. Palm-kernel oil and coconut oil both contain high levels of the medium-chain saturated fatty acids laurate (C12) and myristate (C14), which have excellent foaming properties for production of soaps and other surfactants. Thus, several features of a vegetable oil can impart industrial chemical value. Chemical functionality can alter physical properties or provide reactive sites that allow useful derivatives to be made. Another industrially useful feature is the presence of a highly enriched single component. Some oils also have unique uses as a result of their composition; e.g., cocoa butter is unique in its melting characteristics, which makes it an excellent component of cosmetics in addition to its food uses. Consistent composition is also important for industry, and this is usually closely tied to a high content of a desired component. The goal of developing oil-seeds for industrial use is to introduce one or more of these desirable characteristics into the oil of an agronomically suitable crop.

Seed oils also contain potentially useful fatty acids that have not been introduced into commerce because the plant has not yet been adapted to large-scale planting. Examples of such plants include *Vernonia anthelmintica* and *Euphorbia lagascae*,

which produce oils high in vernolate (octadeca-12,13-epoxy, 9-enoate); *Cuphea* sp., many of which produce oils containing medium-chain fatty acids from caprylate (C8) to myristate at levels up to 95% of a single fatty acid; *Lesquerella* sp., which contain up to 55% lesquerolate (eicosa-14-hydroxy-9-enoate) that can replace ricinoleate in some applications. Each crop has been the target of “New Crops” research, which is aimed at breeding out undesirable agronomic characteristics and introducing desirable traits, such as higher yield or indehiscence. Although considerable progress has been made in each crop, the problem encountered is a “Catch 22”: It is hard to get farmers to grow the crop because there is not yet a significant market for the product, and it is hard to develop a market for the crop, because no reliable source exists.

Breeding programs have expanded the potential uses of vegetable oils over the years. Canola, high oleic safflower, high-oleic sunflower, and low-saturate soybean oil are all the result of extensive traditional breeding programs, based on crossing available crop germplasm. The introduction of mutagenesis provided a new tool to breeders, and it is most often useful in eliminating an undesirable trait. The introduction of genetic engineering greatly expanded the ability of the breeder to introduce desired traits, with genes from incompatible crops, microorganisms, insects, animals, or any other organism being added to the breeders’ toolbox. Moreover, genomic sequencing efforts have revealed the extent of synteny among plants. Synteny refers to a correspondence in genomic arrangement, and this has allowed identification of specific genes associated with agronomically useful traits, e.g., dwarfing. By comparing genomes, useful traits across species and genera can be identified and selected for directly rather than through extensive breeding programs.

Crop genetic engineering holds great promise as a means for developing oilseed crops with unique characteristics that add both commercial and nutritive value, increase utilization, and benefit the environment. Currently, the four genetically engineered (transgenic) crops that have been adopted are all oilseed crops: soy, corn, cotton, and canola. They are a commercial success and account for 99% of transgenic crops planted worldwide. Over 70% of the soy, 50% of the corn, and 70% of the cotton grown in the United States are genetically engineered. Canola is a relatively small crop in the United States, but approximately 60% of the canola planted in the United States is transgenic. Most canola grown in Canada, a leading producer, is transgenic. An increasing number of countries have adopted the technology. The United States, Argentina, Canada, Brazil, China, and South Africa account for 99% of the transgenic crops produced, with an additional 12 countries adopting the technology (1). The growth in planting of transgenic crops is remarkable in that it has all occurred in the last 8 years, from the time the first transgenic crops were introduced in 1996. At this time, each crop has been modified for “input” traits, reducing or eliminating the need for chemical applications by the introduction of genes encoding herbicide tolerance (soy, canola), insect resistance (corn, cotton), or both (cotton). Currently, cotton is the only crop with a significant share of the crop carrying both (stacked) traits. The introduction of genes for input traits is

**TABLE 1. Composition of Transgenic Oilseeds (%).**

Crop	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	X <sup>a</sup>
Cotton-ctrl	27.7	0.6	2.7	15.3	43.2	—	0.2	—	0.2	—	2.3
Cotton-GM	26.8	0.7	2.7	15.5	45.9	—	0.3	—	0.2	—	1.7
Corn-ctrl	9.9	—	1.9	27.4	58.7	1.1	0.4	0.3	0.2	—	—
Corn-GM	9.9	—	1.9	27.5	58.6	1.1	0.4	0.3	0.2	—	—
Soybean-ctrl	11.2	—	4.1	19.7	52.5	8.0	0.4	0.2	0.5	—	—
Soybean GM	11.2	—	4.1	19.7	52.3	8.2	0.4	0.2	0.5	—	—
Canola-ctrl	4.6	0.3	1.6	57.5	19.4	13.8	0.6	1.4	0.3	—	—
Canola-12:0	3.3	0.2	1.1	35.1	14.6	8.8	0.3	0.7	0.2	—	35.5

X<sup>a</sup>: uncommon fatty acid content; for cotton, malvalic, sterculic, and dihydrosterculic; Canola-12:0, 31.3% 12:0 and 4.2% 14:0.

Cotton control is Coker 312; Cotton GM is glyphosate tolerant, selection 1445 (Monsanto), from (2); Corn control is GA21 segregant lacking the gene for glyphosate tolerance; GM variety is GA21 segregant carrying glyphosate tolerance gene, from (3); Soybean control is A5403, and Soybean GM is GTS 40-3-2, derived from particle-bombardment of A5403 with genetic material containing the CP4 EPSP gene for glyphosate tolerance (4); Canola, control (Westar) compared to canola seed with gene for acyl-ACP thioesterase from California Bay laurel (5).

required for maintenance of “substantial equivalence” in the crop. Table 1 displays data for soybean oil, cottonseed oil, and corn oil from plants lacking or including a gene for glyphosate tolerance, and it indicates that the introduced gene has had essentially no effect on composition, especially given the variability that is observed in varietal and climate-related differences in fatty acid composition.

Developments affecting “output” traits, the product of interest in the case of this article being oil, have not yet achieved commercial success. In addition to oilseed crops derived from breeding and mutagenesis, this article will describe the two transgenic oilseeds that have been commercially introduced, briefly describing the biochemical basis of their development and the problems faced in their commercialization. That discussion will be followed by a description of other oils that have been proposed for development through transgenic technology. Finally, the article will discuss issues related to acceptance of transgenic crops.

## 2. TECHNOLOGY FOR ALTERING FATTY ACID COMPOSITION

Several approaches lead to oilseed crops with altered fatty acid composition. The most ancient is evolution, which is a long-term, seemingly random process. Although it is not a practical means for purposefully altering fatty acid composition, especially in a brief time span, evolution has, in fact, yielded a broad range of oilseeds with differing characteristic fatty acid compositions. In the same species and genera, these differences usually consist of varying percentages of the same fatty acids. In some plant families and genera, considerable variation exists as well in the types of fatty acid made in seed oil. These differences provide the variants needed for successfully breeding varieties with altered fatty acid composition. Breeding programs have successfully used available germplasm to develop major



crops soy, corn, rapeseed (Canola), and sunflower that have a more desirable oil content or fatty acid composition. Where evolution may not have provided suitable germplasm, approaches also can be taken to alter fatty acid composition. Random mutagenesis followed by screening and breeding has produced varieties with altered fatty acid composition in oil (6). As the mutagenic approach is geared to eliminating genes, usually this approach has reduced levels of undesirable fatty acid components or increased levels of a desired fatty acid. A recent innovation in this approach is "targeting induced local lesions in genomes" (TILLING), which uses a mutagenic approach but introduces high throughput screening of the  $M_2$  generation (second-generation, mutated lines that have been self-pollinated) to identify specific genes that have been altered or inactivated by mutagenic events (7). Plant selections carrying these mutated genes can then be screened directly for desired characteristics. The TILLING process thus moves most of the screening effort into the laboratory, which considerably reduces the population that would otherwise have to be grown in the field for later screening.

The ability to manipulate fatty acid composition in oilseeds by genetic engineering has resulted from a combination of three approaches. Biochemical characterization has identified most steps in fatty acid biosynthesis (8, 9). Genetic identification and chemical characterization of fatty acid biosynthetic mutants in mutagenized *Arabidopsis thaliana* has provided an extensive genetic map of fatty acid and lipid biosynthetic steps during plant growth and development (10). Identification, characterization, and cloning of enzyme activities in plants that produce nutritionally useful fatty acids, such as  $\gamma$ -linolenic acid, or uncommon, industrially useful fatty acids, such as vernolic acid (12,13-epoxy oleate), have provided the additional information needed to broaden the spectrum of fatty acids available from commodity oilseeds (11). Hundreds of other fatty acids with unusual chemical functionalities are produced by one or more oilseed plants. A considerable amount of research has gone into elucidating the biosynthetic process by which such fatty acids are made, and much enzymology underlying the introduction of unsaturation, conjugated unsaturation, hydroxyl, acetylenic, and epoxy functionality is now understood. The enzymes that carry out each reaction are interrelated, can be interconverted by engineering appropriate amino acid residues, and to a limited extent, can have their specificity for chain length and positional-selectivity altered in a predictable manner (12). The specificity of the chemistry carried out on what is essentially a straight hydrocarbon chain is unprecedented for traditional bench chemistry, and in the future, it may represent the development of green chemistry carried out in plants to produce desired chemical precursors.

### 3. CANOLA FROM TRADITIONAL BREEDING OF OILSEED CROPS

Rapeseed has long been a source of cooking oil and has important industrial uses such as lubricants for high-temperature applications, especially those leading to environmental release of the lubricant; antislip agents in plastics manufacturing; fabric softeners; and additional oleochemical applications. However, the erucic acid

component has been considered a potential health problem, and as a result, a low-erucic acid rapeseed (LEAR) was desired to meet food, feed, and export needs. An intensive breeding program was initiated in the 1950s by R.K. Downey of Agriculture Canada in Saskatoon (13) and B.R. Stefansson (14) of the University of Manitoba to reduce the content of erucic acid and eliminate glucosinolates from the seed, as these were feeding deterrents and impeded use of rapeseed meal for animal feed. Each researcher identified lines of rapeseed with very low erucic acid, based on crossing out *Brassica napus* with *B. juncea*. The low-erucic varieties developed had less than 2% erucic, compared with 55% in rapeseed. The low-erucic, low-glucosinolate “double-low” varieties derived from this research were renamed “Canola” in 1979 by the Western Canadian Oilseed Crushers but are also known as LEAR. Although Canola is a minor crop in the United States, it is the major oilseed grown in Canada and Northern Europe. Although the oil is used primarily for food, its high-oleate content (60%) makes it useful for industrial processes requiring an oxidatively stable oil. Its high yield of oil (per hectare) has resulted in its use as a major source of biodiesel in Europe. Other changes have been bred in Canola selections; these include low linolenate and lower saturate content. Other high-oleate oils have been obtained through traditional breeding in sunflower, safflower, and corn, whereas peanut and olive oil, among others, have a naturally high oleate content.

#### **4. HIGH-OLEIC SUNFLOWER FROM MUTAGENESIS OF OILSEED CROPS**

When available germplasm with desired characteristics is limited, mutagenesis can help to provide additional germplasm. Chemical and radiation mutagenesis have been used in breeding programs to obtain suitable germplasm for generating novel traits. Sunflower has been mutagenized with and screened for fatty acid composition (15). The normal composition of sunflower oil is high in linoleate (60–75%), but some mutagenized lines were found that had higher oleate (>80%). In backcrosses, the high oleate trait remained stable, which indicates a nonreversible mutation had resulted in the high-oleate phenotype. Later research demonstrated that the mutation is in the oleate desaturase gene (16), a single copy gene in sunflower. Biochemically, this process would block conversion of oleate to linoleate and allow oleate to accumulate in the seed (Figure 1). Mutagenesis has altered fatty acid composition of oils derived from other crops, including soybean, cotton, flax, and canola (15).

#### **5. APPLICATIONS OF HIGH-OLEATE OILS**

High-oleate oils are highly desirable for food use. They are stable to oxidation and therefore good for frying and can be stored without spoilage for a longer time than oils with high polyunsaturate content. Oleate is the prevalent fatty acid in the

1. AcetylCoA Carboxylase
2. AcetylCoA ACP Transacylase
3. MalonylCoA ACP Transacylase
4. Condensing Enzymes, KAS III
5. Condensing Enzymes, KAS I
6. Condensing Enzymes, KAS II
7. StearoylACP Desaturase
8. AcylACP Thioesterase
9. Fatty AcylCoA Synthetase
10. Lysophosphatidic Acid AcylCoA Transacylase
11. Oleoyl Desaturase

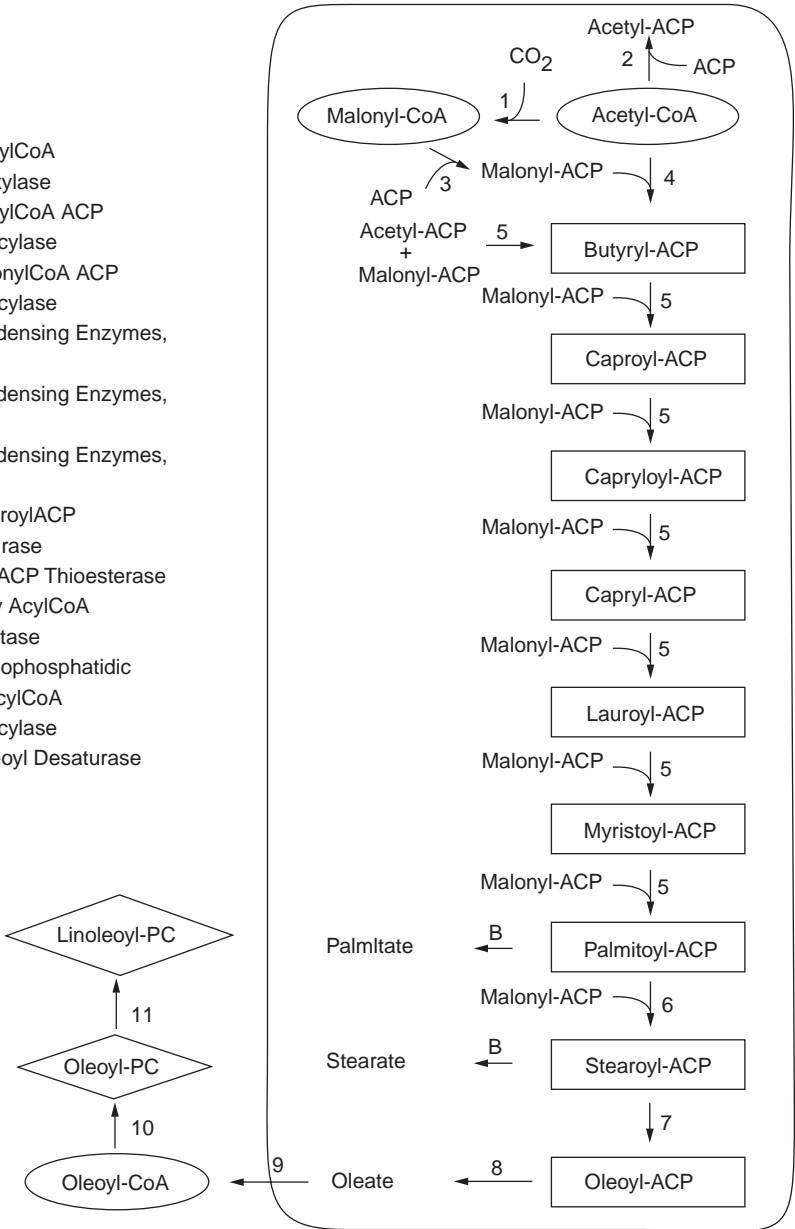


Figure 1. Fatty acid biosynthetic pathway.

“Mediterranean” diet based on olive oil and popularly thought to be the “best” fat to consume for long-range health benefits. The oxidative stability of high-oleate oils also meets industrial needs (17). Such oils are useful in cosmetic applications as they are established to be safe for consumption. They are useful as sources of

oleate, as they can reduce or eliminate the need for purification from other fatty acid components, which adds significant expense to the cost of obtaining pure oleate. As they remain liquid at room temperature and below, and have high-oxidative stability, they are useful in applications such as hydraulic fluids and oil-based insulators. Although problems are associated with using seed oil in these latter applications, the oil has the benefit of being biodegradable and nontoxic in case of a spill (17).

## 6. ALTERED POLYUNSATURATE CONTENT THROUGH MUTAGENESIS

Linseed oil from the flax plant (*Linum usitatissimum* L.) has a high content of  $\alpha$ -linolenic acid, an essential fatty acid for the human diet. Although present in many seed oils at levels from near 0% to 15%, it makes up 55% of the fatty acid content of linseed oil. It is this high content of the oxygen-sensitive linolenate that imparts excellent drying oil qualities to linseed oil, which makes it useful for production of coatings and compound materials such as linoleum. As a source of  $\omega$ -3 fatty acid, it provides an essential fatty acid for the human diet and thus can play an important role in many physiological processes. However, linseed oil is highly susceptible to air oxidation, turning rancid and developing objectionable odors on exposure to air. Because the meal from the flaxseed has been found to contain valuable nutritive components, such as the lignans, it is desirable to have both linseed oil and flax meal that are more stable to air oxidation. To this end, a research group led by Allan Green developed low linolenate strains of flax by mutagenesis of flaxseed followed by screening for low linolenate and high oil content (18). The resulting isolates are high in linoleic acid and less susceptible to rancidity than linseed oil. The normal composition of linseed oil is approximately 13% linoleic and 49% linolenic, whereas the oil derived from these plants, designated "Linola" or "solin," is up to 70% linoleic and 2% or less linolenic (19).

## 7. IMPROVED OIL COMPOSITION OF A TRANSGENIC SOYBEAN

Soybean oil linolenic acid content normally ranges from 5% to 12%, with most in the 8% range, which makes the oil susceptible to oxidation and spoilage. As a result, soy oil is often partially hydrogenated to stabilize the oil by reducing the linolenate content. However, the hydrogenation process introduces *trans*-fatty acids, which are considered undesirable dietary components. It has been shown that reducing the content of linolenic acid in soy oil would significantly stabilize the oil, which makes it useful for frying and other high-temperature cooking operations without the need for hydrogenation (20). A recent introduction, the Vistive soybean, has been bred to contain less than 2%  $\alpha$ -linolenic acid, thereby producing an oil that does not need to be hydrogenated for food use. Interestingly, the low linolenate trait was introduced by traditional breeding into a soybean line genetically engineered

to carry the gene for glyphosate resistance. It is thus a hybrid of traditional and transgenic technology. Vistive will be commercialized in 2005 (21).

## 8. IMPROVED INDUSTRIAL USE FROM GENETICALLY ENGINEERING OILSEED CROPS

### 8.1. Laurate Canola

The first commercial oilseed genetically modified for industrial use is laurate canola, altered to produce lauric acid (22). Laurate oils produce soaps and other surfactants because of the excellent foaming properties of the medium-chain fatty acid, and no temperate climate crop produces laurate. Because the price of laurate oils, derived from coconut and palm kernel, is subject to considerable fluctuation, it was thought that development of a stable, temperate climate crop that could produce laurate would provide a valuable renewable resource to meet a significant domestic need. Moreover, the higher temperature melting properties of laurate oils make them useful in baking and confections where a melting temperature similar to butter or cocoa is desired.

In the usual fatty acid biosynthetic pathway (Figure 1), the major product is oleate with varying amounts of palmitate and stearate produced, depending in part on the relative activities of the acyl-ACP (acyl carrier protein) thioesterases, stearoyl-ACP desaturase, and keto-acyl-ACP synthase II (KAS II). Certain plants that produce uncommon fatty acids have different enzyme(s) present that result in alterations of this pathway. Researchers at Calgene (Davis, CA) identified an enzyme in California Bay laurel seed, which produces oil containing approximately 60% laurate. The enzyme, a lauroyl-ACP thioesterase (FATB gene), specifically releases laurate during the course of fatty acid biosynthesis, which prevents the fatty acid chain from being further elongated and makes the laurate available for incorporation into the triacylglycerol fraction (22). By introducing the gene for this enzyme into Canola, and driving the expression of the gene with a promoter for napin, a seed storage protein that is highly expressed in *Brassica* sp., laurate-producing cultivars averaging 40%, and some up to 60% (on a mole basis), laurate were obtained (Table 1, reference 23). The triacylglycerols in the oil were acylated with laurate in the *sn*-1 and *sn*-3 positions. Because coconut oil contains laurate in all 3 *sn*- positions, the coconut lysophosphatidic acid acyltransferase (LPAAT) was purified and was shown to specifically incorporate laurate into the *sn*-2 position. The gene for the enzyme was cloned, expressed in laurate canola, and resulted in cultivars with a laurate content averaging over 50% (molar basis) (24). Despite the overwhelming scientific success of this approach, and the development of what is logically a valuable industrial crop, the commercialization of laurate canola has not yet been successful. The crop has reduced yields compared with normal canola (25), requires special handling to keep it separate from other canola, has added cost to recoup the research and development of the crop, and includes a premium paid to contracted growers. Given these cost items and the coinciding low price of

laurate oils from tropical sources, laurate canola could not achieve commercial success as a replacement for palm-kernel and coconut oils.

## 8.2. High-Oleate Soybean

High-oleate soybean oil, which contains over 80% oleic acid, was developed and commercialized by the DuPont Corporation (Delaware) (26). The presence of high levels of linoleate in a food oil is undesirable, as the presence of two methylene-interrupted double bonds in a fatty acid makes it more sensitive to oxidation than those high in oleate, which reduces its applicability in certain long-term uses. As linoleate is further desaturated to  $\alpha$ -linolenate in soy, this makes the oil even more sensitive to oxidation. Moreover, oleate is generally considered a more desirable fatty acid for dietary intake. Linoleate is derived from the action of the oleoyl-desaturase; thus, if the oleoyl desaturase activity could be suppressed in soybean, the oil composition should be primarily oleate, which is ideal for food use and some industrial uses mentioned above. Although antisense technology (in which a gene is introduced to be transcribed in the reverse, or antisense, direction) often blocks gene expression, the DuPont group used “gene suppression,” which results in stable reduction of gene expression when the gene is inserted in the sense (same) direction. The cultivars obtained produced oil containing up to 80% oleate, with concomitant reduction in the amount of linoleate, some reduction in the amount of linolenate, and little difference in levels of palmitate and stearate. However, the expense of the seed, the availability of other oilseed crops that can also produce high oleate, and the expense of identity preservation (IP) to keep the seeds separate from normal soybean have inhibited commercial success for the high-oleic transgenic soybean as well (27).

## 9. FUTURE DIRECTIONS FOR TRANSGENIC OILSEEDS

Research efforts are geared to developing oils that meet changing food, feed, and chemical feedstock needs. Hundreds of fatty acids are produced in plant sources, and hundreds more are produced in organisms from microbes to mammals. Some of these would be of great value if they were available in suitable amounts from a crop source. The two general transgenic approaches used to develop such sources are as follows:

- Transgenic crops genetically modified to produce the desired fatty acid
- Transgenic modification of a source plant to make it agronomically suitable

Research groups are pursuing one or both courses to enhance the value and uses of vegetable oil for food and to expand industrial crop production and develop renewable resources that can replace products derived from petroleum. Although none of these have been commercialized yet, the following examples present anticipated new oils.

## 10. POTENTIAL NEW OILS FOR FOOD, FEED, AND INDUSTRIAL USE

### 10.1. New Polyunsaturated Fatty Acid Components

In addition to the essential fatty acids linoleate and  $\alpha$ -linolenate, it is becoming clear that dietary intake of other polyunsaturated fatty acids has important benefits for proper development and health. Eicosapentaenoic acid ( $20:5\Delta^{5,8,11,14,17}$ ) (EPA) and docosahexaenoic acid ( $22:6\Delta^{4,7,10,13,16,19}$ ) (DHA) are known to play an important role in fetal neurological development (28), and they are also associated with reduction of chronic inflammatory diseases and improved psychological mood (29). These  $\omega$ -3 fatty acids are derived from fish oils in the human diet, with algae and phytoplankton providing the original source of the fatty acids for fish. Both EPA and DHA can be produced by humans, via successive elongation and desaturation of  $\alpha$ -linolenate. Linoleate is also subject to the same set of elongation reactions, which leads to production of arachidonic acid ( $20:4\Delta^{5,8,11,14}$ ) (AA). EPA and DHA lead to formation of the  $\omega$ -3 eicosanoids, which tend to be anti-inflammatory, whereas AA leads to the formation of  $\omega$ -6 eicosanoids, which tend to promote inflammation (29). The two types, thus, counterbalance each other. However, the modern diet tends to be richer in linoleate, so there is considerable interest in expanding the availability of EPA and DHA in the diet.

Numerous biosynthetic routes to EPA and DHA exist across the spectrum of organisms that synthesize them (29). One research group (30) has combined genes encoding enzymes from a marine microalgae (*Isochrysis galbana*), from *Euglena gracilis*, and from the oleaginous fungus *Mortierella alpina* to introduce the biosynthetic steps for EPA biosynthesis into *Arabidopsis thaliana*. The resulting triple-transformed plant produced 3% EPA in its leaf tissue and 6.6% arachidonic acid. This successful engineering feat can be followed up to provide seed oil containing AA, EPA, and DHA as a more concentrated product of these fatty acids (31).

An alternative route to EPA and DHA can come from elongation and further desaturation of stearidonic acid ( $18:4\Delta^{6,9,12,15}$ ). Certain plants, including blackberry, borage, and evening primrose, contain up to 25% of  $\gamma$ -linolenic acid ( $18:3\Delta^{6,9,12}$ ) in their seed oil, with considerably smaller amounts of stearidonic acid. The  $\gamma$ -linolenate arises from the action of a  $\Delta$ 6-desaturase on linoleate. Small amounts of  $\omega$ -3-desaturase present in these seeds account for the stearidonate produced. When  $\Delta$ 6-desaturase from borage was introduced into soy, plants producing up to 29%  $\gamma$ -linolenate (precursor to arachidonate), with up to 4% stearidonate in oil, resulted (32). Further desaturation to stearidonate could be promoted with high expression of an  $\omega$ -3-desaturase.

### 10.2. Oils Containing Hydroxy Fatty Acids for Industrial Use

Castor (*Ricinus communis*) is a model industrial crop. The seed is up to 60% oil, which is composed of 90% ricinoleic acid (12-hydroxy oleate), a fatty acid that produces literally hundreds of products, which include lithium grease, low VOC

coatings, plasticizers, Nylon 11, and cosmetics, among others. The laxative effect of the oil proscribes use of castor as a food crop, and it seems to be a monotypic genus. Thus, many concerns expressed for genetic modification of food crops do not apply to castor. However, the presence of a potent allergen and the toxic protein ricin in the seed complicate utilization of the seed meal remaining after oil extraction and prevent widespread cultivation of castor as a crop (33). Initial research efforts were aimed at producing a castor oil substitute in an alternative crop. The gene for the enzyme that made ricinoleate, the oleoyl-12-hydroxylase, was cloned (34) and expressed in plants including *Arabidopsis* and canola (35). However, these transgenic plants never made oil containing more than 20% hydroxy fatty acid. It became apparent that in addition to the oleoyl hydroxylase, other enzymes involved in the biosynthetic pathway for high ricinoleate oil may also have evolved with the pathway, and developed substrate specificities not present in alternative crop plants. This result seem to be the case for the diacylglycerol acyltransferase (DGAT), the terminal step in triacylglycerol biosynthesis. The enzyme from castor displays preference for substrates containing ricinoleate when compared with a DGAT from *Arabidopsis* (36). The biochemical elucidation of castor oil biosynthesis should eventually provide the molecular tools necessary to engineer synthesis of a high-ricinoleate oil in an agronomically suitable crop (37, 38).

As the toxin and allergen are both proteins and have previously been identified and cloned, it is possible to use transgenic technology to block their expression. This approach is being pursued, and has resulted in the development of a transformation system for castor, a plant that had proven to be recalcitrant to transformation and regeneration of intact plants (39).

### 10.3. Oils Containing Novel Monounsaturated Fatty Acids

The fatty acid petroselenic acid (octadeca-cis-6-enoate) is produced in *Umbelliferae* plants such as coriander, with levels approaching 90% in the oil. Unlike oils with high oleate, oils high in petroselenate are solid at room temperature and are a precursor of adipic acid for Nylon 6,6. Although first postulated as arising from a simple variant of the stearyl-ACP desaturase that produces oleate, its production in plants is more complicated. The biosynthesis of the fatty acid occurs by desaturation of palmitoyl-ACP at the C-4 position, followed by elongation to petroselenate (40). Although biochemically analogous to stearyl desaturation, the protein factors, such as ACP and ferredoxin, involved in the reaction appear specific for the petroselenate pathway. It is now thought that, in general, when a plant produces an unusual fatty acid, it may require an entire complement of additional genes to effectively produce the fatty acid (41, 42).

Numerous fatty acids have considerable commercial potential if they can be produced in suitable crops at a high level (42). Some of these are included in Table 2. With perhaps the exception of medium-chain fatty acids, there is not yet a major success in producing commercially useful levels of any uncommon fatty acid in a transgenic crop plant.



**TABLE 2. Industrially Useful Fatty Acids for Transgenic Plant Production.**

Fatty Acid (type)	Source	Use
Eleostearic (conjugated)	Tung, Bitter Melon	Drying oil
Octadeca-9c,11t,13t-trienoic		
Erucic (very long chain)	Rapeseed, Crambe	Lubricants, Anti-slip agent
Docosa -13c-enoic		
$\gamma$ -Linolenic	Borage, Blackberry	Nutraceutical
Octadeca-6c,9c,12c-trienoic		
Medium chain (saturated)	Cuphea, Coconut, Bay Laurel	Detergents
6 to 14 carbons		
Oleic	Many	Hydraulic oil, Oleochemicals
Octadeca-9c-enoic		
Petroselenic (isomer)	Coriander	Nylon 6,6
Octadeca-6c-enoic		
Ricinoleic (hydroxylated)	Castor	Lubricants, Polymers
Octadeca-9c,12-OH-enoic		
Vernolic (epoxy)	<i>Vernonia</i> , <i>Euphorbia lagascae</i>	Coatings, plasticizer
Octadeca-9c,12,13-O-enoic		
Very long chain	Algae	nutraceutical
polyunsaturated VLCPUFA		

## 11. ISSUES RELATED TO TRANSGENIC OILSEEDS

One inhibiting factor in commercial development of transgenic oilseeds with novel traits is public acceptance. The primary principle upon which approval has been based is known as “substantial equivalence,” which means that aside from any introduced changes, the composition of the plant or seed remains essentially unchanged. However, the concept of “unintended consequences” expands the scope of substantial equivalence, which establishes criteria that must be examined and met. Satisfying the concern for unintended consequences broadened the concept of substantial equivalence to include transcripts, the proteome, metabolome, and even genome sameness (43). In the approval process for a transgenic plant, these issues become a key part of the risk assessment both for food crops (44) and for industrial crops (45).

Most transgenic oilseeds with altered fatty acid composition remain research subjects, with commercial introduction limited to two crops, neither of which have yet achieved success in the marketplace. The expected benefits from transgenic crops with altered fatty acid composition include improved stability properties; enhanced nutritive value; expanded use of renewable resources to replace petroleum derived materials; replacement of chemical processes, such as epoxidation of fatty acid double bonds; and gradual expansion of agriculture as a chemical industry, a concept long ago known as “chemurgy.” It is possible to predict some issues that

will arise from commercialization and widespread planting of these crops. It is noteworthy that the major commercially successful transgenic crops are all oilseeds, and some understanding of issues and effects of transgenic oilseeds can be drawn from these crops.

The major transgenic crops grown in the United States and elsewhere are soybean, cotton, maize, and canola. Considerable effort has gone into and continues in optimizing these crops for food use. Industrial applications serve as a supplemental market for vegetable oils. The key to converting oilseeds to enhance food value or expand their use as an industrial feedstock lies in predictable alterations of biosynthetic pathways that will lead to production of the desired product. In the case of these four commercial crops, they have been engineered for the input traits, herbicide tolerance and insect resistance. Studies presenting long-range predictions on profitability of transgenic crops done in the 1980s, before any crops were near commercialization, indicated that sales of seeds for transgenic crops would be the major source of profit. Thus, several manufacturers of agricultural chemicals acquired seed companies and developed research programs that addressed financial and environmental concerns. Some seeds developed under these programs relied on application of a product from the company, thus providing a secure market for the seed and the agrochemical. At the same time, the transgenic crops required considerably less pesticide or herbicide applied, which provided benefit to the farmer via lower capital outlay, an increase in yield, and considerably lower amounts of agrochemicals applied to crops and released into the environment. The secure market for herbicides also provided an economic incentive for carrying out the mandated registration of agrochemicals for each crop.

Although the benefits of transgenic crops to consumers are somewhat abstract, as the level of agrochemical residues on crops is already very low, the benefits to farmers include higher profitability as a result of reduced chemical input and reduced toxic exposure. For example, farmers in some nations experienced a 75% reduction in exposure to the toxic effects of agrochemicals when growing transgenic cotton (46). Reductions in chemical exposure are clearly beneficial to the farmer, farm workers, and wildlife.

Although oilseed crops may be engineered for industrial use, areas of concern relate to the food supply. The introduction of allergens in the form of new proteins is a concern, highlighted by the Starlink episode (47). As yet, no human case of an allergic reaction related to Starlink has been identified. However, any food crop modified to produce a toxic, noxious, or bioactive compound can present a potential health hazard. These hazards would include oilseeds expressing ricinoleate, which is a laxative; vernolate, which might be an irritant; and other fatty acids with unwanted physiological effects. Such crops and components of the crop must be isolated from the food supply by using a sound IP system.

Any process that causes comingling or cross contamination of food and non-food crops is a concern. Cross-pollination with food crops is a particular concern, and several strategies for preventing it have been described (48). For example, crops producing oils for use in industry and containing a non-food fatty acid should not comeingle or cross with related food crops. Such crops would have to be grown

in limited areas and surrounded by a buffer crop to block cross-pollination (or low-probability revertants if male-sterile) (48, 49), a particular concern for canola, which must be buffered from HEAR when grown. An additional concern for nonsterile crops is seed drop during harvesting, which can result in germination and growth of the crop among the crop planted in the next growing season. Recently, some corn seed grown to produce a pharmaceutical protein in one growing season was left in a field and germinated among a crop of soybeans. As a result, the soybeans had to be recovered and destroyed. The end result of this incident was increased regulatory oversight of non-food and industrial transgenic crops to prevent such incidents in the future.

Industrial oilseed crops are beneficial because they are renewable resources, yield biodegradable products, and are environmentally benign. To the extent they supplant products derived from petroleum, they are clearly “green” alternatives to synthetic chemicals and inherently benefit human health by reducing exposure to atmospheric and aqueous emissions from petroleum processing plants. As much controversy about genetic modification is concerned with the safety of food derived from genetically modified plants, genetic modification of industrial crops should be relatively free of controversy. This situation is not the case, for several reasons. Many byproducts of industrial crop processing enter the food supply. For example, after oilseeds, such as industrial rapeseed, have been processed to remove the oil, the remaining meal is protein rich and processed for use as animal feed. In some cases, the meal may produce foods for human consumption; for example, flax meal from linseed oil processing provides nutritional benefit in the form of lignans and omega-3 oil residue (50). Additionally, many crops have dual uses. Soybean is primarily a food crop, but soybean oil and soybean protein are also used for non-food applications, such as inks, coatings, and adhesives. The Starlink maize incident has made it clear that approving a food crop for strictly non-human use (animal feed) is not sufficient to prevent it from entering the human food supply. No genuinely harmful consequences of the Starlink corn to human health were found, only to positive perception of the transgenic crop industry (47). The case described above involving comingling of transgenic corn carrying a therapeutic protein underlines the need for a sound (IP) system and appropriate quarantine (both space and time), if food crops are to produce potentially noxious products, and if food crops are to be planted at a later date in the same field. In the case of Starlink, with animal feed being an inherently cheaper end-use than human food, no economic motivation existed to maintain it separately from other maize. In the case of the transgenic corn, the product would be much higher value than any food use but extremely difficult to prevent some seed from remaining in the harvested field.

For transgenic crop approval in the United States, the action and approval of three Federal agencies is required. The Food and Drug Administration evaluates the crop for direct and indirect food use, the Environmental Protection Agency registers the crop for potential environmental effects, and the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) evaluates genetically modified crops for their potential to disrupt the growth habit of domestic plants. Canada and Australia have similar oversight, and it is likely that this

multipartite regulatory process has engendered consumer confidence in the safety of transgenic crops (44).

IP is the means by which a crop is maintained separately during storage, shipping, and processing, and it has been in practice for several specialty crops that have added value, for example, low-saturated soybean. Insofar as comingling a transgenic industrial crop with other crops could result in health problems, it is essential to segregate transgenic crops from other crops, in the field and postharvest. The concept of IP and crop isolation is also important for market perception. Currently, those persons opposed to eating food from transgenic crops require IP of the nontransgenic crops. The estimated cost of IP is in the range of 5–10% of the crop's value (45). If a given transgenic crop delivers higher value to the end user, at least an economic incentive exists to keep it from contaminating a lower value crop.

### 11.1. Risk Assessment

The genetic engineering of oilseed crops to make them better suited for food and industrial purposes necessitates an evaluation of their potential for hazard. The primary consideration is currently whether the modification to the crop alters it unpredictably; i.e., does the introduced gene maintain the crop as substantially equivalent, or are unintended consequences associated with the introduced gene? In every case, the designed crop, by definition, will produce the desired product. Based on existing toxicological data, it is likely that either the chemical product or related compounds will provide the means to develop a toxicological profile and determine any potential harm arising from production. A procedure based on close comparison of a nontransgenic control with the transgenic has been described (51). A novel product in the plant will require an independent toxicological assessment. The crop residue remaining after extraction of the product will also need to be evaluated for the remaining product, as well as changes in the crop residue that result from the alterations required to make the product. Changes in metabolism brought about to enhance production of a single product can be predicted based on knowledge of biosynthetic pathways affected by the alteration, but a broad-based approach is required to identify unintended changes (51). Regulatory agencies require a demonstration of substantial equivalence depending on the intended uses of the product or the crop residue remaining (47). Finally, migration of the transgene(s) into other crops must be evaluated from the standpoint of potential for harm and the likelihood that it will actually occur. Knowledge of agronomic habit allows assessment of the latter, and toxicological analysis provides the needed information for the former. Any potentially toxic or noxious product can be restricted to sterile strains. Although pollen release from transgenic oilseeds, such as the *Brassica* sp., is a common scenario for concern, a more significant problem may arise from comingling as a result of seed drop during harvest. It is inevitable that crops that are not controlled to prevent gene release will ultimately not be permitted (52). Transgenic technology holds out great promise for expanding the use of renewable resources in production of industrial products.

Accordingly, it is essential that such a benefit be implemented in a way to prevent any harmful effects.

## 11.2. Allergenicity

It is expected that beyond the different product in an “engineered” transgenic oilseed, the crop will retain substantial equivalence to the nontransgenic crop. The genes for given characteristics have been cloned and sequenced; perhaps quantities of the protein have been isolated after being expressed in bacteria or yeast. In many instances, the activity of the native protein has only been demonstrated by the change brought about *in planta*. Therefore, its potential for becoming a problem allergen remains unknown. In the case of oils, where the primary product is free of the transgenic crop protein, the allergenicity of the protein is not a food health issue. However, because most oilseed meals are used as food or animal feed, then protein allergenicity clearly becomes a consideration. In plants that have undergone “metabolic engineering,” the introduced gene(s) is (are) often overexpressed to redirect the flow of metabolites to the desired product, which leads to a relatively high level in the plant (23). Altered timing of expression may also be implemented. Promoter technology is still in a relatively primitive state. In oilseeds, it is common to use promoters that drive the synthesis of storage proteins and to restrict expression of the introduced gene to the seed (23). As storage protein promoters are geared to produce a high level of protein, high levels of the expressed protein can accumulate, and as storage proteins are expressed late into seed development, proteins produced to alter metabolism of the immature seed may persist to a high degree in the harvested seed, whereas they would not normally be present. Methods for demonstrating potential allergenicity exist, for example, model pepsin digestion reactions (47, 51) and a “decision-tree” for assessing allergenicity have been described, with linear epitope analysis and partial sequence identity to allergens as indicators (51). Animal models for allergenicity have also been proposed to supplement the decision-tree. In cases where the protein is not available in isolation, theoretical models to predict allergenicity from the protein sequence are essential to ensure the safety of associated byproducts, such as seed meal.

## 11.3. Pollen Transfer

Ecological concern exists about transmission of pollen from some types of plant, such as the *Brassicac*s and tree crops, either into weedy relatives or into crops grown at some distance. This problem is not limited to transgenic plants. Canola, a rapeseed cultivar bred to produce low glucosinolates and low erucic acid, must be planted in isolation from industrial rapeseed, as each crop will result in seed with altered composition from the ideal: that is, excessive erucic acid in the canola and less erucic acid in the industrial rapeseed. Because the products of industrial crops are not intended for consumption, and may even be noxious, risk management and containment, including the prevention of intercrop cross-pollination, is

essential. The approaches described (53) can address the problem of out-crossing from transgenic crops.

#### 11.4. Economics

A major argument for promoting transgenic technology has been the need to provide more food. Some industrial applications use surplus products, for example, soybean oil, and provide a buffer against surplus crops, to prevent a decline in farm profitability. However, if industrial transgenic farming expands, it is not clear to what extent agriculture may be diverted away from food production, which will result in increased food costs, if industrial crops are grown in higher volume and have a higher value than food crops.

If the success of transgenic industrial oilseeds is to be measured on the basis of their commercial success (see (54) for an economic analysis of genetically modified industrial crop profitability), then the success of such crops can affect the prosperity of the industries they replace, such as chemical manufacturing. Although the overall benefit will be great, as renewable resources replace potentially limited resources, industries and workers may be displaced.

Several developing nations produce specialty crops that meet current industrial needs, such as castor oil, rubber, and lauric acid, all of which are products from Southeast Asia. If these products were to be replaced by transgenic crops grown in temperate regions, economic displacement of the less wealthy countries could occur. However, because transgenic products may also be produced more cheaply in these countries, they should ultimately benefit from the same new technologies. Where new uses, such as biodiesel fuel and fuel additives produced from castor oil and laurate (45), will greatly expand demand, economic disruptions may be offset. Many countries already have research programs in transgenic crop technology. The impact of transgenic technologies in industrial agriculture on the world economy remains to be seen.

Transgenic technology remains a powerful tool for developing a broad range of useful food and industrial oils. To date, attempts to use this technology have been limited to crop input traits, but in the long term, novel crops with altered output traits will fill important niches in the food supply and will help to shift the petroleum economy to renewable resources.

#### REFERENCES

1. C. James. Global Status of Commercialized Transgenic Crops: 2003 Executive Summary International Service for the Acquisition of Agri-Biotech Applications. (2004). Available: <http://www.isaaa.org>.
2. D. L. Nida, S. Patzer, P. Harvey, R. Stipanovic, R. Wood and R. L. Fuchs, *J. Agric. Food Chem.*, **44**, 1967–1974 (1996).
3. R. S. Sidhu, B. G. Hammond, R. L. Fuchs, J. N. Mutz, L. R. Holden, B. George, and T. Olson, *J. Agric. Food Chem.*, **48**, 2305–2312 (2000).

4. S. R. Padgett, N. B. Taylor, D. L. Nida, M. R. Bailey, J. MacDonald, L. R. Holden, and R. L. Fuchs, *J. Nutr.*, **126**, 702–716 (1995).
5. W. E. Neff, T. L. Mounts, and W. M. Rinsch, *Lebensrm. Wiss. u.-Technol.*, **30**, 793–799 (1997).
6. P. F. Knowles, in G. Robbelen, R. K. Downey and A. Ashri, eds., *Oil Crops of the World*, McGraw-Hill, New York, 1985, pp. 260–282.
7. S. Henikoff and L. Comai, *Annu Rev. Plant Biol.*, **54**, 375–401 (2003).
8. J. L. Harwood, *Biochim. Biophys. Acta*, **1301**, 7–56 (1996).
9. T. Voelker and A. J. Kinney, *Annu. Rev. Plant Physiol. Mol. Biol.*, **52**, 335–3361 (2001).
10. J. Browse and C. Somerville, *Annu. Plant Physiol. Plant Mol. Biol.*, **42**, 467–506 (1991).
11. J. J. Thelen and J. B. Ohlrogge, *Metabol. Eng.*, **4**, 12–21 (2002).
12. J. Jaworski and E. B. Cahoon, *Curr. Opin. Plant Biol.*, **6**, 178–184 (2003).
13. R. K. Downey, *Can. J. Plant Sci.*, **44**, 499–504 (1964).
14. B. R. Stefansson, F. W. Hougen, and R. K. Downey, *Can. J. Plant Sci.*, **41**, 218–219 (1961).
15. L. Velasco and J. M. Fernandez-Martinez, *J. Crop Prod.*, **5**, 309–344 (2002).
16. S. Lacombe and A. Berville, *Mol. Breeding*, **8**, 129–137 (2001).
17. S. Erhan and S. Asadauskas, *Ind. Crops Prod.*, **11**, 277–282 (2000).
18. A. G. Green, *Can. J. Plant Sci.*, **66**, 499–503 (1986).
19. A. Green, *Aus. New Crops Newsl.* 3(1). (1995). Available: <http://www.newcrops.uq.edu.au/newslett/ncnl3-92.htm>.
20. T. L. Mounts, K. Warner, G. R. List, W. E. Neff and R. F. Wilson, *JAOCs*, **71**, 495–499 (1994).
21. Monsanto Press Release. (2004). Available: <http://www.monsanto.com/monsanto/layout/media/04/09-01-04.asp>.
22. T. A. Voelker, A. C. Worrell, L. Anderson, J. Bleibaum, C. Fan, D. J. Hawkins, S. E. Radke, and H. M. Davies, *Science*, **257**, 72–74 (1992).
23. T. A. Voelker, T. R. Hayes, A. M. Cranmer, J. C. Turner, and H. M. Davies, *Plant J.*, **9**, 229–241 (1996).
24. D. S. Knutzon, T. R. Hayes, A. Wyrick, H. Xiong, H. M. Davies, and T. A. Voelker, *Plant Physiol.*, **120**, 739–746 (1999).
25. A. E. McCoy, M. Pippin, and R. D. MacNeill IV. Laurate Canola Variety Trial. (1998). Available: <http://www.griffin.peachnet.edu/swvt/1998/rp981rat.htm>.
26. A. J. Kinney, in J. P. Williams, M. U. Khan and N. W. Lem, eds., *Physiology, Biochemistry and Molecular Biology of Plant Lipids*, Kluwer Academic, Dordrecht, the Netherlands, 1997, pp. 298–300.
27. R. Frerichs. (2003). Available: <http://web.aces.uiuc.edu/value/factsheets/soy/fact-oleic-soy.htm>.
28. A. Rodriguez, D. Raederstorff, P. Sarda, C. Lauret, F. Mendy, and B. Descomps, *Eur. J. Clin. Nutr.*, **57**, 727–734 (2003).
29. O. V. Sayanova and J. A. Napier, *Phytochemistry*, **65**, 147–158 (2004).
30. B. Qi, T. Fraser, S. Mugford, G. Dobson, O. Sayanova, J. Butler, J. A. Napier, A. K. Stobart, and C. M. Lazarus, *Nature Biotech.*, **22**, 739–745 (2004).
31. A. G. Green, *Nature Biotech.*, **22**, 680–682 (2004).

32. S. Sato, A. Xing, X. Ye, B. Schweiger, A. Kinney, G. Graef, and T. Clemente, *Crop Sci.*, **44**, 646–652 (2004).
33. D. L. Auld, R. D. Rolfe, and T. A. McKeon, *J. New Seeds*, **3**, 61–69 (2001).
34. F. J. van de Loo, P. Broun, S. Turner, and C. Somerville, *Proc. Nat. Acad. Sci. (USA)*, **92**, 6743–6747 (1995).
35. P. Broun and C. Somerville, *Plant Physiol.*, **113**, 933–942 (1997).
36. X. He, C. Turner, G. Q. Chen, J. T. Lin, and McKeon, *Lipids*, **39**, 311–318 (2004).
37. T. A. McKeon and J. T. Lin, in T. M. Kuo and H. W. Gardner, eds., *Lipid Biotechnology*, Marcel Dekker, New York, 2001, pp. 129–139.
38. M. Smith, H. Moon, G. Chowrira, and L. Kunst, *Planta*, **217**, 507–516 (2003).
39. T. A. McKeon and G. Q. Chen, U. S. Patent 6,620,986 B1, 2003.
40. E. B. Cahoon and J. B. Ohlrogge, *Plant Physiol.*, **104**, 827–837 (1994).
41. M. C. Suh, D. J. Schultz, and J. B. Ohlrogge, *Planta*, **215**, 584–595 (2002).
42. H. Drexler, P. Spiekermann, A. Meyer, F. Domergue, T. Zank, P. Sperling, A. Abbadi, and E. Heinz, *J. Plant Physiol.*, **160**, 779–802 (2003).
43. F. Cellini, A. Chesson, I. Colquhoun, A. Constable, H. V. Davies, K. H. Engel, A. M. R. Gatehouse, S. Karenlampi, E. J. Kok, J.-J. Leguay, S. Lehesranta, H. P. J. M. Noteborn, J. Pedersen, and M. Smith, *Food Chem. Toxicol.*, **42**, 1089–1125 (2004).
44. J. Thomson, *Trends Food Sci. Technol.*, **14**, 210–228 (2003).
45. T. A. McKeon, *Trends Food Sci. Technol.*, **14**, 229–241 (2003).
46. J. Huang, S. Rozelle, C. Pray, and Q. Wang, *Science*, **295**, 674–677 (2002).
47. H. A. Kuiper, G. A. Kleter, H. P. J. M. Noteborn, and E. J. Kok, *Plant J.*, **27**, 503–528 (2001).
48. H. Daniell, S. J. Streatfield, and K. Wycoff, *Trends Plant Sci.*, **6**, 219–226 (2001).
49. P. J. Dale, B. Clarke, and E. M. G. Fonte, *Nature Biotech.*, **20**, 567–574 (2002).
50. B. D. Oomah, *J. Sci. Food Agric.*, **81**, 883–888 (2001).
51. A. Konig, A. Cockburn, R. W. R. Crevel, E. Debruyne, R. Grafstroem, U. Hammerling, I. Kimber, I. Knudsen, H. A. Kuiper, A. A. C. M. Peijnenburg, A. H. Penninks, M. Poulsen, M. Schauzu, and J. M. Wal, *Food Chem. Toxicol.*, **42**, 1047–1088 (2004).
52. S. Smyth, G. G. Khachatourians, and P. W. Phillips, *Nature Biotech.*, **20**, 537–541 (2002).
53. G. van den Eede, H. Aarts, H.-J. Buhk, G. Corthier, H. J. Flint, W. Hammes, B. Jacobsen, T. Midtvedt, J. van der Vossen, A. von Wright, W. Wackernagel, and A. Wilcks, *Food Chem. Toxicol.*, **42**, 1127–1156 (2004).
54. B. Hitz, *Curr. Opin. Plant Biol.*, **2**, 135–138 (1999).



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# *Tree Nut Oils*

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## **1. INTRODUCTION**

Tree nut oils are primarily composed of triacylglycerols, but also contain diacylglycerols, monoacylglycerols, free fatty acids, and other minor components, including natural antioxidants and fat-soluble vitamins. The chemical composition of edible fats and oils largely determines their stability, quality, nutritional value, sensory properties, and potential health effects. Tree nuts, in many cases, provide rich sources of food lipids; up to 75% lipid on a weight basis (1). With a few exceptions, tree nut lipids exist as oils at room temperature. Generally, tree nuts are rich in monounsaturated fatty acids, predominantly oleic acid, but contain much lower amounts of polyunsaturated fatty acids, predominantly linoleic acid and small amounts of saturated lipids (1). In many parts of the world, such as the Middle East and Asia, tree nuts are cultivated for use as oil crops and are important sources of energy and essential dietary nutrients as well as phytochemicals (2). Tree nut oils are also used as components of some skin moisturizers and cosmetic products (3).

Tree nuts, tree nut oils, and tree nut byproducts (defatted meals and hulls) are known to contain several bioactive and health-promoting components. Epidemiological evidence indicates that the consumption of tree nuts may exert several cardioprotective effects, which are speculated to derive from their lipid component that includes unsaturated fatty acids, phytosterols, and tocopherols (4). Recent investigations

have also shown that dietary consumption of tree nut oils may exert even more beneficial effects than consumption of whole tree nuts, possibly due to the replacement of dietary carbohydrate with unsaturated lipids or other components present in the oil extracts (5). Tree nut byproducts are used as sources of dietary protein and as health-promoting phytochemicals such as natural antioxidants. This chapter summarizes the characteristics and potential health effects of several tree nut oils and their byproducts, including almond oil, hazelnut oil, pecan oil, walnut oil, pistachio oil, Brazil nut oil, pine nut oil, and macadamia nut oil, among others. Protein compositions of tree nut byproducts are also discussed collectively at the end of this chapter, with emphasis on the completeness of these proteins based on their amino acid compositions.

## 2. ALMOND

The almond tree (*Prunus delcis* and *Prunus amara*) and its fruit (containing the almond kernel or “almond”) have long been recognized as commercially valuable and nutritionally important. California and Italy are the major almond-producing regions of the world, however, other parts of Europe, Asia, and Australia also contribute to a lower level of production (6). The only other economically important product of almond trees is the almond hull, which is traditionally used in animal feed preparations. Several studies have reported that almond consumption may improve blood lipid profiles by lowering low-density lipoprotein (LDL) cholesterol and raising plasma high-density lipoprotein (HDL) cholesterol levels. Thus, there is much current interest in almond oil as a health-promoting edible oil (7).

The proximate composition of almond includes 50.6% lipid, 21.3% protein, 19.7% carbohydrate, 5.3% water, and 3.1% ash (w/w) (1). The most common method for producing almond oil is hexane extraction that affords high oil yields, however, cold pressing is another commercially used procedure for almond oil production (8). Shi et al. (8) assessed the fatty acid composition of almond oil; oleic acid was major fatty acid present (68%), followed by linoleic acid (25%), palmitic acid (4.7%), and small amounts (<2.3%) of palmitoleic, stearic, and arachidic acids (Table 1). Almond oil is also a rich source of  $\alpha$ -tocopherol (around 390 mg/kg) and contains trace amounts of other tocopherol isomers as well as phyloquinone (70  $\mu$ g/kg) (1). Almond oil contains 2.6 g/kg phytosterols, mainly  $\beta$ -sitosterol, with trace amounts of stigmasterol and campesterol (1).

Sattar et al. (9) examined peroxide formation during light-induced oxidation of several tree nut oils, including almond oil, pine nut oil, and walnut oil. The oils were oxidized for a 7-week period under four different conditions: (1) by direct exposure to light (540 lux), (2) exposure to light in clear glass containers, (3) exposure to light in amber-colored glass containers, and (4) unexposed oils, which were used as controls. The initial peroxide value (PV) of the almond oil was 2.8 meq oxygen/kg oil, which was second only to pine nut oil (9). Results for almond oil peroxidation rate under each condition were expressed as increase in PV/day ( $\Delta$ PV/day); the oxidation rate was highest in almond oil directly exposed to light

**TABLE 1. Fatty Acid Composition of Almond Oil.<sup>a</sup>**

Fatty Acid	(%)
16:0	4.7
16:1	<1
18:0	<1
18:1 (n-9)	68.0
18:2 (n-6)	25.0
18:3 (n-3)	<1
24:0	<1

<sup>a</sup>Adapted from Shi et al. (8).

(0.82  $\Delta$ PV/day), followed by almond oil stored in glass containers (0.43  $\Delta$ PV/day), then almond oil stored in amber-colored containers (0.15  $\Delta$ PV/day), and lowest in unexposed almond oil (0.11  $\Delta$ PV/day). Under all four oxidative conditions, almond oils showed greater oxidative stability than pine nut oil and walnut oil, possibly due to a higher content of tocopherols in almond oil (9). Salvo et al. (10) studied the peroxidation rate and compositional changes of almond oil over a 3-year period at 4°C and ambient temperature. The almond oils used were extracted from sweet (*P. delcis*) and bitter almonds (*P. amara*). The initial composition of the two almond oils were similar, having identical fatty acid compositions and total tocopherol contents; however, sweet almond oil contained only  $\alpha$ -tocopherol (458 mg/kg), whereas bitter almond oil contained 345 mg/kg  $\alpha$ -tocopherol and 113 mg/kg  $\gamma$ -tocopherol. No changes in fatty acid composition were observed during the 3-year storage period; however, the total tocopherol content fell to 245 mg/kg in sweet almond oil and 121 mg/kg in bitter almond oil after one year, and became totally depleted after three years when stored at 4°C (10). Both sweet and bitter almond oils showed similar peroxide formation rates; the initial PV was 9.6 meq oxygen/kg oil and rose to 21.3 after 1 year, 29.6 after 2 years, and 129.5 after 3 years of storage at 4°C. A similar, but faster, trend was observed in almond oils stored at ambient temperatures. Thus, oxidative stability of almond oil (9, 10) depended on the presence of tocopherols and possibly other substances contributing to the stabilization of the oil.

The compositional characteristics of almond oil show that it is rich in several health-promoting nutrients, many of which may be responsible for the observed beneficial effects of dietary almond consumption in cardiovascular diseases (11) and in weight management (12), however, few investigations have explored this topic. Hyson et al. (13) conducted a dietary intervention study to determine whether the consumption of whole almonds or almond oil for 6 weeks would result in similar or different effects on plasma lipids and ex vivo LDL oxidation. Both groups consumed diets with identical almond oil and total fat levels. This study showed that both whole almond and almond oil consumption caused similar reductions in plasma cholesterol and LDL (4% and 6%, respectively) as well as a 14%

**TABLE 2. Phenolic Acid Constituents ( $\mu\text{g/g}$ ) of Selected Tree Nut Meals.<sup>a</sup>**

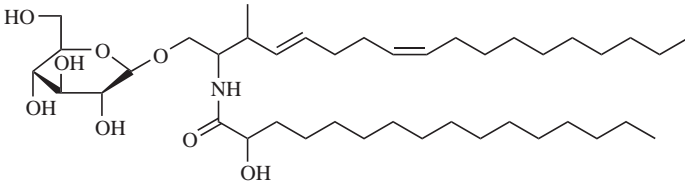
Phenolic Acid	Almond	Hazelnut	Chestnut <sup>b</sup>	Pine Nut	Walnut
<i>p</i> -Hydroxybenzoic	0.30	<0.01	0–0.44	0.16	0.06
Phenyl acetic	0	0	<0.01	<0.01	0–0.02
Vanillic	0.07	<0.01	0	0.14	0.09
Proto-catechuric	0.70	0.36	0.3–0.46	0.28	0.02
Syringic	0	0	0–0.06	0.23	0.02
Gallic	<0.01	<0.01	0.7–4.21	0.08	0.02
Caffeic	0	<0.01	0.16–0.30	0.48	0.10
Ferulic	0	0	0–0.01	<0.01	<0.01
Total	1.08	0.36	1.65–4.97	1.37	0.51

<sup>a</sup>Adapted from Senter et al. (14).

<sup>b</sup>Range of values accounts for three chestnut varieties (American, Chinese, and Hybrid chestnut).

decrease in fasting plasma triacylglycerols. These findings indicate that the lipid component of almond is responsible for its cardioprotective effects and warrants further investigation (13).

The defatted meals and hulls of almonds contain several antioxidative compounds as well as other health-promoting substances. Senter et al. (14) performed a comparative analysis of phenolic acids in selected tree nut meals including almond. The results of this study showed that gallic acid was the predominant phenolic compound in all tree nut meals except pine nut (caffeic acid), almond, and hazelnut (protocatechuric acid). Other phenolic compounds identified included *p*-hydroxybenzoic, *p*-hydroxyphenylacetic, vanillic, syringic, and ferulic acids (Table 2) (14). The antiradical activity of ethanolic extracts of almond and almond byproducts, including brown skins and hulls, have been reported (15). The Trolox equivalent antioxidant activity of brown skins and hulls were 13 and 10 times greater than that of the whole almond extracts. At a concentration of 200 ppm, ethanolic extracts of almond skins and hulls had strong scavenging activities against superoxide radical (95% and 99%, respectively), hydrogen peroxide (91%), hydroxyl radical (100% and 56%, respectively), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (100%) (15). Sang et al. (16) isolated nine phenolic compounds from almond skins and assessed DPPH scavenging activity of each compound; catechin and protocatechuic acid had the greatest antioxidant activity, followed by 3'-*O*-methylquercetin 3-*O*- $\beta$ -*D*-galactopyranoside, then 3'-*O*-methylquercetin 3-*O*- $\beta$ -*D*-glucopyranoside, and 3'-*O*-methylquercetin 3-*O*- $\alpha$ -*L*-rhamnopyranosyl-(1  $\rightarrow$  6)- $\beta$ -*D*-glucopyranoside as well as vanillic and *p*-hydroxybenzoic acids, naringenin 7-*O*- $\beta$ -*D*-glucopyranoside, and, finally, kaempferol 3-*O*- $\alpha$ -*L*-rhamnopyranosyl-(1  $\rightarrow$  6)- $\beta$ -*D*-glucopyranoside (16). Frison-Norrie and Sporns (17) quantitatively assessed the flavonol glycoside composition of blanched almond skins using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), showing the presence of isorhamnetin rutinoside (51  $\mu\text{g/g}$ ), isorhamnetin glucoside (18  $\mu\text{g/g}$ ), kaempferol rutinoside (18  $\mu\text{g/g}$ ), and kaempferol glucoside (6  $\mu\text{g/g}$ ). More recently, Pinelo et al. (18) examined the total phenolics content and DPPH scavenging



**Figure 1.** 1-O- $\beta$ -D-Glucopyranosyl-(2S,3R,4E,8Z)-2-[(2R)-2-hydroxyhexadecanoylamino]-4,8-octadecadiene-1,3-diol.

activity of almond hull ethanolic extracts and reported values of 3.74 mg/g and 58%, respectively. Sang et al. (19) have also isolated potential health-promoting sterols, nucleotides, and one sphingolipid, namely 1-O- $\beta$ -D-glucopyranosyl-(2S,3R,4E,8Z)-2-[(2R)-2-hydroxyhexadecanoylamino]-4,8-octadecadiene-1,3-diol (Figure 1), from defatted almond meals. In light of this data showing that tree nuts, tree nut oils, and tree nut byproducts contain health-promoting phytochemicals, Davis and Iwashii (20) examined the effects of dietary consumption of whole almonds, almond oil, and almond meal on aberrant crypt foci development in a rat model of colon carcinogenesis. This landmark study showed that both almond oil and almond meal reduced aberrant crypt foci development, but whole almonds showed a significantly stronger anticancer effect in this model, implying a synergistic anticancer activity between the lipidic and nonlipidic constituents of almonds (20).

### 3. HAZELNUT

Hazelnuts or filberts (*Corylus sp.*) are a rich source of energy with a 61–63% lipid content (w/w) (1, 21). Other components of hazelnuts are carbohydrate (15.3%), protein (13.0%), water (5.4%), and ash (3.6%) (1). Turkey is the world's largest producer of hazelnuts, accounting for approximately 75% of total hazelnut production, followed by Italy, which accounts for 10% of total global production. In the United States, the state of Oregon is the largest producer and in Canada, southwestern British Columbia produces a small amount of hazelnuts. North America contributes less than 5% to the total world hazelnut production, which is about 850,000 metric tons (unshelled basis) (22).

The fatty acid composition of hazelnut oil is as follows: 78–83% oleic acid, 9–10% linoleic acid, 4–5% palmitic acid, and 2–3% stearic acid as well as other minor fatty acids (Table 3) (1, 22). Parcerisa et al. (23) examined lipid class composition of hazelnut oil, showing that triacylglycerols constituted 98.4% of total lipids, glycolipids comprised 1.4% of total lipids, and trace amounts (<0.2%) of phosphatidylcholine and phosphatidylinositol were also present. Hazelnut oil contains 1.2–1.14 g/kg of phytosterols primarily in the form of  $\beta$ -sitosterol and is a very good source of  $\alpha$ -tocopherol (382–472 mg/kg) (1, 22). The main odorant in

**TABLE 3. Fatty Acid Composition of Hazelnut Oil.<sup>a</sup>**

Fatty Acid	(%)
14:0	0.03
15:0	0.02
16:0	4.85
16:1	0.16
17:0	0.04
17:1	0.07
18:0	2.73
18:1 (n-3)	82.7
18:2 (n-6)	8.89
18:3 (n-3)	0.10
20:0	0.14
20:1 (n-9)	0.16
24:0	0.01
24:1 (n-9)	0.02

<sup>a</sup>Adapted from Alasavar et al. (22).

hazelnut oil responsible for its characteristic flavor is 5-methyl- (E)-2-hepten-4-one or filbertone, which can produce intense hazelnut oil-like aroma at the very low odor threshold of 5-ng/kg oil (24). The oil from unroasted hazelnuts typically contains about 6- $\mu$ g filbertone/kg oil, whereas the oil from roasted hazelnuts contains over 315- $\mu$ g filbertone/kg oil (24). The level of filbertone in hazelnut oil serves as a useful index for assessing possible adulteration of other oils with hazelnut oil and the extent of adulteration (25). Bernardo-Gil et al. (26) studied the composition and oxidative characteristics of hazelnut oils obtained by hexane and supercritical carbon dioxide fluid extraction. Under optimal supercritical fluid extraction conditions (CO<sub>2</sub> density: 880 kg/m<sup>3</sup>, superficial velocity: 0.000642 m/s, time: 240 min, temperature range: 308–321 K, pressure range: 18–23.4 MPa), the total yield and fatty acid compositions of the resultant hazelnut oils did not differ significantly for the two extraction methods; however, the supercritical fluid extract was clearer than the hexane extract, implying some degree of purification by the supercritical fluid (SCF) extraction process (26). Interestingly, the SCF extract contained 17% more tocopherols (458.7 mg/kg) than the hexane extract (382.8 mg/kg). The oxidative stability of both hazelnut oils were assessed using the Rancimat apparatus at 110°C; the SCF extract was more resistant to oxidation (induction periods: 6.7 h and 8.7 h, respectively) (26). Ozdemir et al. (27) studied the oxidative stability of several commercial and experimental hazelnut oils using the stability index calculation ( $[\text{mg}/100\text{-g } \alpha\text{-tocopherol}] \times [\% \text{ saturated fatty acids}/\% \text{ unsaturated fatty acid}]$ ), showing stability index values between 5.4 and 6.3. Similar findings have been reported by other research groups (22, 28, 29). The oxidative stabilities of both stripped and nonstripped hazelnut oil in oil-in-water emulsion and bulk-oil systems have been reported (28). In these systems, oxygen uptake, peroxide value, 2-thiobarbituric acid reactive substances (TBARS), and depletion of  $\alpha$ -tocopherol

levels during a 21-day oxidation cycle at 60°C were assessed. Nonstripped oils were more stable than stripped oils, and both stripped and nonstripped oils were more stable as bulk oils than as oil-in-water emulsions (28). More recently, Romero et al. (29) studied the oxidative stability of stripped and nonstripped hazelnut oils using the Rancimat method at 100°C; the effects of antioxidants in these systems were also evaluated. These researchers studied lipid oxidation in five different hazelnut oil systems: (1) nonstripped cold-pressed hazelnut oil, (2) stripped cold-pressed hazelnut oil, (3) stripped cold-pressed hazelnut oil with 150-mg/kg  $\alpha$ -tocopherol added, (4) stripped cold-pressed hazelnut oil with 140-mg/kg  $\alpha$ -tocotrienol added, and (5) stripped cold-pressed hazelnut oil with 70 mg/kg of  $\alpha$ -tocopherol and 70-mg/kg  $\alpha$ -tocotrienol added (29). The Rancimat studies showed that  $\alpha$ -tocotrienol prolonged the induction period to the greatest extent in the stripped oil system (37.6 h), followed by the mixture of  $\alpha$ -tocopherol and  $\alpha$ -tocotrienol (35.3 h), and finally  $\alpha$ -tocopherol (32.6 h). The induction period in the nonstripped hazelnut oil system (30.8 h) was less than all stripped oils with added antioxidants, and better than the plain stripped oil system (3.5 h). These results collectively show that  $\alpha$ -tocotrienol exhibits a greater antioxidant activity than  $\alpha$ -tocopherol in this system; both  $\alpha$ -tocotrienol and  $\alpha$ -tocopherol exhibited greater antioxidant activities than the minor constituents present naturally in the nonstripped hazelnut oil (29). This research group also assessed the stability of the hazelnut oil systems at 180°C by measuring the formation of polar components and decomposition of tocopherols in all four antioxidant-containing oil systems over an 18 h period; under these extreme conditions  $\alpha$ -tocopherol was the most effective antioxidant (29).

Several reports have shown that hazelnut is a health-promoting food and a contributing factor for the beneficial health effects of the Mediterranean style diet (30); however, few studies have investigated the health effects of hazelnut oil. Balkan et al. (31) examined the effects of hazelnut oil administration on plasma peroxide levels, plasma lipid profiles, plasma LDL and VLDL levels, and atherosclerotic plaque development in male New Zealand white rabbits. In this study, animals were fed either control diets, control diets rich in cholesterol (0.5% w/w), control diets rich in cholesterol (0.5% w/w) with hazelnut oil supplementation (5% w/w), or a control diet with hazelnut oil supplementation (5% w/w) for 14 weeks. The results showed that when supplemented in control diets, hazelnut oil reduced plasma cholesterol and apoB-100 containing lipoprotein levels by an insignificant level. No differences were observed in the high-cholesterol-diet group supplemented with hazelnut oil, which implies that hazelnut oil may be an effective health-promoting agent in diets with normal lipid intake, but cannot reverse the effects of high cholesterol intake (31).

Some researchers have investigated the potential of hazelnuts as a source of natural antioxidants. Yurttas et al. (32) assessed the phenolic composition of methanolic extracts of hazelnuts, showing that gallic acid, *p*-hydroxy benzoic acid, caffeic acid, and sinapic acid were the predominant phenolic acids reported. In addition, quercetin and epicatechins were present. The composition of phenolic acid constituents in hazelnut meal has also been assessed by Senter et al. (14) (Table 2).

#### 4. PECAN

Pecan tree (*Carya illinoensis*) is native to the United States but has also been naturalized for commercial pecan production throughout the world, including Australia, South Africa, and several middle eastern and South America countries (33). Fat is the predominant constituent in all pecan varieties, ranging from 65% to 75% (w/w) (1, 33, 34). Other constituents include 13.9% carbohydrate, 9.1% protein, 3.5% water, and 1.5% ash (w/w) (1). The predominant fatty acids present in pecan oil are oleic (55%), linoleic (33%), linolenic (2%), palmitic (7%), and stearic (2%) acids (Table 4) (34). The most predominant tocol in pecan oil was  $\gamma$ -tocopherol (176 mg/kg), followed by  $\alpha$ -tocopherol (10 mg/kg), and then  $\delta$ - and  $\beta$ -tocopherols (6.2 mg/kg) (1). Pecan oil also contains 0.73 g/kg phytosterols that exist primarily as  $\beta$ -sitosterol (around 90%) (1).

Early studies have shown that pecan oil is a very stable food oil despite its high content of unsaturated fatty acids, thus making it an excellent dietary oil (35). Demir and Cetin (36) examined the total yields, compositions, and oxidative stabilities of expeller-pressed and hexane-extracted pecan oils. Total yields were higher for solvent-extracted batches (67–79%, w/w) than pressed batches (36). The expeller-pressed pecan oil had a significantly higher total tocopherol content when compared with hexane-extracted oil (260 mg/kg and 23 mg/kg, respectively); however, the solvent-extracted oil exhibited a greater oxidative stability with an induction period of 6 h at 100°C, as compared with 5.5 h for pressed oil. These findings may imply that antioxidative constituents, aside from tocopherol, may be contributing to the enhanced oxidative stability of the hexane-extracted oils, however, previous publications (33, 34), using similar solvent-extraction methods, have shown much higher concentrations of tocopherols in pecan oils and thus contradict the findings of Demir and Cetin (36). Toro-Vasquez and Perez-Briceno (37) studied the oxidative stabilities of solvent-extracted pecan oils from 22 Mexican pecan varieties; all varieties tested had high oxidative stability index values (8.5–10.8 h at 110°C).

**TABLE 4. Fatty Acid Composition of Pecan Oil.<sup>a</sup>**

Fatty Acid	(%)
12:0	0.01
14:0	0.05–0.06
14:1	0.02–0.03
16:0	6.49–6.71
16:1	0.20–0.21
18:0	2.23–2.80
18:1 (n-9)	51.1–62.1
18:2 (n-6)	27.2–36.9
18:3 (n-3)	1.52–1.94
20:0	0.12
20:4	0.03

<sup>a</sup>Adapted from Wakeling et al. (34).



Epidemiological findings show that pecan-enriched diets can favorably alter serum lipid profiles in humans and thus reduce cardiovascular disease risk (38); however, the effects of pecan oil intake on human blood lipid profiles have not been reported.

## 5. WALNUT

Walnut (*nux juglandes*) is harvested from walnut tree (*Juglans regia*) and is the most popular nut ingredient in North American cooking. Over 30 varieties of walnut trees are currently harvested that have been developed for various characteristics including pest tolerance, early/late harvest, and shell thickness. The major walnut-producing nations are the United States (California), China, Turkey, India, France, Italy, and Chile (39).

Walnuts contain about 65% lipids, however, considerable differences exist among varieties (range: 52–70%, w/w) (1, 40). Walnuts also contain 15.8% protein, 13.7% carbohydrate, 4.1% water, and 1.8% ash (w/w) (1). The fatty acid composition of walnut oil is unique compared with other tree nut oils for two reasons; walnut oil contains predominantly linoleic acid (49–63%) and a considerable amount of  $\alpha$ -linolenic acid (8–15.5%). Other fatty acids present include oleic acid (13.8–26.1%), palmitic acid (6.7–8.7%), and stearic acid (1.4–2.5%) (Table 5) (40). The tocopherol content of walnut oil varies among different cultivars and extraction procedures and ranges between 268 mg/kg and 436 mg/kg. The predominant tocol isomer is  $\gamma$ -tocopherol (>90%), followed by  $\alpha$ -tocopherol (6%), and then  $\beta$ - and  $\delta$ -tocopherols (41). Nonpolar lipids have been shown to constitute 96.9% of total lipids in walnut oil, whereas polar lipids account for 3.1%. The polar lipid fraction consisted of 73.4% sphingolipids (ceramides and galactosylceramides) and 26.6% phospholipids (predominantly phosphatidylethanolamine) (42). Walnut oil contains approximately 1.8 g/kg phytosterols (1), primarily  $\beta$ -sitosterol (85%), followed by  $\Delta$ -5-avenasterol (7.3%), campesterol (4.6%), and, finally, cholesterol (1.1%) (42).

Several research groups have investigated the oxidative stability of walnut oil and have shown that it is readily oxidizable. Demir and Cetin (36) investigated the oxidative stability of expeller-pressed and hexane-extracted walnut oil at

**TABLE 5. Fatty Acid Composition of Walnut Oil.**<sup>a</sup>

Fatty Acid	(%)
16:0	6.7–8.7
18:0	1.4–2.5
18:1 (n-9)	14–26
18:2 (n-6)	49–63
18:3 (n-3)	8–16

<sup>a</sup>Adapted from Zwarts et al. (40).

100°C by monitoring changes in peroxide value, showing that the induction period of expeller-pressed walnut oil was 3.5 h compared with 4.5 h for hexane-extracted oil. The induction period of different walnut oils was 1.5–2.0 h shorter than values for pecan oil, as expected considering the existing difference in unsaturation between the two oils (36). Savage et al. (41) examined the oxidative stability of walnut oils from 13 different cultivars using the Rancimat method at 100°C; the induction periods were 3.9–7.8 h. One notable trend in this study was that the variation of induction period for various walnut oils was inversely correlated with the levels of linoleic acid and the ratio of total unsaturation to total tocopherol contents (41). Similar findings were reported by Oliveira et al. (43) using walnut oil obtained by supercritical fluid extraction with compressed carbon dioxide. More recently, Amaral et al. (44) examined the oxidative stability of oils from six walnut cultivars obtained by petroleum ether extraction. In one set of experiments, these researchers evaluated oil stabilities using the Rancimat method and showed induction periods between 2.7 h and 3.3 h for walnut oils used. In another set of experiments, the change in peroxide value after one year of storage was assessed; no obvious trends were observed among the various oils (44). Findings from stability studies collectively show that walnut oil has low oxidative stability when compared with other common nut oils, which can be ascribed to its high content of polyunsaturated fatty acids, mostly  $\alpha$ -linolenic acid (44).

Evidence from epidemiologic and intervention studies as well as clinical trials shows that walnut consumption has favorable effects on serum lipid levels in humans, such as lowering LDL, raising HDL, and reducing total serum triacylglycerol levels, all of which reduce the likelihood of suffering from a cardiovascular event (45–47). Many of the beneficial findings associated with walnut consumption have previously been attributed to the polyunsaturated fatty acid intake and have prompted health researchers to investigate which of these effects, if any, can be attributed to the lipid component of walnuts. Lavedrine et al. (48) conducted a cross-sectional study to assess the association between whole walnut and walnut oil consumption and blood lipid levels. This study included 933 men and women aged 18–65 years living in Dauphine, France (a major walnut-producing area). Factors used to assess cardiovascular disease risk included a one-year dietary recall questionnaire and serum levels of HDL, LDL, total cholesterol, and levels of the apolipoproteins apoA1 and apoB. Results from this study showed that higher levels of HDL cholesterol and apoA1 were associated with higher amounts of walnut oil and kernel consumption, with a positive trend existing between the various degrees of walnut oil/kernel consumption in this cohort. Other blood lipids did not show any significant association with walnut consumption; the nature of the cohort group made it impossible to separate the effects of whole walnut and walnut oil consumption (48). More recently, Zibaenezhad et al. (49) examined the effects of walnut oil consumption on plasma triacylglycerol levels in hyperlipidemic men and women. In this trial, 29 patients were given 3g/day walnut oil (six 500-mg capsules per day) for 45 days, 31 patients were given placebo and were used as controls. Supplementation of walnut oil reduced serum levels of LDL, triacylglycerol, and total cholesterol while increasing serum HDL levels, however, only the decrease in serum

triacylglycerol reached significance (49). The fatty acid composition of walnut oil has been suggested as being responsible for its cardioprotective feature, but results from studies, such as that of Espin et al. (50), show that the antioxidative components of walnut oil have significant antiradical properties that may exert a protective effect against the oxidation of biomacromolecules such as LDL, a known risk factor for atheroma development and, thus, heart disease. Therefore, more studies are needed to clarify the putative cardioprotective effects of walnut oil consumption before a casual relationship between the two can be established. The defatted meals of walnuts provide for an excellent source of natural antioxidants at a level of 0.51  $\mu\text{g/g}$  phenolic acids (14) (Table 2).

## 6. PISTACHIO

The pistachio tree (*Pistacia vera*) is native to the Middle Eastern region and has been naturalized in many parts of the world. The world's largest producer of pistachio nuts is Iran (Kerman Province), with an annual output of 300,000 tons. Other major producers are Turkey, the United States (California), and Syria (51). Pistachio contains 44% lipid, 28% carbohydrate, 21% protein, 4% water, and 3% ash (w/w) (1). Other research groups have reported that pistachio nuts contain between 45% and 72% oil, depending on the variety and stage of harvest (52, 53). The main uses of pistachio oil are in the cosmetics and condiment industries. The predominant fatty acid of pistachio oil is oleic acid (56–64%), followed by linoleic acid (23–31%), palmitic acid (9–13%), and small amounts of other fatty acids (Table 6) (54). Pistachio oil contains large amounts of phytosterols (5 g/kg, 85%  $\beta$ -sitosterol) (55), 270 mg/kg of tocopherols (1, 56), and has an acid value higher (2.32-mg KOH/g oil) than other tree nut oils (52, 57). Evidence from several epidemiologic studies suggests that pistachio consumption can reverse several adverse blood lipid parameters such as hypercholesterolemia (58), however, investigations on the health effects of pistachio oil consumption are not readily available or have not been conducted.

**TABLE 6. Fatty Acid Composition of Pistachio Oil<sup>a</sup>**

Fatty Acid	(%)
16:0	9–13
16:1	<1
18:0	6.0
18:1 (n-9)	56–64
18:2 (n-6)	23–31
20:0	<1
24:0	<1

<sup>a</sup>Adapted from Kamangar et al. (54).

**TABLE 7. Fatty Acid Composition of Brazil Nut Oil.<sup>a</sup>**

Fatty Acid	(%)
16:0	14
16:1	0.3
18:0	8.6
18:1 (n-9)	29
18:2 (n-6)	47
20:0	<1
24:0	<1

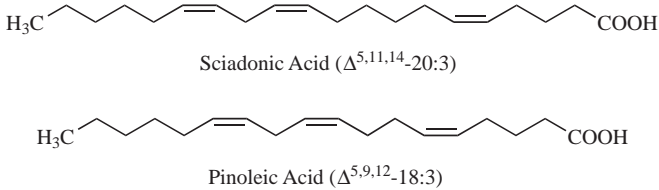
<sup>a</sup>Adapted from Beuchat and Worthington (60).

## 7. BRAZIL NUT

Brazil nuts (*Bertholletia excelsa*) are widely consumed but are produced mainly in South America, with total world production estimated to be about 20,000 metric tons. Bolivia, Brazil, and Peru are the main Brazil-nut-producing nations (59). Brazil nuts are traded mainly in the form of kernels (i.e., shelled) and are used in confectionery, bakery, and health foods. Brazil nuts contain 66–69% lipid, 14.3% protein, 12.2% carbohydrate, 3.5% ash, and 3.5% water (w/w) (1, 60). Brazil nut oil is used in the areas it is produced as cooking oil and is being promoted on the export market (59). As the export value of shelled Brazil nuts is so high, usually only defective Brazil nuts (cracked and partially oxidized) are extracted for their oils that can result in oils with acid values and peroxide values as high as 5.9-mg KOH/g oil and 7.6-meq oxygen/kg oil, respectively (61). The fatty acid composition of Brazil nut oil includes 29–48% oleic acid, 30–61% linoleic acid, 14–15% palmitic acid, 6–8% stearic acid, and 0.5% myristic acid (60, 62) (Table 7).

## 8. PINE NUT

Pine nuts (pinon or pignolia) are the edible seeds within the pine cone of several varieties of pine trees (*Pinus sp.*) but most commonly *Pinus pinea* or “stone pine”. Pine nuts are harvested all over the world, most notably in Russia, China, North Korea, Spain, Italy, and Turkey, among others. Pine nuts contain 48–61% lipids by weight (1, 60). Other constituents of pine nut include carbohydrate (19.3%), protein (11.6%), water (5.9%), and ash (2.2%) (1). Pine nut oil contains predominantly linoleic acid (46.4%) and oleic acid (38.1%). Maritime pine nut (*Pinus pinaster*) oil also contains two fatty acids that are unique among tree nut oils; pinoleic acid and sciadonic acid (Figure 2), which exist at 7% each in pine nut oil and may have antiatherogenic effects (Table 8) (63). The phenolic acid composition of defatted pine nut meal is given in Table 2 and shows that caffeic acid is the predominant phenolic compound (14).



**Figure 2.** Chemical structures of sciadonic and pinoleic acids.

**TABLE 8.** Fatty Acid Composition of Pine Nut Oils.<sup>a</sup>

Fatty Acid	Pine Nut Oil (%)	Maritime Pine Nut Oil (%)
16:0	5.8	3.6
16:1	0.3	0.2
18:0	3.8	2.4
18:1 (n-9)	38.1	18.1
18:2 (n-6)	46.4	55.9
18:3 (n-3)	0.8	1.3
20:0	0.7	ND <sup>b</sup>
20:1	0.9	0.8
$\Delta^{5,9-18:2}$	ND	0.7
$\Delta^{5,9,12-18:3}$	ND	7.1
$\Delta^{5,11-20:2}$	ND	0.8
$\Delta^{5,11,14-20:3}$	ND	7.1

<sup>a</sup>Adapted from Beuchat and Worthington (60) and Asset et al. (63).

<sup>b</sup>None Detected.

## 9. MACADAMIA NUT

Macadamia trees (*Macadamia sp.*) were originally cultivated in Australia, but the United States (Hawaii) is currently the world's largest producer of macadamia nuts. Edible macadamia nuts are from two species; *Macadamia integrifolia* (smooth-shell type) and *Macadamia tetraphylla* (rough-shell type). The macadamia nut industry in Hawaii, Australia, and many other producing areas, is based primarily on the smooth-shell type (64). Oil yields from macadamia nuts range from 59% to 78% (w/w) (1, 65, 66). Macadamia nuts also contain 13.8% carbohydrate, 7.9% protein, 1.4% water, and 1.1% ash (w/w) (1). Compositional studies of macadamia nut oil shows that it is rich in oleic and palmitoleic acids (Table 9) (67), has 18–54 mg/kg tocol isomers (predominantly  $\alpha$ -tocotrienol), and up to 1.5 g/kg phytosterols (predominantly campesterol) (66). Macadamia nut oil has been shown to have a relatively high smoke point of 198°C. The Rancimat method has been used to assess the oxidative stability of several varieties of macadamia nut oil, resulting in induction periods of between 3.6 h and 19.8 h (66).

**TABLE 9. Fatty Acid Composition of Macadamia Nut oil.<sup>a</sup>**

Fatty Acid	(%)
16:0	7.9
16:1	17
18:0	3.3
18:1 (n-9)	57.7
18:2 (n-6)	1.7
20:0	<1
24:0	<1

<sup>a</sup>Adapted from Macfarlane et al. (67).

## 10. CASHEW NUT

The cashew (*Anacardium occidentale* L.) is an evergreen species native to tropical America and contains 47% oil (w/w) (1, 68). Other components of cashew nuts include carbohydrate (27.1%), protein (18.2%), water (5.2%), and ash (2.5%) The predominant fatty acid in cashew nut oil is oleic acid (57.3–65.1%), followed by linoleic (15.6–18.6%), and palmitic (9.0–14.2%) acids (Table 10) (68). Cashew nut oil contains 1.4% unsaponifiable matter (w/w), of which 76.2–82.7% is  $\beta$ -sitosterol. Other sterols present in cashew nut oil include  $\Delta^5$ -avenasterol, campesterol, fucosterol, cholesterol, and stigmasterol (68). Cashew nut oil contains 45.3–83.5 mg/100 g  $\gamma$ -tocopherol; other tocopherols present are  $\alpha$ -tocopherol (2.8–8.2 mg/100g) and  $\delta$ -tocopherol (2.0–5.9 mg/100 g) (68).

## 11. USE OF DEFATTED TREE NUT MEALS AND OTHER BYPRODUCTS AS PROTEIN SOURCES

Defatted tree nut meals and hulls are traditionally used as animal feeds due to their low cost and the high nutritional value of their proteins and other constituents (69). Tree nut byproducts have many food (70) and biochemical applications (71). Tree

**TABLE 10. Fatty Acid Composition of Cashew Nut Oil.<sup>a</sup>**

Fatty Acid	(%)
16:0	9–14.2
16:1	0.3–0.4
18:0	6.3–11.6
18:1 (n-9)	57.3–65.1
18:2 (n-6)	15.6–18.1
20:0	0.3–0.8

<sup>a</sup>Adapted from Toschi et al. (68).

**TABLE 11. Amino Acid Profiles (%) of Tree Nut Proteins.<sup>a</sup>**

Amino Acid	Almond	Hazelnut	Pecan	Walnut	Pistachio	Brazil Nut	Pine Nut	Macadamia Nut	Cashew
Alanine	4.54	4.67	4.46	4.41	4.59	3.71	7.24	3.73	4.18
Arginine	11.2	14.2	13.2	14.4	10.1	13.8	26.9	13.5	10.6
Aspartic Acid	12.4	10.5	10.4	11.6	9.06	8.67	12.6	10.5	8.96
Cystine	1.28	1.51	1.70	1.31	1.78	2.36	2.51	0.05	1.96
Glutamic Acid	23.5	23.3	20.5	17.8	19.1	20.3	23.5	21.8	22.5
Glycine	6.67	4.65	5.09	5.17	4.75	4.62	7.05	4.37	4.68
Histidine	2.69	2.16	2.94	2.48	2.52	2.48	3.31	1.87	2.27
Isoleucine	3.14	3.75	3.77	3.96	4.49	3.32	5.38	3.02	3.94
Leucine	6.68	7.26	6.72	7.42	7.75	7.44	9.98	5.79	7.35
Lysine	2.73	2.63	3.22	2.68	5.74	3.17	5.19	0.17	4.63
Methionine	0.85	1.07	2.05	1.49	1.68	6.49	2.47	0.22	1.80
Phenylalanine	5.22	4.53	4.78	4.50	5.29	4.06	5.30	6.40	4.75
Proline	4.40	3.36	4.08	4.47	4.05	4.23	7.44	4.50	4.05
Serine	4.57	4.41	5.32	5.92	6.11	4.40	5.87	4.03	5.38
Threonine	3.08	2.95	3.44	3.78	3.35	2.33	4.39	3.56	3.44
Tryptophan	0.87	1.42	1.04	1.07	1.36	0.90	1.74	0.64	1.43
Tyrosine	2.41	2.99	2.41	2.57	2.07	2.70	5.07	4.92	2.53
Valine	3.63	4.37	4.61	4.77	6.18	4.87	7.15	3.49	5.46

<sup>a</sup>Values adapted from USDA Nutrient Database for Standard Reference (1).

nut meals are rich in several antioxidative compounds and other health-promoting substances. This has led researchers to investigate their potential as functional food ingredients and as possible sources of nutraceutical extracts (70). The predominant nutritional component of tree nut meals is protein, constituting around 40% of total weight (70). The protein component is of high quality with a reasonably balanced composition of essential amino acids (1). As an example, cashew nut meal contains 42% crude protein and, compared with soybean meal, it has been shown to enhance livestock weight-gain curves and a higher protein score (97 vs. 93, respectively) (72). Similar findings have also been reported for walnut meal (73). The amino acid compositions of proteins from tree nut meals are summarized in Table 11 and show that, in most cases, glutamic acid, arginine, and aspartic acid account for about 40% of the amino acids in these proteins, whereas tryptophan is a limiting amino acid in all tree nut proteins examined in this chapter, except macadamia nut protein, which contains only trace amounts of cystine. Thus, defatted meals of tree nuts serve as excellent sources of high-quality proteins.

## 12. CONCLUDING REMARKS

Several tree nut varieties serve as valuable oil crops due to their high oil yield, unique flavors, and healthful lipid composition. Byproducts of tree nuts also have several uses including functional food ingredients and as sources of nutraceutical extracts and dietary protein. Compared with most other vegetable oils, tree nut oils show high oxidative stability, which is due to high levels of monounsaturated fatty acids rather than polyunsaturated fatty acids and high concentrations of minor components with antioxidant activity. The use of tree nut oils and byproducts in every day cooking is very common in some parts of the world and is becoming more widespread due to increased consumer demand for alternative and health-promoting foods. The consumption of high-fat tree nuts and their oils has been shown to have antiatherogenic effects, which may be related to the known positive cardiovascular health effects of unsaturated fatty acids, phytosterols, and tocol isomers. Other minor phytochemicals present in tree nut oils may also contribute to their observed health effects. Less information is available regarding the health effects of tree nut byproducts.

## REFERENCES

1. United States Department of Agriculture (USDA) Nutrient Database. (2004). Available: [www.nal.usda.gov/fnic/foodcomp/search](http://www.nal.usda.gov/fnic/foodcomp/search).
2. J. S. Bonvehi and F. V. Coll, *Food Chem.*, **48**, 237–241 (1993).
3. N. Madhavan, *Inter. J. Toxicol.*, **20**, 15–20 (2001).
4. F. B. Hu, M. J. Stampfer, J. E. Manson, E. B. Rimm, G. A. Colditz, B. A. Rosner, F. E. Speizer, C. H. Hennekens, and W. C. Willett, *BMJ*, **317**, 1341–1345 (1998).



5. S. M. Grundy, L. Florentin, D. Nix, and M. F. Whelan, *Amer. J. Clin. Nutr.*, **47**, 965–969 (1988).
6. M. Martins, R. Tenreiro, and M. M. Oliveira, *Plant Cell Rep.*, **22**, 71–78 (2003).
7. G. A. Spiller, D. A. J. Jenkins, O. Bosello, J. E. Gates, L. N. Cragen, and B. Bruce, *J. Amer. Coll. Nutr.*, **17**, 285–290 (1998).
8. Z. Shi, Q. Fu, B. Chen, and S. Xu, *Chinese J. Chromatogr.*, **17**, 506–507 (1999).
9. A. Sattar, M. Jan, A. Ahmad, A. Hussain, and A. Khan, *Nahrung*, **33**, 213–215 (1989).
10. F. Salvo, M. Alfa, and G. Dugo, *Rivista Italiana delle Sostanze Grasse*, **63**, 37–40 (1986).
11. J. Sabaté and G. E. Fraser, *Curr. Opin. Lipidol.*, **5**, 11–16 (1994).
12. G. E. Fraser, H. W. Bennett, K. B. Jaceldo, and J. Sabaté, *J. Amer. Coll. Nutr.*, **21**, 275–283 (2002).
13. D. A. Hyson, B. O. Schneeman, and P. A. Davis, *J. Nutr.*, **132**, 703–707 (2002).
14. S. D. Senter, R. J. Horvat, and W. R. Forbus, *J. Food Sci.*, **48**, 798–799 (1983).
15. S. Siriwardhana and F. Shahidi, *J. Amer. Oil Chem. Soc.*, **79**, 903–906 (2002).
16. S. Sang, K. Lapsley, W. S. Jeong, P. A. Lachance, C. T. Ho, and R. T. Rosen, *J. Agric. Food Chem.*, **50**, 2459–2463 (2002).
17. S. Frison-Norrie and P. Sporns, *J. Agric. Food Chem.*, **50**, 2782–2787 (2002).
18. M. Pinelo, M. Rubilar, J. Sineró, and M. J. Nuñez, *Food Chem.*, **85**, 267–273 (2004).
19. S. Sang, H. Kikuzaki, K. Lapsley, R. T. Rosen, N. Nakatani, and C. T. Ho, *J. Agric. Food Chem.*, **50**, 4709–4712 (2002).
20. P. A. Davis and C. K. Iwashita, *Cancer Lett.*, **165**, 27–33 (2001).
21. C. Alasalvar, F. Shahidi, C. M. Liyanapathirana, and T. Ohshima, *J. Agric. Food Chem.*, **51**, 3790–3796 (2003).
22. C. Alasalvar, F. Shahidi, T. Ohshima, U. Wanasundara, H. C. Yurttas, C. M. Liyanapathirana, and F. B. Rodrigues, *J. Agric. Food Chem.*, **51**, 3797–3805 (2003).
23. J. Parcerisa, D. G. Rishardson, M. Rafecas, R. Codony, and J. Boatella, *J. Agric. Food Chem.*, **45**, 3887–3890 (1997).
24. P. Pfner, T. Matsui, W. Grosch, H. Guth, T. Hofmann, and P. Schieberle, *J. Agric. Food Chem.*, **47**, 2044–2047 (1999).
25. M. L. Ruiz del Castillo, M. Mar Caja, M. Herrair, and G. B. Blanch, *J. Agric. Food Chem.*, **46**, 5123–5132 (1998).
26. M. G. Bernardo-Gil, J. Grenha, J. Santos, and P. Cardoso, *Eur. J. Lipid Sci. Technol.*, **104**, 402–409 (2002).
27. M. Ozdemir, F. Ackurt, M. Kaplan, M. Yildiz, M. Loker, T. Gurcan, G. Biringen, A. Okay, and F. Seyhan, *Food Chem.*, **73**, 411–417 (2001).
28. S. F. O’Keefe, V. A. Wiley, and D. A. Knauff, *J. Oil Chem. Soc.*, **70**, 489–492 (1993).
29. N. Romero, P. Robert, L. Masson, J. Ortiz, J. Pavez, C. Garrido, M. Foster, and C. Dobarganes, *J. Sci. Food Agric.*, **84**, 943–948 (2004).
30. P. M. Kris-Erterton, *J. Nutr.*, **129**, 2280–2284 (1999).
31. J. Balkan, A. Hatipoğlu, A. Gülcin, and M. Uysal, *J. Agric. Food Sci.*, **51**, 3905–3909 (2003).
32. H. C. Yurttas, H. W. Shafer, and J. J. Warthesen, *J. Food Sci.*, **65**, 276–280 (2000).

33. L. T. Wakeling, R. M. Mason, B. R. D'Arcy, and N. A. Caffin, *Food Aust.*, **52**, 574–578 (2000).
34. L. T. Wakeling, R. M. Mason, B. R. D'Arcy, and N. A. Caffin, *J. Agric. Food Sci.*, **49**, 1277–1281 (2001).
35. T. M. Pyriadi and M. E. Mason, *J. Amer. Oil Chem. Soc.*, 437–440 (1968).
36. C. Demir and M. Cetin, *Deutsche Lebensmittel Rundschau*, **95**, 278–282 (1999).
37. J. F. Toro-Vazquez and F. Perez-Briceno, *J. Food Lipids*, **5**, 211–231 (1998).
38. S. Rajaram, K. Burke, B. Connel, T. Myint, and J. Sabaté, *J. Nutr.*, **131**, 2275–2279 (2001).
39. Walnut Industry Fact Sheet. (2004). Available: [www.walnut.org/pdfs/walnuts\\_fact-sheet.pdf](http://www.walnut.org/pdfs/walnuts_fact-sheet.pdf).
40. L. Zwarts, G. P. Savage, and D. L. McNeil, *Int. J. Food Sci. Nutr.*, **50**, 189–194 (1999).
41. G. P. Savage, P. C. Dutta, and D. L. McNeil, *J. Amer. Oil Chem. Soc.*, **1999**, 76, 1059–1065.
42. G. Tsamouris, S. Hatzinantiou, and C. Demetzos, *J. Biosciences*, **57**, 51–56 (2002).
43. R. Oliveira, M. Fatima Rodrigues, and M. G. Bernardo-Gil, *J. Amer. Oil Chem. Soc.*, **79**, 225–231 (2002).
44. J. S. Amaral, S. Casal, J. A. Pereira, R. M. Seabra, and B. P. P. Oliveira, *J. Agric. Food Chem.*, **51**, 7698–7702 (2003).
45. M. Abbey, M. Noakes, G. B. Belling, and P. J. Nestel, *Amer. J. Clin. Nutr.*, **59**, 995–999 (1994).
46. D. Zambon, J. Sabate, S. Munoz, B. Campero, E. Casals, M. Merlos, J. C. Laguna, and E. Ros, *Ann. Intern. Med.*, **132**, 538–546 (2000).
47. J. Sabate, G. E. Fraser, K. Burke, S. F. Knutsen, H. Bennet, and K. D. Lindsted, *N. Engl. J. Med.*, **328**, 603–607 (1993).
48. F. Lavedrine, D. Zmirou, A. Ravel, F. Balducci, and J. Alary, *Preventive Med.*, **28**, 333–341 (1999).
49. M. J. Zibaenezhad, M. Rezaezadeh, A. Mawla, S. M. Ayatollahi, and M. R. Panjahshahin, *Angiology*, **54**, 411–414 (2003).
50. J. C. Espin, C. Soler-Rivas, and H. J. Wichers, *J. Agric. Food Chem.*, **48**, 648–657 (2000).
51. Global Pistachio Production and Marketing Challenges. (2004). Available: <http://economics.ca/2003/papers/0460.pdf>.
52. M. Yousfi, B. Nedjmi, R. Bellal, D. B. Bertal, and G. Palla, *J. Amer. Oil Chem. Soc.*, **79**, 1049–1050 (2002).
53. M. Aslan, I. Orhan, and B. Şener, *Int. J. Food Sci. Nutr.*, **37**, 333–335 (2002).
54. T. Kamangar, F. Farrahi, and M. Mehran, *J. Amer. Oil Chem. Soc.*, **52**, 512–513 (1975).
55. M. Yildiz, S. Türcan Gürcan, and M. Özdemir, *Fett/Lipid.*, **3**, 84–86 (1998).
56. A. Danechrad, *Oleagineux.*, **29**, 153–154 (1974).
57. A. M. H. Al-Shaibani, L. D. K. Al-Doori, and O. M. Nori, *Zanco.*, **4**, 93–101 (1986).
58. K. Edwards, I. Kwaw, J. Matud, and I. Kurtz, *J. Amer. Coll. Nutr.*, **18**, 229–232 (1999).
59. Economic Viability of Brazil Nut Trading in Peru. (2004) Available: [www.nri.org/NRET/brazilnuts.pdf](http://www.nri.org/NRET/brazilnuts.pdf).
60. L. R. Beuchat and R. E. Worthington, *J. Fd. Technol.*, **13**, 355–358 (1978).

61. E. M. R. Gutierrez, M. A. B. Regitano-D'Arce, and A. M. O. Rauen-Miguel, *Ciencia e Tecnologia de Alimentos.*, **17**, 22–27 (1997).
62. E. W. Ecky, *Vegetable Fats and Oils*, van Nostrand Reinhold Publishing Co, New York, 1954.
63. G. Asset, E. Baugé, R. L. Wolff, J. C. Fruchart, and J. Dallongeville, *Eur. J. Nutr.*, **40**, 268–274 (2001).
64. H. Ako, D. Okuda, and D. Gray, *Nutrition*, **11**, 286–288 (1995).
65. C. Croy, *Inform*, **5**, 970–971 (1994).
66. A. Kaijser, P. Dutta, and G. Savage, *Food Chem.*, **71**, 67–70 (2000).
67. N. Macfarlane, and R. V. Harris, *AOCS Monograph*, AOCS Press, Champaign, Illinois, 1981, pp. 9, 103–108.
68. T. G. Toschi, M. F. Caboni, G. Penazzi, G. Lercker, and P. Capella, *J. Amer. Oil Chem. Soc.*, **70**, 1017–1020 (1993).
69. D. Harvey, *Commonwealth Bureau of Animal Nutrition*, **19**, 105–113 (1970).
70. M. L. Souza and H. C. Menezes, *Ciencia e Tecnologia de Alimentos*, **24**, 120–128 (2004).
71. W. J. Wolf and S. K. Sathe, *J. Sci. Food Agric.*, **78**, 511–521 (1998).
72. G. Piva, E. Santi, and T. Ekpenyong, *J. Sci. Food Agric.*, **22**, 22–23 (1971).
73. G. P. Savage, *Plant Foods Hum. Nutr.*, **56**, 75–82 (2001).

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## *Germ Oils from Different Sources*

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### **1. INTRODUCTION**

Commercial plant germ oils are mainly obtained from cereal grains, such as corn, wheat, and rice. In most cases, the endosperm of the grain is the part of interest for the industrial use of cereals. Starch and proteins are the major components of the endosperm. Endosperm represents 75–85% of the grain and protects the embryo, which is also referred to as germ.

The germ of a cereal constitutes about 2–3% of the grain and can be separated in a fairly pure form during the milling operation. Although lipids are present in relatively small quantities in grains, they play an important role in cereal processing and nature of the products by affecting the properties of protein and starch. Most of the oil present in the grain is found in the germ fraction of the cereal. For some cereals such as rice, corn, and wheat, oil has economic significance. Some cereals, such as oats, contain a considerable amount of lipid in the endosperm. Lipid content of the germ varies with the grain type, and it can be as high as 60%. Even though a small amount of germ oil is commercially extracted from other grains, such as oats and barley, the most important commercial germ oils are rice, corn, and wheat germ oil (WGO).

**TABLE 1. World Wheat Production.<sup>1</sup>**

Year	Production (x10 <sup>6</sup> Metric Tons)		
	2001	2002	2003
Canada	21	16	24
China, Mainland	94	90	86
India	70	73	65
USA	53	44	64
World	591	574	556

<sup>1</sup>FAOSTAT Database (<http://apps.fao.org>) (11).

Several reviews have been published on wheat (1, 2) corn (3–7), and rice bran oils (8–10) over the years. Therefore, this chapter, with sufficient background information, will emphasize the latest literature on composition, nutritional characteristics, and processing methods of plant germ oils.

## 2. WHEAT GERM

### 2.1. Production and Use

Wheat is one of the leading grain crops produced, consumed, and traded worldwide. About 590 million metric tons of wheat is produced globally each year. China, the United States, and Canada are among the largest wheat growers (Table 1) (11). Statistical data on wheat germ production is not readily available. However it can be estimated that about 10 million tons of wheat germ could be obtained from wheat milling operations worldwide based on the fact that germ constitutes about 2% of the whole wheat grain. Although wheat varieties differ in oil content, in general, the whole kernel contains about 2–4% lipids. Lipid content of endosperm is usually less than 2%. Wheat germ contains about 8–14%, (w/w) oil (12).

Typical proximate composition of commercial wheat germ is shown in Table 2 (2). Wheat germ has great potential as a highly nutritious food supplement. Wheat germ provides three times as much protein, seven times as much fat, 15 times as much sugars, and six times as much mineral content as wheat flour (13). The

**TABLE 2. Wheat Germ Proximate Composition.<sup>1</sup>**

Compound	% (w/w)
Protein	26
Crude Fiber	3
Starch	20
Sugars	16
Oil	10
Moisture	6
Ash	4

<sup>1</sup>Adapted from Barnes (2).

protein content of wheat germ is about 30% (w/w). Although of plant origin, germ protein has similar nutritive value as animal protein (14). All these properties make wheat germ very attractive for enrichment or supplementation of various processed food products.

Wheat germ oil has a number of nutritional and health benefits, such as reducing plasma and liver cholesterol levels, improving physical endurance/fitness, and delaying aging (15). These effects are attributed to the high concentration of bioactive compounds present in the germ. Wheat germ is one of the richest natural sources of  $\alpha$ -tocopherol, which possesses Vitamin E activity (16). The wheat germ market is mainly based on its high Vitamin E content, and WGO is marketed in bottles or in capsules as a dietary supplement. Wheat germ oil is also added to lecithin and cod liver oil. Wheat germ oil has been reported to improve human physical fitness, and this effect is attributed to its high polyacosanol (PC), specifically to its high octacosanol (OC) content (15). There is a growing interest in wheat germ octacosanol as a potential nutraceutical and functional food ingredient.

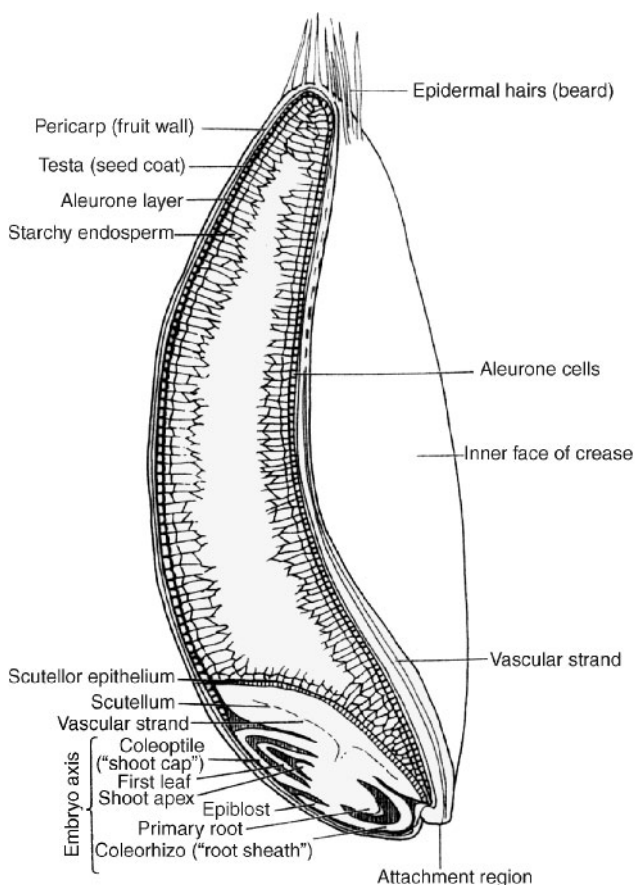
Wheat germ oil is used in products such as foods, biological insect control agents, pharmaceuticals, and cosmetic formulations (15). Wheat oil betaine, aminoaminelactate, sulfosuccinate, and amidopropylamine oxide are some of the WGO-based surfactants used in cosmetics (17, 18). Wheat germ oil and its volatile components, such as C13–C16 saturated and unsaturated hydrocarbons, branched hexylbenzene, octanoic acid,  $\gamma$ -nanolactone, substituted naphthalenes, and cyclic branched ketones, have been reported to have activities as biological insect control agents (19, 20). Wheat germ products are also marketed as dietary supplements for farm animals, racehorses, pets, and mink.

## 2.2. Grain Structure

A sketch of the longitudinal and histological section of a wheat grain is shown in Figure 1 (22). The wheat caryopsis (the fruit of the cereal) contains a single seed. The outermost layer of a seed is the pericarp, which forms a tough protective layer at maturity. The tissue directly surrounding the seed is referred to as testa or seed coat. The whole grain consists of two parts, namely the embryo and the endosperm. The starchy endosperm is the major portion of the seed at maturity and serves as a food reserve to be used by the embryo at germination. The aleurone layer is the outermost layer of the endosperm. The embryo or germ is formed of thin-walled cells and consists of embryo axis and scutellum. The embryo axis develops into the first roots and shoots of the new plant. The scutellum is a tissue layer located between the embryo and the endosperm, and it plays a role in the translocation of hydrolyzed sugars from endosperm to the embryo during germination. Depending on the variety of cereal, either the germ is surrounded by the endosperm or placed laterally and produces a slight protuberance from the grain.

## 2.3. Germ Recovery

Wheat germ is separated from the endosperm during the milling operation. The basic principles of modern wheat milling technology are used worldwide, whereas



**Figure 1.** Structure of wheat grain (21).

slight variations are observed regionally. In general, wheat is transferred from elevator to screen room to separate out chaff, dust, stones, mud balls, glass, nonferrous metal, and grains other than wheat (oats, barley, etc.). Magnets placed at various positions throughout the mill remove ferrous metals. Most mills use dry rather than wet systems to clean wheat before tempering. Tempering is the controlled addition of moisture to wheat to achieve the following objectives: (1) to toughen the skin to resist powdering during milling; (2) to facilitate the physical separation of endosperm and bran; and (3) condition the endosperm for easy size reduction and sieving for flour production. Tempered wheat is then milled. The milling process involves grinding of the grain and separation of the fractions. Milling breaks open the grain and scrapes off as much endosperm from the bran skin as possible. A basic milling operation consists of the following systems: (1) a series of break rolls; (2) grading; (3) purification; (4) sizing (scratch); (5) size reduction; (6) flour dressing; and (7) millfeed (byproducts of wheat flour milling).

In a conventional mill, wheat germ stock occurs primarily in the chop (ground material leaving a break roll from the first two breaks). Germ recovery is completed in a germ separator. The separation and purification of the germ is achieved by taking advantage of two physical characteristics of germ. Germ tends to fragment into particles coarser than bran and endosperm particles because of its plasticity, which is due to its high oil content. Germ also tends to flatten, rather than crush like endosperm. These properties facilitate germ separation by size. The density of germ is greater than that of the other wheat fractions. Thus, wheat germ can also be isolated based on specific gravity differences. In some mills, which are designed to maximize germ recovery, wheat grain is first passed through an impact machine that releases practically the entire germ along with a small quantity of fines. Broken wheat is then sent to the first break rolls after sifting out the fines. Then germ is separated and purified in a germ separator based on the above described principles. After separation, germ is passed through smooth reduction rolls to produce flakes. Flat, thin, and large flakes characterize good-quality wheat germ.

Scutellum is relatively friable and difficult to separate from the other milling fractions. Separation of the embryo axis is easier. After size reduction, the embryo axis goes on to the reduction rolls with coarse middlings where it is flaked and can be separated by sieving. Thus, commercial wheat germ consists predominantly of the embryo axis. The bran and endosperm content of commercial germ may vary with the extent and sophistication of the milling operations. During the flaking process, some oil is pressed out of the germ and transferred to the flour. Hence, the lipid content of the commercial WGO is different than that of the original embryo axis. In mills with no special germ-separation equipment, germ recovery yields are usually very low, about 0.5% for food-grade germ. Yields can be as high as 1.5–2% in mills with advanced germ-recovery systems.

## 2.4. Oil Content

The oil content of wheat germ varies with variety, purity of germ, and extraction method. Pure germ fractions prepared in the laboratory (dissected by hand) contain a much higher amount of oil (~15%) than commercially flaked wheat germ (7–11%), because some oil is lost by expression and transferred to flour during the commercial operations (23). In general, dissected germ contains both embryo and scutellum. It has been reported that scutellum contains about two times more oil than does the embryo (24). The higher oil content in scutellum explains higher oil content of laboratory-dissected germ. Higher oil yields (25–30%) were obtained when total acyl lipid content of wheat germ was determined by acid hydrolysis, which releases oil from the germ matrix (25).

## 2.5. Oil Properties and Lipid Composition

Physicochemical properties of WGO are summarized in Table 3 (26). The specific gravity of WGO varies from 0.925 to 0.938. Typical refractive index of WGO is in the range of 1.469 to 1.483. Iodine and saponification values of the oil are 115–128



**TABLE 3. Physicochemical Properties of Wheat Germ Oil.<sup>1</sup>**

Property	
Specific Gravity	0.928–0.938 (15.5/15.5°C) 0.925–0.933 (25/25°C)
Refractive Index	1.474–1.483 (25°C) 1.469–1.478 (40°C)
Iodine Value	115–128
Saponification Value	179–190
Unsaponifiable (%)	2–5

<sup>1</sup>Adapted from Firestone (26).

and 179–190, respectively. Free fatty acid content of WGO is usually less than 6%; however, it can be as high as 25% if the germ separation, storage, and oil-extraction conditions are not controlled properly. Solvent-extracted crude oil usually has a lower FFA content than that of the mechanically expelled oil. Free fatty acids are not desirable in the oil as a result of their contribution to a bitter and soapy flavor in food products; hence, they have to be removed during the edible oil-refining process. Unsaponifiable matter content of WGO is higher, 1.5–8%, than that of the most other edible oils. Composition of the unsaponifiable content of WGO is discussed later in this chapter.

**2.5.1. Fatty Acid Composition** The fatty acid composition of commercial and laboratory-extracted wheat germ oil has been reviewed by Barnes (2). Significant variations were observed in the fatty acid composition of commercial WGO. These variations were attributed to differences in varieties of wheat, growth conditions, storage conditions of the germ, method of lipid extraction and analysis, adulteration with other vegetable oils, and post extraction treatments such as removal of fatty acids. However, fatty acid compositions of laboratory-extracted oils (hexane extracts) obtained from wheat germ processed at different milling operations were similar (2).

Hexane-extracted wheat germ consisted of about 56% linoleic acid (18:2 n<sub>6</sub>), which is an essential fatty acid (Table 4) (27). Total unsaturated and polyunsatu-

**TABLE 4. Comparison of Fatty Acid Composition<sup>1</sup> of Wheat Germ Oil Extracted with SC-CO<sub>2</sub><sup>2</sup> and Soxhlet<sup>3</sup> Methods.**

Fatty Acid	16:0	18:0	18:1n-9	18:2n-6	18:3n-3	20:0	20:1n-9	20:2n-6	22:1n-9	24:1n-9
SC-CO <sub>2</sub> <sup>2</sup>	16.4	0.6	14.0	56.2	6.1	0.2	1.6	0.2	0.5	0.1
Soxhlet <sup>3</sup>	16.7	0.7	14.6	56.5	6.2	0.2	1.5	0.2	0.4	0.2

<sup>1</sup>GC area percentage.

<sup>2</sup>Extracted at 550 Bar and 40°C.

<sup>3</sup>Extracted with hexane.

rated fatty acid (PUFA) content of wheat germ oil was about 81% and 64%, respectively. It has been well documented that unsaturated fatty acid, especially PUFA, intake reduces coronary heart disease (CHD) (28). Several scientific studies have shown that n-3 fatty acids have health benefits, such as lowering CHD risk (29). It has been also suggested that n-6/n-3 ratio of ten or less results in reduction in fatal CHD risk (29). The n-6/n-3 recommendations of the World Health Organization, Sweden, and Japan are 5–10/1, 5/1, and 2/1, respectively. Wheat germ oil has very high unsaturated and polyunsaturated fatty acid content and an excellent n-6/n-3 fatty acid ratio (9/1). A high concentration of PUFA is a positive attribute in the functional foods and nutraceutical market. However, a high content of C18:3 fatty acid makes the oil susceptible to oxidative rancidity.

Research findings of Dunford and Zhang (27) also demonstrated that there was no significant change in fatty acid composition of the oils extracted with various organic solvents (hexane, ethanol, isopropanol, and acetone) even though the extract yield was affected considerably by the solvent type.

Wang and Johnson (30) have reported that neutralized WGO had higher palmitic, stearic, and oleic acids content and lower linoleic and linolenic acids content as compared with crude and degummed oils. This phenomenon was attributed to two reasons: (1) selective hydrolysis of triacylglycerols (TAG) during germ separation and oil extraction, and (2) removal of phospholipids, which usually contain more PUFA as compared with the neutral oil during the deacidification process.

**2.5.2. Acyl Lipids** The composition of nonpolar acyl lipids in WGO was reported by Barnes (2). Triacylglycerols are the major lipid class in WGO (Table 5). According to Nelson et al. (31), about 30% of the TAG consists of 1-palmito-2,3-dilinolein. Trilinolein (~16%) and 1-palmito-2-linoleo-3-olein (~12%) are the two other major TAGs present in WGO. Details on distribution of specific fatty acids in the TAG of WGO are discussed by Barnes (2) and Nelson et al. (31).

Diacylglycerols (DAGs) content of commercial oils varies from 2% to 11% (2). Monoacylglycerols (MAGs) content of WGO (0.1–1.0%) is usually lower than the DAG content. It had been also reported that fatty acid composition of MAG was significantly different than that of the other acyl lipids (32). Linoleic acid content of MAG was significantly lower than that of the other acyl lipids.

**TABLE 5. Nonpolar Acyl Lipid Composition of Laboratory Extracted Wheat Germ Oil.<sup>1</sup>**

Lipid Class	Composition (%)
Triacylglycerols	63.9–88.5
Diacylglycerols	1.8–6.9
Monoacylglycerols	0.3–1.1
Free Fatty Acids	0.6–22.0
Phytosterol Fatty Acid Esters	5.1–5.8

<sup>1</sup>Adapted from Barnes (1).

**TABLE 6. Effect of Processing on the Phosphorous Content of Wheat Germ Oil.<sup>1</sup>**

Oil Type	Phosphorous (ppm)
Crude	1428
Degummed	1082
Neutralized	99
Bleached	22
Cold-processed	786
Cold pressed	74

<sup>1</sup>Adapted from Wang and Johnson (30).

Wheat germ oil also contains polar lipids. Reported values for WGO polar lipids vary considerably with the extraction method and solvent used for lipid recovery. Hargin and Morrison (33) reported over 20% polar lipids in dissected wheat germ chloroform-methanol extracts. Commercial oils produced by pressure expelling contain 0.2–1.8% (expressed as of total acyl lipids) polar lipids. Commercially solvent-extracted crude WGO consists 0.3–10% polar lipids as of the total acyl lipids (32). Analytical data on WGO phospholipids (PL) is scarce. In dissected wheat germ, phosphatidylcholine (PC) represents about 40–60% of total PL and phosphatidylethanolamine (PE) (9–15%), and phosphatidylinositol (PI) (13–20%) are also present in large amounts (33). The effect of processing on the phosphorous content of the oil was examined by Wang and Johnson (Table 6) (30). Wheat germ oil degumming was very difficult as a result of the presence of a large amount of non-hydratable PL caused by the phospholipase D activity during wheat milling (30).

**2.5.3. Unsaponifiable Components** Wheat germ oil is quite rich in unsaponifiable compounds, in particular phytosterols and tocopherols (Table 7). Small quantities of triterpenols, n-alkanols, carotenoids, and hydrocarbons are also present in the unsaponifiable fraction of the oil. Tocopherols and n-alkanols are commercially the most important unsaponifiable components in WGO.

**2.5.3.1. Tocols** Tocopherols constitute about 18% of the unsaponifiable fraction of WGO (Table 7). Wheat germ oil is one of the richest natural sources of

**TABLE 7. Unsaponifiable Content of Wheat Germ Oil.<sup>1</sup>**

Compounds	% of Unsaponifiables
Tocopherols	18
Hydrocarbons	7
n-alkanols + Triterpenols	9
Methylsterols	17
Phytosterols	35
Others	14

<sup>1</sup>Adapted from Barnes (2).

TABLE 8. Tocol Content of Wheat Germ Oil.

	SC-CO <sub>2</sub> Extraction (550 Bar, 80°C)				Soxhlet Extraction (Hexane)
	15	30	45	60	
Extraction time (min)	15	30	45	60	
α- Tocopherol (ppm)	1353	1320	1365	1176	1377
α-Tocotrienol (ppm)	n.d. <sup>1</sup>	9	n.d. <sup>1</sup>	n.d. <sup>1</sup>	n.d. <sup>1</sup>
β- Tocopherol (ppm)	1005	945	998	1277	1209
β-Tocotrienol +					
γ-Tocopherol (ppm)	23	19	24	31	48
γ- Tocotrienol (ppm)	n.d. <sup>1</sup>	n.d. <sup>1</sup>	n.d. <sup>1</sup>	47	n.d. <sup>1</sup>
δ- Tocopherol (ppm)	4	5	5	16	5
δ- Tocotrienol (ppm)	n.d. <sup>1</sup>	n.d. <sup>1</sup>	n.d. <sup>1</sup>	12	7

<sup>1</sup>Not detected.

α-tocopherols. Synthetic Vitamin E, which is a dl-α-tocopherol, is commercially available. The natural isomer of α-tocopherol occurs in the d-form and possesses greater biological activity than that of the synthetic product. Tocopherols, particularly α-tocopherol, are also known to have antioxidant activity (34). The composition of tocopherol isomers in WGO oil is shown in Table 8. Wheat germ oil is rich in both α- and β-tocopherols. Bran and endosperm contain less tocopherol than that of the germ; hence, their presence reduces tocopherol content in the WGO extracted from germ contaminated with nongerm fractions. It is interesting to note that oil from fresh wheat germ contained similar amounts of tocopherol as the oil from rancid germ (32) indicating that conditions causing lipid deterioration did not have any significant adverse effect on the tocopherols. A commercial form of Vitamin E (α-tocopherol acetate) was detected in some commercial WGO (2). This might be caused by synthetic Vitamin E fortification of these commercial oils because no significant amount of α-tocopherol acetate has been detected either in any plant lipid extracts or laboratory-extracted WGO (2). A very small amount of tocotrienols was also detected in the WGO extracted in our laboratories (Table 8). According to Barnes (1), only α- and β-tocopherols are present in the pure dissected wheat germ. The presence of tocotrienols in the germ oil is attributed to the contaminations from endosperm and bran.

**2.5.3.2. n-Alkanols** n-Alkanols are a group of high-molecular-weight primary fatty alcohols present in many plants. Information on the composition of n-alkanols in WGO is scarce. The major component of wheat wax is octacosanol, which is an n-alkanol with chemical formula CH<sub>3</sub>(CH<sub>2</sub>)<sub>26</sub>CH<sub>2</sub>OH and molecular weight of 410.8 (35, 36). Barnes (1) reported that unrefined, unadulterated, solvent-extracted wheat germ oil contains 80 mg/kg of octacosanol. A U.S. patent, which has expired, describes separation of a mixture of octacosanol and triacontanol from a wheat germ oil unsaponifiable fraction (37).

**2.5.3.3. Sterols** Phytosterols constitute a major fraction of the WGO unsaponifiables (about 35%) (Table 7). According to Itoh et al. (38), WGO contains a

significantly higher amount of phytosterols than do the other common commercial oils. Sitosterol (60–70%) and campesterol (20–30%) are the two major phytosterols present in WGO (38–40). The majority of the phytosterols in WGO are present in an esterified form (41). According to Kiosseoglou and Boskou (41), the total phytosterol content of WGO is about 3–4%. Esterified sterols constitute 2–3% of the oil. The free to esterified phytosterol ratio in the WGO varies between 0.3 and 0.5.

**2.5.3.4. Other Unaponifiable Compounds** Cycloartenol,  $\beta$ -amyrin, and 24-methylene cycloartanol are the major triterpenols, which constitute less than 1% of WGO. Hydrocarbons are minor components in the WGO unsaponifiable fraction (Table 7). According to Kuksis (42), 50% of the hydrocarbons was squalene and the remainder consisted of n-C<sub>29</sub> alkanes. The presence of lutein and cryptoxanthin in WGO were first reported in 1935 and 1940, respectively (43, 44). Recently, Panfili et al. (45), reported that petroleum-ether-extracted WGO contained ~25 ppm lutein, ~23 ppm zeaxanthin, and ~8 ppm  $\beta$ -carotene.

## 2.6. Oil Production

Wheat germ oil can be extracted by mechanical expelling, organic solvent extraction, and supercritical fluid extraction. Mechanical expression and organic solvent extraction are both being used for commercial extraction of wheat germ oil. To our knowledge, supercritical fluid technology has not been commercialized for WGO processing.

Hexane is commonly used for WGO extraction (46). Ethanol and 1,2-dichloroethane are also used to a lesser extent for commercial WGO extraction (1). Although hot solvents are preferred for vegetable oil extraction, it has been reported that at least one company performs solvent extraction at temperatures around 38°C for WGO recovery (1).

Solvent extraction is more efficient than mechanical pressing of wheat germ oil. The residual oil content of solvent-defatted wheat germ can be as low as 1%, (w/w). Solvent-extracted wheat germ is more stable than the mechanically expressed wheat germ because of its lower lipid content. Pressing recovers only 50%, (w/w) of the wheat germ oil, and residual wheat germ requires further stabilization to avoid rancidity and shelf life extension. Mechanical pressing of wheat germ oil can be successful only when the bran contamination is minimized during the milling operation because the oil content of bran is much lower than that of the germ fraction. Pressed wheat germ is perceived as “natural” and usually preferred by consumers.

Barnes (2) reviewed the literature on wheat germ extract yields using various solvents. Hexane and light petroleum ether extraction resulted in yields ranging from 5–15% (w/w). This wide range of variation in oil yield was explained by the degree of contamination of germ by bran, which contains only 5% oil. Diethyl

ether, which is expected to extract more polar components, yielded 7–15% oil. Acetone gave 1.9% more oil yield than light petroleum ether did.

Although most edible oils are refined to remove PL, free fatty acids (FFA), color compounds, and volatile components, WGO is often used in the crude form. Phosphatidylcholine, color, and flavor are desired attributes for the products marketed as “natural” in the health food stores. However, refining improves the stability of the oil. The FFA content of crude WGO can be high, 5–25%, depending on the germ separation conditions, storage, and oil-extraction method. Free fatty acids contribute to bitter and soapy flavors in the product; hence, they are removed from WGO by alkali treatment. However, the alkali deacidification process results in significant losses in oil and more importantly in tocopherols. Wang and Johnson (30) examined the effect of conventional oil-refining processes on the WGO quality. According to this study, tocopherol content of WGO did not change significantly during degumming, neutralization, and bleaching processes. However, deodorization conditions reduced the tocopherol content of WGO significantly. Lower temperature and longer residence time were effective in reducing FFA, peroxide value, and color while retaining tocopherols in WGO during deodorization. Although degumming did not reduce phosphorous content of the crude oil effectively, phosphorous concentration was reduced at every stage of WGO refining. Wang and Johnson (30) have suggested that WGO refining should include acid degumming at high temperatures and high shear for an extended time, as compared with that for the typical vegetable oils, to maximize PL hydration. Although PL, specifically PC, has beneficial health effects for humans, they are removed from the crude oil during the degumming process. Phospholipids tend to precipitate out in the oil during storage and have adverse effects on frying operations due to their emulsification properties. Neutralization of FFA may need excess alkali treatment. Wheat germ oil bleaching requires more bleaching earth than that of the typical vegetable oil refining.

An expired U.S. patent describes molecular distillation of WGO (47). Initially, WGO was degummed by using phosphoric acid and water. Bleaching was carried out with activated clay followed by distillation using a centrifugal molecular distillation unit. Free fatty acids were removed at 140–200°C and below 50 mTorr. It was claimed in the same patent that a Vitamin E concentrate was prepared from purified WGO by a second-stage molecular distillation process carried out at 220–300°C and pressures less than 25 mTorr (47).

Supercritical fluid-extraction technology is an alternative method to conventional hexane extraction. Supercritical fluid extraction of WGO has been reported by several research groups (45, 48–50). Wheat germ oil solubility in SC-CO<sub>2</sub> at 40°C and 200 bar was 0.35% (w/w) (48). Oil extracted with SC-CO<sub>2</sub> has a lighter color and contains less phosphorus than that of the hexane-extracted oil. Although oil extraction rates from the ground and flaked wheat germ were not significantly different, use of flaked wheat germ is recommended for large-scale SC-CO<sub>2</sub> extraction. Ground wheat germ particles can be difficult to handle because of dusting. Furthermore, channeling of SC-CO<sub>2</sub> flow through the ground wheat germ in

the extraction vessel because of compaction may reduce the mass transfer. According to Dunford and Martinez (50) and Taniguchi et al. (48), the  $\alpha$ - and  $\beta$ -tocopherols content of SC-CO<sub>2</sub>-extracted oil were similar to those of hexane-extracted oil. However, Gomez and Ossa (49) reported higher tocopherol content in the SC-CO<sub>2</sub>-extracted WGO as compared with that of the hexane-extracted oil.

Panfili et al. (45) characterized the composition of SC-CO<sub>2</sub>-extracted WGO and defatted cake. According to this study, FFA content and PV of the oils collected during the initial stages of SC-CO<sub>2</sub> extraction (during the first 45 min) were higher than that of the oil fractions collected at the later stages of the process. Similarly, more tocopherols were detected in the oils collected during the first 75 min of 3 h SC-CO<sub>2</sub> extraction. Experiments also indicated that WGO collected during the initial stages of SC-CO<sub>2</sub> extraction had a higher tocopherol content (50). The most abundant carotenoid in SC-CO<sub>2</sub>-extracted WGO was lutein, followed by zeaxanthin and  $\beta$ -carotene. A larger amount of carotenoids was extracted toward the end of SC-CO<sub>2</sub> extraction (45).

Studies carried out with liquid and SC-CO<sub>2</sub> (50–400 bar) at relatively low temperatures (10–60°C) indicated that pressure had a significant effect on the oil yields, whereas the effect of temperature was insignificant (48). Hence, the effect of pressure and temperature on the SC-CO<sub>2</sub>-extraction yields and WGO composition was studied in the range of 100–550 bar and 40–80°C (50). Yields of SC-CO<sub>2</sub> extracts [(weight loss from the sample during the extraction/initial weight of wheat germ used for extraction)\*100] varied significantly with temperature and pressure in the 2% to 20% (w/w) range. The wheat germ oil yield was 11% (w/w) when hot hexane (Soxhlet) was used for extraction. The higher SC-CO<sub>2</sub>-extraction yield (>11%) indicates that SC-CO<sub>2</sub> at high pressures extracted some of the wheat germ components, which are not soluble in hexane. Moisture in the wheat germ might be one of the compounds coextracted with oil resulting in higher extraction yields. The highest SC-CO<sub>2</sub>-extraction yield was obtained at the highest pressure used (550 bar). The temperature dependence of the extract yield was more pronounced at higher temperatures (60°C and 80°C) and the lowest pressure examined in this study (100 bar). This is a result of the significant change in SC-CO<sub>2</sub> density under those conditions.

The fatty acid composition of the extracts was not affected by temperature, pressure, and the extraction method (Table 4). Supercritical carbon-dioxide-extracted oil samples had similar fatty acid composition to that of the Soxhlet-extracted oil (Table 4). All of the wheat germ extracts consisted of about 56% linoleic acid (18:2 n-6), which is an essential fatty acid (Table 4). The total unsaturated and polyunsaturated fatty acid (PUFA) content of the wheat germ oil was about 81% and 64%, respectively. The SC-CO<sub>2</sub> extraction of wheat germ resulted in extracts with similar tocopherol and tocotrienol compositions to those of the Soxhlet extracts (Table 8) (50). These results indicate that SC-CO<sub>2</sub> technology can be used for extraction and fractionation of WGO components to obtain products with high quality.

**TABLE 9. Corn (Maize) Production.<sup>1</sup>**

Year	Production (x10 <sup>6</sup> Metric Tons)		
	2001	2002	2003
China, Mainland	114	122	114
USA	242	229	257
India	13	11	15
Canada	8	9	10
World	615	604	638

<sup>1</sup>FAOSTAT Database (<http://apps.fao.org>) (11).

### 3. CORN GERM OIL

#### 3.1. Production and Use

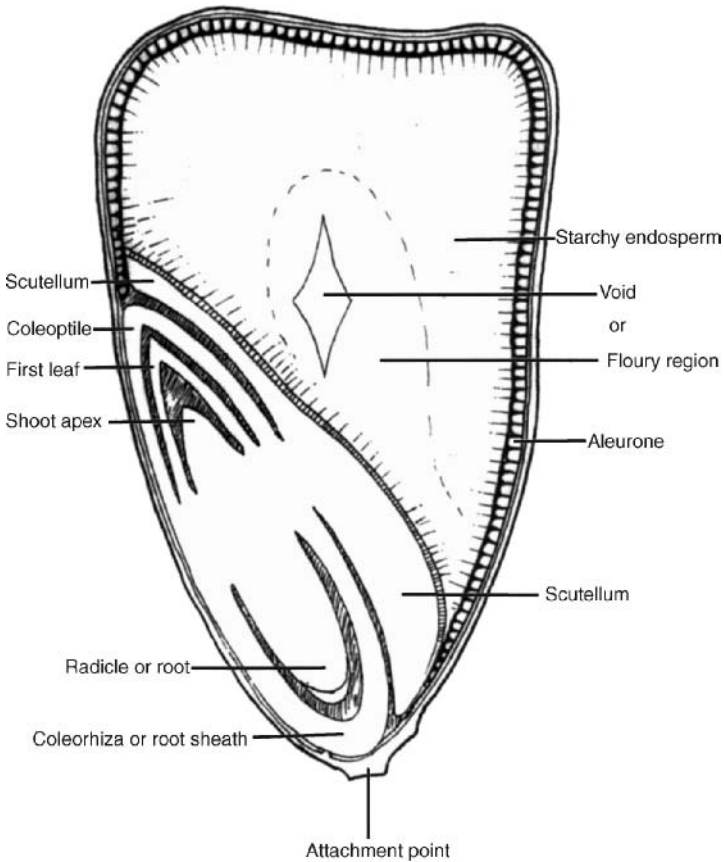
Corn, also referred to as maize (*Zea mays* L.), is one of the most important cereal grains that is a commercial source of vegetable oil. Maize originated in the Americas and was introduced into Europe in 1492. About 600 million tons of maize is produced annually worldwide (Table 9) (11). The United States is by far the largest corn producer in the world followed by Mainland China.

Corn grown in the United States contains about 65% starch and 3–4% oil (5). The corn germ contains 85% of the oil and 80% of the minerals present in the whole grain (6). A large fraction of the maize crop is used as livestock feed. However, in many developing countries, corn is used only for human consumption. Maize is also used for numerous industrial products, in distillation and fermentation industries, and for the production of starch and corn syrup. Corn oil is a byproduct of the corn milling industry and accounts for a relatively small portion of the economic value of the whole plant. Corn germ contains 50–60% oil. Corn oil is used as salad and cooking oil and in margarines.

#### 3.2. Grain Structure

Like the grain of other cereals, corn grain is a caryopsis. The maize grain is a one-seeded fruit and fairly large with an average mass of 285 mg (51). The most important variety of maize is Dent Maize or Indian Corn (*Zea mays* var. *Indentata* Bailey). “Dent” refers to both tooth-like structure and indentation at the end of the grain (Figure 2). Structure of a corn grain is quite similar to any other cereal grain. The endosperm consists of an aleurone layer and horny and floury endosperm. The embryo or germ consists of scutellum and embryonic axis. The plumule, mesocotyl, and the radicle form the embryonic axis. In corn, the germ is located inside the endosperm and a cap that is visible externally protects its end.





**Figure 2.** Structure of corn grain (21).

### 3.3. Germ Separation

Maize milling process has been reviewed by several authors (5, 51, 52). Maize milling can be done by semiwet (20–23% moisture), wet (45% moisture), or dry (~17% moisture) milling operations. Semiwet and dry milling operations require a tempering step, which refers to controlled addition of moisture to the grain before the dehulling and degerming operation. The dry milling process is very similar to the wheat degermination process described earlier in this chapter. The recovered germ contains about 20–25% oil.

The wet milling operation entails a steeping step that involves soaking of cleaned corn in water containing 0.1–0.2% sulfur dioxide at 52°C (5). Then, the whole grain is ground in roller mills. The ground product contains all components of the grain; bran, hulls, starch, gluten, and germ. Various components of ground corn are separated by screening, centrifugation/floatation, and filtration operations. The germ fraction is separated from the other ground grain components either by

centrifugation or floatation operations. Centrifugal separation has replaced the flotation method because it provides improved separation efficiency, cleaner germ, higher operation capacity, and more sanitary conditions. Overflow from the centrifugation process carries lighter weight germ and the underflow contains heavier components such as starch, gluten, and hulls. The germ is then washed, dewatered, and dried. The objective of the degerming operation is to obtain grits with 1% fatty matter. The wet corn milling operation produces germ with 50–60% oil.

### 3.4. Oil Content

An examination of the data on oil content of corn grown between the years 1917 and 1972 showed fairly small variations (4.0% to 4.9%) (53). Since then, plant breeders and genetic engineers altered the starch, protein, and oil content of corn significantly. In 1982, after 82 generation of selection for high and low oil content corn, Illinois High and Low Oil varieties, which contained 19% and 0.3% oil, respectively, were developed (6). A disadvantage of Illinois High Oil Corn was its lower yield than its traditional counterparts. More effective breeding later resulted in 6–8% oil and yields similar to those of a traditional corn crop. Waxy, high amylose, high oil, and high lysine corn have been created to meet the needs of livestock feeders, the food industry, and other industrial users of corn. High oil corn contains 7–8% oil, which is a 2–3% increase over traditional corn. High amylose corn, which was developed to meet the needs of the wet milling industry, contains higher than 50% amylose. This variety also contains a higher amount of oil. Germ of this variety is larger and contains higher quality protein than that of the traditional varieties. High lysine corn contains higher levels of essential amino acids lysine and tryptophane. These varieties are not grown as widely as traditional dent type corn.

There are several agronomic factors, such as drought, leaf blight, and fertilizer application, that affect oil content in the crop (54–56). For example, fertilizer application was shown to improve oil content of the corn grain only slightly. However, an increase in the total grain yield was significant, consequently increasing the oil produced per acre (57). Drought also increased protein content of the grain while decreasing oil content (54). An epidemic of southern maize leaf blight did not affect the starch and protein content of the grain, but the oil content was decreased significantly (55).

Germ is particularly susceptible to mechanical damage, which causes oil deterioration by enzymatically catalyzed fatty acid oxidation and TAG hydrolysis. Fungi are the main factor responsible for the production of FFA in stored grains (6). Damaged pericarp makes the grain susceptible to fungal invasion. Fungi grow preferentially on the germ causing oil depletion. High drying temperatures have been linked to difficult and incomplete grinding, poor germ separation leading to inefficient oil recovery, and high FFA content in the oil (56, 58). When maize is harvested at a moisture content of over 25%, it should be dried at temperatures below 60°C to avoid adverse affects on the oil (59).

**TABLE 10. Physicochemical Properties of Maize Germ Oil.<sup>1</sup>**

Property	
Specific Gravity	0.917–0.925 (20°/20°C)
Refractive Index	1.470–1.473 (25°C) 1.465–1.468 (40°C)
Iodine Value	107–135
Saponification value	187–195
Unsaponifiable (%)	1–3

<sup>1</sup>Adapted from Firestone (26).

### 3.5. Oil Properties and Lipid Composition

Physicochemical properties of maize oil are summarized in Table 10. Specific gravity of maize oil varies between 0.917 and 0.925 at ambient temperature (20°C/20°C). Unsaponifiable content of corn oil is lower (1–3%) than that of the WGO.

**3.5.1. Fatty Acid Composition** Corn oil fatty acid composition can vary depending on the seed type and the climatic conditions. Oil obtained from maize grown in the northern hemisphere had higher iodine value than that of the maize oil grown in the southern hemisphere. U.S. grown corn had higher linoleic acid and lower oleic acid content as compared with South African corn (Table 11) (60). The fatty acid compositions of various grain fractions also show differences (Table 12). Corn germ oil contains more linoleic and oleic acids and a lower amount of palmitic acid than those of the endosperm oil (61). There is a wide variability in fatty acid composition of maize oil. It was found that oleic and linoleic acids content of 788 maize varieties ranged from 14% to 64% and from 19% to

**TABLE 11. Fatty Acid Composition of Maize Oil.<sup>1</sup>**

Fatty Acid	South Africa (%)	U.S.A. (%)
12:0	0.4	0.1
14:0	0.2	0.2
16:0	11.5	11.0
16:1	0.1	—
18:0	2.0	2.0
18:1	38.7	24.1
18:2	44.3	61.9
18:3	1.1	0.7
20:0	0.6	1.7
22:0	0.1	
22:1	0.3	
24:0	0.3	

<sup>1</sup>Adapted from Leibovitz and Ruckenstein (60).

**TABLE 12. Fatty Acid Composition of Corn Grain Fractions.<sup>1</sup>**

Grain Fraction	Fatty Acid Composition (% w/w)				
	16:0	18:0	18:1	18:2	18:3
Germ	11	1	24	63	1
Endosperm (Non-starch lipids + aleurone lipids)	17	2	19	58	4

<sup>1</sup>Adapted from Tan and Morrison (61).**TABLE 13. Fatty Acid Composition of Different Corn Varieties.<sup>1</sup>**

Fatty Acid	High Oleic	Maize	Low Saturate
12:0	—	0–0.3	—
14:0	—	0–0.3	—
16:0	10–16	9.2–16.5	6–8
16:1	—	0–0.4	—
18:0	2	0–3.3	1
18:1	44–64	20–42.2	25–31
18:2	20–38	39.4–65.6	58–64
18:3	0.8–1.0	0.5–1.5	0.8–0.9
20:0	1	0.3–0.7	0.5
20:1	—	0–0.4	—
20:2	—	0–0.1	—
22:0	—	0–0.5	—
22:1	—	0–0.1	—
24:0	—	0–0.4	—

<sup>1</sup>Adapted from Weber (64).

71%, respectively (62). Polyunsaturation of maize oil fatty acids increased 5–8% over the past 20 years (63). Weber discussed the effect of breeding and genetic modifications on the fatty acid composition of maize (6, 64). Maize, high oleic, and low saturate corn oil fatty acid compositions are shown in Table 13 to illustrate the potential of biotechnology for modification of oil composition in plants.

**3.5.2. Acyl Lipids** Acyl lipid composition of maize oil is shown in Table 14. Triacylglycerols are the main components of maize oil (about 90%). Traditional

**TABLE 14. Acyl Lipid Composition of Maize Oil.<sup>1</sup>**

Lipid Type	% of Total Acyl Lipid
Sterol Fatty Esters	2
Triacylglycerols	89
Free Fatty Acids	1
Glycolipids	2
Phospholipids	4

<sup>1</sup>Adapted from Weber (63).

**TABLE 15. Unsaponifiable Composition of Commercial Corn Oils.<sup>1</sup>**

	Mazola	Kroger
Hydrocarbons	0.039	0.039
Sterols	1.42	0.95
Sterol esters	0.37	0.32

<sup>1</sup>Adapted from Worthington (67).

maize germ oil contains approximately 58% triunsaturated followed by about 40% diunsaturated TAG's (60). The majority of the TAG species were PLL (palmitic-linoleic-linoleic) (20%), POL (palmitic-oleic-linoleic) (15%), LLL (linoleic-linoleic-linoleic) (26%), OLL (oleic-linoleic-linoleic) (27%), and OOL (oleic-oleic-linoleic) (14%) (65).

Although FFA content of the oil varies considerably depending on the preharvest and postharvest conditions, it is usually in the range of 1–1.5% of the total acyl lipids. Wet milled corn germ oil contains higher FFA content than that of the dry milled oil, 1.5–4% and about 2%, respectively (7, 66). The PL content of corn germ oil varies with the extraction process. Expelled oil contains about 120 ppm PL as compared with 670 ppm in hexane prepress commercial products (66).

**3.5.3. Unsaponifiable Content** The unsaponifiable content of maize oil ranges from 1% to 3% of the crude oil. Tocopherols and sterols are the main components of the corn oil unsaponifiables (Table 15). Worthington (67) examined the sterol and hydrocarbon content of two commercial corn oils (Table 14). Squalene was the major hydrocarbon found in the corn oil unsaponifiable fraction (35–60%). Weber (68) reported that 2–4% of the carotenoids present in corn kernel are localized in the germ. Lutein and zeaxanthin are the most abundant carotenoids in corn kernels. The levels of carotenoids in corn germ oil are relatively low as a result of low concentrations in the germ and losses during the oil bleaching process.

**3.5.3.1. Tocols** The tocol content of maize oil varies from 26 ppm to 102 ppm of grain (6). Traditionally,  $\gamma$ -tocopherol has been considered the predominant tocol in maize oil. However, some varieties contain  $\alpha$ -tocopherol as a main isomer. Weber (6) reported that  $\gamma$ -tocopherol content of maize inbreds ranged from 36% to 88% (as a percent of total tocopherols), whereas  $\alpha$ -tocopherol content varied from 2.1% to 45%. Milling is known to have a significant effect on the tocopherol content of corn germ oil. Oil from wet milled corn germ contained only 18% of the tocopherol present in whole grain; however, 73% of the tocopherols was recovered from the dry milled corn germ (69).

**3.5.3.2. Sterols** The total phytosterol content (free and esterified phytosterols) of corn germ oil is higher than in most of the other vegetable oils (3, 70). Corn germ oil contains about 1.1% (w/w) phytosterol esters (70). Sitosterol is the major sterol

**TABLE 16. Effect of Processing on Phytosterol Content of Corn Germ Oil.<sup>1</sup>**

	(mg/g)		
	Esterified Sterols	Free Sterols	Total Sterols
Crude corn oil	548	439	976
Physical refining			
Degummed	480	448	910
Bleached	460	461	898
Deodorized	491	257	743
Chemical refining			
Degummed	461	488	930
Neutralized	473	390	869
Bleached	447	388	845
Deodorized	455	336	793

<sup>1</sup>Adapted from Verleyen et al. (72).

in corn germ oil. Sitosterol, campesterol, stigmasterol, and  $\Delta^5$ -avenasterol consist of 90–95% of all corn oil sterols (6, 71). Itoh et al. identified a new sterol, 24-methyl-E-23-dehydrolophenol, in maize germ oil. The effect of corn oil refining process on the free, esterified, and total sterols is shown in Table 16 (72).

### 3.6. Oil Processing

Corn germ oil processing is similar to that for other vegetable oils. Dry germ is initially subjected to high pressure in an expeller that breaks down the cell structure and releases the oil. The expelling operation reduces the oil content of the corn from about 50% to 15%. The residue “germ cake” is flaked then hexane extracted. These processes reduce the oil content of the germ to less than 1.5%. The meal (defatted germ) is used as animal feed. Solvent is removed from the miscella by distillation. The oil is then filtered to remove suspended solid material. Recently, extrusion of corn germ has been employed as a preparation step for solvent extraction to produce a crude corn oil of high quality and high yield (73). Germ quality significantly affects the oil recovery. As an example, blight infection decreased oil recovery from 53% in the uninfected corn to less than 30% in infected corn (74). Also, FFA content of blight-infected crude oil was very high, about 12%, which caused higher refining losses.

According to Mounts and Anderson (5), a few processors use water degumming, otherwise PL are removed during alkali refining. The conventional caustic soda refining has been used by the majority of refiners in the United States for neutralization of crude corn oil. Physical or steam refining can also be used for crude corn oil neutralization. Physical refining is recommended only for high-quality oil, otherwise the oil becomes dark during the physical refining process. A degumming process should precede physical refining. Free fatty acids can also be removed by liquid-liquid extraction or by a new method, which involves solvent extraction in a perforated rotating disc column (75). Undesirable odor and flavor components of

**TABLE 17. Effect of Processing on the Tocopherol, Phosphorous (P), Iron (Fe), and FFA content of Corn Germ Oil.<sup>1</sup>**

	Tocopherols (ppm)			Total	P (ppm)	Fe (ppm)	FFA (%)
	$\alpha$	$\gamma$	$\delta$				
Crude corn oil	266	1009	54	1329	110	0.9	1.3
Physical refining							
Degummed	261	968	52	1281	1	<0.1	1
Bleached	262	963	50	1275	0.3	<0.1	1
Deodorized	142	506	23	671	0.3	<0.1	0.08
Chemical refining							
Degummed	219	842	47	1108	7.5	0.2	0.3
Neutralized	217	811	45	1073	0.6	<0.1	0.2
Bleached	198	798	42	1039	0.1	<0.1	0.1
Deodorized	83	458	19	560	0.1	<0.1	0.09

<sup>1</sup>Adapted from Verleyen et al. (72).

corn oil are removed during the deodorization process at temperatures above 200°C and under 2–10 mm Hg vacuum. The deodorization process removes a significant amount of tocopherol (Table 17) and phytosterols (Table 16) from the oil (3). Pigments are usually removed by treating the oil with acid-activated bleaching clay (4). Dewaxing or winterization is usually carried out at 5–10°C. Wax precipitate that forms during the winterization process is removed by filtration (60). Effect of refining process on the tocol, FFA, iron, and phosphorous content is summarized in Table 17.

A process to extract whole flaked corn using ethanol was reported in 1992 (76, 77). This sequential extraction process (SEP) involved the following steps: (1) extraction of crude oil and removal of water from ethanol and (2) extraction of food-grade protein using an alkali-alcohol mixture. Corn was extracted with 95% ethanol at 76°C in a countercurrent mode while simultaneously dehydrating the ethanol. A small amount of zein was coextracted along with oil (76). The oil extracted using the above explained SEP contained a higher concentration of FFA, DAG, PL, and carotenoids and a smaller amount of TAG and had darker red color than the hexane-extracted corn oil. A modified SEP used a 30% hexane and 70% ethanol mixture at 56°C for corn extraction (77). The modified SEP resulted in products with smaller amounts of FFA, DAG, and PL and larger concentration of TAG and carotenoids than the original SEP oil. However, refining losses for SEP oil was higher than that of the hexane-extracted oil. A U.S. patent also describes recovery of oil and zein by ethanol extraction of dry milled corn followed by a membrane process to fractionate oil and zein (78).

Karlovic et al. (79) examined the aqueous enzymatic extraction of corn germ oil. Hydrothermal pretreatment, grinding, and enzymatic treatment of corn germ improved extraction efficiency. Although the energy cost for enzymatic corn germ oil extraction was lower than that of the conventional extraction, the enzyme cost made the process more expensive (79).

Supercritical fluid extraction of corn germ oil has been also reported (66, 80, 81). Refining losses and FFA content of dry milled corn germ oil extracted with supercritical carbon dioxide (SC-CO<sub>2</sub>) at 5000–8000 psi and 50°C oil was lower and it had a lighter color than those of the commercial expeller-milled crude oil (80). Total unsaponifiable and tocopherol contents were similar for both oils. Wet and dry milled corn germs were also extracted with SC-CO<sub>2</sub> at higher temperatures and pressures 50–90°C and 8000–12000 psi (66). Experimental data indicated that increasing extraction temperature and pressure did not adversely affect the oil quality, i.e., FFA, color, phosphorus refining loss, and unsaponifiable matter content of the extracts did not increase significantly. Although tocopherol content of dry milled corn oil decreased with increasing SC-CO<sub>2</sub> temperature, it was similar to that of the expeller oil. Supercritical carbon-dioxide-extracted wet milled corn oil contained less tocopherol than commercial prepress hexane-extracted oil. Unsaponifiable content of SC-CO<sub>2</sub> extracted wet and dry milled corn germ oil was similar to that of the commercial corn oil, 1.2–1.4%. The SC-CO<sub>2</sub> extraction process yields more TAG in the oil (98.4–99%) than commercially extracted corn germ oil (95.8% and 97.2% for wet and dry milled corn germ oil, respectively) as a result of very low PL content of SC-CO<sub>2</sub>-extracted corn germ oil (1–5 ppm) (66). It was also reported that SC-CO<sub>2</sub>-extracted wet milled corn germ oil had better flavor profile than commercially extracted corn germ oil.

## 4. RICE BRAN OIL

### 4.1. Production and Use

China is the largest producer of rice grain, followed by India, Japan, and the United States. About 600 million metric tons of rice is grown worldwide annually (Table 18). The main consumers of rice bran oil (RBO) are Asian countries, such as Japan, Korea, China, Taiwan, Thailand, Pakistan, and India (82). In the United States RBO production started in the 1950s but was discontinued in 1980s because it appeared uneconomic (8). Interest in RBO was renewed in 1990s due to export opportunities and its nutritional benefits. Rice bran oil has been commercially produced for food use in the United States since 1994.

**TABLE 18. Rice (Paddy) Production.<sup>1</sup>**

Year	Production (x10 <sup>6</sup> Metric Tons)		
	2001	2002	2003
China, Mainland	179	176	166
India	140	108	132
Japan	11	11	10
USA	10	10	9
World	598	570	589

<sup>1</sup>FAOSTAT Database (11).



Rice bran oil has several unique properties that make it very appealing as a specialty oil in niche markets. It has a nut-like flavor and is quite stable after extraction. Rice bran oil contains high levels of bioactive components, such as phytosterols, tocopherols, and tocotrienols, which have nutraceutical value. Although RBO can be used virtually in any application to replace other vegetable oils, it is well suited for use where both functionality and health benefits are important. For example, RBO exhibits excellent frying performance because of its good storage stability and fry life and contributes a pleasant flavor to the fried foods. Rice bran oil is also used in margarines. Its natural tendency to form stable  $\beta'$  crystals and its high palmitic acid content results in a good balance of plasticity, creaminess, and spreading properties. Rice bran oil processed to retain high levels of tocopherols may be used as a natural antioxidant source and can be used as coatings for a wide range of products and snacks such as crackers and nuts. Rice bran oil can be blended with other oils to improve their stability (83). Rice bran oil is an attractive ingredient, which can be incorporated into functional foods and nutraceuticals to provide health benefits. Industrial uses of RBO include an additive to animal feed, glycerine, and soap production. Rice wax can be used in confectionery products, cosmetics, shoe polish, and auto wax (84).

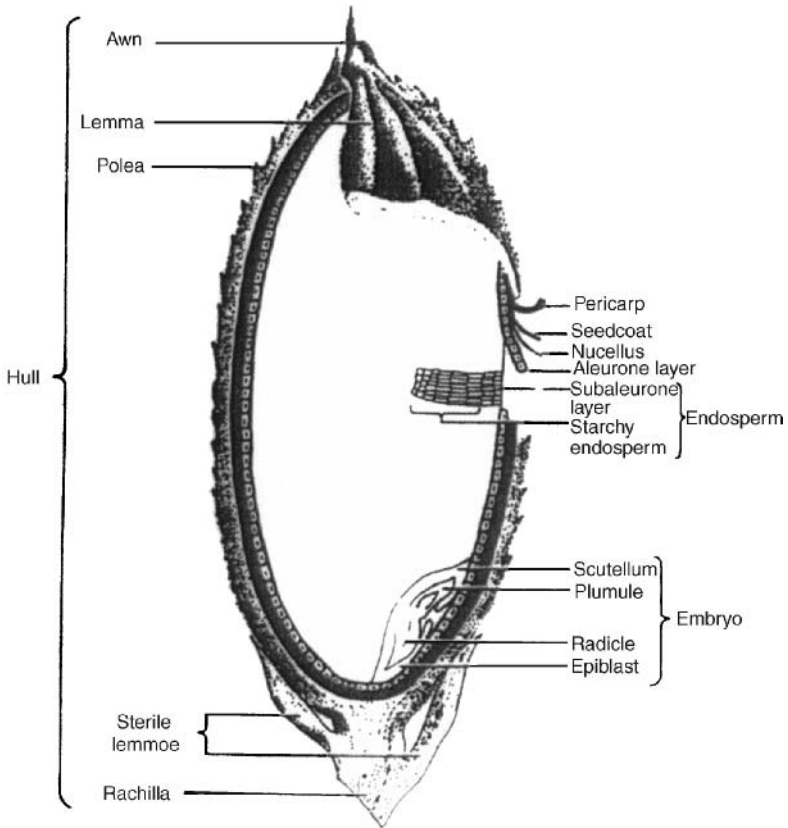
## 4.2. Grain Structure

Rice grain structure is similar to that of most of the other cereal grains. The grain consists of the edible portion, caryopsis or brown rice, and the hull (Figure 3). Removal of the hull from the rice grain by dehulling exposes the caryopsis. Pericarp, seed coat, nucellus, and embryo comprise the bran portion of the rice grain. The bran portion is 5–8% of the brown rice weight (85). The embryo alone is 1–2% of the weight of brown rice. Commercial rice germ includes the outer layers enclosing the embryo and represents about 3% of the grain. Commercial bran usually includes the germ. Thus, commercial RBO contains both bran and germ oil. The lipids are present in the grain in the form of lipid bodies or spherosomes (86). The lipid bodies are very small,  $\leq 0.7 \mu\text{m}$  in all tissues of the embryo. The lipid content of rice germ is higher (34–37%) than the bran fraction (19–26%).

## 4.3. Grain Processing

The purpose of rice milling is to produce an edible, polished product (white rice) from rough rice. Rice bran is a byproduct of rice milling industry. Huller type rice mills, where dehulling and milling is a single processing step, produces “rice mill feed,” whereas “raw rice bran” is produced during a process (cone-type mills) where dehulling is a separate process, which separates germ and bran. Germ contains about four times more oil than the rice mill feed ( $\sim 4\%$ ) (10). The total amount of rice bran available from rice milling operations throughout the world is estimated to be 30 million tons (10).

The rough rice or paddy undergoes a precleaning process prior to milling. Foreign materials, such as stones, mud balls, straw, and weed, are separated with the use of screens, aspirators, and gravity separators. Cleaned rough rice is then



**Figure 3.** Structure of rice grain (85).

dehulled. Most modern rice mills use rubber roll shellers to remove the hull from the kernel so that brown rice is obtained (87). Unshelled kernels are separated by density from the brown rice stream in the paddy separators prior to bran separation. In the United States, rice bran consists of pericarp, aleurone, subaleurone layer, germ or embryo, and a small amount of endosperm. The germ can be removed from the kernel as a relatively intact particle and typically large enough to be retained on a U.S. 18 mesh screen (87). However, germ separation from the bran is not a common practice in the United States. Bran is removed from the milling chamber by air suction and collected in cyclone separators and secondary cloth filters. Then bran is screened through 16–18 mesh to remove fine particles. The amount of lipids present in the bran is inversely proportional to the degree of milling.

Ideally, bran should be stabilized within a few minutes after removal from the kernel. Stabilization process inactivates enzyme lipase that causes rapid hydrolysis of TAG. Three methods developed for brown rice stabilization are: (1) heat denaturation and inactivation of lipases, (2) extraction with an organic solvent to remove

**TABLE 19. Rice Bran Proximate Composition.<sup>1</sup>**

Compound	% (w/w)
Protein	15
Oil	18
Carbohydrates	50
Ash	7
Crude fiber	7
Total dietary fiber	
Soluble	2
Insoluble	26

<sup>1</sup>Adopted from Orthoefer (8).

oil that serves as a substrate for lipase, and (3) ethanolic denaturation and inactivation of lipases and lipase-producing bacteria and mold. The rice bran stabilization process has been discussed in detail by Champagne (88).

#### 4.4. Oil Content

A typical proximate analysis for rice bran is shown in Table 19. Rice bran typically contains 16–32% oil. However, the oil content of the rice kernel and bran might vary for several reasons. An early or late cropping season, crop variety, and degree of milling are some of the factors affecting oil content of rice kernel and the bran (9). Rao et al. (89) examined the oil content of rice bran from four varieties of brown rice grown in India and polished to different degrees (1–10% of its weight as bran). The oil content of bran did not change significantly up to 6% polishing, after which there was a gradual decrease. This phenomenon was explained by the presence of higher amounts of endosperm and inner aleurone material in the bran after more polishing. Recently, 204 genetically diverse rice cultivars were evaluated for oil content in the bran fraction (90). Genotype and environment had a significant effect on the lipid content of rice bran. The variations suggest that breeding material is available for modifying oil content and composition in rice bran (90).

#### 4.5. Oil Properties and Lipid Composition

The typical compositions of crude rice bran and germ are shown in Table 20. Bran and germ oil compositions are very similar. Crude rice oil tends to contain higher levels of non-TAG components, which causes higher oil refining losses than for other vegetable oils. Physical properties of RBO are summarized in Table 21. In the United States, characteristics of quality RBO are described as: maximum 0.1% FFA, maximum peroxide value 1 meq/kg, 0.05% moisture, iodine value 95–110, saponification value 180–195, and Lovibond color value of 3.5R (91). Japanese standards for refined RBO are similar to those in the United States: cloud point,  $\leq 15^{\circ}\text{C}$ ; unsaponifiable matter,  $\leq 5\%$ ; saponification value, 180–195; iodine

**TABLE 21. Physicochemical Properties of Rice Bran Oil.<sup>1</sup>**

Property	
Specific Gravity	0.916–0.921 (25°C/25°C)
Refractive Index	1.470–1.473 (25°C) 1.465–1.468 (40°C)
Iodine Value	92–108
Saponification value	181–189
Unsaponifiable (%)	3–5
Smoke Point	231°C
Fire Point	352°C
Cloud Index	17°C

<sup>1</sup>Adapted from Firestone (26).

value, 92–115; specific gravity (25°C/25°C), 0.913–0.919; refractive index, 1.470–1.473; and cold test at 0°C for 1 h (9).

**4.5.1. Acyl Lipids** The lipids of rice bran and germ are quite similar and mainly consist of TAG, FFA, ASG, PE, and PC (Table 22). TAGs are the main components of the RBO (Table 23). The monoacylglycerol content of RBO is higher than in other vegetable oils (6–7%). The wax content of RBO varies significantly with the variety. Waxy varieties can produce an RBO that contains up to 8% wax. The amount of wax in the RBO can be reduced to 0.5% by adjusting the extraction conditions (i.e., low-temperature extraction) (92). Rice bran oil waxes are esters of C16–C26 fatty acids and saturated C24–C30 fatty alcohols. Rice waxes are

**TABLE 22. Lipid Composition of Rice Germ and Bran.<sup>1</sup>**

Lipid composition (%)	Germ	Bran
NL	91–92	88–90
GL	2–3	4–5
PL	6–7	7–8
Lipid classes (% of total)		
TAG	77–79	75–76
FFA	4	4–5
ASG	1	2
SG	<1	<1
PE	3–4	3
PC	3–4	3–4
LPE	<1	<1
Others	8–9	8–9

<sup>1</sup>Neutral lipids (NL), Glycolipids (GL), Phospholipids (PL), Triacylglycerols (TAG), Free fatty acids (FFA), Acylsterylglucosides (ASG), Sterylglucosides (SG), Phosphatidylethanolamine (PE), Phosphatidylcholine (PC), Lysophosphatidylethanolamine (LPE). Adapted from Juliano (9).

**TABLE 23. Lipids Classes of Rice Bran Oil.<sup>1</sup>**

Lipid Class	Composition (%)
Triacylglycerols	88–89
Diacylglycerols	3–4
Monoacylglycerols	6–7
Free Fatty Acids	2–4
Waxes	3–4
Glycolipids	6–7
Phospholipids	4–5

<sup>1</sup>Adapted from Juliano (9).

classified as soft and hard waxes based on their melting points, 75°C and 80°C, respectively. Two thirds of the waxes are present in polymeric form with the remainder in monomeric form (92). Waxes are separated from the crude oil during the refining process as a result of their adverse effects during frying and opaque appearance in the oil.

**4.5.2. Fatty Acid Composition** The fatty acid content of RBO is mainly palmitic, oleic, and linoleic acid (Table 24). The low linolenic acid content of RBO makes it stable to oxidation. Several studies reported variations in fatty acid composition of RBO (90, 95–96). Goffman et al. (90) studied the fatty acid composition of 204 rice varieties. Genotype and environment significantly affected stearic, oleic, linoleic, and linolenic acids but not palmitic acid content of the RBO. The ratio of saturated to unsaturated acid ratio (S/U) was correlated to the palmitic acid content of the oil. Japonica lines had low palmitic acid content and S/U ratio, whereas Indica lines were characterized by high palmitic acid content and high S/U ratio (90).

**TABLE 24. Fatty Acid Composition of Rice Bran Oil.<sup>1</sup>**

Fatty Acid	(%)
14:0	0.5–0.7
16:0	16–28
16:1	0.5
18:0	2–4
18:1	38–48
18:2	16–36
18:3	0.2–2.2
20:0	0.5–0.8
20:1	0.3–0.5
22:0	0.1–0.5
24:0	0–0.5

<sup>1</sup>Adapted from Juliano (9).

**TABLE 25. Unsaponifiable Composition of Rice Bran Oil.<sup>1</sup>**

Component	%
Hydrocarbons	18
Phytosterols	43
Sterol esters	10
Triterpene alcohols	28
Tocopherols	1

<sup>1</sup>Adopted from Orthoefer (8).

**4.5.3. Unsaponifiables** Rice oil has a significantly higher concentration of unsaponifiables compared with other vegetable oils (Table 25). The bioactive components of rice bran are associated with the oil fraction (97) and concentrated in the unsaponifiable fraction of the oil (8).

**4.5.3.1. Tocols** Along with the protein and edible oil, rice bran is also a rich source of tocopherols (500 ppm-4% bran oil). About 30% of the total tocopherols in RBO are  $\alpha$ -tocopherols. Over 95% of the total tocopherols is contained in the rice germ. The tocopherol content of commercially available rice bran varies significantly (98). In general, increasing degree of brown rice milling resulted in higher tocopherol content in the bran (98).

Rice bran oil and palm oil are the only readily available oils that contain significant levels (about 1000 ppm) tocotrienols (99). Tocotrienols belong to the Vitamin E family and have similar chemical structures. According to Tomeo et al. (100), tocotrienols are powerful antioxidants. Commercially available RBO may contain 980 ppm  $\gamma$ -tocotrienol (101).

**4.5.3.2. Sterols** The unsaponifiable content of RBO is mainly phytosterols (Table 25). These include free sterols, sterol esters, sterylglucosides, and acylsterylglucosides.  $\beta$ -Sitosterol is the main sterol present in RBO. Other sterols present in the RBO include 4-desmethylsterol, 4-monomethylsterol, and 4,4-dimethylsterol (10). Crude RBO contains about 1.5% oryzanol (102). Oryzanol, a major component of rice unsaponifiables, is a group of compounds containing ferulate (4-hydroxy-3-methoxycinnamic acid) esters of triterpene alcohols and plant sterols. Cycloartenol, 24-methylene cycloartenol, campesterol, and  $\beta$ -sitosterol are present as ferulate esters in the RBO. Oryzanol was reported to contribute to the hypocholesterolemic activity of RBO in rats (103) and hamsters (104). Studies carried out with human subjects also support hypocholesterolemic activity of RBO (105). The oryzanol content of the RBO varies with the technique used for oil refining. Crude oil contains  $\sim$ 2% oryzanol. The degumming process reduces the oryzanol content to 1.7%. Physically refined oil contains 1.0–1.5% and alkaline refined oil has 0.1% oryzanol (8).

#### 4.6. Oil Production

A major problem with rice bran oil extraction is the high lipase activity, which results in FFA formation within a few days of milling particularly at high temperature and humidity. Free fatty acid content in rice bran increases during storage, i.e., 2–4% in a fresh crop, 5–8% in 1-year grain, and >10% in a 2-year-old crop (105). Thus, lipase is inactivated to stabilize rice bran prior to oil extraction (88). Heat-stabilized bran may be stored up to three months. However, oil extraction should be carried out within the first month to obtain better efficiency and higher quality oil.

Mechanical expression of rice bran yields less oil, 10–12%, than solvent extraction, 16–18%. Rice bran is treated with steam and dried prior to pressure expression. Prepressing is usually carried out at 70 kg/cm<sup>2</sup> followed by oil expulsion at 105–316 kg/cm<sup>2</sup> (9). As a result of the low yield of oil from mechanical extraction, residual oil in the bran is recovered with hexane. Hexane extraction can be performed by batch or a continuous operation. Continuous operation uses countercurrent flow to improve mass transfer. Solvent extraction at high temperatures results in higher crude oil yield, but the crude oil is of lower quality. A new oil-extraction process, which involves premolding of rice bran at 14% moisture content and ≤40°C followed by hexane extraction at ≤15°C, was reported to yield a light-colored crude oil with no wax (9).

Rice bran oil refining involves very similar unit operations as for other vegetable oils. Alkali refining is widely used for neutralization of RBO. However, some refiners use molecular distillation at high temperature and low pressures. Rice bran oil refining usually involves wax removal, which is usually achieved by gradual cooling of the crude oil in settling tanks followed by filtration or centrifugation of the sludge at low temperature (84). Water is used for phospholipid removal. Colored compounds are removed by using either activated carbon or bleaching earth. Although a common practice for oil deodorization has been steam stripping, modern refiners use steam-vacuum deodorization. According to Orthoefer (8), total losses for crude RBO refining might be as high as 18–22%.

There have been several studies of SC-CO<sub>2</sub> extraction of RBO. Taniguchi et al. (107) noted the presence of oryzanol in SC-CO<sub>2</sub>-extracted RBO, which had a lighter color and was less phosphorous than hexane-extracted oil. Zhao et al. (108) showed that fractions obtained at high extraction pressures contained low FFA, waxes, and unsaponifiables. Ramsay et al. (109), comparing yields and sterol content of the hexane- and SC-CO<sub>2</sub>-extracted RBO, showed that total sterol content of the SC-CO<sub>2</sub>-extracted RBO was less than that found in the hexane-extracted oil. Pilot scale SC-CO<sub>2</sub> extraction of RBO was examined by Shen et al. (110). The same research group also determined the apparent partition coefficients of oil components between the oil and CO<sub>2</sub> phases. A two-stage SC-CO<sub>2</sub> process that involved extraction of RBO in the first stage, followed by continuously feeding the initial extract to a second stage expansion column to achieve further fractionation of the oil components, was also reported by the same group (110). The rate of RBO extraction with SC-CO<sub>2</sub> has been correlated with dimensionless Sherwood, Schmidt, and Reynolds numbers by Kim et al. (111).

Dunford and King (102, 112, 114) developed a phytosterol-enrichment process using supercritical fluid fractionation (SFF) technology. Enrichment of the phytosterol esters was achieved during oil processing rather than by isolation from the byproducts and readdition to the oil. Using a high-pressure-packed fractionation column, the researchers were able to obtain RBO fractions with a similar phytosterol ester content to that in commercially available phytosterol-enriched margarines. Commercial phytosterol-enriched margarines contain mainly fatty acid esters of phytosterols. However, the SFF product contained both fatty acid esters of phytosterols and oryzanol. Higher oryzanol content of the SFF-processed oil is an additional feature of the SFF process. Hexane-extracted RBO was used for this study; however, oil extracted with SC-CO<sub>2</sub> can also be used as a starting material.

## 5. OAT AND BARLEY OIL

The annual productions of barley and oats average about 141 and 25 million metric tons in the world (11), respectively. Like corn, most barley and oat grain is used for animal feed (about 70% of world production) (115). Today, the use of oat and barley in human foods is very limited. However, recent interests in oat- and barley-derived dietary fibers enriched in  $\beta$ -glucan create a great potential for functional foods, nutraceuticals, and other value-added product development from these grains.

### 5.1. Grain Processing

The barley kernel is comprised of the caryopsis and the enclosing hull or husk. Barley hull is strongly attached to the pericarp. Thus, it is very difficult to dehull, instead barley is usually pearled. The caryopsis consists of the pericarp, integuments, aleurone layer, endosperm, and germ or embryo (116). The embryo is located at the attachment end of the caryopsis on its dorsal side. Barley is milled to make blocked barley, pearl barley, barley groats, barley flakes, and barley flour. A barley milling operation consists of the following operations: cleaning, conditioning, bleaching (used in some countries), blocking (shelling), aspiration (husk removal), sifting, cutting (on groat cutter), and pearling (rounding). Barley flakes are made from groats or pearl barley on flaking rolls. Barley flour is produced in a roller mill. Jadhav et al. (117) reported a processing scheme that is used for barley processing by one manufacturer in the United States. This process involved the following steps: carter disc separators, flat stone mill (removes hull tips that makes up 5% of the grain), decorticator I (removes hulls and bran, which is 25% of the grain), decorticator 2 (removes bran and germ), roller milling (grinding and sifting to remove crease, 10% of the grain), and decorticator 3 (removes crease bran, aleurone, and outer endosperm, 10% of the grain). Commercially available barley products are pot and pearled barley, grits, flakes, and malt flour (117).



The oat grain consists of the groat (actual caryopsis of the oat) and a surrounding hull or husk. The use of whole, unmilled oats is limited due to the high cellulose content in the hulls. Thus, the hull is separated and removed by dehulling (shelling) (118). The oat groat contains an active lipase, which has to be inactivated before milling to avoid lipid hydrolysis. A stabilization step to inactivate the enzyme is an essential operation in an oat milling operation. There are two oat milling systems (118). The traditional, or dry-shelling, system consists of the following steps: width grading, stabilization, kiln-drying, length grading, and shelling on stones. The modern green-shelling system involves the following unit operations: width grading, shelling by impact, stabilization, kiln-drying, and length grading. Both milling systems also employ cutting, grinding (for oatmeal, oat flour, and oat bran), steaming, and flaking (for rolled oats). Steel-cut groats, old-fashioned flakes, quick flakes, instant flakes, and flour are all common forms of commercial oat products (119). Based on the American Association of Cereal Chemists' definition, oat bran can be up to 50% of the groat. The FDA also adopted this oat bran definition by some modifications: Oat bran can be up to 50% of the groat, but it has to provide at least 5%  $\beta$ -glucan and 16% total dietary fiber (119).

## 5.2. Lipids

**5.2.1. Barley** The data on barley and oat lipids is very limited in the literature. Barley differs in chemical characteristics, because of genotype and environment and the interaction between the two. Large variations in chemical composition of barley have been reported (120). Oil is a minor component of barley and constitutes 2–7% (w/w, based on dry matter) of the grain weight. The barley varieties Risø 1580 and Hiproly contain more lipids than the average barley (117).

Ko et al. (121) reported the fractional proportions of barley grain as follows: germ 0.3%, endosperm 72.2%, pearling flour 4.1%, bran 12.6%, and hull 10.1% (w/w). Based on this fractionation, the oil content of germ, endosperm, pearling flour, bran, and hull were 13%, 0.7%, 10.7%, 5.6%, and 2.6%, respectively. Similar oil content in barley fractions (bran: 4.6–5.3%, germ: 14.7%) were also reported by Seog et al. (122). However, the oil content of milled barley products may vary depending on the milling system used. The oil contents of milled barley products are shown in Table 26. Barley bran had the highest oil content among the milled

**TABLE 26. Oil Content of Milled Barley Products.<sup>1</sup>**

Products	Oil Content (% w/w, on dry matter basis)
Pearl barley	1.1
Barley flour	1.9
Barley husk	0.3
Barley bran	4.0
Barley dust	2.5

<sup>1</sup>Adapted from Kent and Evers (118).

barley products (118). Wang et al. (123) used a Miag Multomat 8 roller dry mill and a laboratory-type pearler to fractionate Waxbar (two-rowed) and Azhul (six-rowed) waxy hullless barley cultivars. Fractions from the first break flour through the fourth middling were combined, mixed, and designated as flour for the roller mill products. The six roller mill fractions obtained were flour, fifth middling, red dog, reduction shorts, break shorts, and bran. Fifth middling (4.5%, w/w), followed by red dog (3.8%), had the highest oil contents among the fractions obtained from the roller mill. Pearling flour contained a substantially larger amount of oil (8.2%, w/w) as compared with that of the whole grain (2.8%, w/w).

The greatest portion of the lipids in barley kernel is nonpolar lipids (67–78%). The compositions of lipids in the embryonic axis, bran endosperm, and hull fractions of hullless barley caryopses were determined by Price and Parsons (124) (Table 27). Neutral lipids were predominant in all fractions. Phospholipid content of barley hull was lower than that of the bran endosperm and embryonic axis. The hull fraction contained the highest glycolipid amount among the grain fractions.

Linoleic acid (C18:2) was the predominant component of barley neutral lipids (Table 28). Oleic (18:1) and palmitic acid (16:0) were the other major fatty acids in all the barley fractions. A significant amount of polyunsaturated acid, linolenic (18:3), was also detected in all the barley fractions. Arachidic acid (C20:0) was present in measurable amounts in hull fraction of barley.

**5.2.2. Oats** The geographical location and weather can alter the total lipids of oats, although the effect of environment is small compared with that of the varietal effect (125). In general, the oil content of oats ranges from 2% to 11% (125). The higher oil content is associated with *Avena* species other than *Avena sativa*. Oat embryo contains significantly higher oil than that of bran endosperm and hull (Table 27). Oat hull has relatively lower oil content. Practically all the lipid is in

**TABLE 27. Lipid Content and Composition in Grain Fractions of Barley and Oats.<sup>1</sup>**

Grain Fraction/Lipid Type		Lipid Composition (% of total lipid)	
		Barley	Oats
Embryo axis	NL	75.8	87.4
	GL	6.4	3.8
	PL	17.8	8.8
Bran-endosperm	NL	64.4	56.9
	GL	12.5	21.4
	PL	23.1	21.7
Hull	NL	75.9	66.9
	GL	18.2	27.6
	PL	5.9	5.5

<sup>1</sup>Adapted from Price and Parsons (124).

NL: Neutral lipids, GL: Glycolipids, PL: Phospholipids.

TABLE 28. Fatty Acid Composition of Neutral Lipids of Barley and Oats.<sup>1</sup>

Fatty Acid	Barley			Oats		
	Embryonic Axis	Bran-endosperm	Hull	Embryonic Axis	Bran-endosperm	Hull
C12:0	—	—	1.7	—	—	0.6
C14:0	0.2	0.2	5.3	0.1	0.3	3.4
C16:0	19.5	20.6	26.1	17.1	16.4	32.4
C16:1	—	0.1	2.0	0.6	0.1	1.7
C18:0	0.6	1.3	5.4	0.6	1.5	4.7
C18:1	20.4	16.6	21.5	32.1	36.1	18.2
C18:2	49.7	56.8	30.6	45.8	43.9	27.6
C18:3	9.6	4.4	5.0	3.7	1.7	8.8
C20:0	—	—	2.4	—	—	2.6

<sup>1</sup>Adapted from Price and Parsons (124).

the dehulled grain. Although the oil content of the oat milling products varies with the milling system used, a typical range is as follows: oatmeal, 7.5%; rolled oats, 7.6%; oat flour; 7.9%; oat husk; 0.4%; oat dust; 5.0%; meal seeds; 3.8%; and oat feed meal; 1.5% (w/w) (118).

Oat lipids may contain 55–90% neutral lipids depending on the grain fraction (Table 27). Oat bran endosperm contains significantly higher amounts of both glycolipids and phospholipids. The phospholipid content of oat hull is lower than that of the other fractions. There is considerable variation in the reported proportions of individual lipid classes, but the major component of the neutral lipids is TAG (35–85%) (125). The free fatty acid content of oat lipids is in the range of 2–11%.

The fatty acid composition of oat lipids is similar to that of the barley oil (Table 28). Linoleic acid is the major fatty acid in all the grain fractions. Oat lipids are also rich in oleic acid. Palmitic acid is the third major fatty acid in oat oil.

### 5.3. Lipophilic Bioactive Components

**5.3.1. Barley** Barley oil is a good source of tocols. All eight tocol isomers were detected in barley germ (134 ppm), endosperm (12 ppm), bran (117 ppm), and hull (34 ppm) (121). Tocols were uniformly distributed in the grain. Although the level of total tocols was highest in the pearling flour (240 ppm),  $\gamma$ - and  $\delta$ -tocopherols and tocotrienols were not detected in this fraction. Wang et al. (123) also reported that the barley pearling flour contained the highest amount of tocopherols and tocotrienols.  $\alpha$ -Tocopherol was the predominant isomer in the barley germ (92 ppm) and pearling flour (170 ppm), whereas  $\alpha$ -tocotrienol was the most abundant isomer in endosperm, bran, and hulls (121). Peterson (126) could not detect any  $\alpha$ -tocotrienol in the barley germ; however, Ko et al. (121) reported a presence of  $\alpha$ -tocotrienol in the barley germ (8 ppm). The disparity was attributed to different genotypes of barley and different milling processes used for these studies.

The data on phytosterols in barley oil is not available in the literature. The most recent data on phytosterols in barley grain was reported by Piironen et al. (127). Total phytosterol content of barley varies between 40–80 mg/100 g grain. Sitosterol consists about 50% of the total sterols.

**5.3.2. Oats** Oat grain and oat oil contain both tocopherols and tocotrienols. The major tocols are  $\alpha$ -tocopherol and  $\alpha$ -tocotrienol. Significant variations occur in tocol composition of oat oil due to the oat variety and milling process used for the study. Total tocol content of oat oil varies from 175 ppm to 640 ppm (125).  $\alpha$ -Tocopherol (20–82% of the total tocols),  $\alpha$ -tocotrienol (16–51% of total tocols),  $\delta$ -tocotrienols (0–12% of total tocols), and  $\gamma$ -tocopherol (0–36% of total tocols) are the predominant tocol isomers reported in the oat oil.

Presence of phytosterols in oat oil was first reported by Idler et al. in 1953 (128). Oat grain contains 35–60 mg phytosterols/100 g grain (127). Phytosterol content of oat oil varies between 0.19% to 0.32% (125).  $\beta$ -Sitosterol (40–70% of total sterols) is the major phytosterol in oats.  $\Delta^5$ - and  $\Delta^7$ -Avenasterol are the two other phytosterols that present in significant quantities in oats. Campesterol, stigmasterol,  $\Delta^7$ -stigmasten-3 $\beta$ -ol,  $\Delta^7$ -cholesten-3 $\beta$ -ol, and cholesterol were also present in oat grain.

## 6. CONCLUSIONS

Plant germ oils of high nutritional quality can be obtained from cereal grains. Germ oil processing presents a challenge because of the high content of biologically active heat labile components. Physical refining methods improve retention of these compounds in the final product. Currently plant germ oils, except corn germ oil, are produced for specialty markets in the United States. However, increasing consumer demand for healthy food products may change the market trends in the future.

## REFERENCES

1. P. J. Barnes, in P. J. Barnes, ed., *Lipid in Cereal Technology*, Academic Press, New York, 1983, p. 389.
2. P. J. Barnes, *Fette Seifen Anstrichmittel*, **84**, 256 (1982).
3. F. T. Orthoefer and R. D. Sinram, in S. A. Watson and P. E. Ramstad, eds., *Corn: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minnesota, 1987, p. 535.
4. L. R. Strecker, M. A. Bieber, G. Mazza, T. Grossberger, and W. J. Doskoczynski, in T. H. Hui, ed., *Bailey's Industrial Oil and Fat Products, Edible Oil and Fat Products; Oils and Oilseeds*, 5th ed., vol. 2, John Wiley and Sons, New York, 1996, p. 125.
5. T. L. Mounts and R. A. Anderson, in P. J. Barnes, ed., *Lipid in Cereal Technology*, Academic Press, New York, 1983, p. 373.

6. E. J. Weber, in P. J. Barnes, ed., *Lipids in Cereal Technology*, Academic Press, New York, 1983, p. 353.
7. R. A. Reiners and C. M. Gooding, in G. E. Inglett, ed., *Corn: Culture, Processing, Products*, Avi Publishing Co., Westport, Connecticut, 1970, p. 241.
8. F. T. Orthofer, *Food Technol.*, **50**, 62 (1996).
9. B. O. Juliano, in P. J. Barnes, ed., *Lipids in Cereal Technology*, Academic Press, New York, 1983, p. 305.
10. F. T. Orthofer, in T. H. Hui, ed., *Bailey's Industrial Oil and Fat Products, Edible Oil and Fat Products; Oils and Oilseeds*, 5th ed., vol. 2, John Wiley and Sons, New York, 1996, p. 393.
11. FAO. <http://apps.fao.org>.
12. N. O. V. Sonntag, in D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, 4th ed., vol. 1, John Wiley & Sons, New York, 1979, p. 289.
13. P. H. Rao, G. V. Kumar, R. G. C. P. Rao, and S. R. Shurpalekar, *Lebensmittel Wissenschaft und Technologie*, **13**, 302 (1980).
14. D. B. Jones and K. D. Widness, *J. Nutr.*, **31**, 675 (1946).
15. T. S. Kahlon, *Cereal Foods World*, **34**, 872 (1989).
16. N. T. Dunford, *Food Technol.*, **55**, 38 (2001).
17. M. Berdick, *J. Am. Oil Chem. Soc.*, **49**, 406 (1972).
18. T. Schoenberg, *Soaps Cosmetics Chem. Specialties*, **61**, 37 (1985).
19. J. M. Nara, R. C. Lindsey and W. E. Burkholder, *J. Agric. Food Chem.*, **29**, 68 (1981).
20. J. M. Nara and W. E. Burkholder, *Environ. Entomol.*, **12**, 703 (1983).
21. R. E. Angold, in P. J. Barnes, ed., *Lipids in Cereal Technology*, Academic Press, New York, 1983, p. 1.
22. B. Godon, in B. Godon and C. Willm, eds., *Primary Cereal Processing*, VCH Publishers Inc., New York, 1994, p. 61.
23. W. R. Morrison and K. D. Hargin, *J. Sci. Food Agric.*, **32**, 579 (1981).
24. J. J. C. Hinton, *Biochem. J.*, **38**, 214 (1944).
25. W. R. Morrison, S. L. Tan, and K. D. Hargin, *J. Sci. Food Agric.*, **31**, 329 (1980).
26. D. Firestone, *Physical and Chemical Characteristics of Oils, Fats and Waxes*, AOCS Press, Champaign, Illinois, 1999, pp. 152.
27. N. T. Dunford and M. Zhang, *Food Research International*, **36**, 905 (2003).
28. A. P. Simopoulos, *Amer. J. Clin. Nutr.*, **70**, 560S (1999).
29. F. B. Hu, *Nutrition*, **17**, 741 (2001).
30. T. Wang and L. A. Johnson, *J. Amer. Oil Chem. Soc.*, **78**, 71 (2001).
31. J. H. Nelson, R. L. Glass, and W. F. Geddes, *Cereal Chem.*, **40**, 343 (1963).
32. P. J. Barnes and P. W. Taylor, *J. Sci. Food Agric.*, **31**, 997 (1980).
33. K. D. Hargin and W. R. Morrison, *J. Sci. Food Agric.*, **31**, 877 (1980).
34. A. Azzi and A. Stocker, *Progress In Lipid Research*, **39**, 231 (2000).
35. A. Pollard, A. C. Chibnall, and S. H. Piper, *Biochem. J.*, **27**, 1889 (1933).
36. A. P. Tulloch and L. L. Hoffman, *Phytochemistry*, **12**, 2217 (1973).
37. E. Levin, V. K. Collins, D. S. Varner, J. D. Mosser, and G. Wolf, U.S. Patent 3,031,376, 1962.

38. T. Itoh, T. Tamura, and T. Matsumoto, *J. Amer. Oil Chem. Soc.*, **50**, 122 (1973).
39. T. Itoh, *J. Amer. Oil Chem. Soc.*, **50**, 122 (1973).
40. R. J. Anderson, R. L. Shriner, and G. O. Burr, *J. Amer. Oil Chem. Soc.*, **48**, 2987 (1926).
41. B. Kiosseoglou and D. Boskou, *Oleagineux*, **42**, 169 (1987).
42. A. Kuksis, *Biochemistry*, **3**, 1086 (1964).
43. J. C. Drummond, E. Singer, and R. J. MacWalter, *Biochem. J.*, **29**, 456 (1935).
44. O. Gisvold, *J. Amer. Pharm. Assoc.*, **29**, 312 (1940).
45. G. Panfili, L. Cinquanta, A. Fratianni, and R. Cubadda, *J. Amer. Oil Chem. Soc.*, **80**, 157 (2003).
46. Anonymous, *Inform*, **13**, 282 (2002).
47. L. Singh and W. K. Rice, U.S. Patent 4,298,622, (1981).
48. M. Taniguchi, T. Tsuji, M. Shibata, and T. Kobayashi, *Agric. Biol. Chem.*, **49**, 2367 (1985).
49. A. M. Gomez and E. M. de la Ossa, *J. Amer. Oil Chem. Soc.*, **77**, 969 (2000).
50. N. T. Dunford and J. Martinez, 6th International Symposium on Supercritical Fluids, Versailles, France, 2003.
51. C. Willm, in B. Godon and C. Willm, ed., *Primary Cereal Processing*, VCH Publishers, Inc., New York, 1994, p. 319.
52. R. A. Anderson, in G. E. Inglett, ed., *Corn: Culture, Processing, Products*, AVI Publishing Company, Inc., Westport, Connecticut, 1970, p. 253.
53. F. R. Earle, *Cereal Chem.*, **54**, 70 (1977).
54. S. K. Jurgens, R. R. Johnson, and J. S. Boyer, *Agron. J.*, **70**, 678 (1978).
55. J. E. Freeman, *Trans. Amer. Soc. Agric. Engr.*, **16**, 671 (1973).
56. C. Vojnovich, R. A. Anderson, and E. L. J. Griffin, *Cereal Foods World*, **20**, 333 (1975).
57. L. F. Welch, *Agron. J.*, **61**, 890 (1969).
58. M. M. MacMaster, M. D. Finkner, M. M. Holzapfel, J. H. Ramser, and G. H. Dungan, *Cereal Chem.*, **36**, 247 (1959).
59. R. B. Brown, G. N. Fulford, L. Otten, and T. B. Daynard, *Cereal Chem.*, **58**, 75 (1981).
60. Z. Leibovitz and C. Ruckenstein, *J. Amer. Oil Chem. Soc.*, **60**, 347 (1983).
61. S. L. Tan and W. R. Morrison, *J. Amer. Oil Chem. Soc.*, **56**, 531 (1979).
62. M. D. Jellum, *J. Agric. Food Chem.*, **18**, 365 (1970).
63. E. J. Weber, *Cereal Chem.*, **55**, 572 (1978).
64. E. J. Weber, *Biochemical Genetics*, **21**, 1 (1983).
65. N. K. Andrikopoulos, *Food Reviews International*, **18**, 71 (2002).
66. G. R. List, J. P. Friedrich, and D. D. Christianson, *J. Amer. Oil Chem. Soc.*, **61**, 1849 (1984).
67. R. E. Worthington, *J. Amer. Oil Chem. Soc.*, **59**, 317A (1982).
68. E. J. Weber, in S. A. Watson and P. E. Ramstad, eds., *Corn: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minnesota, 1987, p. 311.
69. G. W. Grams, C. W. Blessin, and G. E. Inglett, *Cereal Chem.*, **48**, 356 (1971).
70. R. A. Ferrari, W. Esteves, K. D. Mukherjee, and E. Schulte, *J. Agric. Food Chem.*, **45**, 4753 (1997).
71. T. Itoh, N. Shimizu, T. Tamura, and T. Matsumoto, *Phytochemistry*, **20**, 1353 (1981).

72. T. Verleyen, U. Sosinska, S. Ioannidou, R. Verhe, K. Dewettinck, A. Huyghebaert, and W. De Greyt, *J. Amer. Oil Chem. Soc.*, **79**, 947 (2002).
73. A. Maza, U.S. Patent 6,201,142, (2001).
74. R. A. Anderson, J. J. Ellis, and E. L. J. Griffin, *Cereal Sci. Today*, **17**, 41 (1972).
75. C. G. Pina and A. J. A. Meirelles, *J. Amer. Oil Chem. Soc.*, **77**, 553 (2000).
76. M. P. Hojilla-Evangelista, L. A. Johnson, and D. J. Myers, *Cereal Chem.*, **69**, 643 (1992).
77. F. Feng, D. J. Myers, M. P. Hojilla-Evangelista, K. A. Miller, L. A. Johnson, and S. K. Singh, *Cereal Chem.*, **79**, 707 (2002).
78. M. Cheryan, U.S. Patent 20,020,183,490, (2002).
79. D. J. Karlovic, M. Bocevaska, J. Jakovlevic, and J. Turkulov, *Acta Alimentaria*, **23**, 389 (1994).
80. D. D. Christianson, J. P. Friedrich, G. R. List, K. Warner, E. B. Bagley, A. C. Stringfellow, and G. E. Inglett, *J. Food Sci.*, **49**, 229 (1984).
81. E. Ronyai, B. Simandi, S. Tomoskozi, A. Deak, L. Vigh, and Z. Weinbrenner, *J. Supercritical Fluids*, **14**, 75 (1998).
82. T. S. Kahlon, R. M. Saunders, R. N. Sayre, F. I. Chow, M. M. Chiu, and A. A. Betschart, *Cereal Chem.*, **69**, 485 (1992).
83. J. B. Taylor, T. M. Richard, C. L. Whelm, M. M. Chrysam, M. Otterburn, and G. A. Leveille, U.S. Patent 15,552,167, (1996).
84. S. C. Chang, R. M. Saunders, and B. S. Luh, in B. S. Luh, ed., Avi Publishing Co., Westport, Connecticut, 1980, p. 764.
85. B. O. Juliano, and D. B. Bechtel, in B. O. Juliano, ed., *Rice: Chemistry, and Technology*, American Association of Cereal Chemists, St. Paul, Minnesota, 1985, p. 17.
86. D. B. Bechtel, and B. O. Juliano, *Ann. Bot.*, **45**, 503 (1980).
87. K. L. J. Hargrove, in W. E. Marshall, and J. I. Wadsworth, eds., *Rice Science and technology*, Marcel Dekker, Inc., New York, 1993, p. 381.
88. E. T. Champagne, in W. E. Marshall and J. I. Wadsworth, eds., *Rice Science and Technology*, Marcel Dekker, Inc., New York, 1993, p. 17.
89. S. N. R. Rao, T. K. Ananthachar, and H. S. R. Desikachar, *J. Food Sci. Technol.*, **2**, 115 (1965).
90. F. D. Goffman, S. Pinson, and C. Bregman, *J. Amer. Oil Chem. Soc.*, **80**, 485 (2003).
91. D. R. McCaskill and F. Zhang, *Food Technol.*, **53**, 50 (1999).
92. L. Gingras, *Inform*, **11**, 1196 (2000).
93. J. C. Lugay and B. O. Juliano, *J. Amer. Oil Chem. Soc.*, **41**, 273 (1964).
94. H. Taira, M. Nakagahra, and T. Nagamine, *J. Agric. Food Chem.*, **36**, 45 (1988).
95. H. Taira, *J. Amer. Oil Chem. Soc.*, **66**, 1326 (1989).
96. S. C. Deka, D. R. Sood, and S. K. Gupta, *J. Food Sci. Technol.*, **37**, 217 (2000).
97. R. Sharma and C. Rukmini, *Lipids*, **21**, 715 (1986).
98. A. G. G. Krishna, J. V. Prabhakar, and D. P. Sen, *J. Food Sci. Tech.*, **21**, 222 (1984).
99. R. R. Eitenmiller, *Food Technol.*, **51**, 78 (1997).
100. A. C. Tomeo, M. Geller, T. R. Watkins, A. Gapor, and M. L. Bierebaum, *Lipids*, **30**, 1179 (1995).

101. E. J. Roger, S. M. Rice, R. J. Nicolasi, D. R. Carpenter, C. A. McClelland, and L. J. Romanczyk, *J. Amer. Oil Chem. Soc.*, **70**, 301 (1993).
102. N. T. Dunford and W. J. King, *J. Food Sci.*, **65**, 1395 (2000).
103. G. S. Seetharamiah, T. P. Krishnakantha, and N. Chandrasekhara, *J. Nutr. Sci. Vitaminol.*, **36**, 291 (1990).
104. R. J. Nicolasi, E. J. Roger, D. Kritchevsky, J. Scimeca, and P. Huth, *Artery*, **22**, 266 (1997).
105. G. Yoshino, *Current Therapeutic Res.*, **45**, 543 (1989).
106. K. Yokochi, Rice By-Products Utilization, International Conference, Valencia, Spain, 1974.
107. M. Taniguchi, E. Tsuji, H. Morimoto, M. Shibata, and T. Kobayashi, *Nippon Shokuhin Kogyo Gakkaishi*, **34**, 102 (1987).
108. W. Zhao, A. Shishikura, K. Fujimoto, K. Arai, and S. Saito, *Agric. Biol. Chem.*, **51**, 1773 (1987).
109. M. E. Ramsay, J. T. Hsu, R. A. Novak, and W. J. Reightler, *Food Technol.*, **30**, 98 (1991).
110. Z. Shen, M. V. Palmer, S. S. T. Ting, and R. J. Fairclough, *J. Agric. Food Chem.*, **44**, 3033 (1996).
111. H. Kim, S. Lee, K. A. Park, and I. Hong, *Sep. Purif. Technol.*, **15**, 1 (1999).
112. N. T. Dunford, J. A. Teel, and J. W. King, *Food Research Intenational*, **36**, 175 (2002).
113. N. T. Dunford and J. W. King, *J. Amer. Oil Chem. Soc.*, **78**, 121 (2001).
114. N. T. Dunford and J. W. King, U.S. Patent 6,677,469 B1, (2004).
115. S. Kim and B. E. Dale, *Biomass and Bioenergy*, **26**, 361 (2004).
116. N. L. Kent, *Technology of Cereals*, Pergamon Press, Oxford, United Kingdom, 1983, pp. 154.
117. S. J. Jadhav, S. E. Lutz, V. M. Ghorpade, and D. K. Salunkhe, *Crit. Rev. Food Sci.*, **38**, 123 (1998).
118. N. L. Kent and A. D. Evers, *Kent's Technology of Cereals*, Elsevier Science, Ltd., Oxford, United Kingdom, 1994, pp. 334.
119. K. Salisbury, Grains for the Health of It: Increasing Grains and Whole Grains Consumption as a Strategy for Health, Minneapolis, Minnesota, 2001.
120. A. A. M. Andersson, C. Elfverson, R. Andersson, S. Regner, and P. Aman, *J. Sci. Food Agric.*, **79**, 979 (1999).
121. S-N. Ko, C-J. Kim, H. Kim, C-T. Kim, S-H. Chung, B-S. Tae, and I-H. Kim, *J. Amer. Oil Chem. Soc.*, **80**, 585 (2003).
122. H-M. Seog, M-S. Seo, Y-S. Kim, and Y-T. Lee, *Food Sci. Biotechnol.*, **11**, 623 (2002).
123. L. Wang, Q. Xue, R. K. Newman, and C. W. Newman, *Cereal Chem.*, **70**, 499 (1993).
124. P. B. Price and J. Parsons, *J. Agric. Food Chem.*, **27**, 813 (1979).
125. E. G. Hammond, in P. J. Barnes, ed., *Lipids in Cereal Technology*, Academic Press Inc., London, United Kingdom, 1983, p. 331.
126. D. M. Peterson, *Cereal Chem.*, **71**, 442 (1994).
127. M. L. Piironen, J. Toivo, and A. M. Lampi, *Cereal Chem.*, **79**, 148 (2002).
128. D. R. Idler, S. W. Nicksic, D. R. Johnson, V. W. Meloche, H. A. Schuette, and C. A. Baumann, *J. Amer. Oil Chem. Soc.*, **75**, 1712 (1953).



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## *Oils from Herbs, Spices, and Fruit Seeds*

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### **1. INTRODUCTION**

Edible seed oils are important common food ingredients. Fatty acids are primary nutritional components found in edible seed oils. Growing evidence has suggested that individual fatty acids may play different roles in human health. Diets rich in a specific fatty acid may provide potential prevention of a number of health problems or diseases. For instance,  $\omega 3$  (n-3) unsaturated fatty acids may have health benefits including the prevention of cancer, heart disease, hypertension, and autoimmune disorders. Currently, consumer's growing interest in improving their dietary nutrition is driving the development of novel seed oils having unique fatty acid profiles and other beneficial components, including phytosterols and natural antioxidants. It is the purpose of this chapter to summarize the edible fruit, spice, or herb seed oils with unique fatty acid profiles. Physicochemical properties and other beneficial components of these oils, such as phytosterols and tocopherols, may also be included. The seed oils are presented according to their primary or distinguishing fatty acid (s), including oleic, linoleic,  $\alpha$ -linolenic, and  $\gamma$ -linolenic acids. Seed oils containing only small amounts of beneficial fatty acids but significant quantities of other valuable components (natural antioxidants) are also included.

## 2. EDIBLE SEED OILS RICH IN $\alpha$ -LINOLENIC ACID (18:3n3)

Alpha-linolenic acid (18:3n-3) is an 18-carbon fatty acid with three double bonds at carbons 9, 12, and 15. It is an essential n-3 fatty acid that is a required nutrient for human beings and can be obtained through diets including both plant and animal sources. Alpha-linolenic acid can be converted by elongases and desaturases to other beneficial n-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are implicated in normal brain development, normal vision, and a decreased risk of heart disease. Novel dietary sources of n-3 fatty acids are desired for those who do not consume adequate amounts of fish or fish-based food products rich in long-chain n-3 fatty acids. This section summarized fruit, spice, and herb seed oils rich in  $\alpha$ -linolenic acid (18:3n-3). These include black raspberry, red raspberry, boysenberry, marionberry, blueberry, cranberry, sea buckthorn, basil, and hemp seed oils.

### 2.1. Black Raspberry Seed Oil (*Rubus occidentalis* L., cv Jewel)

Black raspberry is a member of the genus *Rubus* from the Roseacea family, which is also known as caneberries. The majority of black raspberry crops are located in the Northwest region of the United States, predominantly in Oregon. The annual harvests for black raspberries in Oregon in 2002 and 2003 were 3.02 million pounds and 2.70 million pounds, respectively. Nearly 99.5% of the total crop goes into postharvest production (<http://www.nass.usda.gov/or/berries03.pdf>), and seeds are a major byproduct thereof.

The fatty acid profile of two cold-pressed black raspberry seed oils demonstrated high concentrations of both n-3 and total unsaturated fatty acids. The concentration of  $\alpha$ -linolenic acid (18:3n-3) was 35% of total fats, and unsaturated fatty acids comprised 98–99% (Table 1). Linoleic acid was the predominant fatty acid (Table 1); however, the ratios of n-6 to n-3 fatty acids were very low at 1.6:1. The other measurable fatty acids included oleic (18:1n-9) and palmitic (16:0) acids (Table 1). The overall fatty acid composition of black raspberry seed oil was very similar to red raspberry seed oil (1) (Table 1).

### 2.2. Red Raspberry Seed Oil (*Rubus idaeus*)

Red Raspberry is a production crop grown throughout the world, and the total worldwide annual production is typically around 250,000 metric tons (<http://www.oregon-berries.com>). The majority of commercial raspberries are grown in Eastern Europe, followed by Northern and Western Europe, the United States, and Chile. Like black raspberries, red raspberries are also grown in the Northwest region of the United States, and total production in the years 2002 and 2003 was 42.2 metric tons (MT) and 38 MT, respectively (<http://www.nass.usda.gov/or/berries03.pdf>).

Red raspberry seed oils, extracted by either hexane (2) or cold-pressing (3), were examined for their fatty acid compositions. Both methods detected very similar

**TABLE 1. Fatty Acid Compositions (g Fatty Acid/100-g Oil) of Fruit Seed Oils Relatively High in  $\alpha$ -Linolenic Acid (18:3n-3).<sup>a</sup>**

Fatty Acid	Black Raspberry <sup>(1)</sup>	Red Raspberry <sup>(2, 3)</sup>	Boysenberry <sup>(3)</sup>	Marionberry <sup>(3)</sup>	Blueberry <sup>(3)</sup>	Cranberry <sup>(3-5)</sup>	Buckthorn <sup>(6)</sup> <i>sinensis</i>	Buckthorn <sup>(6)</sup> <i>ramnoides</i>	Buckthorn <sup>(7)</sup> <i>mongolica</i>
16:0	1.2–1.6	1.2–2.7	4.2	3.3	5.7	3.0–7.8	7.7–9.6	6.7–8.2	8.6
18:0	trace	1.0	4.5	3.1	2.8	0.2–1.9	2.1–3.3	2.3–4.1	3.3
18:1	6.2–7.7	12.0–12.4	17.9	15.1	22.8	20.0–27.8	12.9–26.1	13.7–20.0	17.9
18:2n-6	55.9–57.9	53.0–54.5	53.8	62.8	43.5	35.0–44.31	38.2–43.6	36.7–43.0	38.6
18:3n-3	35.2–35.3	29.1–32.4	19.5	15.7	25.1	22.3–35.0	20.2–36.3	25.4–36.0	29.1
others	nd	nd	nd	nd	nd	2.58	1.9–2.5	1.8–3.8	2.1
n-6/n-3	1.59–1.63	1.64–1.87	2.75	3.99	1.73	1.16–2.0	1.07–2.00	1.02–1.62	1.33

<sup>a</sup>Black raspberry, Red raspberry, Boysenberry, Marionberry, Blueberry, Cranberry, Buckthorn *sinensis*, Buckthorn *ramnoides*, and Buckthorn *mongolica*, stand for black raspberry, red raspberry, boysenberry, marionberry, blueberry, cranberry, buckthorn *sinensis*, buckthorn *ramnoides*, and buckthorn *mongolica* seed oil, respectively. Numbers correspond to the references cited. “nd” stands for not detected.

fatty acid profiles and high concentrations of  $\alpha$ -linolenic acid, an n-3 fatty acid (Table 1). The crude oil from the hexane extract contained 29.1%  $\alpha$ -linolenic acid and the extra virgin cold-pressed seed oil had 32.4%  $\alpha$ -linolenic acid. Both of these samples were also very comparable in their fatty acid compositions compared with the black raspberry seed oil discussed above (Table 1). In addition to its  $\alpha$ -linolenic acid content, red raspberry seed oil may contain a significant level of tocopherols and other natural antioxidants (2, 3). Total tocopherol was 97-mg/100-g oil and 61-mg/100-g oil in the hexane-extracted and the cold-pressed oils, respectively (2, 3), whereas the antioxidant activity, measured as the oxygen radical absorbing capacity (ORAC), was 48.8- $\mu$ moles trolox equivalents per gram of oil (3). Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, is a water-soluble analog of  $\alpha$ -tocopherol and widely used as a standard antioxidant compound.

### 2.3. Boysenberry Seed Oil (*Rubus* hybrid)

Like the other caneberries (black raspberry, red raspberry, marionberry), boysenberry also prefers the growing conditions found in the Northwest region of the United States. However, aside from Oregon, boysenberry is also grown in Northern California as a production crop. In 2002 and 2003, the total boysenberry production in the United States was 2665 tons and 2350 tons, respectively.

Also, like the other cold-pressed caneberry seed oils, boysenberry seed oil had a high percentage (19.5%) of n-3  $\alpha$ -linolenic acid and a low n-6 to n-3 ratio of 2.8:1. Total unsaturated fatty acids constituted over 91% of the seed oil and polyunsaturated fatty acids (PUFA) were very high at 73.3%, but stearic, palmitic, and total saturated fatty acids were higher than all other caneberry seed oils (Table 1). Interestingly, the boysenberry seed oil demonstrated the best antioxidative potential using the oxygen radical scavenging capacity (ORAC) test compared with eight other seed oil samples, including blueberry, black raspberry, and red raspberry seed oils, which are known to be rich in antioxidants (3).

### 2.4. Marionberry (*Rubus* hybrid) Seed Oil

Marionberry is a blackberry hybrid. It is another member of the caneberry family and is also grown in the Northwest United States, specifically in Oregon. The production in 2002 was 15,000 MT and in 2003 it was 12,910 MT. Marionberry comprises almost one-half of the total caneberry production in Oregon.

In 2004, Parry et al. (3) examined the chemical composition and physicochemical properties of cold-pressed marionberry seed oil. The oil was shown to contain a relatively high percentage of n-3 fatty acids in the form of  $\alpha$ -linolenic acid (15.7%) (Table 1). This amount was lower than that of other caneberry seed oils, including black raspberry, red raspberry, and boysenberry seed oils, tested under the same conditions. The n-6 to n-3 fatty acid ratio was 4:1, which was the highest among the tested caneberry group.

## 2.5. Blueberry Seed Oil (*Vaccinium corymbosum*)

Blueberries are grown in temperate climates throughout the world; however, the largest producers are the United States and Canada. Approximately 42,000 MT are produced annually outside of the United States and Canada. In 2002 and 2003, the United States harvested 87.3 MT and 86.200 MT, respectively. (<http://usda.mannlib.cornell.edu/reports/nassr/fruit/pnf-bb/ncit0104.pdf>, <http://www.ushbc.org/blueberry.htm>).

The cold-pressed blueberry seed oil investigated by Parry and Yu. (3) demonstrated a high concentration of n-3 fatty acids. Alpha linolenic acid was the sole source of the n-3 and comprised 25.1% of the total fatty acids (Table 1). The ratio of n-6 to n-3 fatty acids was 1.7:1. Linoleic acid (18:2n-6) was the most prevalent fatty acid in the blueberry seed oil followed by  $\alpha$ -linolenic, oleic, palmitic (16:0), and stearic (18:0) acids (Table 1). The blueberry seed oil also showed a significantly higher antioxidant capacity compared with marionberry, black raspberry, cranberry, and pumpkin seed oils using the oxygen radical absorbance capacity (ORAC) test. Therefore, blueberry seed oil may serve as an excellent dietary source of n-3 fatty acids and natural antioxidants.

## 2.6. Cranberry (*Vaccinium macrocarpon*) Seed Oil

The North American cranberry, *Vaccinium macrocarpon*, is best adapted to grow at higher latitudes and in bog terrains. It is grown for production in Wisconsin, Maine, New Jersey, Oregon, and Washington in the United States, and British Columbia and Quebec in Canada. Cranberries are also grown in Europe, but are a different species of *Vaccinium*. The total production in the United States for the year 2002 was 284,200 tons and was projected to be 291,500 tons in 2003 (<http://usda.mannlib.cornell.edu/reports/nassr/fruit/zcr-bb/cran0803.pdf>).

Several studies have confirmed that the seed oil from the North American variety of cranberry contains significant levels of  $\alpha$ -linolenic acid. In a U.S. patent, Heeg et al. (4) reported the  $\alpha$ -linolenic acid content of cranberry seed oil to be between 30% and 35% of total fatty acids. In 2003, Parker et al. (5) found 22.3%  $\alpha$ -linolenic acid in the cold-pressed cranberry seed oil, and in 2004, Parry et al. (3) determined the oil to contain 32.0%  $\alpha$ -linolenic acid from two different lots of the seed oil. The ratios of n-6 to n-3 fatty acids in all were low from 1.2:1 to 2:1. Also, all of the studies documented similar ratios among the rest of the common fatty acids found in cranberry seed oil, including, in order of higher amount present: linoleic, oleic, palmitic, stearic, and eicosadienoic (20:2) acids (Table 1). In addition to  $\alpha$ -linolenic acid, cranberry seed oil is rich in natural antioxidants (8). These antioxidants may directly react with free radicals and prevent lipid oxidation in human low-density lipoprotein.

## 2.7. Sea Buckthorn (*Hippophae rhamnoides* L.) Seed Oil

Sea buckthorn is native to Asia and Europe. It is a hardy plant that is also being considered as a major commercial crop in Canada. It has been used in Tibetan, Mongolian, and Chinese traditional medicine for more than 1000 years, and has demonstrated many beneficial health attributes (6). The fruit has a good flavor and is rich in nutrients. The whole berries contain a higher concentration of

Vitamin C than strawberry, kiwi, orange, and tomato, and the fruit also contains a higher concentration of Vitamin E than wheat embryo, safflower, maize, and soybean (9).

In 2001, Yang and Kallio (9) investigated the lipid compositions of two subspecies of *Hippophae rhamnoides* L. The subspecies were *H. rhamnoides* L. "sinensis" and *H. rhamnoides* L. "rhamnoides". Twelve samples of *sinensis* and nine samples of *rhamnoides* were grown at different locations in China and Finland, respectively. Among the twenty-one samples there was some variation in the compositions of the seed oils; however, they all had relatively high percentages of  $\alpha$ -linolenic acid,  $\gamma$ -linolenic acid (18:3n-6), and oleic acid (18:1n-9). All seed oil samples also had an n-6 to n-3 fatty acid ratio under 2:1 (Table 1). Other constituent fatty acids included palmitic, stearic, and vaccinic (18:1n-7) acids. Kallio et al. (7) examined the fatty acid composition of the subspecies *sinensis*, and *mongolica* of *Hippophae rhamnoides* L. Both displayed fatty acid profiles very similar to those found in the previous study. The fatty acid composition of *Hippophae rhamnoides* L. *mongolica* is shown in Table 1.

## 2.8. Basil (*Ocimum* sp.) Seed Oil

Basil is a popular herb grown throughout the world and is an ingredient in many recipes. There are more than 50 species, but sweet basil, *Ocimum basilicum*, is the most common variety (10).

In 1996, Angers et al. (10) investigated the fatty acid composition of the seed oils of four species of basil, including *Ocimum basilicum*, *Ocimum canum*, *Ocimum gratissimum*, and *Ocimum sanctum*. Also, four total different varieties of *Ocimum basilicum* were tested. All samples were compared with flaxseed oil and had similar fatty acid profiles in regard to  $\alpha$ -linolenic, palmitic, and stearic acids. The flaxseed oil had 52%  $\alpha$ -linolenic acid, and the basil seed oils had 57.4–62.5%  $\alpha$ -linolenic acid (Table 2). The n-6 to n-3 fatty acid ratio of the flaxseed oil was 1:3.2, and

**TABLE 2. Fatty Acid Compositions (g Fatty Acid/100-g Oil) of Herb Seed Oils and Other Oils with Relatively High Concentrations of  $\alpha$ -Linolenic Acid (18:3 n-3).\***

Fatty Acid	<i>O. Basilicum</i> (basil) <sup>(10)</sup>	Hemp <sup>(5, 11, 12)</sup>
16:0	6.8–8.8	5.8–6.7
16:1	0.2–0.3	0–0.2
18:0	2.0–2.8	2.6–3.2
18:1	8.7–11.6	9.9–15.6
18:2	18.3–21.7	53.4–60.0
18:3 n-3	57.4–62.5	15.1–19.4
18:3 n-6	0.1–0.3	0–3.6
20:0	< 0.2	0.8–1.0
20:1	trace	nd
others	trace	0–1.8
n-6/n-3	0.3–0.4	2.8–3.5

\**O. basilicum* (basil) and Hemp stand for *O. basilicum* (basil) and hemp seed oil, respectively. Numbers correspond to the references cited. "nd" stands for not detected.

the basil seed oils were 1:1.6–1:3.6. Complete fatty acid profiles are shown in Table 2.

## 2.9. Hemp (*Cannabis sativa*) Seed Oil

Hemp is an ancient crop that is still cultivated by many societies and has many different uses. The fiber from hemp has been used to make rope, paper, and clothing; hemp is used for medicinal purposes, and its seed oil is commercially available (11).

Recent investigations of hemp seed oil (5, 11, 12) reported similar findings in fatty acid compositions. The n-3 fatty acid,  $\alpha$ -linolenic acid, was determined to constitute between 15.1% and 19.4% of total fat (Table 2). Gamma-linolenic acid (18:3n-6) was also detected in two of the studies, and comprised up to 3.6% of total fatty acids (11, 12) (Table 2). The most prevalent fatty acid was linoleic in all of the studies, which was between 53.4% and 60.0% of total fatty acids and was followed by  $\alpha$ -linolenic, oleic, palmitic,  $\gamma$ -linolenic, and stearic acids. Eicosadienoic, arachidic (20:0), and behenic (22:0) acids were also detected in small quantities.

## 3. EDIBLE SEED OILS RICH IN $\gamma$ -LINOLENIC ACID (18:3n6)

Gamma-linolenic acid (18:3n-6) is an important unsaturated fatty acid. It is the precursor for biosynthesis of arachidonic acid that is a precursor for prostaglandin formation. Recently,  $\gamma$ -linolenic acid has been recognized for its potential health benefits in prevention and treatment of cardiovascular disorders, premenstrual syndrome, atopic eczema, rheumatic arthritis, and alcoholism (13, 14). Seed oils of blackcurrant and other *Ribes* species, as well as evening primrose seed oils, are rich sources of natural  $\gamma$ -linolenic acid.

### 3.1. Black Currant and Other *Ribes* Seed Oils

Blackcurrant (*Ribes nigrum*) is cultivated for the production of its berries (15). It is rich in ascorbic acid and exhibited high levels of antioxidant activity (16). Blackcurrant is mainly consumed in the form of juice and the seeds are the byproduct of juice production. Blackcurrant seed oils were analyzed for fatty acid composition, tocopherols, and their prostaglandin E<sub>2</sub> production reduction potential (15, 17–19). Blackcurrant seed oil is an excellent dietary source of both  $\gamma$ -linolenic (18:3n-6) and  $\alpha$ -linolenic (18:3n-3) acids. Gamma-linolenic acid constituted 12–25% of the total fatty acids, whereas  $\alpha$ -linolenic acid comprised the other 10–13% (Table 3). The fatty acid composition was dependent on genotype and growing conditions. The seed oils also had significant levels of tocopherols (18). The total tocopherol content was 1.2–2.5-mg/g oil, with a mean value of 1.7-mg/g oil for ten oil samples. The major tocopherol in the blackcurrant seed oil was  $\gamma$ -tocopherol, but  $\beta$ -tocopherol was not detected in the blackcurrant seed oil. In 1999, Wu et al. (17) investigated the effect of dietary supplementation with blackcurrant seed oil on the immune response of healthy elderly subjects.

**TABLE 3. Fatty Acid Composition (g Fatty Acid/100-g Oil) of Seed Oils Relatively High in  $\gamma$ -Linolenic Acid.\***

Fatty Acid	Blackcurrant <sup>(21)</sup>	Blackcurrant <sup>(19)</sup>	Evening primrose ( <i>Oenothera Spp.</i> ) <sup>(20)</sup>	Evening primrose ( <i>Oenothera biennis</i> ) <sup>(14)</sup>	Evening primrose ( <i>Oenothera lamarckiana</i> ) <sup>(13)</sup>
16:0	5.3	6.0–6.3	7–10	9.1	5.8–7.2
18:0	1.5	1.3–1.6	1.5–3.5	3.1	1.5–3.1
18:1	14.7	8.9–9.6	6–11	17.7	9.2–20.1
18:2n-6	47.0	42.7–43.5	65–80	64.3	62.0–74.6
18:3n-6	12.2	22.0–24.6	8–14	4.9	5.5–9.6
18:3n-3	13.2	10.0–11.5	nd	trace	nd
n-6/n-3	4.5	5.7–6.8	N/A	N/A	N/A

\*Blackcurrant and Evening primrose stand for Blackcurrant and Evening primrose seed oil, respectively. Numbers correspond to the references cited. "nd" stands for not detected, whereas "N/A" stands for not applicable.



The seed oil moderately enhanced immune function through reducing the production of prostaglandin E<sub>2</sub>, suggesting that blackcurrant seed oil may have potential in preventing cancer, cardiovascular disease, and other health problems.

Other *Ribes* species, including *R. grossularia* (red-black gooseberries), *R. grossularia* (yellow gooseberries), *R. nigrum* (blackcurrants), *R. rubrum* (red currants), and *R. nigrum* × *R. hirtellum* (jostaberries), were also examined for  $\gamma$ -linolenic acid concentration and tocopherol content in the seed oils. Among the tested samples, blackcurrant seed oil had greatest level of  $\gamma$ -linolenic acid, and all three species of currant had of total concentration of tocopherols over 1.0 mg/g oil (18).

### 3.2. Evening Primrose Seed Oil

Evening primrose (*Oenothera* spp.) belongs to the Onagraceae family and produces a large number of highly fertile seeds. The roots of evening primrose are used in human diet, whereas its bark, leaves, and essential oil are used for medicinal purposes (<http://botanical.com/botanical/mgmh/p/primro70.html>) (13, 20). Evening primrose seed oil is a natural source of  $\gamma$ -linolenic acid (18:3n-6). Hudson (20) evaluated 192 evening primrose (*Oenothera* spp.) seed oil samples for their fatty acid compositions. The normal range of  $\gamma$ -linolenic acid concentration was 8–14% and the extreme range was 2–20% of total fatty acids, with a median of 10.4% (Table 3) (20). Linoleic acid normally accounted for 65–80% of total fatty acids and the median was 73%, which was as high as that of any known vegetable oil. Another study showed that common evening primrose (*Oenothera biennis*) seed oil contained 4.9% of  $\gamma$ -linolenic acid, along with 64% linoleic acid (14). In addition, the growing conditions were found to alter the  $\gamma$ -linolenic acid content in the seed oil. The concentration of  $\gamma$ -linolenic acid ranged from 5.5–9.6% of total fatty acids (Table 3) (13). Ratnayake et al. (14) reported that evening primrose seed oil from Canada contained 64.3% of linoleic acid and 4.9% of  $\gamma$ -linolenic acid (Table 3). These previous studies indicate that evening primrose (*Oenothera* spp.) seed oil contains a significant level of natural  $\gamma$ -linolenic and oleic acids, and linoleic acid is the predominant fatty acid. This topic is discussed in another chapter in this series.

## 4. EDIBLE SEED OILS RICH IN LINOLEIC ACID (18:2n6)

Linoleic acid (18:2n-6) is an essential fatty acid that must be obtained through diets. In this section, fruit, spice, and herb seed oils rich in linoleic acids are summarized. These seed oils include watermelon, melon (*Cucumis melo* and *Colocynthis citrullus*), goldenberry, grape, rose fruit, paprika, red pepper, onion, black cumin, and Onagraceae seed oils. Several seed oils may be listed in other sections if they contain significant level of a special fatty acid. For example, pumpkin seed oils rich in both oleic acid and linoleic acid, are listed under the section named, “Edible seed oils rich in oleic acid (18:1n-9).”

#### 4.1. Watermelon (*Citrullus vulgaris*) Seed Oil

Watermelon (*Citrullus vulgaris*) is taxonomically classified as a member of the Cucurbitaceae family, which is also known as the gourd family. Other gourds include pumpkins, cucumbers, squash, and other melons. It prefers warm climate growing conditions and is produced worldwide where conditions permit. Over 1200 varieties have been cultivated and about 250 varieties are grown in North America, <http://www.watermelon.org/index.asp?a=dsp&htype=about&pid=39>. The world's production of watermelons in 2002 was over 81 million metric tons (MMT) and approximately 71% of that was grown in China. The U.S. production in both 2002 and 2003 was over 1.7 MMT (<http://usda.mannlib.cornell.edu/reports/nassr/fruit/pvg-bban/vgan0104.txt>). Watermelon seeds are consumed as snack food worldwide and are used to prepare edible oil in some countries.

Watermelon seed oil was prepared and evaluated for its physicochemical properties (22, 23). The seed oil consisted of 59.6% linoleic acid (18:2n-6) and 78.4% total unsaturated fatty acids (Table 4). The predominant fatty acid in the oil was linoleic acid, which was followed by oleic, palmitic, and stearic acids. Linolenic, palmitoleic, and myristic acids were minor constituents. The refractive index, acid value, peroxide value, and free fatty acids of watermelon seed oil were determined to be 1.4696 (25°C), 2.82 (mg KOH/g oil), 3.40 (mequiv oxygen/kg oil), and 1.41 (% as oleic acid), respectively. The saponification value of watermelon seed oil was 201 (mg KOH/g oil), and its iodine value was 115 (g iodine/100-g oil), which was significantly higher than pumpkin at 109 (g iodine/100-g oil) (22, 23).

#### 4.2. Melon (*Cucumis melo*) Seed Oil

Melon, *Cucumis melo*, is a member of the Cucurbitaceae family and grows best in tropical regions. The pulp of the fruit has pleasant flavor and taste, and the seeds are generally treated as waste; however, medicinal effects have been reported for the seeds (24, 25). Hexane-extracted seed oil of *Cucumis melo* hybrid AF-522 was determined to contain 64 g of linoleic acid per 100 g of total fatty acids (Table 4) (24). Significant amounts of oleic, palmitic, and stearic acids were also detected in the melon seed oil. The specific gravity (28°C), refractive index (28°C), and iodine value of the seed oil were 0.9000, 1.4820, and 112, respectively, under the experimental conditions (24). Earlier in 1986, Lazos (25) extracted the oil from *Cucumis melo* seeds and examined its physicochemical properties (25). Linoleic acid was the primary fatty acid and accounted for 64.6% of the total fat (w/w), along with 20.1% oleic acid, and 14.7% total saturated fatty acids (Table 4). Iodine value and refractive index (40°) of the seed oil were 124.5 and 1.4662, respectively.

#### 4.3. Melon (*Colocynthis citrullus* L.) Seed Oil

*Colocynthis citrullus* L (melon) is a tropical vine that is native to West Africa. The flesh from the fruit of this melon is bitter and inedible; the edible part of the fruit is the seed (33). Nwokolo and Sim (26) examined the fatty acid composition of

**TABLE 4. Fatty Acid Composition (g Fatty Acid/100-g Oil) of Fruit Seed Oils Relatively High in Linoleic Acid.\***

Fatty Acid	Watermelon <sup>(22, 23)</sup>	Melon ( <i>cucumis melo</i> ) <sup>(24, 25)</sup>	Melon ( <i>Colocynthis citrullus</i> L.) <sup>(26, 27)</sup>	Goldenberry <sup>(28)</sup>	Grape <sup>(29, 30)</sup>	Rose <sup>(31)</sup>	Paprika <sup>(22, 23, 32)</sup>
16:0	11.3	9.0–9.5	11.8–12.1	7.3	5.8–14.2	1.7–3.1	11.2–13.8
18:0	10.2	4.9–5.6	9.0–10.7	2.5	≤ 8.6	1.7–2.5	3.2–3.7
18:1	18.1	19.4–20.1	13.5–14.5	11.7	13.7–31.9	14.7–18.4	9.8–14.6
18:2n-6	59.6	64.1–64.6	57.7–65.4	76.4	50.1–77.8	48.6–54.4	67.8–74.4
18:3n-3	0.4	0.2–0.3	≤ 2.1	0.3	≤ 5.0	16.4–18.4	nd
Total saturated	21.5	14.7–15.2	21.1–25.3	11.9	8.4–14.4	11.6–18.1	15.0–17.6
Total unsaturated	78.4	84.4–85.1	74.6–77.5	88.1	85.5–91.5	81.8–88.3	82.5–84.9

\* Watermelon, Melon (*Cucumis melo*), Melon (*Colocynthis citrullus* L.), Goldenberry, Grape, Rose, and Paprika stand for Watermelon, Melon (*Cucumis melo*), Melon (*Colocynthis citrullus* L.), goldenberry, grape, rose, and paprika seed oil, respectively. Numbers correspond to the references cited. “nd” stands for not detected.

*Colocynthis citrullus* seed oil and found that it contained a relatively high percentage of linoleic acid that accounted for 57.7% of total fatty acids (Table 4) (26). Oleic acid was the second major fatty acid (14.5%). The seed oil contained about 25.3% saturated fatty acids (Table 4). Moussata and Akoh (27) also reported a similar fatty acid profile of *Colocynthis citrullus* L. seed oil. The primary fatty acid was linoleic acid, contributing 65.4% of total fats. The other significant fatty acids included oleic (13.5%), palmitic (12.1%), and stearic (9.0%) acids (Table 4).

#### 4.4. Goldenberry (*Physalis peruviana* L.) Seed Oil

Goldenberry, (*Physalis peruviana* L.), also known as cape gooseberry, is a perennial native to the Andes. It is also cultivated in the United States, South Africa, East Africa, India, New Zealand, Australia, and Great Britain (34). It is related to both tomatoes and chile peppers and prefers growing in well draining soils like tomatoes. Goldenberry has a pleasant flavor that is similar to tomatoes and is eaten in many ways, including in salads, cooked dishes, chocolate covered desserts, jams, preserves, and natural snacks (28). The fruit is an excellent source of Vitamins A and C as well as minerals. Goldenberry seed oil was prepared by extracting lyophilized ground seed meal with chloroform-methanol and was characterized for fatty acid composition (28). The fatty acid composition of the seed oil is shown in Table 4. Linoleic acid was the predominant fatty acid and constituted 76.1% of total fat. Combined monounsaturated fatty acids were 12.2%, linolenic acid was 0.33%, and total polyunsaturated fatty acids were 76.1%. These data suggest that goldenberry seed oil may serve as an excellent dietary source for linoleic acid, the essential n-6 fatty acid, and may be a good choice for consumers seeking a greater intake of total unsaturated fatty acids.

The fat-soluble Vitamins E and K, carotene, and phytosterols were also detected in the goldenberry seed oil (28). Total tocopherols were 29.7 mg/g oil, including 0.9-mg  $\alpha$ -, 11.3-mg  $\beta$ -, 9.1-mg  $\gamma$ -, and 8.4-mg  $\delta$ -tocopherols. The total Vitamin K content was 0.12-mg/g oil, and the  $\beta$ -carotene concentration was 1.30-mg/g oil. In addition, significant levels of phytosterols were also detected. The major phytosterol in the goldenberry seed oil was campesterol, having a concentration of 6.5-mg/g oil. Other phytosterols, including ergosterol, stigmasterol, lanosterol,  $\beta$ -sitosterol,  $\Delta$ 5-avenosterol, and  $\Delta$ 7-avenosterol, were also detected in the seed oil.

#### 4.5. Grape Seed Oil (*Vitis* spp.)

World grape production was 61.2 million tons in 2001 (<http://www.winetitles.com.au/awol.overview/world.asp>). Grape seeds are byproducts from the manufacturing of grape juice, jam, jelly, and wine. In 1998, Abou Rayan et al. (29) investigated the characteristics and composition of Egyptian-grown Cabarina red grape seed oil. Crude grape seed oil was extracted with hexane at room temperature. Linoleic acid was the major fatty acid detected and comprised more than 50% of

the total fatty acids (Table 4) (29). Oleic acid was the second major fatty acid in the seed oil, along with significant levels of palmitic and stearic acids. This finding is consistent with a previous observation in which linoleic acid accounted for 62% of the total fatty acids in grape seed oil (Table 4) (30). Iodine value (IV) and peroxide value (PV) were also determined according to the methods described in AOCS, 1983. The measured IV was 130-g iodine/100-g oil, and the PV was determined to be 2.92-mequiv peroxide/kg oil.

#### 4.6. Rose Fruit (*Rosa canina* L.) Seed Oil

Rose, *Rosa canina* L., also known as dogberry or hop fruit, is in the Rosaceae family. The fruit of this particular species of rose is generally used to prepare a stew. The seeds from *Rosa canina* L. were investigated for their chemical composition and nutritional values for medicinal purposes. Seed oils were prepared from fruits grown at three locations in Turkey and evaluated for their fatty acid composition (31). Linoleic acid was the primary fatty acid detected, which ranged from 48.6–54.4% of total fatty acids, followed by  $\alpha$ -linolenic acid (16.4–18.4%) and oleic acid (14.7–18.4%) (Table 4). The seed oil contained approximately 85% total unsaturated fatty acids, indicating that *Rosa canina* L. seed oil may be an excellent source for unsaturated and essential fatty acids.

#### 4.7. Paprika (*Capsicum annuum*) Seed Oil

Paprika (*Capsicum annuum*) is a commonly used flavor enhancer, and following production, the seeds are treated as waste. Paprika seed oils have been evaluated for their physicochemical properties (22, 23, 32). Paprika seed oil contained more than 82% of total unsaturated fatty acids, with polyunsaturated fatty acids comprising 67.8% of total fatty acids (Table 4) (22, 23). Oleic acid was the second major fatty acid at approximately 15% of the total. This fatty acid profile was consistent with a previous observation by Domokos et al. (32) on the fatty acid profile of Hungarian paprika seed oils. Linoleic acid comprised 74.4% of the total fat, whereas oleic and palmitic acid made up 9.8% and 11.2% of total fat, respectively (32). The paprika seed oil was determined to contain 870 mg/kg oil total tocopherols, 380 mg/kg oil carotenoids, and 0.92% phytosterols (32).

#### 4.8. Apple Seed Oil

In 1997, the production of apples was 44.7 MMT worldwide, and 84% of that was processed (<http://www.geocities.com/perfectapple/prod.html>). In 2000–2001, the worldwide apple production reached a record high of 48 MMT (<http://www.fas.usda.gov/http/circular/2003/3-7-03%2520Web%2520Art.%Updates//World%2520Apple%2520Situation%25202002-03.pdf>). Apple seeds are a byproduct of processing. In 1971, Morice et al. (35) investigated the seed oils from three different varieties

**TABLE 5. Fatty Acid Composition (g/100-g Fatty Acids) of Apple Seed Oils.**<sup>(35)</sup>

Fatty Acid	Granny Smith	Sturmer	Dougherty	Golden Delicious
16:0	6.8–7.1	4.8–6.4	5.7–6.8	8.5
16:1	0.1–0.2	0.1	0.1–0.2	0.5
18:0	1.0–2.1	1.5–2.5	1.3–2.1	nd
18:1	24.4–27.4	32.8–36.6	34.6–42.1	31
18:2	62.1–64.1	52.1–58.3	48.2–56.1	59
18:3	0.2–0.4	≤ 0.5	≤ 0.6	0.5
20:0	0.6–1.1	0.7–1.7	0.6–0.9	0.5
20:1	0.2–0.3	0.2–0.4	0.2–0.3	nd
20:2	0.1–0.7	0.1–0.7	0.0–0.3	nd
22:0	0.1–0.2	0.1–0.3	0.1	trace

<sup>a</sup>Granny Smith, Sturmer, Dougherty, and Golden Delicious stand for seed oil of four varieties of apple. Numbers correspond to the references cited. "nd" stands for not detected.

of apples: Granny Smith, Sturmer, and Dougherty, and compared them with the seed oils prepared from other apple varieties. The results showed similarities in the fatty acid profiles among the varieties (Table 5). Oleic and linoleic acids consisted of 85–95% of the total fatty acids in all tested samples (35). The investigators also examined other physicochemical properties of apple seed oils. The Granny Smith apple seed oil had an iodine value of 127-g iodine/100-g oil; the Sturmer had an IV of 122.4-g iodine/100-g oil, and the Dougherty's IV was 119-g iodine/100-g oil. Apple seed oils may be useful as a dietary source for linoleic and oleic acids.

#### 4.9. Red Pepper Seed Oil

Chili peppers, *Capsicum sp.* are members of the Solanaceae family. They originated in South America but are now grown worldwide. They are eaten in many dishes throughout the world and provide the feeling of "hotness" when eaten. There is a very large range of hotness among the *Capsicum* species, which depends on their concentration of capsaicin. Some selected peppers ranging from lowest in concentration of capsaicin include bell, Anaheim, jalapeno, Hungarian wax, serrano, cayenne, and habanero. Red pepper seeds are byproducts from the production of red pepper powder. For centuries in South America, peppers have been used to treat such ailments as gastrointestinal disorders, and it is also thought to benefit those suffering from circulatory diseases. Capsaicin, the spicy chemical in peppers, has also been shown to be a potent anti-inflammatory in vivo (36).

The roasted red pepper seed oil contained an extremely high concentration of linoleic acid, approximately 74%, and a high total unsaturated fat level (Table 6) (37). The fatty acid profile was very similar to that of both goldenberry seed (*Physalis peruviana* L.) and safflower oils (36). The iodine value of roasted red pepper seed oil was determined to be 137-g iodine/100-g oil. This shows that there is a high degree of unsaturation in the oil. Oxidative stabilities of the roasted red

**TABLE 6. Fatty Acid Composition (g Fatty Acid/100-g Oil) of Herb Seed Oils Relatively High in Linoleic Acid.\***

Fatty Acid	Red Pepper <sup>(37)</sup>	Onion <sup>(38)</sup>	Onion <sup>(3)</sup>	Black Cumin <sup>(39)</sup>	Onagraceae <sup>(40)</sup>
16:0	13.4	9.1	5.6–6.2	13.0–13.1	8.0–10.9
18:0	2.1	4.4	0.6–3.5	3.2	2.4–3.5
18:1	10.2	34.3	26.7–30.1	24.0	8.7–13.1
18:2n-6	73.9	44.6	63.7	57.0–57.3	71.5–80.0
18:3n-3	0.4	0.3	nd	nd	nd

\*Red pepper, Onion, Black cumin, and Onagraceae stand for red pepper, onion, black cumin, and Onagraceae seed oil. Numbers correspond to the references cited. "nd" stands for not detected.

pepper seed oil were tested at different roasting times. As roasting time increased, the oxidative stability of the oil increased significantly.

#### 4.10. Onion Seed Oil

Onion (*Allium cepa*) seeds contained about 23.6% crude fat. The seed oil was analyzed for its chemical composition. The onion seed oil contained 44.6% linoleic acid and 34.3% oleic acid (Table 6) (38). The total unsaturated fatty acids comprised of 79% of the oil. A greater concentration of linoleic acid was determined in the cold-pressed onion seed oil obtained from Botanical Oil Co. (Spooner, WI). Linoleic acid accounted for 63.7% of total fatty acids, and oleic acid ranged from 26.7–30.1%. The total unsaturated fatty acids were about 90% (3). In summary, onion seed oil may serve as a dietary source of essential n-6 fatty acid and oleic acid.

#### 4.11. Black Cumin (*Nigella sativa* L.) Seed Oil

Black cumin (*Nigella sativa*) is an annual spicy herb. It has been used for many years as a food preservative and a traditional medicine for protection against and a therapeutic remedy against a number of health disorders (41). Black cumin seed and its oil have also been used for medicinal purposes (39). According to Ramadan and Mörsel (39), the seed contained about 28–35% oil. Black cumin seed oil contained a relatively high level of unsaturated fatty acids (~84%) of the total fatty acids (Table 6). The major fatty acid in the seed oil was linoleic acid, which accounted for about 57% of the total fatty acids, followed by oleic acid from 23.9–24.1%, along with a small amount of palmitic and stearic acids. However, the iodine value of the seed oil was only 48.4-g iodine/100-g oil, which is much lower than the expected value relating to the level of unsaturation.

#### 4.12. Seed Oils of Some Onagraceae Rich in Linoleic Acid

Onagraceae contains about 36 genera and 300 species; it can grow in mud, sand, rocks, or on grassy plains and occurs chiefly in the temperate zone of the New

World, primarily on the Pacific coast. Family members include evening primrose, fushia, suncups, willowherb, and clarkia (<http://1.1911encyclopedia.org/O/ON/ONAGRACEAE.htm>).

The seeds of selected Onagraceae, including *Oenothera picensis*, *O. indecora*, *Ludwigia longifolia*, and *O. L. peruviana* were analyzed for their physicochemical characteristics (40). Linoleic acid was the predominant fatty acid in all tested seed oils, comprising 71.5–80.0% of the total fatty acids (Table 6) (40). These Onagraceae seed oils may be excellent dietary sources of the essential n-6 fatty acid (18:2n-6).

## 5. EDIBLE SEED OILS RICH IN OLEIC ACID (18:1n-9)

Oleic acid is an n-9 monounsaturated fatty acid (MUFA). Growing evidence suggests that diets rich in oleic acid may serve as an alternative choice to a low-fat blood cholesterol reducing diet, modulate immune function, and may delay the development of atherosclerosis (42–44). Oleic acid is the predominant fatty acid in olive, canola, peanut, and specially produced sunflower seed oils. Oleic acid is not an essential fatty acid; it is synthesized in vivo through the desaturation of stearic acid (18:0). Oleic acid is also rich in a number of other edible oils, including mango, cherry, date, pumpkin, naked seed squash, fluted pumpkin, carob bean germ, American ginseng, *Khaya senegalensis*, and *Moringa oleifera* seed oils.

### 5.1. Mango Seed Kernel Oil

Mango seed kernels contain about 4–12% total fat (45–47). Mango seed kernel oil is rich in oleic acid (Table 7), and exhibited 42% (47), 34–59% (45), and 41–44% of total fatty acids (46). Stearic acid is the other major fatty acid in mango seed kernel

**TABLE 7. Fatty Acid Composition (g/100-g Fatty Acids) of Fruit Seed Oils Rich in Oleic Acid.\***

Fatty Acid	Mango <sup>(45–47)</sup>	Cherry <sup>(48)</sup>	Date <sup>(49)</sup>	Fluted Pumpkin <sup>(26)</sup>	Carob Bean Germ <sup>(50)</sup>
12:0	nd	nd	16.9–17.8	nd	nd
14:0	nd	nd	10.8–12.1	0.6	< 0.1
16:0	6–18	6.8–9.4	10.2–10.4	17.1	7.8–14.2
16:1	nd	0.4–0.6	0.2	nd	nd
18:0	26–57	1.6–2.1	2.8–2.8	15.0	3.0–10.0
18:1	34–59	23.9–37.5	43.5–45.0	35.4	20.4–38.5
18:2n-6	1–13	40.0–48.9	8.7–8.2	27.1	43.6–59.2
18:3n-3	nd	< 1	0.6	1.2	0.3–1.3
20:0	< 4	< 1.3	0.5–0.6	1.7	nd
Other FA	nd	10.3–13.3	3.6–4.1	nd	nd

\*Mango, Cherry, Date, and Fluted pumpkin stand for mango, cherry, date, and fluted pumpkin seed oil, respectively. Carob bean germ stands for carob bean germ oil. Numbers correspond to the references cited. "nd" stands for not detected.



oil and may account for up to 57% of the total fat. In addition, palmitic and linoleic acids were detected in the oil along with trace amounts of  $\alpha$ -linolenic acid (45, 47).

## 5.2. Cherry Seed Oil

The cherry tree (*Prunus avium* L.) is a member of the Rosaceae family. Cherry seed contains about 18% oil on a dry weight basis (48). Significant levels of oleic acid were detected in the cherry seed oils prepared by hexane extraction using a Soxhlet apparatus. Oleic acid comprised 24–38% of the total fatty acids from three different varieties of cherry fruits (Table 7) (48). Linoleic acid was the major fatty acid in the cherry seed oil, and ranged 40–49% in the seed oil, along with  $\alpha$ -eleostearic (18:n-5), palmitic, stearic, arachidonic, and  $\alpha$ -linolenic acids (Table 7). alpha-eleostearic acid ( $\Delta^{9c,11t,13t}$ ), comprising 10–13% of cherry seed oil, is a conjugated isomer of  $\alpha$ -linolenic acid ( $\Delta^{9c,12c,15c}$ ). alpha-eleostearic acid was not detected in other previously studied seed oils from prunoids including peach, apricot, and plum seed oils.

## 5.3. Date Seed Oil

Dates (*Phoenix dactylifera* L.) are popular in most Middle Eastern countries and serve as a major source of food and nutrients (51, 52). Oil contents and fatty acid profiles of date seeds may vary among individual varieties. Date seeds contained 20–24% total fat (49). Oleic acid was the primary fatty acid in the date seed oil and had a concentration of 43.5–45% of total fatty acids. This was followed by lauric (12:0), myristic (14:0), palmitic (16:0), linoleic (18:2n6), capric (10:0), and stearic (18:0) acids along with trace amounts of other fatty acids (Table 7). Date seed oil may serve as an excellent dietary source of oleic acid with a minor amount of linoleic acid.

## 5.4. Fluted Pumpkin (*Telfaria occidentalis*) Seed Oil

The fluted pumpkin (*Telfaria occidentalis*) is a tropical gourd native to West Africa. It is taxonomically classified as a member of the Cucurbitaceae family. The fruits are very large and weigh up to 13 kg, but only the seeds are edible (33). The seeds are very rich in both protein and fat, containing approximately 28% and 55%, respectively, from whole oven-dried fluted pumpkin seeds (26). The fatty acid profile of fluted pumpkin seeds demonstrated a high oleic acid content of 35.4% and a total saturated fatty acid concentration over 34% (Table 7) (26). Significant level of linoleic acid (18:2n-6) was also detected in the seed oil.

## 5.5. Carob Bean Germ Seed Oil

Carob (*Ceratonia siliqua* L.), a tree in the Leguminosae family, is widely cultivated in the south and east of the Mediterranean region. The pulp tastes sweet and is used as a food source (50). The seed germ contains about 5% oil. Oleic acid

contributed to 20–39% of the total fatty acids, but only a small portion of that was on the sn-2 position of the triacylglycerol. Linoleic acid ranged 44–59%, whereas palmitic and stearic acids accounted for 8–14% and 3–10% of the total fatty acids, respectively, along with minor amount of linolenic and myristic acids (Table 7). In addition,  $\beta$ -sitosterol was the primary sterol compound and contributed to 74% of the total sterols. Other sterol compounds included stigmasterol (17% of total sterol), campesterol (6%), and cholesterol (4.4%).

## 5.6. Pumpkin (*Curcubita* sp.) Seed Oil

Pumpkin, *Curcubita* sp., is a member of the gourd family, Cucurbitaceae, that also includes melons, cucumbers, squash, and gac. In 2003, the United States' production of pumpkins was approximately 335,000 MT (<http://usda.mannlib.cornell.edu/reports/nassr/fruit/pvg-bban/vgan0104.txt>). In some mid-eastern African countries, dried pumpkin seeds have been used to treat tapeworm when eaten on an empty stomach (53). Also, for many years in Europe, pumpkin seeds have been used as a remedy for micturition. Pumpkin seed oil has also shown possible beneficial affects in retarding the progression of hypertension (54), potential anti-inflammatory activity in arthritis (55), and may be effective in reducing the risk of bladder-stone disease (56).

The fatty acid compositions of the seed oils prepared from a combination of two different pumpkin species (*pepo* and *mixta*) are shown in Table 8 (25). The seed oil contained 37.8% oleic acid and 43.1% linoleic acid and was fairly high in unsaturated fats (81%). Among the four pumpkin seed oil samples analyzed by Spangenberg and Orgrinc (57), oleic acid content was consistent and ranged 30.2–33.9% of total fatty acids (57), along with 24.5–47.9% linoleic acid (Table 8). In addition, the oil of unroasted pumpkin seed kernel (*Cucurbita mixta*) contained 21.4% oleic acid and 58.9% linoleic acid (58). A recent study of pumpkin seed oil detected 55.6% linoelic acid and 20.4% oleic acid in the total fatty acids, with a total unsaturated fatty acid concentration of 76.5% (22, 23). The iodine value for the pumpkin seed oil was 103-g iodine/100-g oil (25), and the refractive index was 1.4616 (40°C) (25), 1.4706 (25°C) (22, 23), and 1.4615 (60°C) (58). The ORAC value of roasted pumpkin seed oil was determined to be 1.1- $\mu$  moles TE/g oil, and was the lowest in comparison with six other fruit seed oils tested that included blueberry, red raspberry, black raspberry, boysenberry, and cranberry seed oils (3). Phytosterols were also detected in pumpkin seed oil (22, 23).

The seed oils from African pumpkin (squash) (*Cucurbita pepo* L.) were evaluated for their fatty acid profiles and the presence of other phytochemicals (53). The seed oils contained 28–36% oleic acid. The primary fatty acid was linoleic acid, along with palmitic and stearic acids, with a total unsaturated fatty acid concentration of 77–83%. Alpha-tocopherol was determined at a concentration up to 3.0 mg/100 g. These data suggest that pumpkin seed oil may be a better choice for consumers who prefer high unsaturation, or both linoleic and oleic acids. Seed oils from species of *Cucurbita* with minimal pericarp, called “naked seed squash,” are discussed below.

**TABLE 8. Fatty Acid Composition (g Fatty Acid/100-g Oil) of Cucurbita Seed Oils Relatively High Oleic Acid.\***

Fatty Acid	Pumpkin <sup>(25)</sup>	Pumpkin (C. Pepo) <sup>(57)</sup>	Pumpkin <sup>(58)</sup>	Pumpkin (Cucurbita sp) <sup>(22, 23)</sup>	African Pumpkin <sup>(53)</sup>	Pumpkin <sup>(3)</sup>	Squash <sup>(60)</sup>	Squash <sup>(59)</sup>
16:0	12.7	13.6–49.2	13.8	13.4	11.1–14.0	6.6	6.6–24.4	12.8–15.8
18:0	5.4	4.8–11.2	5.9	10.0	4.8–8.2	3.5	1.2–10.2	5.2–8.3
18:1	37.8	30.2–33.9	21.4	20.4	28.3–35.5	42.0	10–31.6	46.6–60.4
18:2n-6	43.1	24.5–47.9	58.9	55.6	43.0–51.9	47.8	39.3–77.2	9.6–27.9
18:3n-3	0.3	0.4–0.9	nd	nd	0.3	nd	nd	0.1–0.8

\*Pumpkin and Squash stand for pumpkin and squash seed oil, respectively. Numbers correspond to the references cited. “nd” stands for not detected.

## 5.7. Naked Seed Squash (*Curcubita pepo* L.) Seed Oil

Squash (*Curcubita foetidissima*, *C. pepo*, and *C. lagenaria*) has been consumed worldwide for thousands of years. The fruits are used as vegetables and desserts, and seeds are consumed as nuts or used to prepare edible oils (61). Normally, each plant produces 1–9 fruits weighing 0.47–12.67 kg, and each fruit may have 16–393 seeds (59). The weight of the individual naked seeds ranged from 46–223 mg. Seeds of the naked seed squash varieties are preferred for direct consumption and further processing. In 1996, Idouraine et al. (59) reported that the crude oil content in the nine selected naked seed squash lines (*C. pepo* L.) ranged 34–44% on a dry weight basis. The seed oil of the selected naked seed squash was rich in oleic acid, which made up 47–60% of the total fatty acids. Other major fatty acids were linoleic, palmitic, and stearic acids, along with a small amount of other long-chain fatty acids. The total unsaturated fatty acids ranged from 70–79% for the nine tested seed oils (59). These were in consistent with earlier observations that oleic acid was the major fatty acid and constituted 46–50% of the seed oils for all progeny lines (62, 63). In summary, the seed oil of *Curcubita pepo* may serve as edible oil rich in unsaturated fatty acid and oleic acid.

In addition, other studies reported that linoleic acid was the predominant fatty acid in the seed oils of squash varieties (*Curcubita foetidissima*) with a range of 39–77%, whereas the level of oleic acid was 10–32% (25, 60, 64). Interestingly, a significant level of conjugated dienoic acids was detected in the seed oils of *Curcubita foetidissima*. It is widely accepted that conjugated linoleic acids may have a number of health benefits, such as anticarcinogenesis, reducing body weight, antiatherosclerotic activity, and antioxidant activity (65). Future research is required to further confirm the presence of the conjugated dienoic acids in the seed oils of *Curcubita foetidissima* and identify their chemical structures. In summary, seed oil of *Curcubita foetidissima* may serve as edible oil rich in unsaturated fatty acid and linoleic acid.

## 5.8. American Ginseng Seed Oil

American ginseng (*Panax quinquefolium* L.), native to North America, is one of the most widely used medicinal herbs. American ginseng seed oil prepared by hexane or methylene chloride extraction at ambient temperature was analyzed for fatty acid profile and phytosterol content (66). The seed oils contained about 86.8–87.5% oleic acid, 10.0–10.5% linoleic acid, and 2.6% total saturated fatty acids (Table 9). Significant levels of phytosterols were observed in the American ginseng seed oils prepared with hexane and  $\text{CH}_2\text{Cl}_2$  (66). Squalene was the major phytosterol compound with a concentration of 502–514-mg/100-g oil, followed by  $\beta$ -sitosterol and stigmasterol at levels of 164–177-mg/100-g oil and 93–95-mg/100-g oil, respectively. Phytosterols are thought to benefit human health through lowering cholesterol and increasing antioxidant activity (71, 72). American ginseng seed oil would therefore be a desirable dietary source for oleic acid, squalene, and total phytosterols.

**TABLE 9. Fatty Acid Composition (g/100-g Fatty Acids) of Seed Oils Rich in Oleic Acid.\***

Fatty Acid	American Ginsengo <sup>(66)</sup>	<i>K. Senegalensis</i> <sup>(67)</sup>	<i>M. Oleifera</i> <sup>(68, 69)</sup>	<i>M. Oleifera</i> Mbololo <sup>(70)</sup>
14:0	nd	nd	< 0.1	0.1
16:0	2.2–2.3	21.4	5.9–6.5	5.7–6.0
16:1	nd	nd	1.0–1.8	0.1
18:0	0.3–0.4	10.4	5.7–7.2	1.42–1.57
18:1n-9	86.8–87.5	64.6	66.9–76.0	73.6–75.4
18:2n-6	9.95–10.5	nd	0.6–1.3	0.7
18:3n-3	nd	nd	< 0.2	0.2
20:0	nd	nd	3.0–4.0	2.5–2.7

\*American ginsengo, *K. senegalensis*, *M. Oleifera*, and *M. oleifera* Mbololo stand for American ginsengo, *K. senegalensis*, *M. oleifera* Mbololo, and *M. Oleifera* seed oil, respectively. Numbers correspond to the references cited. "nd" stands for not detected.

### 5.9. *Khaya senegalensis* Seed Oil

*Khaya senegalensis* is a dry land mahogany that grows to about 30 m in height and 3 m in girth; it is widely distributed in the savanna region of Nigeria. The seed of *Khaya senegalensis* contains about 53% oil. The seed oil has been used to treat certain local ailments. Oleic acid was the predominant fatty acid in the seed oil and accounted for 65% of the total fatty acid, along with 21% palmitic and 10% stearic acids (Table 9) (67). The seed oil had a saponification value of 186-mg KOH/100-g oil, a density of 0.962, a refractive index of 1.4690 (20°C), and an iodine value of 68.0-g iodine/100-g oil (67).

### 5.10. *Moringa oleifera* Seed Oil

*Moringa oleifera*, native to the western and sub-Himalayam tracts of India and other countries in Asia, Africa, the Middle East, Central America, and the Caribbean islands, is the most common and broadly distributed species of the Moringaceae family (73). The leaves, flowers, fruits, and roots of the tree are used as vegetables (70). Each fruit generally contains about 20 seeds, which have an average weight of 0.3 g, with the kernel responsible for 70–75% of the weight. The seed oils of *Moringa oleifera* were extracted and analyzed for chemical compositions (68, 69). The seed oil was a rich source of monounsaturated fatty acid, especially 18:1 that contributed to 74.4–76.0% of the total fatty acids (Table 9) (68, 69). A significant amount of phytosterols was detected in the seed oil (68). The major sterols were  $\beta$ -sitosterol (46.65% of total sterol), stigmasterol (19%), campesterol (16%), and  $\Delta^5$ -avenasterol (10.7%), along with a number of others.

The seeds of *M. oleifera* variety Mbololo yielded 26%, 31%, and 36% crude oil by cold-pressing, hexane extraction, and chloroform-methanol extraction (1:1, v/v), respectively (70). The seed oil was rich in total monounsaturated fatty acids and contained 74–75% oleic acid (Table 9). The total saturated fatty acids were

19–21% in the seed oil, with small amounts of linoleic and  $\alpha$ -linolenic acids. The density of the seed oils prepared by solvent extraction and cold-pressing ranged from 0.8809–0.9182 g/mL at 24°C, which is similar to that of olive oil at 0.915 g/mL at the same temperature. The refractive index ( $N_D^{40^\circ\text{C}}$ ) was 1.4549–1.4591 and the smoke point was 198–202°C for the seed oils. The seed oil prepared by cold-pressing had a greater viscosity of 103 mPa·s, whereas the oil prepared by solvent extraction exhibited a viscosity of 57–66 mPa·s, which is more comparable with that of olive oil (74 mPa·s) (70). In addition, the seed oil of *M. oleifera* variety Mbololo contained greater concentrations of total sterols and tocopherols than olive oil. The primary sterol in the Mbololo seed oil was  $\beta$ -sitosterol with significant levels of stigmasterol, campesterol, and  $\Delta^5$ -avenasterol, as well as small amounts of other sterol compounds (70). Thus, seed oil of *M. oleifera* may serve as a dietary source of oleic acid, sterols, and tocopherols.

## 6. OTHER SPECIAL SEED OILS OF FRUIT, SPICE, AND HERB

### 6.1. Gac (*Momordica cochinchinensis*)

Gac (*Momordica cochinchinensis* Spreng) is another member of the gourd family (Curcubitaceae). The gac fruit is round and about 18–22 cm in diameter. It is native to Asia and is both consumed as food and used in traditional medicine. The seeds comprise about 16% of the total fresh weight of the fruit.

Ishida et al. (74) examined gac seed oil for its fatty acid composition. The oil was determined to contain an average of 60.5% stearic acid. Palmitic and arachidic acids were two other saturated fats found in the gac seed oil, and the total percent of saturated fatty acids ranged from 60.5% to 79.2%. The most prevalent unsaturated fatty acid was linoleic acid at 20.3%, with an interesting variety of others including palmitoleic (16:1), oleic (18:1n-9), *cis*-vaccenic (18:1n-11),  $\alpha$ -linolenic acid (18:3n-3), eicosa-11-enoic acid (20:1n-11), and eicosa-13-enoic acid (20:1n-13) (Table 10).

**TABLE 10. Fatty Acid Composition (g/100-g Fatty Acids) of Gac and Pomegranate Seed Oils.**

Fatty Acid	Pomegranate <sup>(75)</sup>	Gac <sup>(74)</sup>
16:0	4.8	5.2–6.2
18:0	2.3	54.5–71.7
18:1	6.3	4.8–11.2
18:2n-6	6.6	11.2–25.0
18:3n-3	nd	0.4–0.6
18:3n-5	65.3	nd
others	nd	3.0–4.1

<sup>†</sup>Pomegranate and Gac stand for pomegranate and gac seed oil, respectively. Numbers correspond to the references cited. “nd” stands for not detected.

## 6.2. Pomegranate Seed Oil

Pomegranate (*Punica granatum*), of the Punicaceae family, is a small tree grown in Iran, India, and the United States, as well as in most Near and Far East countries (75). Pomegranate is used as a table fruit and is also processed into juice. Pomegranate preparations, including the juice of the fruit, the dried pericarp, the bark, and the roots, have been used in folk medicines to treat colic, colitis, dysentery, diarrhea, menorrhagia, oxyuriasis, parasitosis, headache, vermifugal, carminative, antispasmodic, taenicial, and emmenagogue (75). Seeds are byproducts from the juice manufacture. Cold-pressed pomegranate seed oil was prepared and analyzed for fatty acid composition, inhibitory effects against both cyclo-oxygenase and lipoxigenase, antioxidant properties, and total phenolic content (75). The major fatty acid was punicic acid (18:3n-5), which comprised 65% of total fatty acids, along with linoleic, oleic, palmitic, and stearic acids (Table 10). The seed oil contained about 150 ppm total phenolics on an oil weight basis. The oil extract, at a concentration of 5  $\mu\text{g/mL}$  total phenolics, exhibited 37% inhibition of the sheep cyclo-oxygenase activity under experimental conditions (75). On a same weight concentration basis, the oil extract resulted in 75% inhibition of the soybean lipoxigenase activity, whereas butylated hydroxy anisole (BHA) had a 92% inhibition under the same experimental conditions. The oil extract also showed strong antioxidant activity in the coupled oxidation system of carotene and linoleic acid, and the antioxidant capacity was comparable with that of BHA and green tea extract on the same weight concentration basis (75). These data suggest the potential application of pomegranate seed oil as anti-inflammatory agent and for general health promotion.

## 7. SUMMARY

There is an increasing demand for edible oils with special fatty acid profiles and other beneficial components for improving nutritional status. A number of studies have been conducted to screen for and evaluate the chemical composition and potential nutraceutical applications of fruit, spice, and herb seed oils. Among the discussed edible seed oils, some have unique fatty acid compositions, such as black raspberry and hemp seed oils rich in  $\alpha$ -linolenic acid and date and naked seed squash seed oils rich in oleic acid, whereas blackcurrant seed oil is rich in  $\gamma$ -linolenic acid. The oils of selected fruit, spice, and herb seeds may also contain significant levels of phytosterols, tocopherols, carotenoids, and natural antioxidants. The chemical composition of edible seed oil determines the potential health benefit and applications for the oil. Individual edible seed oils may be preferred by special groups of consumers for preventing and treating a selected health problem or for general health promotion. Great opportunities are available in the research and development of specialty seed oils and the oil-based nutraceutical products from fruit, spice, and herb seeds for improving human health. More research is required to screen and characterize the fatty acids and bioactive components in the fruit, spice, and herb seeds to develop novel edible seed oils for optimum human nutrition.

## REFERENCES

1. J. W. Parry and L. Yu, *J. Food. Sci.*, **69**, 188–192 (2004).
2. B. D. Oohmah, S. Ladet, D. V. Godfrey, J. Liang, and G. Benoit, *Food. Chem.*, **69**, 187–193 (2000).
3. J. W. Parry and L. Yu, Abstracts of Papers, 228th National Meeting of the American Chemical Society, Philadelphia, Pennsylvania, August 22–26, 2004.
4. H. Heeg, T. Lager, and G. Bernard, U.S. Patent 6,391,345. 2002.
5. T. D. Parker, D. A. Adams, K. Zhou, M. Harris, and L. Yu, *J. Food. Sci.*, **68**, 1240–1243 (2003).
6. B. Yang and H. P. Kallio, *J. Agric. Food Chem.*, **49**, 1939–1947 (2001).
7. H. Kallio, B. Yang, P. Peippo, R. Tahvonen, and R. Pan, *J. Agric. Food Chem.*, **50**, 3004–3009 (2002).
8. L. Yu, K. Zhou, and J. Parry, *Food Chem.*, Accepted for publication.
9. T. S. C. Li, in J. Janick, ed., *Perspectives on New Crops and New Uses*, ASHS Press, Alexandria, Virginia, 1999, pp. 335–337.
10. P. Angers, M. R. Morales, and J. E. Simon, in J. Janick, ed, *Progress in New Crops*, ASHS Press, Arlington, Virginia, 1996, pp. 598–601.
11. B. D. Oohmah, M. Busson, D. V. Godfrey, and J. C. G. Drover, *Food Chem.*, **76**, 33–43 (2002).
12. D. Pierce and G. Brooks, U.S. Patent 6,063,369, 2000.
13. Z. Yaniv, C. Ranen, A. Levy, and D. Palevitch, *J. Exp. Bot.*, **40**, 609–613 (1989).
14. W. M. N. Ratnayake, D. G. Matthews, and R. G. Ackman, *J. Amer. Oil Chem. Soc.*, **66**, 966–969 (1989).
15. M. L. Ruiz Del Castillo, G. Dobson, R. Brennan, and S. Gordon, *J. Agric. Food Chem.*, **52**, 948–952 (2004).
16. C. E. Lister, P. E. Wilson, K. H. Sutton, and S. C. Morrison, *Act. Hor.*, **585**, 443–449 (2002).
17. D. Wu, M. Meydani, L. S Leka, Z. Nightingale, G. J. Handelman, J. B. Blumberg, and S. N. Meydani, *Amer. J. Clin. Nutr.*, **70**, 536–43 (1999).
18. F. D. Goffman and S. J. Galletti, *J. Agric. Food Chem.*, **49**, 349–354 (2001).
19. M. L. Ruiz Del Castillo, G. Dobson, R. Brennan, and S. Gordon, *J. Agric. Food Chem.*, **50**, 332–335 (2002).
20. B. J. F. Hudson, *J. Amer. Oil Chem. Soc.*, **61**, 540–543 (1984).
21. U. Ollson, P. Kaufmann, and B. G. HerslÖf, *J. Chromatogr.*, **505**, 385–394 (1990).
22. T. A. El-Adaway and K. Taha, *J. Agric. Food Chem.*, **49**, 1253–1259 (2001).
23. T. A. El-Adaway and K. Taha, *Food Chem.*, **74**, 47–54 (2001).
24. M. L. S. De Melo, N. Narain, and P. S. Bora, *Food Chem.*, **68**, 411–414 (2000).
25. E. S. Lazos, *J. Food Sci.*, **51**, 1382–1383 (1986).
26. E. Nwokolo and J. S. Sim, *J. Sci. Food Agric.*, **38**, 237–246 (1987).
27. C. O. Moussata and C. C. Akoh, *J. Amer. Oil Chem. Soc.*, **74**, 177–179 (1997).
28. M. F. Ramadan and J. T. Morsel, *J. Agric. Food Chem.*, **51**, 969–974 (2003).
29. M. A. Abou Rayan, A. A. Abdel-Nabey, O. R. Abou Samaha, and M. K. Mohamed, *Alex. J. Agric. Res.*, **43**, 67–79 (1998).



30. M. G. Massanet, J. A. Montiel, E. Pando, and F. L. Rodriguez, *Grasas Aceites.*, **37**, 233–236 (1986).
31. M. Özcan, *J. Med. Food.*, **5**, 137–140 (2002).
32. J. Domokos, J. Perédi, and J. Bernáth, *Acta Hort.*, **331**, 49–52 (1993).
33. G. I. D. Ogunsua and A. O. Ogunsua, *Plant Foods Hum. Nutr.*, **41**, 35–44 (1991).
34. R. McCain, in J. Janick and J. E. Simon, eds., *New Crops*, Wiley, New York, 1993, pp. 479–486.
35. I. M. Morice, F. B. Shorland, and E. Williams, *Sci. Food Agric.*, **22**, 186–188 (1971).
36. R. Sancho, C. Lucena, A. Macho, M. A. Calzado, M. Blanco-Molina, A. Minassi, G. Appendino, and E. Muñoz, *Eur. J. Immunol.*, **32**, 1753–1763 (2002).
37. M. Y. Jung, J. Y. Bock, S. O. Baik, J. H. Lee, and T. K. Lee, *J. Agric. Food Chem.*, **47**, 1700–1704 (1999).
38. P. U. Rao, *Food Chem.*, **50**, 379–382 (1994).
39. M. F. Ramadan and J. T. Mörsel, *Nahrung.*, **46**, 240–244 (2002).
40. J. A. Zygadlo, R. E. Morero, R. E. Abburra, and C. A. Guzman, *J. Amer. Oil Chem. Soc.*, **71**, 915–916 (1994).
41. M. L. Salem and M. S. Hossain, *Int. J. Immunopharmacol.*, **22**, 729–740 (2000).
42. R. L. Hargrove, T. D. Etherton, T. A. Pearson, E. H. Harrison, and P. M. Kris-Etherton, *J. Nutr.*, **131**, 1758–1763 (2001).
43. R. J. Nicolosi, T. A. Wilson, G. Handelman, T. Foxall, J. F. Keaney, and J. A. Vita, *J. Nutr. Biochem.*, **13**, 392–402 (2002).
44. P. Yaqoob, *Eur. J. Clin. Nutr.*, **56**(Suppl. 3), S9–S13 (2002).
45. G. Lakshminarayana, T. Chandrasekhara Rao, and P. A. Ramalingaswamy, *J. Amer. Oil Chem. Soc.*, **60**, 88–89 (1983).
46. M. A. Ali, M. A. Gafur, M. S. Rahman, and G. M. Ahmed, *J. Amer. Oil Chem. Soc.*, **62**, 520–523 (1985).
47. Rao P. Udayasekhara, *Food Chem.*, **50**, 379–382 (1994).
48. F. Comes, M. Farines, A. Aumelas, and J. Soulier, *J. Amer. Oil Chem. Soc.*, **69**, 1224–1227 (1992).
49. W. N. Sawaya, J. K. Khalil, and W. J. Safi, *J. Food Sci.*, **49**, 617–619 (1984).
50. M. P. Maza, R. Zamora, M. Alaiz, F. J. Hidalgo, F. Millán, and E. Vioque, *J. Sci. Food Agric.*, **46**, 495–502 (1989).
51. S. A. Salem and S. M. Hegazi, *J. Sci. Food Agric.*, **22**, 632 (1971).
52. W. N. Sawaya, H. A. Khatchadourian, J. K. Khalil, W. M. Safi, and A. Al-Shalhat, *J. Food Sci.*, **47**, 1489 (1982).
53. Y. M. H. Younis, S. Ghirmay, and S. S. Al-Shihry, *Phytochem.*, **54**, 71–75 (2000).
54. H. A. Zuhair, A. A. Abd El-Fattah, and M. I. El-Sayed, *Pharmacol. Res.*, **41**, 555–563 (2000).
55. A. T. Famhim, A. A. Abd-EL Fattah, A. M. Agha, and M. Z. Gad, *Pharmacol. Res.*, **31**, 73–79 (1995).
56. V. Suphakarn, *Amer. J. Clin. Nutr.*, **45**, 115–121 (1987).
57. J. E. Spangenberg and N. Ogrinc, *J. Agric. Food Chem.*, **49**, 1534–1540 (2001).
58. B. S. Kamel, J. M. DeMan, and B. Blackman, *J. Food. Technol.*, **17**, 263–269 (1982).

59. A. Idouraine, E. A. Kohlhepp, and C. W. Weber, *J. Agric Food Chem.*, **44**, 721–724 (1996).
60. J. A. Vasconcellos, J. M. Berry, C. W. Weber, and W. P. Bemis, *J. Amer. Oil Chem. Soc.*, **57**, 310–313 (1980).
61. E. S. Lazos, *Plant Foods Hum. Nutr.*, **43**, 257–273 (1992).
62. W. P. Bemis, J. W. Berry, M. J. Kennedy, D. Woods, M. Morah, and A. J. Deutchman, *J. Amer. Oil Chem. Soc.*, **44**, 429–430 (1967).
63. M. S. El-Gharbawi, *Libyan J. Agric.*, **6**, 199–203 (1977).
64. J. A. Vasconcellos, W. P. Bemis, J. M. Berry, and C. W. Weber, *J. Amer. Oil Chem. Soc.*, **59**, 55–68 (1981).
65. L. Yu, D. Adams, and M. Gabel, *J. Agric. Food Chem.*, **50**, 4135–4140 (2002).
66. T. H. J. Beveridge, Th. S. C. Li, and J. C. G. Drover, *J. Agric. Food Chem.*, **50**, 744–750 (2002).
67. F. E. Okieimen and C. O. Eromosele, *Bioresource Technol.*, **69**, 279–280 (1999).
68. F. Anwar and M. I. Bhangar, *J. Agric. Food Chem.*, **51**, 6558–6563 (2003).
69. G. Vlahov, P. K. Chepkwony, and P. K. Ndalut, *J. Agric. Food Chem.*, **50**, 970–975 (2002).
70. J. Tsaknis, S. Lalas, V. Gergis, V. Dourtoglou, and V. Spiliotis, *J. Agric. Food Chem.*, **47**, 4495–4499 (1999).
71. W. H. Ling and P. J. Jones, *Life Sci.*, **57**, 195–206 (1995).
72. G. S. Kelly, *Altern. Med. Rev.*, **4**, 29–36 (1999).
73. J. F. Morton, *Econ. Bot.*, **45**, 318–333 (1991).
74. B. Ishida, C. Turner, M. Chapman, and T. McKeon, *J. Agric. Food Chem.*, **52**, 274–279 (2004).
75. S. Y. Schubert, E. P. Lansky, and I. Neeman, *J. Ethnopharmacol.*, **66**, 11–17 (1999).

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## *Marine Mammal Oils*

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### **1. INTRODUCTION**

Marine oils are obtained from the flesh of fatty fish, liver of lean white fish, and blubber of marine mammals. Although lipids from marine fish have been used as food and medicine, traditional uses of blubber lipids of marine mammals were mostly industrially oriented, except for Innus and Eskimos. Marine mammal oils were lubricants or “train” oils as well as fuel and used for lighting (1). However, recent research findings on the importance of long-chain polyunsaturated fatty acids (LC PUFA) in human health have opened new channels for their value-added use in food and pharmaceutical industries (2). During the last three decades, it has been established that Greenland Eskimos living on their traditional diet have a lower incidence of coronary heart disease than do Danes living on a western-style diet (3, 4). It has been recognized that polyunsaturated fatty acids could be useful in controlling serum triacylglycerols, but the fatty acids provided by the food industry were often of the  $\omega 6$  family (1).

Lipids from marine mammals such as seal, whale, and walrus are primarily stored as subcutaneous fat or blubber. Seal blubber comprises 29% of the carcass

**TABLE 1. Lipid Content (g/100 g tissue) of Selected Tissues from Four Species of Seals.<sup>1</sup>**

Tissue	Harp	Gray	Ringed	Hooded
Blubber	93.88 ± 1.64	91.93 ± 1.07	93.55 ± 1.98	89.43 ± 1.82
Muscle	1.92 ± 0.03	1.82 ± 0.03	1.85 ± 0.53	2.36 ± 0.74
Brain	8.10 ± 0.32	10.25 ± 0.10	6.86 ± 1.01	7.40 ± 0.79
Kidney	2.97 ± 0.18	3.42 ± 0.04	3.58 ± 0.07	3.14 ± 0.05
Heart	2.19 ± 0.31	1.81 ± 0.38	2.32 ± 0.01	2.04 ± 0.01
Liver	3.83 ± 0.19	5.60 ± 0.94	3.71 ± 0.07	3.66 ± 0.03
Lung	2.24 ± 0.46	2.04 ± 0.03	2.05 ± 0.07	1.76 ± 0.01

<sup>1</sup>Data from Ref. (6).

weight and is considered a valuable component of it (5). Blubber lipids are mobilized in times of energy need and replenished when food is in excess (2). Although the blubber is the major site of lipid in the body of marine mammals, lipid is also found in the muscles, liver, kidney, heart, lung, brain, and other organs. Lipid content of different tissues in different species of seals varies (Table 1) (6). In addition, milk contains a high content of fat. A study carried out on hooded seals showed that females may secrete up to 10 kg of milk on a daily basis, which contains 60% fat (7). The milkfat of marine mammals resembles the composition of the depot fats of these animals (8). The mobilization of maternal fat reserves and transfer of milkfat from mother to pup occurs at very high rates (9). Lipids in marine mammals function as a source of energy, structural components of cells and tissues, and provide buoyancy (10). The blubber of marine mammals, especially harp seal, because of its economic importance, has been the subject of numerous studies on marine oils (6).

## 2. LIPID CLASSES

The oils from marine mammals are of very different composition. In the characterization of marine oils, many chromatographic techniques have been employed. These techniques include thin layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), and supercritical fluid chromatography (SFC). These techniques have advantages and disadvantages depending on the goal of the analysis. TLC has been an excellent tool for qualitative analysis of components present in marine oils (11).

The various lipid classes of marine mammals include triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids, wax esters, cholesterol, cholesterol esters, hydrocarbons, vitamins, and ether lipids. Some marine oils are very simple, containing almost exclusively triacylglycerols (TAGs), whereas others contain a variety of lipid classes (11), as shown in Table 2. Seal blubber, the depot lipid, is mostly composed of neutral lipids (98.9%), in contrast to intramuscular lipids (78.8% neutral and 21.1% polar lipids) (13). TAGs are the predominant components of seal blubber, whereas organ lipids include both TAGs and phospholipids, and

**TABLE 2. Composition of Arterial Lipid, as wt% of Total, for Three Species of Whales.<sup>1</sup>**

Species	Tissues	Hydrocarbons	Wax and Sterol		Wax Alcohol and Sterol	Monoacylglycerol	Phosphatide	Free Fatty Acid
			Ester	Triacylglycerol				
Sperm whale	Aortic lesions	—	16.5	6.4	13.8	—	57.8	5.5
	Aortic intima	—	13.1	4.4	13.0	0.4	66.1	2.7
	Aortic media	—	3.3	2.8	14.8	0.3	76.9	1.9
Killer whale	Fibrous aortic atheroma	—	17.3	11.4	12.6	—	58.7	—
	Aortic intima	0.3	7.4	29.5	17.0	0.2	45.5	—
	Aortic media	0.6	4.2	23.9	16.4	0.6	54.3	—
	Fatty coronary atheroma	—	56.4	20.9	4.1	—	18.6	—
	Coronary intima media	0.7	1.7	50.4	17.3	0.9	25.8	—
Pilot whale	Aortic lesions	2.8	17.1	9.2	28.6	0.4	35.9	6.0
	Aortic intima media	0.4	13.5	4.6	20.5	0.6	57.5	3.1

<sup>1</sup>From Ref. (12).

differences exist that originate from their varying proportions in different tissues. In marine mammals such as whales and seals that have enormous layers of fat under skin, TAGs serve as insulating material, which permits survival even in the cold waters of the Arctic and the Antarctic (14).

In addition to TAGs, wax esters (long-chain alcohols esterified to fatty acids) are another important group of neutral lipids found in marine mammals. Most species of marine mammals have C32, C34, C36, and C38 (total of alcohol plus acid) as major components (15). Whale oils are especially interesting because some contain fatty acids that are largely in the form of wax esters (16). The oils from the blubber of the Physeteridae may consist mainly of wax esters. The sperm whale blubber oil consists of a mixture of about 79% wax esters and 21% TAGs (17). The dwarf sperm whale (*K. simus*) blubber oils consist of 42% wax esters and 58% TAGs (18). The blubber fat of beaked whales (*Berardius*, *Hyperoodon*, and *Ziphius*) is composed almost entirely of wax esters (94–99%) along with low levels of TAGs (2–6%) (19). Several of possible functions for wax esters in marine mammals has been proposed; these functions include their role as a reserve energy store, buoyancy, metabolic water, thermal insulation, and biosonar (20–22).

Among unsaponifiable matters, hydrocarbons, especially long-chain hydrocarbons, are found in detectable amounts in marine mammal oils. Some marine oils contain less than 0.1% hydrocarbons, whereas others contain as much as 90% (23). In the liver of the seal, *Arctocephalus* (*Pinnipedia*), liver squalene was 0.50% of the oil (24). High squalene contents (90%, 91%, and 92.8%) occur in shark liver oils (23–25). The 16:0, 16:1, and pristine were found in the bottlenose whale (*Berardius bairdi*); (26) pristine is a highly unsaturated long-chain hydrocarbon (C49) occurring in the liver oil of sei whale (*Balaenoptera borealis*) (27) and sperm whale (*Physeter catodon*) (28). In the blubber of the sei whales, pristine was present at 11.3% and squalene at 13.1% of the total unsaponifiable fraction (29). Total hydrocarbons were present at 0.3% of dry matter weight of the blubber, 1.6% in liver, and 1.3% in the muscle (30). Among cetaceans, limited data for two dolphins have been published: In *Delphinus longirostris* liver, very long-chain hydrocarbons (C44) were detected (31), and zamene was present in *Langenorchus acutus* (32).

### 3. FATTY ACID COMPOSITION

The fatty acid composition of marine lipids varies significantly, especially when compared with vegetable oils. The fatty acid composition of blubber oil of marine mammals is generally similar to fish oils as it contains a large proportion of long-chain highly unsaturated fatty acids. However, the proportion of fatty acids in fish and marine mammals varies considerably (2).

A marine oil typically contains some 40 different fatty acids, with carbon numbers varying from 10 to 24, which results in many different TAGs with the same carbon number, but with different levels of unsaturation (11). The fatty acids

present in marine mammal oils can be classified as saturated and unsaturated fatty acids. The fatty acids C12:0, C14:0, C16:0, and C18:0 are among the common saturated fatty acids. In addition, marine oils usually contain detectable ( $\leq 0.2\%$ ) amounts of C20:0, and sometimes recognizable C24:0, but very little C22:0; the total is normally 0.5% or less for these three fatty acids (33). Even-numbered carbon fatty acids make up about 97% of the total fatty acids, with a few notable exceptions (17). Some fatty acids with odd-numbered carbon chain such as C15:0 and C17:0, along with traces of C13:0 and C19:0, have also been found in marine oils (33). Besides, monomethyl branched fatty acids have been isolated from marine oils, such as 3-methyldodecanoic acid from blubber of the sperm whale physter catodon (34).

In contrast to relatively small amounts of saturated fatty acids, marine mammal oils have been characterized by high amounts of monounsaturated fatty acids (MUFAs) and  $\omega 3$  polyunsaturated fatty acids (PUFAs) (35, 36). For instance, the content of MUFAs in neutral and polar lipids in seal blubber is more than 60% and 46%, respectively (37). Most fatty acids are long-chain with 20 to 22 carbon atoms and have  $\omega 3$  configurations. Ackman et al. (38) have pointed out that the total C20 and C22 monounsaturated and polyunsaturated fatty acids in each layer of whale blubber is nearly constant, but the ratios of the monounsaturated to polyunsaturated fatty acids change significantly. The most common long-chain PUFAs in marine lipids are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as a smaller amount of docosapentaenoic acid (DPA), all of which belong to the  $\omega 3$  family (10). The high content of  $\omega 3$  fatty acids in marine lipids is suggested

**TABLE 3. Fatty Acid Composition (g/100 g) of Blubber of Various Species of Seal.<sup>1</sup>**

Fatty Acid	Bearded	Gray	Harbor	Harp	Hooded	Ringed
14:0	3.05	3.83 ± 0.03	4.52 ± 0.13	4.66 ± 0.49	4.40 ± 0.38	3.36 ± 0.66
16:0 DMA	ND	ND	ND	ND	ND	ND
16:0	10.14	6.61 ± 0.08	8.03 ± 0.38	6.24 ± 0.44	9.81 ± 1.57	4.82 ± 2.07
16:1 $\omega 7$	17.77	12.77 ± 0.09	19.26 ± 0.53	14.93 ± 0.46	10.09 ± 0.35	23.12 ± 0.18
18:0 DMA	ND	ND	NNND	ND	ND	ND
18:1 $\omega 9$ DMA	ND	ND	ND	ND	ND	ND
18:1 $\omega 7$ DMA	ND	0.45 ± 0.01	ND	0.46 ± 0.00	ND	ND
18:0	2.15	0.94 ± 0.02	0.85 ± 0.02	0.95 ± 0.03	1.83 ± 0.31	0.42 ± 0.19
18:1 $\omega 9$	16.76	24.50 ± 0.44	18.61 ± 0.55	18.59 ± 1.01	22.77 ± 2.66	19.72 ± 1.33
18:1 $\omega 7$	9.49	4.95 ± 0.09	5.16 ± 0.44	3.57 ± 0.36	3.75 ± 0.47	5.03 ± 0.46
18:2 $\omega 6$	2.30	1.28 ± 0.00	1.27 ± 0.04	1.36 ± 0.20	1.63 ± 0.20	2.58 ± 0.02
20:1 $\omega 9$	5.08	12.50 ± 0.43	9.06 ± 0.33	12.56 ± 2.92	13.00 ± 1.86	6.71 ± 2.17
20:4 $\omega 6$	0.94	0.51 ± 0.00	0.44 ± 0.00	0.36 ± 0.96	0.31 ± 0.03	0.30 ± 0.02
20:5 $\omega 3$	8.28	4.85 ± 0.13	9.31 ± 0.21	6.82 ± 0.69	5.21 ± 1.65	8.72 ± 1.06
22:0	0.63	<0.3	1.19 ± 0.02	<0.3	<0.3	0.75 ± 0.67
22:1 $\omega 11$	0.27	0.62 ± 0.03	0.31 ± 0.01	0.77 ± 0.61	0.86 ± 0.33	0.34 ± 0.01
22:5 $\omega 3$	4.26	5.06 ± 0.05	4.22 ± 0.14	4.78 ± 0.25	2.29 ± 0.08	5.46 ± 0.47
22:6 $\omega 3$	7.22	8.91 ± 0.29	7.76 ± 0.98	10.48 ± 1.98	9.56 ± 2.36	9.45 ± 1.74

DMA: dimethyl acetal.

ND: not detected.

<sup>1</sup>From Refs. (6) and (40).

TABLE 4. Fatty Acids Composition of Blubber Expressed as a Percentage by Weight of Fatty Acids Present from both Sexes of Four Species of Phocid Seals.<sup>1</sup>

Fatty Acids	<i>P. vitulina largha</i>		<i>P. fasciata</i>		<i>P. hispida</i>		<i>P. barbatus</i>	
	Male	Female	Male	Female	Male	Female	Male	Female
12:0	0.03	0.04	0.02	0.02	0.03	0.02	0.06	0.05
12:1	tr	tr	tr	tr	0.01	tr	tr	0.01
13:0	tr	0.01	0.01	0.01	0.01	0.01	0.01	0.01
13:1 ω9	tr	tr	tr	tr	tr	tr	tr	tr
14:0	1.89	2.45	2.34	3.00	3.36	1.68	1.77	2.76
14:1	0.38	0.42	0.43	0.39	0.83	0.56	0.29	0.26
15:0	0.31	0.41	0.33	0.33	0.25	0.22	0.39	0.40
15:1 ω6	0.07	0.05	0.06	tr	0.01	0.01	0.06	0.05
16:0	6.82	7.96	5.38	7.67	5.25	3.23	7.27	9.96
16:1 ω7	10.96	10.63	9.26	8.51	19.04	9.52	12.46	14.05
16:2 ω4	0.24	0.39	0.33	0.36	0.45	0.32	0.32	0.44
16:3 ω6	—	—	—	—	0.06	0.04	—	tr
16:3 ω3	0.11	tr	0.16	—	0.25	—	0.13	—
17:0	0.54	0.47	0.42	0.35	0.47	0.41	1.88	0.80
17:1 ω8	0.39	0.32	0.25	0.27	0.39	0.27	0.57	0.33
18:0	0.93	1.10	1.05	1.75	0.66	0.73	1.66	2.30
18:1 ω9	29.75	24.15	21.31	20.91	15.79	16.21	21.25	21.69
18:2 ω6	0.89	1.10	0.82	1.07	0.81	0.59	0.62	0.82
18:2 ω4	0.21	0.22	0.23	0.16	0.20	0.17	0.21	0.16
18:3 ω6	tr	0.05	tr	—	0.27	0.07	tr	0.11
18:3 ω3	0.13	0.32	0.30	0.88	0.52	0.22	0.11	0.21
18:4 ω3	0.52	0.83	0.80	0.73	1.03	0.75	0.72	0.93
19:0	tr	0.27	0.26	tr	0.73	0.35	0.35	0.52
19:1	0.02	0.07	0.15	0.11	0.10	0.08	0.41	0.32
20:0	0.06	0.09	tr	0.36	tr	0.07	tr	0.23
20:1	—	—	—	—	—	—	3.75	—
20:1 ω9	13.83	8.94	14.99	13.58	5.01	8.17	3.94	8.99
20:2 ω6	0.15	0.16	0.20	0.29	0.13	0.13	0.24	0.24
20:2 ω4	0.11	tr	tr	tr	0.04	0.10	0.08	0.12
20:3 ω6	tr	tr	tr	0.09	0.08	0.06	0.11	0.11
20:3 ω3	tr	tr	tr	tr	tr	tr	tr	tr
20:4 ω6	0.54	0.57	0.63	1.21	0.33	0.39	1.31	0.76
20:4 ω3	0.26	0.37	0.56	0.68	0.34	0.37	0.38	0.51
20:5 ω3	6.54	10.56	8.47	8.24	11.96	10.57	14.01	9.27
21:0	0.08	—	tr	tr	—	—	0.32	0.29
21:5 ω3	—	—	—	0.06	0	0.21	0.58	0.51
22:0	—	—	—	—	—	0.05	—	—
22:1 ω11	2.69	2.42	5.07	6.49	0.68	1.57	0.82	3.26
22:2 ω6	—	—	—	—	0.16	0.20	—	—
22:4 ω6	0.68	0.81	0.81	0.64	—	1.16	1.44	0.83
22:4 ω3	—	—	—	0.21	0.22	—	—	—
22:5 ω3	7.81	7.67	7.74	6.51	11.62	14.55	9.82	4.76
22:6 ω3	12.62	16.66	17.06	14.54	17.79	26.19	12.05	13.38
24:1	0.42	0.43	0.59	0.57	1.12	0.76	0.59	0.56

<sup>1</sup>From Ref. (44).



**TABLE 5. Fatty Acid Composition of Lipids (g/100-g lipid) from Different Tissues of Harp Seal.<sup>1</sup>**

Fatty Acid	Blubber	Muscle	Brain	Kidney	Heart	Lung	Liver
14:0	4.66 ± 0.49	2.46 ± 0.71	0.48 ± 0.07	1.15 ± 0.88	0.91 ± 0.03	2.40 ± 0.06	1.21 ± 0.20
16:0 DMA	ND	1.86 ± 0.15	1.66 ± 0.19	3.09 ± 0.67	3.74 ± 0.10	1.93 ± 0.02	0.15 ± 0.01
16:0	6.24 ± 0.44	12.29 ± 0.91	15.45 ± 0.64	12.71 ± 0.19	10.56 ± 0.81	24.45 ± 0.67	13.63 ± 0.76
16:1 ω7	14.93 ± 0.46	7.30 ± 0.79	1.33 ± 0.22	4.05 ± 0.09	4.32 ± 0.12	2.54 ± 0.08	5.34 ± 0.13
18:0 DMA	ND	0.78 ± 0.08	4.38 ± 0.43	1.65 ± 0.28	2.69 ± 0.33	1.68 ± 0.08	0.16 ± 0.04
18:1 ω9 DMA	ND	0.84 ± 0.09	1.82 ± 0.08	0.74 ± 0.06	2.58 ± 0.05	0.92 ± 0.02	0.19 ± 0.06
18:1 ω7 DMA	0.46 ± 0.00	0.97 ± 0.11	2.69 ± 0.18	0.79 ± 0.07	1.74 ± 0.03	1.01 ± 0.00	0.13 ± 0.04
18:0	0.95 ± 0.03	5.93 ± 0.19	18.08 ± 0.69	12.30 ± 0.59	11.09 ± 0.34	8.72 ± 0.26	19.55 ± 0.87
18:1 ω9	18.59 ± 1.01	18.48 ± 0.68	14.02 ± 0.89	12.52 ± 0.45	16.09 ± 0.17	13.41 ± 0.28	11.80 ± 1.45
18:1 ω7	3.57 ± 0.36	4.88 ± 1.39	4.60 ± 0.28	5.50 ± 0.35	4.56 ± 0.25	3.96 ± 0.27	6.10 ± 0.84
18:2 ω6	1.36 ± 0.20	1.54 ± 0.12	0.15 ± 0.09	3.33 ± 0.03	3.86 ± 0.08	1.14 ± 0.02	2.04 ± 0.38
20:1 ω9	12.56 ± 2.92	11.75 ± 1.58	1.83 ± 0.24	2.20 ± 0.57	4.51 ± 0.05	2.68 ± 0.62	3.40 ± 0.58
20:4 ω6	0.36 ± 0.96	3.87 ± 0.63	5.29 ± 0.22	10.11 ± 1.08	9.90 ± 0.13	5.81 ± 0.14	9.50 ± 0.57
20:5 ω3	6.82 ± 0.69	5.56 ± 0.60	0.70 ± 0.60	9.86 ± 0.53	9.74 ± 0.13	4.86 ± 0.31	8.07 ± 1.74
22:0	<0.3	1.81 ± 0.32	0.25 ± 0.09	0.99 ± 0.67	0.58 ± 0.32	0.58 ± 0.27	0.58 ± 0.04
22:1 ω11	0.77 ± 0.61	0.93 ± 0.23	0.21 ± 0.13	0.21 ± 0.04	ND	0.81 ± 0.00	0.23 ± 0.13
22:5 ω3	4.78 ± 0.25	2.21 ± 0.32	2.92 ± 0.26	2.13 ± 0.05	1.17 ± 0.06	2.35 ± 0.04	2.16 ± 0.05
22:6 ω3	10.48 ± 1.98	6.73 ± 1.30	15.56 ± 0.64	3.29 ± 0.83	4.11 ± 0.59	5.80 ± 1.07	8.17 ± 1.83

DMA: dimethyl acetal.

ND: not detected.

<sup>1</sup>From Refs. (6) and (40).

as a consequence of cold temperature adaptation, because at lower habitat temperatures,  $\omega 3$  PUFA remain liquid and oppose any tendency to crystallize (33). Most long-chain PUFAs are formed in unicellular phytoplankton and multicellular sea algae and eventually pass through the food web and become incorporated into the body of fish and other higher marine species, including marine mammals, which often eat fish (39). The fatty acid composition of oils from most species of marine mammals has been summarized (1). Seal oils, because of the increasing interest in seal fishery and product development, have been in focus and frequently studied by researchers. The fatty acid composition of oils from different species of seal has been reviewed (2). Table 3 shows the fatty acids and their contents in blubber lipid from six species of seals.

The fatty acid composition of blubber of marine mammals such as seals is regulated by the diet (41), location (42), season, as well as physiological conditions such as age (43) and sex (44) of the animal. Table 4 presents the fatty acid compositions of seals of different species and sexes. In some marine mammals, the depot fats are largely dietary fatty acids laid down with a minimum change, but the fatty acids of the lipids of the essential organs have terrestrial characteristics (1). Fatty acid composition also depends on tissue and species of the animal. However, differences are most apparent among tissues (Table 5). Seal blubber, for example, had a high content of monounsaturated fatty acids, but it was low in arachidonic acid, dimethyl acetals, and DHA. Lung tissue lipids were high in palmitic acid, and heart tissue lipids had a higher content of linoleic acid. The proportions and fatty acid constituents in different tissues are different, most probably because of their varying functional requirements (6). The lipids of vital organs of seals and whales contain high proportions of fatty acids of the  $\omega 6$  family, similar to those of terrestrial animals. The distinction between the fatty acids of functional organs such as liver, heart, and other organs with depot fat has been discussed (6, 45).

The fatty acid distribution in the TAG molecules in blubber oil of marine mammals is different from that of fish oils. The  $\omega 3$  fatty acids (EPA, DPA, DHA) in blubber oil of marine mammals tend to be located primarily in the *sn-1* and *sn-3*

**TABLE 6. Positional Distribution of Fatty Acids in Triacylglycerols from Blubber Fat of Marine Mammals.<sup>1</sup>**

	Position	Fatty Acids (mol %)												
		14:0	16:0	16:1	18:0	18:1	18:2	20:1	22:1	20:5	22:5	22:6	18:4	20:4
Harbor seal	1	4	11	15	1	29	1	18	8	3	2	3	—	—
	2	11	13	30	1	30	3	3	1	1	1	1	—	—
	3	1	4	14	1	26	1	16	7	8	6	10	—	—
Harp seal	1	1	7	9	1	27	1	17	4	6	4	15	—	—
	2	6	9	27	2	36	5	4	1	2	1	3	—	—
	3	1	5	11	1	20	2	7	1	12	11	26	—	—
Sei whale	1	3	13	3	4	14	1	33	10	3	1	6	1	5
	2	12	6	12	1	29	5	10	2	5	1	3	4	6
	3	4	6	2	2	7	1	28	16	6	3	16	1	3

<sup>1</sup>From Ref. (14).

**TABLE 7. Fatty Acid Distribution in Different Positions of Triacylglycerols of Harp Seal Blubber Oil.<sup>1</sup>**

Fatty Acid	<i>sn</i> -1	<i>sn</i> -2	<i>sn</i> -3
Total saturates	6.34	25.56	4.32
Total unsaturates	90.51	73.25	94.32
Total monounsaturates	62.91	65.98	51.09
Total polyunsaturates	27.60	7.27	43.23
Eicosapentaenoic acid (EPA)	8.36	1.60	11.21
Docosapentaenoic acid (DPA)	3.99	0.79	8.21
Docosahexaenoic acid (DHA)	10.52	2.27	17.91
Total omega-3	25.65	5.56	38.87
Total omega-6	0.75	1.58	3.34

<sup>1</sup>From Ref. (2).

positions of TAGs (Tables 6 and 7), whereas in fish oils, they are located abundantly in the *sn*-2 position of TAGs (46). In harp seal oil, as measured by <sup>13</sup>C NMR, only 3.2% of DHA and 4.6% of EPA were esterified to the *sn*-2 position of the TAG (47). During digestion, the fatty acids are liberated from *sn*-1 and *sn*-3 positions of the TAG by a position-specific enzyme such as pancreatic lipase, whereas the fatty acids attached to the *sn*-2 position are distributed in the body in the form of chylomicron (2).

#### 4. OXIDATIVE STABILITY

Marine oils, like other highly unsaturated oils, are susceptible to oxidation and are more sensitive to oxidative deterioration than vegetable oils. Furthermore, they contain only insignificant amounts of natural antioxidants, such as tocopherols (48). The long-chain PUFAs (EPA, DPA, DHA) contain five or six double bonds that render them prone to atmospheric oxidation accompanied by the development of a fishy taste and smell (49). The secondary products of oxidation may give rise to unacceptable flavors and odors in the oil, impair digestibility of the oil, and can damage or destroy the body's cells, as a result of free radical attack (48). Although antioxidants are generally available for prevention of lipid oxidation in foods, natural antioxidants, such as tocopherols, unfortunately are not effective in inhibiting oxidation of marine oils (50). The autoxidation rate of PUFA depends on the type and structures of fatty acids in lipids (51). For instance, seal oil is more stable than fish oil and less vulnerable to the natural process of oxidation because of its fatty acid composition and distribution and location of fatty acids in the triacylglycerol molecules as well as because of its minor components (48). Removal of minor components from oil may result in lower oxidative stability of oils (52–55). Interesterification may change the oxidative stability of marine oils. Oxidative stability of minke whale blubber oil was reduced after redistribution of fatty acids with lipase or NaOCH<sub>3</sub> (50). The lower oxidative stability of interesterified whale oil seemed to

**TABLE 8. Changes of Tocopherol and Acid Value of Seal Blubber Oil during Processing.<sup>1</sup>**

Sample	$\alpha$ -Tocopherol (mg/100g)	Acid Value (mg KOH/g oil) (as % Oleic Acid)
Crude	2.8 $\pm$ 0.18	2.72 $\pm$ 0.10 (1.367%)
Alkali-refined	3.20 $\pm$ 0.11	0.08 $\pm$ 0.00 (0.040%)
Refined-bleached (RB)	3.1 $\pm$ 0.12	0.03 $\pm$ 0.01 (0.015%)
Refined-bleached and deodorized (RBD)	2.4 $\pm$ 0.09	0.04 $\pm$ 0.01 (0.020%)

<sup>1</sup>From Ref. (56).

be caused by displacement of PUFAs located at the *sn*-1 and *sn*-3 positions in TAG to the *sn*-2 position. PUFAs located at the *sn*-1 and *sn*-3 positions in whale oil might become more susceptible to free radical oxidation when they are transferred to 1,2- or 2,3-positions (50). Furthermore, overprocessing of marine oils may adversely affect their keeping quality by the removal of their endogenous natural antioxidants (Table 8), and it is recommended that processing be minimized or that important minor components with antioxidant activity be returned to the oil to improve their quality (56).

Control of autoxidation of unsaturated fatty acids is vital to preserve integrity, nutritional value, and functionalities of marine oils. Several reports in the literature have reviewed these matters in detail. Controlling of the availability of essential reactants in the oxidation process, such as oxygen, light, and other factors, may provide a means of retarding autoxidation. The level of available oxygen for reactions can be controlled by reducing the partial pressure of oxygen (57, 58) or replacing the headspace of the container with a nonreactive gas such as nitrogen. Proper packaging of lipid is also necessary to prevent contact of oxygen with unsaturated fatty acids. Microencapsulation that has been practiced as a packaging technique for oils can coat oil droplets and prevent their contact with atmospheric oxygen (59). In addition, hydrogenation controls autoxidation by reducing the reactivity of lipid molecule, but at the cost of reducing or eliminating PUFA and compromising the nutritive value of the oil. Moreover, antioxidants may be added at very low concentrations to control oxidation without changing the color and flavor to preserve the nutritive value of oils (2).

## 5. PROCESSING

The basic processing steps for the manufacturing of marine oils for human consumption involve cooking or rendering to release the oil followed by possible degumming, alkali refining, bleaching, and finally deodorization as well as possible

addition of antioxidants (2). During these processing steps, free fatty acids, mono- and diacylglycerols, phospholipids, sterols, vitamins, hydrocarbons, pigments, proteins and their degradation products, suspended mucilaginous and colloid-like matter, and oxidation products of fatty acids are removed from the oil (60). Processing steps of marine oils are similar to those for vegetable oils; however, the quality of crude marine oils is less uniform than that for crude vegetable oils. To obtain high-quality crude marine oils, proper handling and chilling of raw material to minimize oxidative damage after landing is vital (2).

The rendered, crude oil from blubber of marine mammals can also be treated with silica at low temperature under vacuum followed by bleaching and deodorization, as described by Mag (48). The resulting oil, which is completely bland, is essentially free of proteinaceous materials, phosphatides and mucilage, and prooxidant metals and very low in colored compounds, peroxides, and secondary oxidation products. This method avoids the use of acids and bases that are required in conventional degumming and alkali refining of marine oils, thus eliminating the risk of contamination as well as reducing the number of processing steps. The method is also environmentally friendly because it does not require soapstock and waste water processing (48). Another approach for preparing and stabilizing food-grade marine oil has been proposed by Kendrick and Macfarlane (49). This method includes treating the oil with silica, optionally in the presence of carbon, and with vacuum steam deodorization at 140–210°C in the presence of antioxidants (49). Regardless of the processing method employed, the resultant product must be stabilized by addition of food-grade antioxidants, particularly mixed tocopherols.

## 6. PRODUCTION OF $\omega$ 3 FATTY ACID CONCENTRATES

Supplementaion of  $\omega$ 3 fatty acids has been recommended in addition to making attempts to substitute saturated fatty acids with PUFA in dietary lipids. Marine oils serve as a rich source of  $\omega$ 3 fatty acids and may be used as the raw material for preparation of  $\omega$ 3 fatty acid concentrates. It has been suggested that PUFA concentrates devoid of more saturated fatty acids are much better than marine oils because they allow keeping the daily intake of total lipids as low as possible (61). Enriched fatty acids or esters can be produced by fractional vacuum distillation (62), low-temperature crystallization (63), chromatographic separation, including HPLC (64–66), silver resin chromatography (67), supercritical fluid extraction (68), urea complexation (69, 70), and enzyme hydrolysis (71), among others.

Fractional vacuum distillation takes advantage of differences in the boiling points of fatty acids under vacuum. This method is a an old one and requires high temperature. The fractionation of marine oil esters is difficult because separation of such components becomes less effective with increasing molecular weight (72).

Low-temperature crystallization is based on the fact that the melting point of fatty acids changes considerably with the type and degree of unsaturation (73). At low temperatures, long-chain saturated fatty acids that have higher melting

points crystallize out and PUFAs remain in the liquid form. Briefly, the process consists of cooling the oil or fatty acids in a solvent, which holds for a specified period of time, and of removing the crystallized fraction by filtration. This method requires the least amount of equipment and the simplest apparatus and has been an indispensable method for preparing pure fatty acids (74, 75).

Fatty acids could also be separated according to their carbon number or degree of unsaturation with appropriate adsorbents (63). Chromatographic techniques such as HPLC and silver resin chromatography have been successfully employed to prepare  $\omega$ 3 fatty acid concentrates. However, these methods have certain shortcomings, including use of organic solvents, loss of resolution of the column upon repeated use, and difficulties in scaling up the process for commercial production (76).

Supercritical fluid extraction (SFE) is a method that circumvents some problems associated with conventional separation techniques. Carbon dioxide, as an inert, inexpensive, nonflammable, and environmentally acceptable gas is the solvent of choice because of its moderate critical temperature and pressure (76). SFE has been used effectively to refine marine oils and remove cholesterol, polychlorinated biphenyls (PCB), Vitamin E, and other components (77). The disadvantages of this process include the use of extremely high pressures and the high capital cost.

The simplest and most efficient technique for obtaining  $\omega$ 3 PUFA concentrates in the form of free fatty acids is urea complexation. This technique is well established for elimination of saturated and monounsaturated fatty acids (70). In this method, the saturated and monounsaturated fatty acids can easily complex with urea after hydrolysis of TAG with alkaline, and crystallize out on cooling and may subsequently be removed by filtration (70). This method is favored by many researchers because complexation depends on the configuration of the fatty acid moieties because of the presence of multiple double bonds, rather than of pure physical properties such as melting point or solubility (10).

It is generally considered that PUFA in the acylglycerol form is nutritionally more favorable than the corresponding methyl or ethyl esters as impaired intestinal absorption of alkyl esters of  $\omega$ 3 fatty acids has been observed in laboratory animals (78–81). Although most methods produce PUFA concentrates in the form of free fatty acids or their corresponding alkyl esters, enzyme hydrolysis is a technique proposed to produce  $\omega$ 3 fatty acids concentrates in the form of acylglycerols by hydrolyzing the TAG with lipase. Saturated and monounsaturated fatty acids can be easily hydrolyzed because they do not present any barriers to lipases such as the commercial microbial lipases (71).

Commercial marine mammal oils, such as seal blubber oil products, are available in form of soft gel capsules as nutritional supplements (82). The quality parameters of three commercial seal oil capsules are listed in Table 9.

Marine mammal oils or their  $\omega$ 3 concentrates can also be modified for different applications. Modifications include the changing of the fatty acid composition and/or their location in the glycerol backbone. Structured lipids containing both  $\omega$ 3 long-chain PUFAs, possibly from seal blubber oil, or their  $\omega$ 3 concentrates, and medium-chain fatty acids (MCFAs), which are saturated fatty acids with 6–12

**TABLE 9. Quality Comparison of  $\omega$ 3 Seal Oil Capsules (Laboratory Analysis).<sup>1</sup>**

Quality Indicators	Brand A	Brand B
EPA w/w %	6.89 $\pm$ 0.07	6.43 $\pm$ 0.07
DPA w/w %	4.11 $\pm$ 0.02	3.76 $\pm$ 0.00
DHA w/w %	8.16 $\pm$ 0.01	8.50 $\pm$ 0.10
18:3 $\omega$ 3 w/w %	0.48 $\pm$ 0.01	0.48 $\pm$ 0.00
18:4 $\omega$ 3 w/w %	1.10 $\pm$ 0.04	1.35 $\pm$ 0.06
Tocopherols mg/100 g	Up to 400	Up to 1400
Acid value mg KOH/g oil	3.21 $\pm$ 0.05	1.69 $\pm$ 0.03
Conjugated dienes	5.80 $\pm$ 0.25	7.63 $\pm$ 0.13
TBARS value $\mu$ mol/g oil	4.20 $\pm$ 0.05	4.50 $\pm$ 0.15

<sup>1</sup>From Ref. (56).

carbon atoms, have been produced. Enzyme-catalyzed synthesis of structured lipids has been proposed, with commercial lipase preparations (83). The final products, with reduced calorie, exhibit the combined health benefits of long-chain PUFAs and MCFAs, which are believed to possess many unique nutritional and metabolic characteristics (83).

## 7. APPLICATIONS

Marine oils have been widely used in food and pharmaceutical industries as well as in nonedible applications. The nonedible uses of marine oils primarily exploit their highly unsaturated nature. In leather manufacturing, sulfated marine oils are used to treat leather to prevent its brittleness and dryness. Oleochemicals (fatty acids, fatty alcohols, esters of methyl and other alcohols, nitrogen derivatives) derived from marine oils find a wide range of industrial applications, including use in lubricants, corrosion inhibitors, plastic and rubber compounding, floatation agents, personal care products, cleaners, textile and paper additives, asphalt additives, and tableting, among others. In addition, marine oils have long been used as an alternative fuel to petroleum-based products (2). Other industrial uses of marine oils are in the manufacturing of polyurethane resins, cutting oils, caulks and sealants, printing ink formulations, insecticides, and buffing compounds (84). Refined marine oils have also been used in skin and hair care products. Marine oils may be used in the manufacturing of animal and poultry feed. Traditionally, marine oils have been used as an economic source of calories to stimulate growth of farmed animals. However, current knowledge about successful inclusion of EPA and DHA to mitochondria, microsomes, and lipoprotein membranes of chicken by feeding marine oil supplemented diets has provided novel uses for marine oils in the animal feed industry (2). It has been demonstrated that chicken has a natural predisposition to accumulate EPA and DHA from the precursor C18:3 ( $\omega$ 3) (85). Thus, inclusion of fish meal in chicken diet enhanced the accumulation of EPA and DHA in chicken flesh

(85). Feeding seal blubber oil at 1.25% to hens was found to increase long-chain  $\omega$ 3 PUFA and decrease arachidonic acid in the egg yolk lipids without any detriment to their sensory properties (82). This result is not surprising because deodorized oil was used in this study and the level of inclusion of seal oil was modest. In the food industry, the major global use of marine oils has been in the manufacturing of margarine and other edible oil products. In this, hydrogenated marine oils were a low-priced alternative to vegetable oils. However, hydrogenation reduces the unsaturation of fatty acids and negates the potential health benefits of PUFA; if not fully hydrogenated, introduction of trans-fats to product formulation is also of concern. Therefore, incorporation of long-chain fatty acids into the diet continues to be a topic of interest for food manufacturers, scientists, and consumers (2).

## 8. HEALTH BENEFITS AND DISEASE PREVENTION

Recognition of the health benefits associated with consumption of seafoods ( $\omega$ 3 fatty acids) is one of the most promising developments in human nutrition and disease prevention research in the past three decades. According to our current knowledge, long-chain  $\omega$ 3 PUFAs play an important role in the prevention and treatment of coronary artery disease (86), hypertension (87), diabetes (88), arthritis and other inflammatory (89), autoimmune disorders (90), as well as cancer (91, 92) and are essential for normal growth and development, especially for brain and retina (93). The most direct and complete source of  $\omega$ 3 oils is found in the blubber of certain marine mammals, especially in the harp seal. Among its advantages is that the body's absorption of  $\omega$ 3 fatty acids from marine mammal blubber may be faster and more thorough than is the case with flaxseed and fish oils (48). As marine mammal oils contain a high concentration of MUFAs, it is possible that some of their beneficial effects may be ascribed to their MUFAs or to the combined effect of MUFA and  $\omega$ 3 PUFA (94). A pilot study indicated that a low dose of seal oil supplementation can reduce atherogenic risk indices in young healthy persons, and the effects are strongly dependent on the integrated  $\omega$ 3 fatty acids dose (95,96). The essential fatty acids found in seal oil include a high level of DPA (up to ten times that of fish oils). There is growing evidence that DPA is the most important fatty acid that keeps artery walls soft and plaque free (48). Marine oils are also attractive from a nutritional point of view because they are thought to provide specific physiological functions against thrombosis, cholesterol buildup, and allergies (50). Oils from the blubbers of seal and whale have beneficial effects on selected parameters that play a role in cardiovascular disease; it has been hypothesized that the effect of whale oil is not mediated by its  $\omega$ 3 fatty acids alone (97). The difference in the beneficial effects of whale and seal oils on cardiovascular disease may argue against the distribution of  $\omega$ 3 fatty acids in TAG as being relevant to the superiority of whale oil, because the  $\omega$ 3 fatty acids are mainly in the *sn*-1 and *sn*-3 positions of both oils. The effect of whale oil is probably not mediated by  $\omega$ 3 fatty acids alone as the content of these fatty acids is relatively low in whale oil. Thus, in addition to



$\omega$ 3 fatty acids, other dietary factors may play a role in the protective effects against atherosclerosis and thrombosis in Greenland Eskimos (97).

The beneficial effects of PUFA have also been ascribed to their ability to lower serum TAG, to increase membrane fluidity, and to reduce thrombosis by conversion to eicosanoids (98). Both EPA and DHA induced increases in the serum concentrations of the corresponding fatty acids as well as in their relative contents in platelets (99). However, distribution of  $\omega$ 3 PUFA in TAG molecules influences glycerolipid metabolism and arachidonic acid contents of serum and liver phospholipids as well as thromboxane (TX)  $A_2$  production. In rats that were fed marine oils, for instance, plasma and liver TAG concentrations were more effectively reduced by dietary seal oil than by fish oil. Furthermore, dietary seal oil reduced arachidonic acid content in liver phosphatidylcholine and phosphatidylethanolamine and serum phosphatidylcholine more effectively than fish oil. Activities of fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH), and the malic enzyme were significantly lowered when hamsters were fed seal oil (100). The predominant effect of seal oil was caused by the suppression of fatty acid synthesis in the liver (101). In addition, reduction of TX  $A_2$  production of platelets and whole blood platelet aggregation by seal oil has been observed (102, 103).

## 9. HEALTH EFFECTS OF DPA

Docosapentaenoic acid (DPA) is an elongation product of EPA. Unlike other  $\omega$ 3 fatty acids, DPA has not been studied in any detail. Because of its availability, DPA is present in a much lower concentration compared with that of EPA and DHA in marine oils, and because of the difficulty in purifying it from mixtures containing EPA and DHA with similar physicochemical properties (104). Although only less than 1% DPA can be found in most fish oils, it is relatively more abundant in seal oil. Harp seal oil contains 4–6% of DPA. DPA is almost as important as either EPA or DHA. About one-third of the long-chain  $\omega$ 3 fatty acids circulating in human blood are attributed to DPA as the effective agent (105). DPA may have pharmacological effects different from those of EPAs and DHAs, and it has recently appeared as a focus topic (104).

It has been demonstrated that angiogenic activity in endothelial cells induced by vascular endothelial growth factor (VEGF) can be suppressed by  $\omega$ 3 PUFA treatment. Among LC PUFA, DPA was the most potent inhibitor of angiogenesis; the inhibitory activity by DPA pretreatment was approximately six-fold in comparison with that of EPA and DHA, which indicates that DPA could be developed as a novel drug or supplement against angiogenesis-related diseases (106). Angiogenesis plays a major role in tumor growth and metastasis, and blocking angiogenesis can restrict tumor growth. In addition, the stimulative effect of EPA on endothelial cells migration may be caused by DPA. In vitro studies have revealed that the activity of DPA to stimulate endothelial cell migration is ten times higher than EPA (107). Therefore, it is possible that the antiarteriosclerotic function of seal oil is mainly caused by DPA rather than by EPA and DHA (104). Evidence suggests that DPA is the

most important fatty acid in keeping artery walls soft and plaque-free (105). A recent study published by Tokyo Medical and Dental University indicates that DPA can be more than ten times as effective as EPA in helping to heal damaged blood vessels (105). Moreover, arachidonic acid-stimulated blood platelet aggregation was inhibited by  $\omega 3$  fatty acids in a dose-dependent manner, among which DPA was the most potent inhibitor (105). DPA exhibits considerable activity for interfering with the cyclooxygenase pathways, thus inhibiting platelet aggregation most effectively (108). In addition, it has been suggested that the DPA concentration in platelet is inversely associated with coronary artery disease in women (109), and a high proportion of DPA in serum is associated with a decreased risk of acute coronary events in middle-aged men (110).

## 10. COMPARISON OF FISH OIL AND MARINE MAMMAL OIL

Fish oils and marine mammal oils are generally characterized by a large group of saturated and unsaturated fatty acids, which are commonly associated with their mix of TAGs (16). However, differences exist between the oils from fish and marine mammal sources.

Fish oils may generally be described as flesh oil, liver oil, or oil of the whole fish (111). The livers of white lean fish are known to be high in oil content. The fish livers of cod, halibut, and shark contain approximately 50% oil and serve as an important source of Vitamin A and Vitamin D (112). TAG is the major component of depot fats of fish. Ether lipids, however, are restricted to the liver oils of deep sea sharks (113). Like marine mammal oils, fish oils are rich in MUFA and PUFA, and they are good sources of  $\omega 3$  PUFA such as EPA and DHA. However, DPA, which is abundant in blubber of marine mammals, especially seals, is found in much lower level or is absent in fish oils. Furthermore, the molecular configurations of EPA and DHA in fish oil vary slightly from that found in marine mammal oils (48). Research has shown that seal oil may be more beneficial than fish oil in reducing the risk of heart disease and diabetes, which is likely because of the relative absence of DPA in fish oil and possibly the slower rate at which the body can use EPA and DHA from fish oil (105). Fish oils vary considerably in the type and level of their fatty acids depending on the particular species and their diets. For example, fish species raised by aquaculture often have a lower level of  $\omega 3$  fatty acids than those in the wild (48), and freshwater fish contain higher levels of the  $\omega 6$  fatty acids than do marine fish (112). The fatty acid distribution in TAG of fish oil is also different from that of marine mammal oil. In fish oil, PUFAs occupy the *sn*-2 position of TAG, saturated and MUFAs the *sn*-1 position, and MUFAs the *sn*-3 position. In marine mammals, however, the *sn*-1 and *sn*-3 positions are occupied by LC PUFA such as EPA and DHA, and especially the *sn*-3 position, as noted earlier. The *sn*-2 position is esterified to saturated fatty acids and especially to C16 and C18 MUFAs (14, 112). The different distribution of fatty acids might be a factor for lower oxidative stability of fish oils compared with seal oil (48).

## REFERENCE

1. R.G. Ackman, and F. Lamothe, in R. G. Ackman, ed., *Marine Biogenic Lipids, Fats, and Oils*, vol. 2, 1989, CRC Press, Boca Raton, Florida.
2. F. Shahidi, in F. Shahidi, ed., *Seal Fishery and Product Development*, Science Tech, NF, Canada, 1988.
3. P. Bjerregaard and J. Dyerberg, *Int. J. Epidemiol.*, **17**, 514–519 (1988).
4. P. Bjerregaard, G. Mulvad, and H. S. Pederson, *Int. J. Epidemiol.*, **26**, 1182–1190 (1997).
5. R. Gales, D. Renouf, and E. Noseworthy, *Can. J. Zool.*, **72**, 545–551 (1994).
6. E. Durnford and F. Shahidi, *J. Amer. Oil Chem. Soc.*, **79**, 1095–1102 (2002).
7. O. T. Oftedal, W. D. Bowen, and D. J. Boness, *Physiol. Zool.*, **66**, 412–436 (1993).
8. R. G. Ackman and R. D. Burgher, *Can. J. Biochem. Physiol.*, **41**, 2501–2505 (1963).
9. S. J. Iverson, *Symp. Zool. Soc. Lond.*, **66**, 263–291 (1993).
10. U.N. Wanasundara, Ph.D. dissertation, Memorial University of Newfoundland, 1997.
11. C. Borch-Jensen, and J. Mollerup, *Chromatographia*, **42**, 252–258 (1996).
12. S. Hashimoto, S. Dayton, and J. C. Roberts, Jr., *Comp. Biochem. Physiol.*, **20**, 975 (1967).
13. F. Shahidi, J. Synowiecki, R. Amarowicz, and U. Wanasundara, in C. T. Ho, and T.G. Hartaman, eds., *Lipids in Food Flavors*, ACS Symposium Series 558, American Chemical Society, Washington, D.C., 1994, p. 233–243.
14. G. K. Holmer, in R. G. Ackman, ed., *Marine Biogenic Lipids, Fats, and Oils*, vol. 1, CRC Press, Boca Raton, Florida, 1989.
15. R. F. Lee and J. S. Patton, in R. G. Ackman, ed., *Marine Biogenic Lipids, Fats, and Oils*, vol. 1, CRC Press, Boca Raton, Florida, 1989.
16. E. H. Gruger, Jr., in M. E. Stansby, ed., *Fish Oils*, AVI Publishing, Westport, Connecticut, 1967.
17. I. A. Hansen and C. C. Cheah, *Comp. Biochem. Physiol.*, **31**, 757 (1969).
18. C. Litchfield, A. J. Greenberg, D. K. Caldwell, M. C. Caldwell, J. Sipos, and R. G. Ackman, *Comp. Biochem. Physiol. B*, **50**, 591 (1975).
19. C. Litchfield, A. J. Greenberg, and J. G. Mead, *Cetology*, **23**, 10 (1976).
20. J. C. Nevenzel, *Lipids*, **5**, 308 (1970).
21. J. R. Sargent, R. F. Lee, and J. C. Nevenzel, in P. Kolattukudy, ed., *Chemistry and Biochemistry of Natural Waxes*, Elsevier, Amsterdam, 1976.
22. J. R. Sargent, *Sci. Prog. Oxford*, **65**, 437 (1978).
23. J. H. Heller, M. S. Heller, S. Springer, and E. Clark, *Nature*, **179**, 919–920 (1957).
24. M. L. Karnovsky and W. S. Rapson, *J. Soc. Chem. Ind. London*, **66**, 124 (1947).
25. E. Andre and H. Canal, *Bull. Soc. Chim.*, **45**, 498 (1929).
26. Y. Toyama and T. Isihawa, *J. Chem. Soc. Jpn.*, **59**, 1193 (1938).
27. T. Tsuchiya and A. Tanaka, *Chem. Abstr.*, **50**, 11035 (1956).
28. T. Tsuchiya, *Chem. Abstr.*, **47**, 12569 (1953).
29. T. Hashimoto and H. Mamuro, *Chem. Abstr.*, **68**, 51165 (1968).
30. N. R. Bottino, *Lipids*, **13**, 18 (1978).
31. T. Tsuchiya and A. Kato, *Chem. Abstr.*, **46**, 11717 (1952).

32. M. Blumer and D. W. Thomas, *Science*, **148**, 370 (1965).
33. R. G. Ackman, in R. G. Ackman, ed., *Marine Biogenic Lipids, Fats, and Oils*, vol. 1, CRC Press, Boca Raton, Florida, 1989.
34. P. K. Christensen and R. A. Gehe, *Acta Chem. Scand.*, **16**, 1153 (1965).
35. H. O. Bang, J. Dyerberg, and N. Hjoorne, *Acta Med. Scand.*, **200**, 69–73 (1976).
36. H. O. Bang, J. Dyerberg, and H. M. Sinclair, *Am. J. Clin. Nutr.*, **33**, 2657–2661 (1980).
37. F. Shahidi, U. N. Wanasundara, and R. Amarowicz, in O. Mills, H. Okai, A. M. Spanier, and M. Tamura, eds. *Chemistry and Novel Foods*, American Chemical Society, Washington, D.C., 1996.
38. R. G. Ackman, C. A. Eaton, and P. M. Jangaard, *Can. J. Biochem.*, **43**, 1513–1520 (1965).
39. W. Yongmanichai and O. P. Ward, *Prog. Biochem.*, **24**, 117–125 (1989).
40. E. Durnford and F. Shahidi, *J. Amer. Oil Chem. Soc.*, **80**, 405 (2003).
41. O. Grahl-Nielsen and D. Mjaavatten, *Marine Biol.*, **110**, 59–64 (1991).
42. G. C. West, J. J. Burns, and M. Modafferi, *Can. J. Zool.*, **57**, 1249–1255 (1979).
43. F. R. Engelhardt and B. L. Walker, *Comp. Biochem. Physiol. B*, **47**, 169 (1974).
44. G. C. West, J. J. Burns, and M. Modafferi, *Can. J. Zool.*, **57**, 189–195 (1979).
45. R. G. Ackman, S. N. Hooper, and J. Hingley, *Can. J. Biochem.*, **50**, 833 (1972).
46. U. N. Wanasundara, and F. Shahidi, in F. Shahidi and K. K. Cadwallader, eds. *Flavor and Lipid Chemistry of Seafoods*, American Chemical Society, Washington, D.C., 1997.
47. M. Aursand, L. Jorgensen, and H. Grasdalen, *J. Amer. Oil Chem. Soc.*, **72**, 293–297 (1995).
48. T. Mag, Patent WO00/44862A1, PCT International Patent Application 2000.
49. A. Kendrick and N. Macfarlane, U.S. Patent 6623774B2, 2003.
50. H. Kimoto, Y. Endo, and K. Fujimoto, *J. Amer. Oil Chem. Soc.*, **71**, 469–473 (1994).
51. K. Miyashita and T. Takagi, *J. Amer. Oil Chem. Soc.*, **65**, 1156 (1988).
52. F. Shahidi, F. Hamam, and M. Ahmad, Khan, *J. Amer. Oil Chem. Soc.*, in press.
53. F. Hamam and F. Shahidi, *J. Agric. Food Chem.*, **52**, 2900–2906 (2004).
54. F. Hamam and F. Shahidi, *Food Chem.*, **91**, 583–591 (2005).
55. F. Hamam and F. Shahidi, *J. Amer. Oil Chem. Soc.*, **81**, 1–7 (2004).
56. F. Shahidi and U. N. Wanasundara, *J. Food Lipids*, **6**, 159–172 (1999).
57. R. C. Lindsay, *Food Prod. Dev.*, **11**, 93–96 (1977).
58. D. B. Josephson, R. C. Lindsay, and D. A. Stuibler, *J. Food Sci.*, **55**, 5–9 (1985).
59. F. Shahidi and R. B. Pegg, *J. Food Sci.*, **56**, 1500–1504 (1991).
60. A. P. Bimbo and J. B. Crowther, *Infofish Int.*, **6**, 20–25 (1991).
61. N. Haagsma, C. M. Gent, J. B. Luten, R. N. Jong, and E. Doorn, *J. Amer. Oil Chem. Soc.*, **59**, 117–118 (1982).
62. R. G. Ackman, P. J. Ke, and P. M. Jangaard, *J. Amer. Oil Chem. Soc.*, **50**, 1 (1973).
63. J. B. Brown and D. K. Kolb, *Prog. Chem. Fats Lipids*, **3**, 57–94 (1955).
64. M. I. Aveldano, M. van Rollins, and L. A. Horrocks, *J. Lipid Res.*, **24**, 83 (1983).
65. J. Halgunset, E. W. Lund, and A. Sunde, *J. Chromatogr.*, **237**, 496 (1982).
66. J. P. Roggero and S. V. Coen, *J. Liq. Chromatogr.*, **4**, 1817 (1981).

67. R. O. Adolf and E. A. Emken, *J. Amer. Oil Chem. Soc.*, **62**, 1592 (1985).
68. W. B. Nilsson, E. J. Gauglitz, J. K. Hudson, V. F. Stout, and J. Spinelli, *J. Amer. Oil Chem. Soc.*, **65**, 109 (1988).
69. M. A. Grompone, *Fett. Wissenschaft. Technologie.*, **94**, 388–394 (1992).
70. U. N. Wanasundara and F. Shahidi, *Food Chem.*, **65**, 41–49 (1999).
71. U. N. Wanasundara and F. Shahidi, *J. Amer. Oil Chem. Soc.*, **75**, 945–951 (1998).
72. H. Breivik, *Modern Applications of Marine Oils*, Toronto, Ontario, Canada, May 7–8, 1992.
73. G. G. Haraldsson, *J. Amer. Oil Chem. Soc.*, **61**, 219–222 (1984).
74. H. Schlenk, *J. Amer. Oil Chem. Soc.*, **38**, 728–736 (1961).
75. K. S. Markley, in K. S. Markey, ed., *Fatty Acids*, 2nd ed, Part 3, Interscience Publishers, New York, 1964, pp. 1983–2123.
76. V. K. Mishra, F. Temelli, and B. Ooraikul, *Food Res. Int.*, **26**, 217–226 (1993).
77. S. S. H. Rizvi, J. A. Daniels, A. L. Bernado, and J. A. Zollweg, *Food Technol.*, **40**, 57–64 (1986).
78. T. Hamazaki, A. Hirai, T. Terano, J. Sajiki, S. Koado, T. Fujita, Y. Tamura, and A. Kumagai, *Prostaglandins*, **23**, 557–567 (1982).
79. S. El-Boustani, C. Colette, L. Monnier, B. Descomps, C. A. Paulet, and F. Mendey, *Lipids*, **22**, 711–714 (1987).
80. L. D. Lawson and B. G. Hughes, *Biochem. Biophys. Res. Comm.*, **152**, 328–335 (1988).
81. D. D. Hong, Y. Takahashi, M. Kushiro, and T. Ide, *Biochim. Biophys. Acta*, **1635**, 29–36 (2003).
82. M. Schreiner, H. W. Hulan, E. Razzazi-Fazeli, J. Boehm, and C. Iben, *Poultry Sci.*, **83**, 462–473 (2004).
83. S. P. J. N. Senanayake and F. Shahidi, *Food Res. Int.*, **35**, 745–752 (2002).
84. P. M. Jangaard, Report No. 115, Program Development, Fisheries Branch, Halifax, Nova Scotia, 1986.
85. R. G. Ackman, M. F. Lamothe, H. W. Hulan, and E. Proudfoot, *N-3 News*, **111**, 1–4 (1988).
86. M. L. Wahlqvist, *Nutrition*, **14**, 627–633 (1998).
87. P. R. C. Howe, *Ann. NY Acad. Sci.*, **827**, 339–352 (1997).
88. I. Krishna Mohan and U. N. Das, *Nutrition*, **17**, 126–151 (2001).
89. T. Babcock, W. S. Helton, and N. J. Espat, *Nutrition*, **16**, 1116–1118 (2000).
90. D. S. Kelly, *Nutrition*, **17**, 669–673 (2001).
91. D. P. Rose and J. M. Connolly, *Pharmacol. Ther.*, **83**, 217–244 (1999).
92. T. Akihisa, H. Tokuda, M. Ogata, M. Ukiya, M. Iizuka, T. Suzuki, K. Metori, N. Shimizu, and H. Nishino, *Cancer Lett.* **2–5**, 9–13 (2004).
93. G. J. Anderson, W. E. Connor, and J. D. Corliss, *Pediatr. Res.*, **27**, 89–97 (1990).
94. J. C. Hansen, H. Sloth Pedersen, and G. Mulvad, *A Review Arctic. Med. Res.*, **53**, 4–17 (1994).
95. E. C. Bonefeld Jorgensen, S. M. Moller, and J. C. Hansen, *Int. J. Circumpolar. Health*, **60**, 25–33 (2001).

96. B. Deutch, E. C. Bonefeld Jorgensen, and J. C. Hansen, *Nutr. Res.*, **20**, 1065–1077 (2000).
97. B. Osterud, E. Elvevoll, H. Barstad, J. Brox, H. Halvorsen, K. Lia, J. O. Olsen, R. L. Olsen, C. Sissener, O. Rekdal, and E. Vognild, *Lipids*, **30**, 1111–1118 (1995).
98. J. E. Kinsella, *Food Technol.*, **40**, 89–97 (1986).
99. E. Vognild, E. Elvevoll, J. Brox, R. L. Olsen, H. Barstad, M. Aursand, and B. Osterud, *Lipids*, **33**, 427–436 (1998).
100. H. Yoshida, I. Ikeda, M. Tomooka, M. Mawatari, K. Imaizumi, A. Seto, and H. Tsuji, *J. Nutr. Sci. Vitaminol.*, **47**, 242–247 (2001).
101. H. Yoshida, M. Mawatari, I. Ikeda, K. Imaizumi, A. Seto, and H. Tsuji, *J. Nutr. Sci. Vitaminol.*, **45**, 411–421 (1999).
102. I. Ikeda, H. Yoshida, M. Tomooka, A. Yoset, K. Imaizumi, H. Tsuji, and A. Seto, *Lipids*, **33**, 897–904 (1998).
103. J. Brox, K. Olaussen, B. Osterud, E. O. Elvevoll, E. Bjornstad, T. Brenn, G. Brattebo, and H. Iversen, *Lipids*, **36**, 7–13 (2001).
104. K. Yazawa, *World Rev. Nutr. Diet.*, **88**, 249–252 (2001).
105. C. Ho, *Omega 3: the Seal Connection 2003*, Flanker Press Ltd., St. John's, Newfoundland, Canada.
106. M. Tsuji, S. Murota, and I. Morita, *Prostaglandins, Leukotrienes and Essential Fatty Acids*, **68**, 337–342 (2003).
107. T. Kanayasu-Toyoda, I. Morita, and S. Murota, *Prostaglandins, Leukotrienes and Essential Fatty Acids*, **54**, 319–325 (1996).
108. S. Akiba, T. Murata, K. Kitatani, and T. Sato, *Biol. Pharm. Bull.*, **23**, 1293–1297 (2000).
109. J. M. Hodgson, M. L. Wahlqvist, J. A. Boxall, and N. D. Balaz, *Am. J. Clin. Nutr.*, **58**, 228–234 (1993).
110. T. Rissanen, S. Voutilainen, K. Nyyssonen, T. A. Lakka, and J. T. Salonen, *Circulation*, **9**, 7–2679 (2000).
111. E. Aaes-Jorgensen, in M. E. Stansby, ed., *Fish Oils*, AVI Publishing, Westport, Connecticut, 1967.
112. F. B. Padley, F. D. Gunstone, J. L. Harwood, in F. D. Gunstone, J. L. Harwood, and F. B. Padley, eds., *The Lipid Handbook*, Chapman and Hall Ltd. New York, 1986.
113. C. Litchfield, A. J. Greenberg, C. E. Eaton, and R. G. Ackman, *Lipids*, **13**, 860 (1978).

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## *Fish Oils*

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### **1. INTRODUCTION**

At one time “fish oils” were low-cost industrial materials for the paint and linoleum industries. After World War II (WWII), these industries switched to chemicals and plastics, and therefore, much information in older books became obsolete by 1960. Hydrogenation of fats to produce margarines and shortenings, starting about 1900, led to improved oil refining and better quality and included whale oils when these animals were still plentiful. Two factors have recently impacted negatively on large-scale and continued use of marine oils in our food supply.

Because of one of the earliest media-stimulated public health panics in the late 1970s, that of the erucic acid (22:1) of rapeseed and mustard oils, alleged to damage hearts, food use of partially hydrogenated fish oils in that form petered out because of their content of both natural and artifact 22:1 isomeric fatty acids, as described in Barlow and Stansby (1). As millions of healthy Germans and Poles had thrived on rapeseed, and the fish-eating Scandinavians were universally healthy, this fear was based on scanty evidence. The major result of this scare against sources of very long-chain monoethylenic fatty acids was acceleration of development of low-erucic-acid rapeseed oils such as canola (2). The desirable physical properties of partially hydrogenated fish oils in some margarines and in shortening for baking purposes continued but depended primarily on conversion of most of the

natural *cis*-ethylenic bonds of the fatty acids to *trans*-ethylenic bond configurations, rather than to saturated fatty acids (3). Recently, a media storm against *trans*-acids followed initial scientific research on the health aspects of small changes in serum cholesterol, despite long-term human exposure (4), and again partially hydrogenated fish oil use was condemned. Despite the adverse image, the baking industry was conservative and this usage persisted in technically advanced countries such as Denmark until recently (5). Dairy products and ruminant meat products continue to include “natural” *trans*-acids (6).

In a positive turn of fate, by 1980, the observations of Dyerberg and Bang (7) on the excellent cardiac health of the Eskimo population of Greenland, who had a high intake of dietary omega-3 (n-3) fatty acids from seal and fish fats, were also noted in the media. This publication in 1979 created an astounding public interest in fish oils as nutrition supplements, usually taken in capsules. In 1985, another positive media bombshell exploded when a long-term study in Zutphen in the Netherlands showed that a large male population group had reduced their cardiovascular mortality rate by eating fish regularly (8). Obviously, fish omega-3 (n-3) fatty acids were beneficial.

By 1994, the United Kingdom had followed up on such reports and released an official government medical report recommending that people eat fish at least twice a week, one meal being of fatty fish (9). Belatedly the American Heart Association released a “Scientific Statement” in 2002, with a similar recommendation (10).

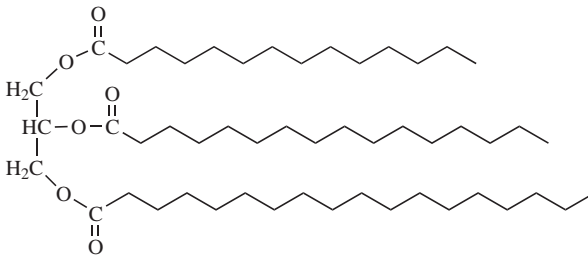
Through this exciting period the dietary intake of fish in western society had declined as other modern foods were easier to produce, store, and distribute. Fish were also beginning to be more scarce as fisheries were overexploited and fish populations diminished or were even wiped out.

In this review, only fish “oils” can be considered. The fat in edible parts of fish ranges from about 16% down to 0.7%, the latter being almost exclusively the basic muscle phospholipids (Figure 1). These lipids are an excellent source of the highly unsaturated C20 and C22 omega-3 fatty acids of medical interest, and the content of omega-3 fatty acids is stable at roughly 0.5-g/100-g muscle. However, it is the muscle triacylglycerols that are variable for quantity and quality. This subject is conveniently reviewed for the food industry in one medium-length paper (11) in a journal available in most major libraries, being a part of a whole issue (No. 4) on seafood. A chapter on marine fatty acids in a reference book associating all types of fatty acids with specific health problems could also be useful (12). As for the actual health benefits of the omega-3 fatty acids, a special supplement issue of the *American Journal of Clinical Nutrition* (13) is also highly recommended as it draws together the thoughts and facts from several dozen distinguished authors active in the field of omega-3 fatty acids.

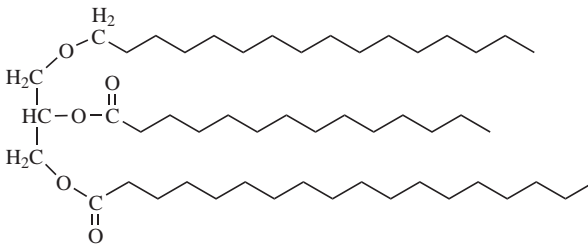
It must be noted that the promotional bandwagon for fish oils and seafood omega-3 fatty acids has been neatly hijacked by those oilseed companies offering a vegetable oil omega-3 fatty acid, alpha-linolenic acid (18:3n-3). Only about 10% of this fatty acid in the diet survives immediate catabolism and is needed for “essential” roles in the skin and elsewhere (14). Whether enough survives to be elongated and to enrich brain and retinal tissues and thus to perform the vital



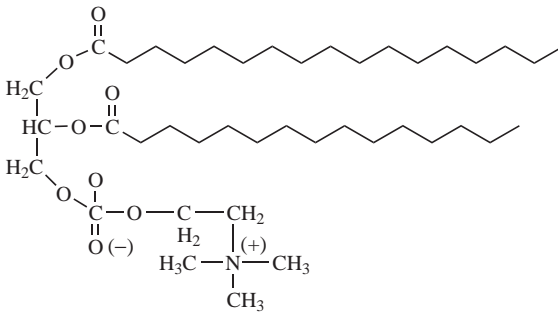
## Glycerol-Based Fish Lipid Classes



Triacylglycerols



Diacyl-1-glyceryl ether



Phosphatidyl Choline

**Figure 1.** Three classes of lipids found in fish bodies and sharing in common glycerol fatty acid linkages.

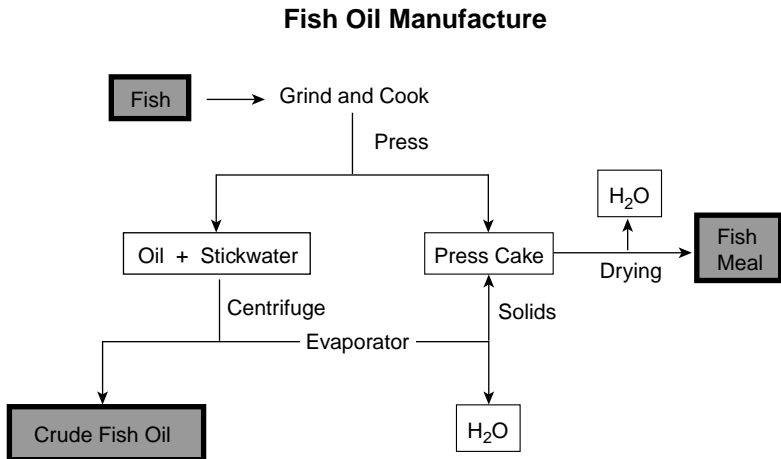
functions of the fish DHA or docosahexaenoic acid (22:6n-3) and EPA or eicosapentaenoic acid (20:5n-3) is not known, but the distinction by chain lengths should always be made. The term “conditionally dispensable” coined by one expert (15) is perhaps suitable for 18:3n-3 at this time and in this context of recommending marine oils rich in long-chain n-3 fatty acids. Only human trials can be trusted to be definitive, and modest doses of vegetable (C18) or fish (C20 + C22) fatty acids for 12 weeks, although informative (16), cannot accurately predict the life expectancy

and good health desired by the public. These trials are often linked to modifications in diet, for example, reduction in intake of n-6 fatty acids. Critical reexamination of published results is highly desirable and useful (17, 18).

Throughout the last 250 years, cod liver oil has steadily maintained a health role well known to be inclusive of vitamins A and D. Our aging population is increasingly faced with osteoporosis and mobilization of calcium intake to strengthen bones, now a high priority in the adult population that requires Vitamin D, whereas formerly the benefit target was rapidly growing children. Cod liver oil remains a nutritional supplement staple for vitamins, but it also contains omega-3 fatty acids (19). However, it is a specific staple outside the scope of this book except for a brief mention on oil production and refining.

## 2. WHY DO WE STILL HAVE FISH OILS?

Fish oils are a byproduct of the production of fish meal. Both commodities are subject to price fluctuations that are only indirectly related. The meal has been historically used for terrestrial animal feedstuffs. The production process can be condensed to apply to two basic functions: take fish or fish waste, cook it, and squeeze it (Figure 2). Subsequent steps are varied for each commodity, but they essentially are well described by Young (20), whereas the desirable properties of fish meal are concisely described by Bimbo and Crowther (21). Similar summaries, especially on sources and production, are provided by various chapter authors in a book on pelagic fish (22), because most fish caught for meal and oil are pelagic (migratory) and subject to seasonal exploitation. The principal competition to fish meal is soybean meal and other oilseed meals (23).



**Figure 2.** A simplified diagram of the fish meal manufacturing process. Shading denotes the principle products.

The gloomy chronology in Section 1 for food and health aspects of fish oils since WWII would have ruined most basic industries, but as a byproduct of fish meal production, that of oil has continued. The human (and pet) nutritional supplement markets would absorb only a small amount of the total world production, which has remained at approximately 1.2–1.4 million tons for the last decade (23).

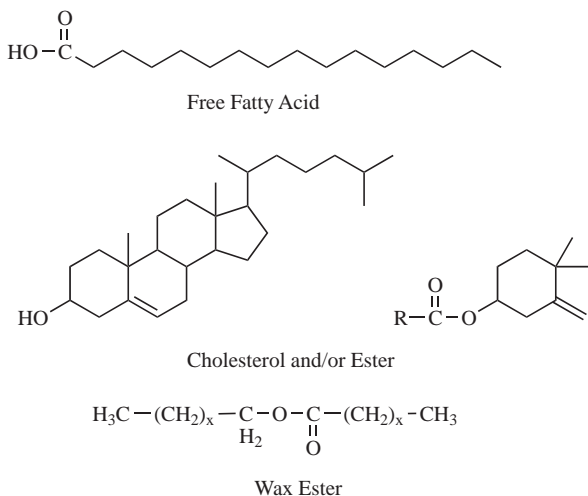
Aquaculture has provided part of the solution, which is particularly true for salmonids, and to trout farmed in freshwater, a very large amount of the former industrial grade (unrefined) production of fish oil goes to Atlantic salmon (*Salmo salar*) production in places as far away from their origin in the North Atlantic as New Zealand and Chile. Other species are now coming into aquaculture production, usually higher priced fish such as Atlantic halibut, grouper, sea bass, tilapia, and so on. Fish oil is not just a cheap fat, as most of the fish will grow on diets of fish meal plus other fats, but around 1–2% of the total dietary fatty acids should be the long-chain polyunsaturated fatty acids (20:5n-3 and 22:6n-3) that are basic to the functional membranes of new cells when combined with the protein of the fish meal. Fish meal usually contains approximately 8–10% of fat, and it is often a good source of this minimum requirement of omega-3 fatty acids. Excessive fat in fish diets is thought to spare protein degradation for energy, which results in more growth, and the 20% of total dietary fat for the salmon diets common a decade ago is now frequently replaced with a total of 26–28%, the so-called high-energy diets. Peru has been a major producer of industrial grade fish oil for the aquaculture industry, but the anchovy fished there fails to return to the coast occasionally for Pacific Ocean and climate reasons (an EI Niño event), and reduced production can drive the world price of fish oil to above \$500 (US)/tons compared with more normal prices of \$250–\$400/ton (23).

The new factor in the fish oil industry is as a built-in supplement in the human diet instead of a separate food additive. It is in the form (usually) of highly refined fish oil added in a microencapsulated format. The oils used must be highly refined to meet Food and Drug Administration (FDA) standards, especially in the United States, where menhaden oil was the first oil approved for this purpose. There, the intake of the oil must not exceed 3 g/day of the two fatty acids 20:5n-3 and 22:6n-3 in a designated list of typical foodstuffs. Although ethyl esters and concentrates of oils and esters will eventually be approved, it is almost certain that the starting materials will be those fish oils essentially very low in 20:1 and 22:1. As will be discussed below, these oils typically have 20:5n-3 > 22:6n-3, i.e., EPA > DHA in the popular terminology. The initial omega-3 publicity in the 1980s favored EPA for superior blood vessel function, but gradually this public image has changed to favor DHA as superior for heart muscle function and neural problems. Infant nutrition is yet another popular field for debate on omega-3 fatty acids, and it should be noted that the Martek Biosciences Corp., Columbia, nw, was the first to develop algal (i.e., vegetarian) sources of 22:6n-3 and 20:4n-6 (arachidonic acid) as preformed long-chain fatty acids representative of human breast milk when added to enriched infant formulas. Revenues for this company were forecast to exceed \$100 million in 2003.

### 3. FISH OIL FATTY ACIDS AND GAS-LIQUID CHROMATOGRAPHY

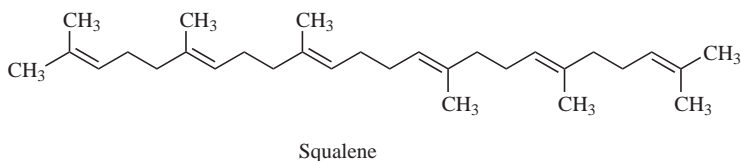
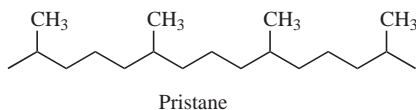
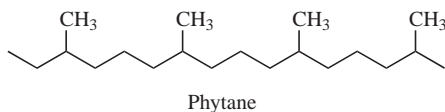
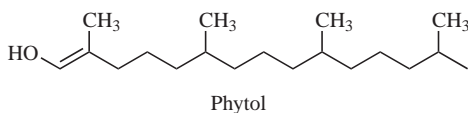
Fish oils mostly contain triacylglycerols of fatty acids (Figure 1), recovered by fairly simple technology (Figure 2) from the whole bodies of fish, often from species considered inedible in contemporary Western society. In fact, many exceptions to these generalizations exist, because in addition to the triacylglycerols, there can be wax esters (1/2 fatty alcohols), diacylglyceryl ethers (2 fatty acids, Figure 1), cholesterol and cholesteryl esters (Figure 3), and even the hydrocarbon squalene (Figure 4). Also, the marine mammals are to be considered. The depot fats of baleen whales and seals yield oils similar to the above general description of fish oil including fatty acid composition, but the triacylglycerols differ in fatty acid molecular arrangements and warrant a separate discussion. The depot fats of the toothed whales can include wax esters (sperm whales), or even triacylglycerols and wax esters incorporating short-chain fatty acids such as isovaleric (the dolphins and similar small species). These topics are covered elsewhere (24). Marine invertebrates also will have to be excluded from this discussion as there is no large-scale or industrial use of their triacylglycerols. "Krill" oil produced from small Antarctic crustacea such as *Euphausia superba* is now offered in small amounts for the nutritional health product market, but the investment prospects are daunting (25). The two volumes of *Marine Biogenic Lipids, Fats and Oils* offer lipid class and lipid

#### Fish Oil Miscellaneous Natural Lipids



**Figure 3.** Lipids that may be found in marine oils. Free fatty acids are artifacts of postmortem processes in fish bodies and may range up to 5% or even 10% of oils. Cholesterol and its esters are usually in the 0.5–1.0% range, but wax esters may be major oil components in oils from certain fish.

## Neutral Fish Oil Compound

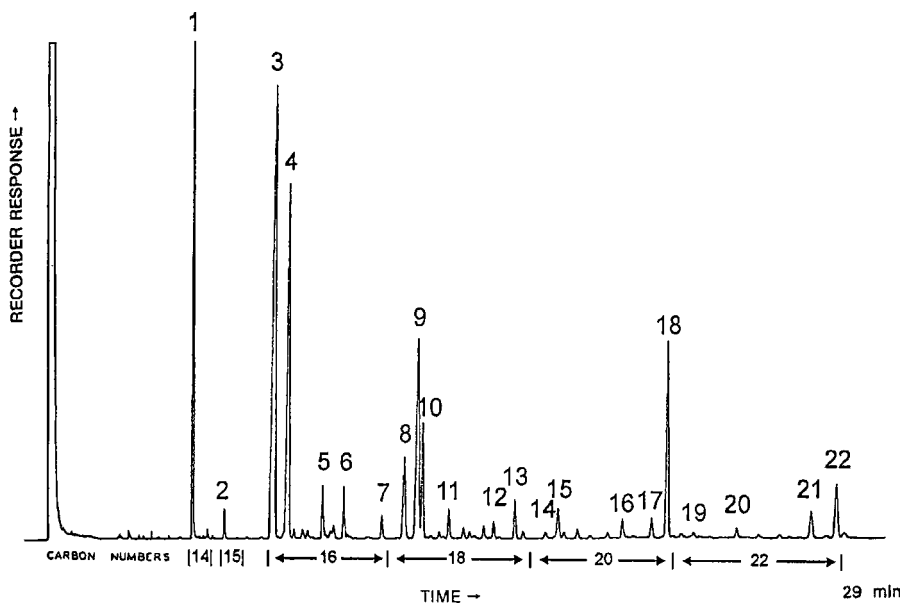


**Figure 4.** Phytol and phytol-related hydrocarbons, pristane and phytane, are associated and may be found in some fish oils. Phytanic, pristanic, and 4,8,12-trimethyltridecanoic acids are common fish oil components derived from phytol. Squalene is usually of animal origin and a feature of some shark liver oils.

component composition information on these topics from resource and biochemical points of view (24).

Although traditional quality factors such as iodine value, free fatty acids (Figure 3) and unsaponifiable content (Figures 3 and 4) are still important in trading fish oils, the fatty acid composition determined by gas-liquid chromatography (GLC) of methyl esters is now frequently required. Figure 5 is a typical example of what is desirable, and it may help to explain the fatty acids to be expected. This analysis is on a special class of liquid phases based on polyglycols (e.g., SUPEL-COWAX-10, DURA-WAX, Stabilwax, Omegawax-320, etc.), which limits even chain length overlaps to two C24 fatty acids and 22:6n-3. These chain lengths are marked accordingly.

Figure 6 is an extensive list of the fatty acids typical of the triacylglycerols of fish oils and includes one baleen whale oil. These were all of interest to industrial and food fats and oils companies two decades ago (1), but even later were of limited interest in human nutrition (26). For practical purposes, marine oils can be defined by 12 fatty acids that add to about 90% of the array of peaks in Figure 5 and determine the properties of a given oil. These are (in a shorthand giving the chain-length,



**Figure 5.** Analysis of menhaden (fish) oil on an Omegawax-320 capillary column. Equipment: Varian 3400 GLC, splitless injection helium carrier gas. Initial temperature 69°C for 1.4 min; ramp to 170°C at 50°C/min; hold for 8 min at 170°C; ramp to 220°C at 3°C/min; hold 15 min; total time 43 min. Note FAME chain lengths below baseline. Peaks identified are as follows: 1 = 14:0; 2 = 15:0; 3 = 16:0; 4 = 16:1n-7; 5 = 16:16:2n-4; 6 = 16:3n-4; 7 = 16:4n-1; 8 = 18:0; 9 = 18:1n-9; 10 = 18:1n-7; 11 = 18:2n-6; 12 = 18:3n-3; (followed by 18:3n-1); 13 = 18:4n-3 (followed by 18:4n-1); 14 = 20:0; 15 = 20:1n-9 (followed by 20:1n-7); 16 = 20:4n-6; 17 = 20:4n-3; 18 = 20:5n-3; 19 = 22:1 group; 20 = 21:5n-3; 21 = 22:5n-3; 22 = 22:6n-3 (with 24:0 preceding and 24:1n-9 following). Analysis time, 30 min. Reproduced by permission of *Anal. Chem. Acta.*

number of *cis*-methylene-interrupted ethylenic bonds, and position of the ethylenic bond nearest the methyl end of the chain):

14:0	16:1n-7	18:2n-6
16:0	18:1n-9	18:3n-3
18:0	20:1	18:4n-3
	22:1	20:5n-3
		22:6n-3

After considering the many analyses of fish oils available, the author concluded that there was only one "basic" fatty acid composition of fish oils from coldwater or from northern latitudes (27). Generally this is typified by menhaden oil, a species that feeds exclusively by filtering phytoplankton out of the ocean water in the Gulf of Mexico or in the Atlantic Ocean off the east coast of the United States or the

anchovy oil from Peru (Figure 6), which also feeds close to the plant base of the food chain.

Menhaden oil has a fatty acid composition that provides a good example of the "basic" marine fish oil fatty acid system. For example, it is characterized (Figure 6) by low values of 20:1 and particularly 22:1. The origin of these two fatty acids has been discussed in reviews of freshwater lipids (28) as well as of marine lipids (29, 30). In principle the extension by one acetate unit of the plentiful 18:1n-9 and 18:1n-7 will give 20:1n-9 and 20:1n-7, and a second step leads to some 22:1n-9 and 22:1n-7. Generally the process stops there, and only small amounts of these are found relative to 22:1n-13 and especially 22:1n-11. In addition to a little 24:0, a more obvious peak for 24:1 is found, which accounts for that in Figure 6. The marine 24:1n-9 includes nervonic acid, functional in various organs of animals, and may accumulate from food fatty acids. Other isomers are possible. The 22:1 peak is actually resolved by efficient open-tubular GLC into four distinct peaks (Figure 7), of which the major peak is primarily 22:1n-11 but also includes some 22:1n-13, followed by 22:1n-9, 22:1n-7, and 22:1n-5. Similarly the 20:1 array (Figure 7) includes a frontal shoulder of 20:1n-11, sometimes difficult to see on the dominant 20:1n-9 peak, which is followed by 20:1n-7 and 20:1n-5 and some other peaks of non-methylene-interrupted dienoic (NMID) acids that might occur as discussed below.

The origin of the unexpected 22:1n-13 and 22:1n-11 fatty acid isomers (Table 1) is now not a mystery (29, 30). The pioneering work of many scientists some decades ago on marine lipids led to those of a group of tiny marine crustaceans called copepods. They found that one major lipid class in several species known to be important as food for fish in the North Atlantic was wax esters (31). In fact, lipids of a copepod sample examined in Halifax contained 61.2% wax esters and only 31.6% triacylglycerols (29). The distribution of ethylenic bond positions in the copepod lipid wax ester fatty alcohols is compared with the alcohols recovered from the body depot fats of several regional fish species known to feed directly on copepods in Table 1. It is believed that the copepod fatty alcohols are converted directly by the fish to the corresponding fatty acids, which accounts for the very high proportion of 22:1 usually observed in fish feeding at this trophic level (Table 1). However, it is fair to point out that the copepod fatty acids of both the triacylglycerols and the wax esters had modest contents of the same isomers (29). The dominance of the unusual 22:1 wax ester isomer n-11 in the copepod is not readily explained, but it may be based on physical properties such as melting point, specific gravity, and so on.

It will be noticed that in most tables of fish oil, fatty acid compositions 20:1 and 22:1 are simply listed as such and do not include isomer details. They are perfectly acceptable in diets for aquaculture fish (32). As reviewed earlier, their presence in fish oils did contribute to false alarms about 22:1 fatty acids and alleged heart damage in animals consuming rapeseed oil or fish oils, the latter usually being in partially hydrogenated form for margarines and shortening. This was independent of the much more recent possibilities of *trans*-fatty acids of any origin leading to cholesterol and atheroma problems in the human population (33, 34).

Fatty acid composition of six samples of fish oil and one whale oil in commercial trade. Unpublished data reproduced by courtesy of W. Schokker and H. Boerma, Unilever Research, Vlaardingen.

	Herring North Sea	Anchovy Peru	Whale Antarctic	Pilchard South Africa	Sardine Portugal	Menhaden U.S.A	Pilchard ?
12	0.10	0.10	0.20	0.20	0.10	0.15	0.10
14	6.10	7.45	7.45	7.75	6.70	7.30	7.30
14:1	0.15	—	0.75	0.15	—	—	—
16: Branched	0.40	0.40	0.40	0.40	0.50	0.45	0.55
15:0	0.40	0.60	0.65	0.40	0.75	0.65	0.60
16:0	10.75	17.45	13.40	15.65	17.80	19.00	15.60
16:1	7.30	9.00	10.50	8.50	6.00	9.05	9.00
16:2 ω7	0.20	0.20	0.20	0.55	0.40	0.50	0.40
16:2 ω4	0.40	1.00	0.65	1.45	0.65	1.25	1.55
16:3 ω4	6.70	2.05	0.10	2.00	0.40	1.45	1.70
16:3 ω3	—	—	0.20	—	0.20	0.20	0.15
16:4 ω4	0.10	—	—	—	0.10	0.15	0.20
16:4 ω1	1.20	2.45	0.95	3.20	1.60	2.30	2.60
17: Br	0.30	0.35	0.25	0.25	0.20	0.20	0.15
17:0	0.35	0.55	0.95	0.80	0.80	0.90	0.85
17:1	0.30	—	0.25	—	0.30	—	—
18: Br	0.80	0.70	1.10	0.60	0.60	0.45	1.00
18:0	1.40	4.00	2.70	3.65	3.60	4.20	3.45
18:1	10.30	11.55	27.60	9.25	13.00	13.20	10.40
18:2 ω9	tr.	0.10	0.10	0.15	0.15	0.30	0.20
18:2 ω6	0.95	1.20	1.90	0.80	1.20	1.30	1.30
18:2 ω4	0.10	0.60	0.20	0.50	0.30	0.40	0.50
18:3 ω6	0.05	0.30	0.20	0.35	0.20	0.25	0.30
18:3	tr.	0.20	0.10	0.30	0.10	0.30	0.20
18:3 ω3	2.00	0.75	0.85	0.45	1.00	1.30	0.65
18:4 ω3	3.15	3.05	1.05	2.05	3.15	2.75	2.65
18:4	0.15	0.20	0.20	0.15	0.10	0.15	0.20
19: Br	—	0.10	—	—	0.20	0.20	—
19:0	0.20	0.10	0.60	0.20	0.40	0.40	0.10
19:1	0.10	—	0.40	—	—	—	—
20:0	0.10	0.30	0.20	0.60	0.40	0.35	0.30
20:1	13.40	1.55	6.75	2.50	4.30	2.00	1.45
20:2 ω9	—	0.30	0.10	0.40	0.15	0.45	0.15
20:2 ω6	0.15	0.35	0.15	0.25	0.20	0.35	0.30
20:3 ω6	0.10	0.10	0.20	0.30	0.10	0.15	0.20
20:3 ω3	0.30	1.10	0.60	1.35	0.85	0.80	1.00
20:4 ω6	tr.	0.10	0.25	0.10	0.10	0.15	0.15
20:4 ω3	0.75	0.70	1.30	0.70	1.05	1.35	0.80
20:5 ω3	7.45	17.00	4.70	19.30	11.00	11.00	18.30
21:0	0.10	tr.	0.05	0.15	0.10	0.05	0.10
21:5 ω2	0.25	0.70	0.15	0.90	0.50	0.60	0.90
22:0	0.05	0.05	0.10	0.20	0.20	0.20	0.15
22:1	21.25	1.15	2.40	3.10	3.80	0.55	1.55
22:2	0.20	0.10	0.05	0.05	0.10	0.20	0.10
22:3 ω3	—	0.15	0.25	0.15	0.15	0.15	0.20
22:4 ω3	0.25	0.55	0.20	0.40	0.70	0.50	0.60
22:5 ω3	0.75	1.60	2.40	2.35	1.30	1.90	1.80
22:6 ω3	6.75	8.75	5.70	6.45	13.00	9.10	9.60
23:0	0.10	0.05	0.05	0.10	0.10	0.10	0.15
24:0	0.15	0.05	tr.	0.15	0.10	0.15	0.10
24:1	0.75	0.50	0.30	0.50	0.60	0.35	0.70
Wijs I.V.	135.7	181.0	121.7	182.0	169.7	162.1	189.4
GLC- I.V.	138.0	181.5	122.1	183.9	170.0	161.5	190.7

Figure 6. Reproduction of a personal communication to R. G. Ackman from scientists of Unilever Research, Vlaardingen, the Netherlands, as published in (1). Reproduced by permission of Academic Press.





**Figure 7.** Part of gas-liquid chromatographic analysis of methyl esters of cod liver oil on an Omegawax-320 column,  $0.32 \times 30 \times 0.25$ . Temperature  $160^{\circ}\text{C}$  for 8 min,  $3^{\circ}\text{C}/\text{min}$  to  $220^{\circ}\text{C}$ , hold. Peaks identified are as follows: 1 =  $18:4n-3$ ; 2 =  $18:4n-1$ , unmarked probably  $18:5n-3$ ; 3 =  $20:0$ ; 4 =  $20:1n-9$  with frontal shoulder of  $20:1n-11$  after peak 3; 5 =  $20:1n-7$ ; 6 =  $20:1n-5$ ; unknown; 7 =  $20:2n-6$ ; unknown; 8 =  $20:3n-6$ ; 9 =  $20:4n-6$ ; 10 =  $20:3n-3$ ; 11 =  $20:4n-3$ ; 12 =  $20:5n-3$ ; small peak for  $22:0$ ; 13 =  $22:1n-13 + 22:1n-11$ ; 14 =  $22:1n-9$ ; 15 =  $22:1n-7$ ; 16 =  $22:1n-5$  (Ackman, unpublished).

As of the time of writing (mid-2003), the FDA has announced that *trans*-acid contents of foods will soon be required on food composition labels. Fish oils, now omega-3 nutritional supplements in some foods, are essentially excluded from such considerations because they contain almost exclusively *cis*-ethylenic bonds.

**TABLE 1. Principal n-3 Fatty Acids, Saturated, and Monoethylenic Fatty Acid Isomers (w/w%) in Triacylglycerols and Wax Esters of Copepods and Commercial Oils of Pelagic Species of North Atlantic Fish Likely to be Consuming Copepods.**

	Copepod		Commercial Oils			
	Triacylglycerol	Wax Esters	Total Acids	Wax Esters	Mackerel Total Acids	Herring Total Acids
14:0	19.84	38.42	7.85	5.23	7.81	8.77
16:0	28.98	11.15	8.81	8.36	15.93	14.84
18:0	1.04	0.35	0.72	1.03	1.73	0.97
16:1n-9	0.05	0.35	0.03	1.58	0.29	ND
16:1n-7	8.89	12.33	15.42	18.16	8.20	7.22
16:1n-5	0.73	1.04	0.73	0.12	0.54	0.52
18:1n-9	3.14	3.46	4.40	5.95	8.61	12.27
18:1n-7	0.91	0.56	3.43	1.69	3.78	3.66
18:1n-5	0.41	0.15	0.62	0.65	0.54	0.64
18:2n-6	0.97	0.83	0.78	0.86	1.28	0.78
18:3n-3	1.08	1.00	0.20	0.36	0.99	0.39
18:4n-3	3.23	5.63	1.36	1.87	2.47	0.93
20:1n-11	0.33	0.36	1.20	0.46	0.24	0.50
20:1n-9	4.12	4.37	14.53	9.34	10.59	14.37
20:1n-7	0.34	0.55	1.84	0.92	1.13	0.94
20:1n-5	0.02	0.01	0.23	0.10	0.09	0.19
20:4n-6	0.29	0.19	0.29	0.60	0.36	0.24
20:5n-3	8.38	5.81	9.35	22.38	7.84	2.85
22:1n-11(13)	5.16	4.59	17.45	5.90	12.74	20.92
22:1n-9	0.34	0.65	1.70	0.92	1.00	1.36
22:1n-7	0.11	0.11	0.42	0.19	0.19	0.33
22:5n-3	0.60	0.23	0.60	0.74	0.57	0.37
22:6n-3	4.90	0.64	2.70	5.32	7.66	2.70
24:1	0.49	0.24	0.59	0.13	0.69	0.52

ND = not detected.

From Ratnayake and Ackman (30).

#### 4. SATURATED, ISOMERIC MONOENOIC, AND UNUSUAL FATTY ACIDS

The "basic" fish oil (27) also included a generous amount of saturated fatty acids. As can be seen from Figure 5 and Table 1, the saturated fatty acids are dominated by the 16:0 (palmitic acid), usually accompanied by about half as much or less of 14:0 (myristic acid) and much less of 18:0 (stearic acid). Usually the saturated fatty acid totals are at least 20%, especially as the odd chain (15:0, 17:0) and methyl-branched (iso, anteiso, pristanic, phytanic) fatty acids (compare Figure 4) are saturated and will total around 2–3%. An unsaturated peak that is often observed is 17:1n-8, which is roughly equal to 17:0. The details of these peaks are discussed in other publications, but those researchers attempting modern open-tubular gas chromatography analyses should be aware of their presence and influence on peak identification and quantitation. As can be seen from Figure 6, there is an

inverse relation among classes of fatty acids, so oils rich in 20:1 and 22:1 generally have lower levels of saturated fatty acids.

The peaks for C14 monounsaturated fatty acids follow 14:0 and are usually a jumble of peaks for 14:1 isomers, mixed up with those for iso and anteiso 15:0 and the isoprenoid 4,8,12-trimethyltridecanoic acid, followed reasonably clearly by that for 15:0 (Table 2). Little interest exists in these details, and the only obvious next peak before 16:0 should be iso-16:0, and sometimes another isoprenoid acid, pristanic or 2,6,10,14-tetramethylpentadecanoic, is found just ahead of 16:0.

**TABLE 2. Fatty Acid Composition (w/w%), with Relative Retention Times, for Japanese Sardine Oil (36), Compared with That of Triacylglycerols of Cultured Cells of the Marine Diatom *Phaeodactylum tricornutum* (39).**

Peak No.	Fatty Acid	RRT	Sardine	<i>P. tricornutum</i>
1	10:0	0.057	0.02	—
2	12:0	0.119	0.11	0.4
3	iso-13:0	0.144	—	—
4	13:0	0.166	0.02	1.1
5	iso-14:0	0.208	—	—
6	14:0	0.245	7.25	6.9
7	14:1 (n-9)	0.260	0.25	—
8	14:1 (n-7)	0.275	0.06	—
9	14:1 (n-5)	0.287	0.07	—
10	iso-15:0	0.296	0.15	—
11	anteiso-15:0	0.313	0.08	—
12	15:0	0.348	0.31	2.0
13	iso-16:0	0.421	—	—
14	anteiso-16:0	0.437	0.08	—
15	16:0	0.502	19.42	21.2
16	16:1 (n-11)	0.529	0.84	—
17	16:1 (n-9)	0.541	—	4.4
18	16:1 (n-7)	0.564	8.64	23.0
19	iso-17:0	0.600	0.07	—
20	anteiso-17:0	0.631	0.32	—
21	16:2 (n-7)	0.643	0.05	—
22	16:2 (n-4)	0.686	1.23	3.4
23	17:0	0.704	0.33	—
24	17:1 (n-10)	0.732	0.52	—
25	16:3 (n-4)	0.762	1.57	3.3
26	17:1 (n-8)	0.785	0.13	0.4
27	17:1 (n-6)	0.830	0.05	—
28	iso-18:0	0.844	0.09	—
29	anteiso-18:0	0.875	0.03	—
30	16:4 (n-1)	0.892	2.55	0.2
31	18:0	1.000	2.62	2.3
32	18:1 (n-13)	1.041	0.11	—
33	18:1 (n-9)	1.110	6.38	6.5
34	18:1 (n-7)	1.131	3.04	0.8
35	18:1 (n-5)	1.169	0.23	1.2

(Continued)

TABLE 2. (Continued)

Peak No.	Fatty Acid	RRT	Sardine	<i>P. tricorutum</i>
36	iso-19:0	1.222	—	—
37	18:2 (n-6)	1.291	0.85	2.5
38	18:3 (n-6)	1.371	0.47	0.3
39	19:0	1.415	0.14	—
40	19:1 (n-8)	1.515	0.11	—
41	18:3 (n-3)	1.575	0.40	1.3
42	18:4 (n-3)	1.730	2.06	0.2
43	18:4 (n-1)	1.768	0.22	—
44	20:0	1.995	0.11	0.1
45	20:1 (n-11)	2.142	2.37	—
46	20:1 (n-9)	2.176	1.33	—
47	20:1 (n-7)	2.235	—	—
48	20:2,5,11	2.403	—	—
49	20:2 (n-6)	2.783	—	0.4
50	20:4 (n-6)	2.928	1.26	0.3
51	20:4 (n-3)	3.396	0.74	0.4
52	20:5 (n-3)	3.608	17.01	8.7
53	22:1 (n-11 + 13)	4.205	2.09	—
54	22:1 (n-9)	4.288	0.27	—
55	22:2,7,11	4.902	—	—
56	22:2,7,13	4.985	—	—
57	21:5 (n-3)	5.158	0.60	—
58	22:5 (n-3)	7.150	2.17	0.9
59	22:6 (n-3)	7.510	10.09	2.3
60	24:1 (n-9)	8.408	1.09	—

The C16 polyunsaturated fatty acids are often confusing because two series can coexist and overlap in GLC analyses, basically the familiar n-3 and n-6 series with 16:2n-6, 16:3n-3, and 16:4n-3. Superimposed on these acids are members of an n-1, n-4, and n-7 series. The latter are well documented (35), and they may be prominent in fish lipids where algal fatty acids are deposited more or less directly, for example, in Japanese sardine oil (Table 2). All should be included in extensive tabulations of marine fatty acids, and their methyl ester peak relative retention times for the low-polarity GLC phase SILAR-5CP (36) are included in Table 2, which will clarify their positions after 16:1n-7 for similar GLC columns, but on higher polarity GLC columns they will overlap with C18 fatty acids. In default of GC-MS, older techniques of plotting or separation factors may help in identifications (37, 38), although these require an isothermal GLC analysis. To illustrate how the n-1, n-4, and n-7 fatty acids can be transferred to fish oils, the fatty acids in the triacylglycerols of a well-known unicellular alga, *Phaeodactylum tricorutum*, are included in Table 2, because it is a basic diatom in that geographic location (39). It may be noted that 16:4n-1 is a minor fatty acid presumably because very little 18:4n-3 exists. The 16:4n-1 would be generated in algae by the enzymatic process that produces 18:4n-3, but acting on a C16 chain length instead of a C18 chain length.

Other sources must provide the amount obvious in the sardine oil, and similar or lesser contents are common in other fish oils.

Curiously, in fish oils, 18:4n-1 is frequently clearly visible after the peak for 18:4n-3 (No. 12) in Figure 5 and is usually about one-quarter of its size. Another unusual fatty acid of algal origin is 18:5n-3 (40). It is known from GLC analyses of both phytoplankton and zooplankton lipids. In Figure 7, it is probably the small sharp peak between peak 2 (18:4n-1) and peak 3 (20:0), approximately as for the moderately polar SILAR-5CP liquid phase of (40). All unusual fatty acids should be neatly handled by the mammalian body degradation process and even by those in fish, and they are seldom reported in fish oils. Incidentally, peak 3 is wider than expected, a characteristic of methyl esters of saturated fatty acids in such analyses. Of the other unusual fatty acids in fish oils, the 21:5n-3 (20 in Figure 5) is common and well documented (41). It is of interest if 23:0 (tricosanoic acid methyl ester) is used as an internal standard in GLC (42). The latter may coincide with 21:5n-3, and the Omegawax-320 was a slight modification of SUPELCOWAX-10 to avoid this problem. On the other hand, the NMID referred to earlier (both C20 and C22) appear in mollusc lipids, possibly in physical imitation of the polyunsaturated fatty acids of membranes (43), and they are not apt to be observed in industrial pelagic fish oils. On tropical reefs or among fish-consuming mollusks, they should be considered as likely if the normal resolution among the later-eluting C20 and C22 monoethylenic isomers (n-7, n-5) is obscured on polyglycol capillary columns.

## 5. POLYUNSATURATED FATTY ACIDS

The C18 polyunsaturated fatty acids include the familiar terrestrial fatty acids 18:2n-6 and 18:3n-3, along with some 18:3n-6, but in most marine fish oils, these are  $\leq 2\%$  of each (Figure 6, Table 1). The amount of 18:4n-3 is in the 2–4% range. Among the C20 polyunsaturates, there will be found very small proportions of 20:2n-6, 20:3n-6, 20:3n-3, and 20:4n-3. The 20:4n-6 (arachidonic acid) peak is generally the same size as these except in tropical fish lipids, where it can be more important. The latter are, however, not usually commercial fish oil sources. Although of nutritional importance in mammals, 20:4n-6 is usually grossly overshadowed by the C20 and C22 omega-3 polyunsaturated fatty acids in marine lipids. One reason for avoiding higher polarity gas-liquid chromatographic columns is that with the chain length overlap, this usually puts 22:1 and 20:4n-6 in juxtaposition or coincidence.

The polyunsaturated fatty acids were intimately associated with early (ca. 1960) attempts to lower serum cholesterol with varying degrees of success (44). In that particular study, with ethyl ester concentrates given to provide up to 4 g/day n-3 PUFA, a side effect of “an increased feeling of well-being coupled with improved cerebration” was reported only for the group receiving omega-3 fatty acids. About the same time, up to 26 mL/day of seal oil with a content of about 5 g of omega-3 fatty acids was fed for cholesterol-lowering effect without ill effects (45). Today

this simplistic dietary approach to one cardiac risk factor, cholesterol, is no longer acceptable in some circles because of the multiplicity of risk factors for "heart disease" (46). Essentially this recent review rules out arachidonic acid (20:4n-6) as having little effect, and it considers EPA (20:5n-3) as being more effective than DHA (22:6n-3) in the lowering of serum triacylglycerols, another independent risk factor (47). Another study also suggested that EPA was more effective than DHA in lowering blood pressure (48).

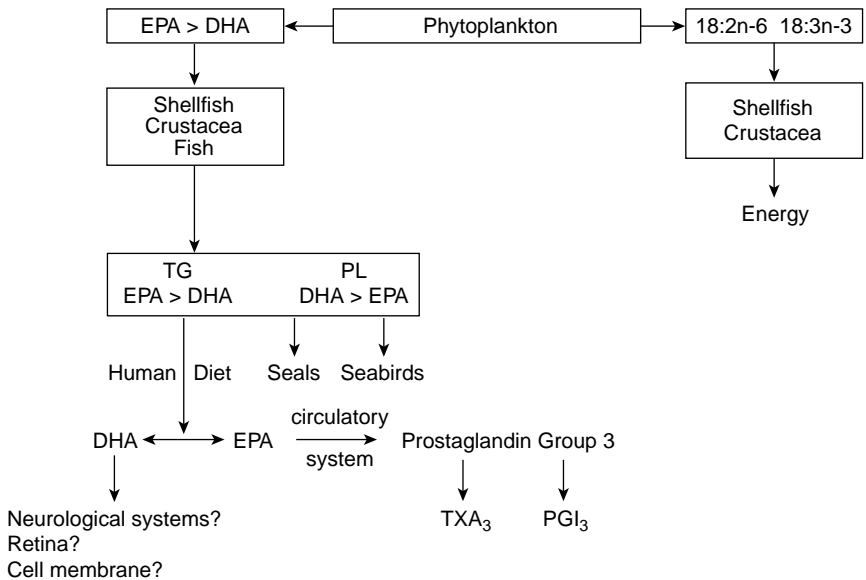
Tables 1 and 2 and Figures 5 and 6 show a third long-chain omega-3 fatty acid, 7,10,13,16,19-docosapentaenoic acid (or 22:5n-3), one of two DPA isomers. The other is the n-6 isomer, 22:5n-6, eluting just before 22:5n-3 on GLC. In Figure 5, the 22:5n-3 is peak 21 and a 22:4n-3 may be present after peak 20. In 1996, two papers (49,50) gave this 22:5n-3 fatty acid considerable potency in respect to endothelial cells. Considering that the related 20:5n-3 was originally considered to keep the blood vessel walls elastic through providing a prostaglandin (51), this could well be a beneficial role for EPA and even DPA in circulation separate from the heart, but research on the relative roles of the three omega-3 fatty acids in the aortic endothelium seems to have fallen into abeyance, perhaps because they are often said to be freely interconverted (52). This statement is not necessarily true, but the conversion of EPA to n-3 DPA and the reverse process, by one acetate unit, is generally accepted. The critical step, the conversion of 22:5n-3 to 22:6n-3, is not so easy, involving elongation to 24:6n-3 and peroxisomal shortening to 22:6n-3, and some biochemists now think that exogenous supplies of DHA are the preferable route to increasing the amount available in the body, especially in late pregnancy and lactation. This digression into the biochemistry of the logical beneficiaries of fish oils, humans, cannot cover the maternal/infant problems discussed at length by various authors in Ref. 13, a topic not without controversy (53, 54).

More emphasis has been placed on the loss of heart function taking place through arrhythmia (55), and it is possible that DHA is the more functional omega-3 fatty acid in the heart muscle. Unfortunately, the available fish oils divide into the menhaden/anchovy type, richer in EPA, an omnivorous group such as cod with EPA  $\approx$  DHA, and the tuna oils, both body and orbital, with 20–25% DHA. In fact, the first research on the beneficial aspects of omega-3 fatty acids in arrhythmia was conducted in Australia (56), conveniently near an Asian source of tuna oil rich in DHA, which lead to misunderstandings over a role for this omega-3 fatty acid in particular for preventing arrhythmia.

The health and welfare interest in long-chain omega-3 fatty acids inevitably raises the question of "where do they come from" and "are they safe." The latter question applies to oils and will be addressed in a production and quality section, but in reality, most fatty acids are of plant origin and perfectly safe.

The phytoplankton produce toxins dangerous even to fish, usually observed as "red tides" (57). These are fish kills in the oceans and near the shores, and fish oils are not made from fish found dead. A different issue is shellfish toxins where the digestive assimilation of unstable algal toxins does not kill the host (58), but fish oils are not made from such filter-feeding animals.

## Origin of Marine Fatty Acids



**Figure 8.** Origin of unsaturated fatty acids in phytoplankton, followed by discrimination by invertebrates leading to accumulation of EPA and DHA in oils and eventually in higher animals.

Each new discovery of unusual fatty acids in marine organisms leads to concerns that eventually dissipate. An example is the furanoid fatty acids that were once well documented as common in most oils (26). More recent papers and books on omega-3 fatty acids simply ignore the subject.

Figure 8 is a simplification of much work by many scientists over decades. The phytoplankton in the ocean produce all fatty acids necessary for fish oil and with somewhat similar compositions in places as remote as Australia and Scotland. Specific differences exist among them, but broadly the fatty acid patterns are related to colors familiar to people who see the macrophytes growing on the edges of the sea: red, green, and brown. However, even the accumulation of the amount of triacylglycerols is controlled by the available nutrients, especially nitrogen, and light intensity (59). The 22:6n-3 is not universal in phytoplankton, but it is found all over the world in benthic algae (60) or phytoplankton (61,62). For unknown reasons, fatty acid phytoplankton biosynthesis often stops at 20:5n-3, which was presumably the reason for the original (*ca.* 1980) ratio of 18:12 for 20:5n-3 and 22:6n-3 in oil from the filter-feeding menhaden fish (Figure 5), repeated on hundreds of labels for bottles of fish oil capsules, and repeated again in most concentrates prepared from it or from anchovy oil (Fig. 6). Actually the production of 18:2n-6 and 18:3n-3 is limited in a total lipid context for brown and red macrophyte

algae, but it is more common in the green, *Ulva pertusa* being a familiar and much studied example (60).

As already remarked, various invertebrates feed on the phytoplankton, and smaller carnivorous fish feed on those vegetarian species as well as on carnivorous intermediate invertebrates. Available 22:6n-3, with its neural and visual implications, is probably conserved at these lower levels and is vital in fish muscle phospholipids (30–40% of fatty acids). In oils, 18:4n-3 and 18:4n-1 are preserved as shown in Figures 5 and 6. Recent progress in the lipid biochemistry of fish shows that the rainbow trout can perform biosynthesis of 22:6n-3 (DHA) from 18:3n-3 (alpha-linolenic acid). Only a small part of that provided is converted to DHA (63), and surprisingly this was substantially converted by the pyloric cecum as well as by the liver (64), which raises an interesting point about adaption of fish biochemistry to circumstances. The marine fish ingest preformed EPA and DHA, but there was a curious change in diet for North American freshwater fish such as the rainbow trout. The recent glaciations should have wiped out the resident invertebrates, and after salmonids returned from the ocean, recolonization of food species for freshwater fish would have been based on insect life introduced from Central America. Thus, deprived of an excess of marine long-chain omega-3 fatty acids, adaption to elongate the available insect C18 fatty acids would have been necessary once the returning salmonids penetrated waters remote from the ocean. A similar situation for insect lipids and fatty acids is known to come from Britain (65). These sources could provide EPA but not DHA for freshwater fish. Freshwater fish, although beneficial in most respects among our sources of both n-3 and n-6 fatty acids (12,66,67), are not apt to produce large volumes of fish oils of distinctive character. An exception is the U.S. farmed catfish industry, which is subject to an excess of n-6 dietary fatty acids from local aquaculture diets. The production of the visceral oil has been described, in 2003 in JAOCS but with confusion in published fatty acids. The northern lakes of Canada support fisheries with more potential for accumulating longer-chain omega-3 fatty acids in the oils (67) or muscle. In four oils, the longer chain omega-3 fatty acids (including 18:4n-3) totaled 9.6%, 13.3%, 17.0%, and 18.6% of total fatty acids. The corresponding totals for the longer chain n-6 fatty acids arachidonic acid (20:4n-6), 22:4n-6, and 22:5n-6 were 3.3%, 2.5%, 3.7%, and 4.2%, mostly of 20:4n-6. Thus, a generally higher level of n-6 fatty acids over marine oils is found, but overall a favorable balance exists between preformed C20 and C22 n-3 and n-6 longer chain PUFA contents in the oil. The fatty acids of the edible muscle, widely eaten locally and also exported, are similarly skewed in favor of omega-3 fatty acids, but with the additional n-6 fatty acids already mentioned (R.G. Ackman, unpublished).

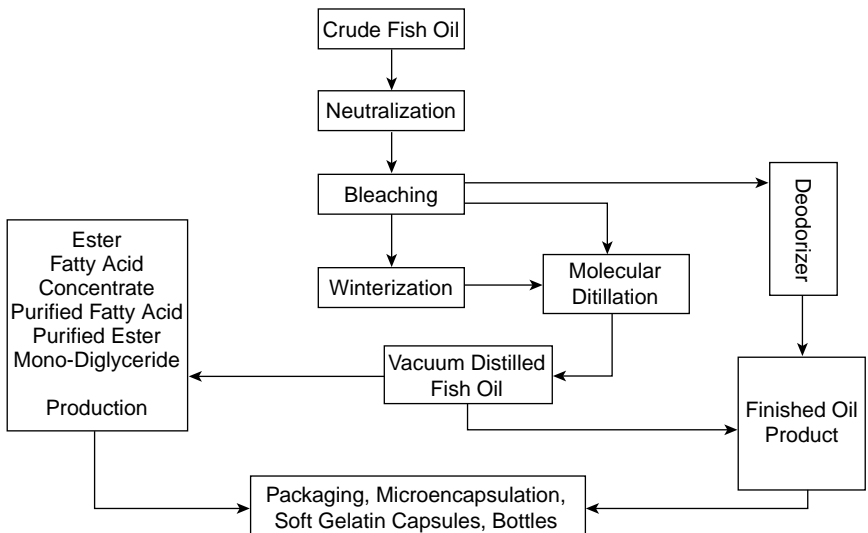
## 6. FISH OIL PRODUCTION AND QUALITY

A paramount concern in maintaining oil (and meal) quality is speedy processing after catch. As 100 tons or more could be involved, chilling is not always practical, but fish pumps can transfer the catch quickly from boats to the processing plant.



Even these delays must be avoided. Preferably no more than 24–30 hours should elapse, depending on temperature before fish reduction. The fish enzymes, both of muscle tissue and of digestive tissue, and those of gut bacteria, combine to break down protein. The oil degradation is basically from lipolytic enzymes, but some of the oil-soluble free fatty acids may come from partially digested food and phospholipids and not necessarily from triacylglycerols. The free fatty acids, abbreviated to FFA, are one of the oldest and simplest guides to fish oil quality. In one detailed review (68), it is pointed out that the oil stored in fat cells (adipocytes) illustrated for salmon by Zhou et al. (69) will be set free by 50°C, although cooking with steam usually reaches 95°C. Separation of the oil is achieved with presses and with very expensive and very efficient centrifuges. Once cooled, the oils are stable, provided no protein particulates are carried over from the first separations of oil. A second “polishing” centrifuge can handle this matter. The oil should be cooled before storage in clean, dry tanks. These tanks should be filled as full as possible and provided with provision for drainage of any sediments and water (foots). Complex flow diagrams are provided by various authors (20,23,26,70). They are complex because the thermodynamics, mainly for water removal, dictate costs. References 20, 23, 26, and 70 provide additional detailed diagrams for those interested. Subsequent technology leading to consumer products is summarized in Figure 9.

The basic crude fish oils are exemplified by the quality details of Table 3. Ranges are given because these are specifications for crude oils, produced at the level of over a million tons per year. The first six properties are traditional wet chemistry assays and the American Oil Chemists’ Society (AOCS) Official and Tentative



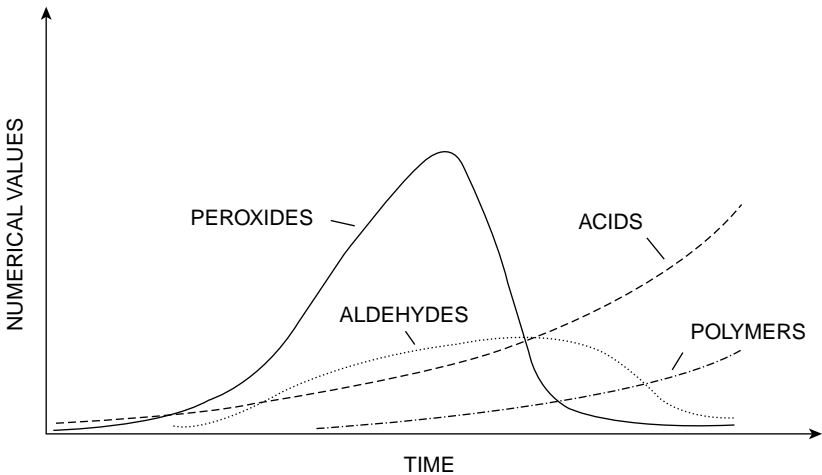
**Figure 9.** Production of pharmaceutical-grade fish oil or nutritional supplements. Reproduced from (70) by permission of the American Oil Chemist’s Society.

**TABLE 3. Crude Fish Oil Quality Guidelines and Physical Characteristics.**

<b>Quality Guidelines</b>	
Moisture and impurities, %	usual basis 0.5 up to 1% maximum
Free fatty acids, % oleic	range 1–7% but usually 2–5%
Peroxide value, meq/kg	3–20
Anisidine number 4–60	
Totox value	10–60
Iodine value	
Capelin	95–160
Herring	115–160
Menhaden	120–200
Sardine	160–200
Anchovy	180–200
Jack mackerel	160–190
Sand Eel	150–190
Color, Gardner scale	up to 14
Iron, ppm	0.5–7.0
Copper, ppm	less than 0.3
Phosphorus, ppm	5–100
<b>Physical characteristics</b>	
Specific heat, cal/g	0.5–0.55
Heat of fusion, cal/g	about 54
Caloric value, cal/g	about 9,500
Slip melting point, °C	10–15
Flash point, °C	
As triglycerides	about 360
As fatty acid	about 220
Boiling point, °C	greater than 250
Specific gravity	
At 15°C	about 0.92
At 30°C	about 0.91
At 45°C	about 0.90
Viscosity, cp	
At 20°C	60–90
At 50°C	20–30
At 90°C	about 10

From A. P. Bimbo (70).

Methods, Champaign, IL, or the Association of Official Analytical Chemists (AOAC), Gaithersburg, MD, are the usual sources for North America to access recognized, standardized, and detailed analytical methods. Traditionally, even different staff members in one laboratory can get somewhat different results. Technical spectral methods are now becoming useful, but the search for some exact and rapid replacement fish oil technology goes on. Some problems are discussed in Appendix 1, courtesy of *Codex Alimentarius*. A problem peculiar to the peroxide value, the anisidine number, and hence the totox value is that it is a moving target. Figure 10 shows that oxidation of highly unsaturated fatty acids proceeds, but at the



**Figure 10.** Time relationship among peroxides and their degradation products after oxidation of marine oils. No quantitative relationship is implied. Described in text as a “moving target” for analytical methods.

same time degradation of the peroxides can also proceed, with degradation to aldehydes producing the familiar “fishy” flavor of both oils and fish muscle lipids undergoing development of rancidity. Many candidate molecules are offered for consideration (26), some being unstable themselves. The acids produced can be volatile, and one ending to a peroxide free radical’s career can be to lead to polymerization, either within a triacylglycerol or between triacylglycerols. The splitting of an oxidized fatty acid chain can take place anywhere, but one-half of the product(s) will still be attached to the glycerol molecule. Thus, removal of free volatile aldehydes, for example, reduces the aroma from rancidity, but after their removal, refining can leave the anisidine value for the remaining glycerol-bound aldehydes as a real number of 5 or more. Addition of antioxidants depends on the value of the raw material and of the final product, so it is not likely to be added to crude fish oils. These already include the natural antioxidant benefit of natural alpha-tocopherol. Sometimes cheaper vegetable oil deodorization mixtures of tocopherols may be added after refining because the alpha-tocopherol may be lost in that step. The products of oxidation of oils protected by mixed tocopherols may then differ somewhat, but they have recently been studied in detail (71).

As already mentioned, the iodine value has been largely replaced by exact fatty acid composition from gas-liquid chromatography of the methyl esters of the single fatty acids. In addition to the AOCS Ce 1b-89 and AOAC 991.39 methods, European Pharmacopeia 4 method 01/02/1352 includes their standards for “omega-3 acidorum triglycerida” and the GLC analysis for EPA and DHA in triacylglycerols and an associated method for ethyl ester products (see below), as does the Voluntary Monograph (October 2002) on Omega-3, DHA, EPA and DHA + EPA of the Council for Responsible Nutrition of Washington. This body also gives

**TABLE 4. Council for Responsible Nutrition Quality Standards for Nutraceutical Grade Fish Oils in the United States.**

CRN Quality Standards for Nutraceutical Grade Fish Oils	
<b>Measures of Oxidation</b>	
Peroxide Value (PV), meq/kg	5 Max
Anisidine Value (AV)	20 Max
TOTOX ((2 × PV) + AV)	26 Max
<b>Purity</b>	
Dioxins (PCDDs, PCDFs)	2 pg/g WHO-TEQ Max
PCB's	<0.09 mg/kg (ppm)
Lead	<0.10 mg/kg (ppm)
Cadmium	<0.10 mg/kg (ppm)
Mercury	<0.10 mg/kg (ppm)
Arsenic	<0.10 mg/kg (ppm)
Omega 3 Fatty Acids	Expressed on a weight/weight basis (mg/g)
Acid Value	3 mg KOH/g Max

recommended maxima for heavy metals, dioxins and PCBs, and wet chemistry value maxima (Table 4).

New regulations require new technology, which is especially true for food use. Bleaching with activated clays has been a long-established practice to remove chlorophyll green or a brownish tint acquired from heating oils in the presence of other materials, and the objective of a clear yellow oil is usually possible. Recently, the purification target has been extended to removal of organochlorine materials, polyaromatic hydrocarbons, and most recently dioxins in particular. Activated carbon is recommended for dioxins, typically at 0.5–1.5% carbon for 20–45 minutes at 80–100°C. Some operators add this to the bleaching earth to reduce handling steps. Various associated matters, such as disposal of the oil-coated clay, develop that should be considered, and all are dealt with in a recent conference report (72).

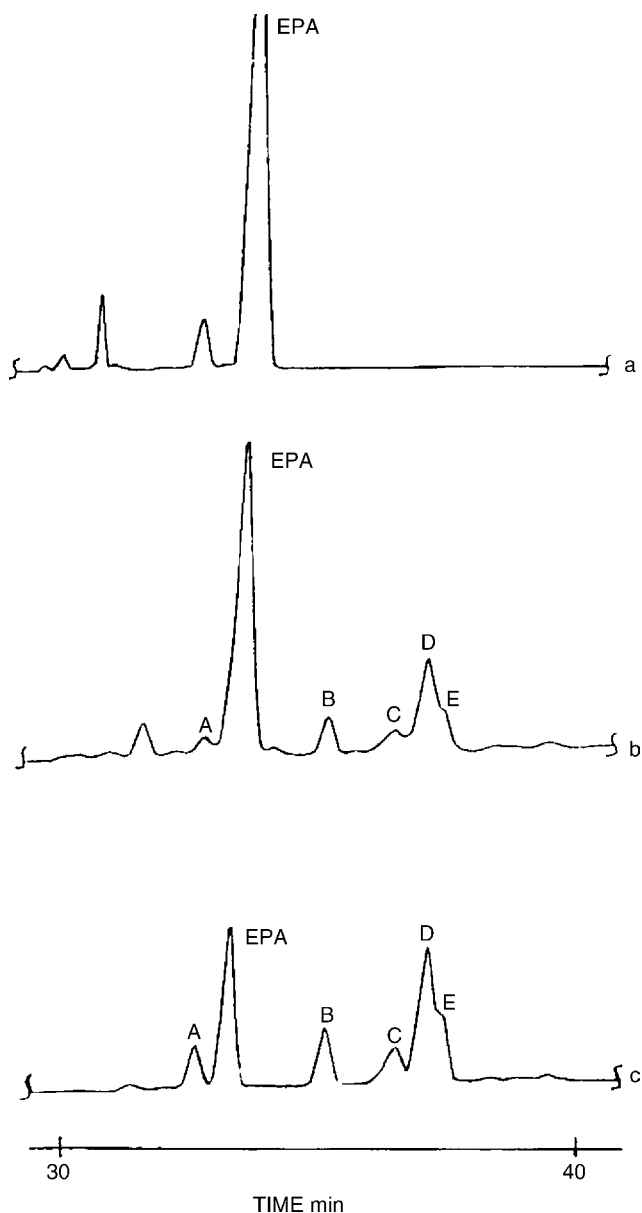
Occasionally, a highly purified fish oil is required for research purposes. Such oils can be prepared by large-scale chromatography (73) for use in studying oxidation products. The recent paper based on such technology is instructive in illustrating the variety of products produced from oxidation of even purified fish oil (71). Modern technology is beginning to investigate such materials in situ (74).

For crude fish oil, deodorization has changed considerably, with new technology designed to reduce temperature and/or exposure times. In 1990, a review chapter in a book (75) described classic batch tray technology with gravity cascade transfer of the oil and steam sparging to carry away volatiles. This process was satisfactory for hydrogenated fish oils, but thermal damage to one of the highly unsaturated fatty acids of vegetable oil had been recognized nearly 20 years earlier (76). A critical temperature of about 185°C was observed, and at 230°C, severe isomerization of ethylenic bonds (*cis* to *trans*) was observed in 18:3n-3 with prolonged heating. These isomers were also found in retail oil samples and so were produced by

standard deodorizers of the type mentioned and illustrated in a chapter in a book on fish oils (75). A thin-film design, the Campro unit is designed for a very short residence time, and with oil transfer as a thin film by gravity and by high-velocity sparge steam, and it is described as suitable for fish oil refining at 210°C. In fact, this is satisfactory for fish oils based on recent experience with this unit for seal oil (private communication). Designs for throughputs of up to 300 tons/day are available. The damage done to fish oil fatty acids at 220°C was investigated in depth with open-tubular gas-liquid chromatography (77), and it illustrated the problems of prolonged heat exposure (Figure 11). This case is a time-temperature relationship, so operating flexibility is desirable.

A true molecular still ejects a low-molecular-weight molecule from a less volatile liquid surface. If a very good vacuum exists ( $\leq 10^{-3}$  mm), this molecule may bounce around with a few residual air molecules but eventually will either fall back or attach to a cold condenser. Sometimes this is described as short-path distillation, which is not usually an efficient separation process as shown by a comparison of removal of DDT from cod liver oil and concurrent losses of Vitamin A (78). The term *molecular* still has now become attached to units of a different design applied to fish oils, for example, from the Pope Company of Menomonee Falls, WI, or the Pfaudler company of Rochester, NY. The Pope model of molecular still illustrated (75, 79) shows this wiped-wall concept. There is an outer cylindrical shell with heat applied on the outside. An interior centered post is actually a cold condenser, and wiper blades rotate continuously against the inner walls of the outer shell as the oil is fed in at the top, spreading the oil as a film of <1 mm thickness. The constant agitation of the film moves fresh volatile oil materials to the surface, and they pass into the evacuated space. The desired vacuum is sufficient to cause the volatiles, especially organochlorine materials (MW  $\leq 358$ ), but even cholesterol (MW 386), to pass over to the condenser at moderate (ca. 200°C) temperatures. Also removed are squalene (MW 410) and most of the volatile fatty acid oxidation products such as 2,4-decadienal (MW 152) and other lower molecular weight molecules contributing to objectionable flavors (73,74). Unfortunately, the natural alpha-tocopherol present (MW 430) may suffer the same fate. The antioxidant value of it and of squalene (80) may be lost. That these materials are effectively removed from triacylglycerol oils with molecular weights in the 800–1000 range is a result of the repeated turnover and mixing of the oil during its descent of several meters in very large units. The low viscosity at high temperatures helps refining by such units, and very large surface areas permit flowthroughs of tons of fish oil per hour.

As desirable products have similar molecular weights (ethyl docosahexaenoate is 356, and ethyl eicosapentaenoate is 330), it is clear that this type of fish oil product cleanup is best done at the oil stage to avoid losses at the same time as contaminant removal, which is carried out as described above. However, dimers and/or other polymeric and/or colored materials may be left behind if wiped wall equipment is used in its capacity of a simple and inefficient short-path still for the ethyl esters only. This process will produce a water-white distillate product. Regrettably, it is not an efficient way to separate ethyl-EPA from ethyl-DHA.



**Figure 11.** The GLC C20 region of a menhaden omega-3 PUFA concentrate (ethyl ester): (a) before and (b) after heat treatment at 220°C, and (c) the 20:5 region of an artifact concentrate isolated by AgNO<sub>3</sub> column chromatography. Peaks A–E refer to artifacts formed after heat treatment. Analysis on a SUPELCOWAX-10 fused-silica capillary column operated isothermally at 195°C. Note that components B–E fall into the region where several 22:1 isomers may be found (cf. Figs. 5 and 7). From (77).

## 7. CONCENTRATES OF FISH OIL OMEGA-3 PRODUCTS

Table 5 shows the marketing and label strategies for some current marine omega-3 products sold in Halifax, Canada. Obviously samples No. 1 and No. 6 are natural oils. Perhaps No. 5 is simply winterized oil, a process demonstrated in Table 6 for menhaden oil, which follows from the tendency for DHA to be in the 2-position of fish oil triacylglycerols (81). EPA is reputed to be somewhat less specific. In the absence of 20:1 and 22:1, the outer 1- and 3-positions may, in some molecules, present two saturated fatty acids from 14:0, 16:0, and 18:0 in one triacylglycerol molecule, which leads to the stearine composition of Table 6.

Simple biochemical rules are made more complex by other factors, and in fish oil triacylglycerols, the 20:1 and 22:1 fatty acids of a high melting point confuse the issue (82). The traditional enzymatic approach to fatty acid distribution will soon be replaced by nondestructive instrumental methods, particularly nuclear magnetic resonance (NMR). It can distinguish the proportion of DHA between the 1,3- and 2-positions (74) and otherwise provide the details shown in Table 7 for a few oils, which shows verification of the method through an international exchange (83). A

**TABLE 5. Some Recent Omega-3 Product Retail Labels in Canada.**

No	Description	Label Content in mg		Capsule size or liquid intake
		EPA	DHA	(1000 mg basis)
1	Wild Sockeye	(Total 90)		1000
2	Wild Harvested Pacific Salmon	180	111	1000
3	O3mega	400	200	1065
4	Natural Sea*	775 (517)	500 (333)	1500 (1000)
5	Holista Premium Fish Oil**	180	120	1000
6	Omega Gold (liquid)	900 (180)	600 (120)	5000 (1000)

\*Other (undefined) 225 mg.

\*\*Each capsule is said to be equivalent in omega-3 content to 2.5 oz (70 g) portion of cooked salmon.

**TABLE 6. Fatty Acid Composition (w/w%) of Menhaden Oil with Olein and Stearine Fractions.**

	Menhaden Oil	Olein Fraction	Stearine Fraction
14:0	9	8	11
16:0	21	18	31
16:1	11	12	9
18:0	3	3	5
18:1	12	12	10
20:1	2	2	2
20:5	14	15	11
22:5	2	2	1
22:6	10	11	7

From A. P. Bimbo (26).

**TABLE 7. Comparison of DHA Content from the Interlaboratory  $^1\text{H}$  NMR Analysis Between Japan and Norway Together with GC Data. Ethylene Glycol Dimethyl Ether was Used as Internal Standard ( $^1\text{H}$  NMR Analysis with 30s Pulse Repetition Time).**

Sample Oil Data		DHA Content (mg/g)			DHA Proportion (mol%)			Proportion (mol%) n-3 Fatty Acids		
		Norway	Japan	GC	Norway	Japan	GC	Norway	Japan	GC
No. 1	Average	248.17	276.1	267.6	27.03	27.5	24.5	32.05	32.1	33.6
Bonito	CV	0.64	1.33	1.67	0.78	0.46	2.49	0.08	0	2.03
No. 2	Average	123.79	122.6	123.4	11.93	12	11	22.56	22.2	21.6
Tuna	CV	4.01	1.38	0.73	2.98	0.43	1.71	0.85	0.4	1.35
No. 3	Average	214.51	208.3	215.7	20.65	20.7	20.2	29.08	28.8	29.9
Tuna	CV	2.72	0.91	0.2	0.66	2.02	1.43	0.05	0.73	0.89
No. 4	Average	217.97	206.7	215.4	21.18	20.8	19.7	32.22	31.8	32.1
Tuna	CV	3.84	0.07	0.72	1.59	0.47	1.35	0.23	0.34	1.07
No. 5	Average	111.07	111.1	106	10.65	10.8	10.2	28.03	28.1	28.6
Salmon	CV	3.27	0.95	1.47	0.88	0.32	1.15	0.32	0.33	0.74

From S. Wada (74).

recent enzymatic examination of tuna oil (EPA 6.69%, DHA 26.4%) showed 8.7% EPA and 56.3% DHA in the 2-position (84).

Retail product No. 1 of Table 5 is likely to be simply salmon waste oil, the fish name conferring an elite status. Our research (Ackman, unpublished) suggests that many "salmon oil" encapsulated oils are unrelated to any salmon oil in fatty acid composition. In 1989, our analysis showed many products of this type to be exaggerated as to omega-3 fatty acid content (85), and a more recent European survey in 1998 gave comparable results and reported on quality (86).

In the menhaden oil winterization of Table 6, the increase in polyunsaturated fatty acids is modest in the olein fraction, and the commercial objective may have been the stearine fraction, 50% richer in saturated acids of commercial interest. As already remarked for polyunsaturated fatty acids, 18/12 (in implied percents) or 180/120 (in mg/g) were obtained as triacylglycerols from menhaden and/or anchovy oils with minimal trouble and technology. Retail product No. 2 is also a product like No. 5. Winterization also would prevent either capsules or oil turning cloudy if refrigerated. Strangely, hardly anybody challenges label claims as to chemical nature, although the word *oil* may be carelessly used because concentrates are almost always ethyl esters, but numbers should always be expressed in the free acid form.

Among the various laboratory procedures used for studying fatty acids, concentrations by chromatography on silver nitrate impregnated silica gel, or equivalent (87), are too expensive to scale up; although effective for fish oils (88), they and mercuric adducts (89) would not be acceptable for health and safety reasons.

Before 1980, there was a now forgotten industrial technology for concentrating fish and vegetable oils called the Solaxol process. It can be described as the first large-scale use of supercritical gases, in this case, propane. A report with much convenient detail based on iodine values was published in 1949, but as a historical record (90), because several large plants were closed during the war and the prime



markets such as paints, linoleum, and oil cloth disappeared after the war. Two decades later supercritical carbon dioxide was to be the panacea in this field, but early promises, including fish oil fractionation, were seldom realized (91, 92).

Much research was conducted to prepare concentrates when the U.S. National Marine Fisheries Service provided funds for exploring most existing technologies for concentrating fish oil fatty acids. This exploration would provide omega-3 concentrates for the medical research sponsored by the National Institutes of Health and included supercritical fractionation of esters. Unlike commercial research, results were available to all interested parties. A book chapter by authors located in the Charleston, Seattle, and Gloucester NMFS laboratories, and in the Hormel Institute, Austin, Minnesota (93), provides detail on all methods considered for fractionating methyl or ethyl esters, because menhaden oil was clearly intractable. For supercritical CO<sub>2</sub>, the differences in factors such as the influence of chain length (i.e., molecular weight) and unsaturation, including explanations of results achieved by others, are helpful in explaining why this fractionation process has not been commercially developed. The final total enrichment process is described below.

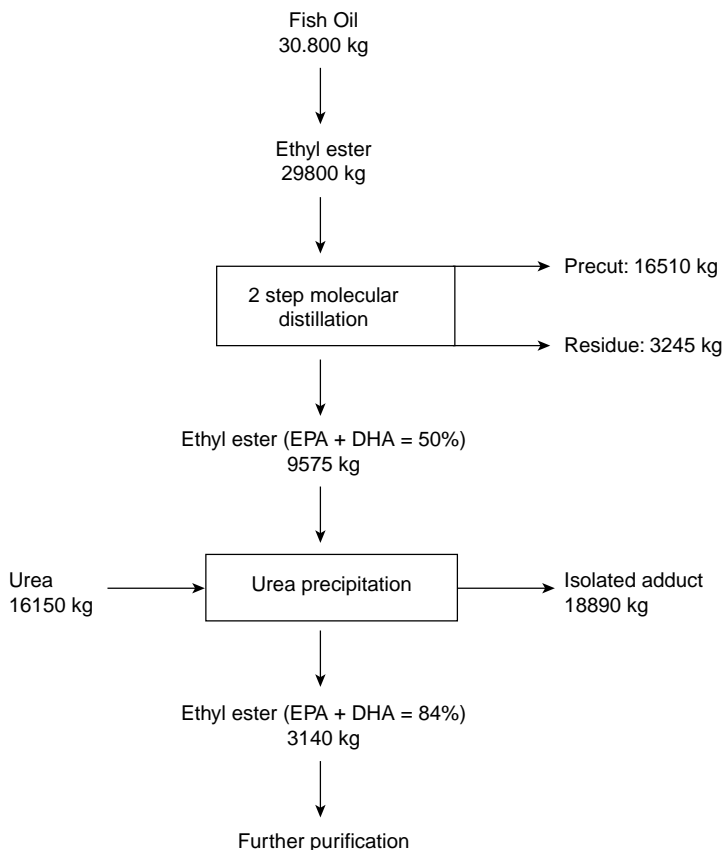
Urea complexing was demonstrated for fractionation of fatty acids of a marine oil (as methyl esters) as early as 1963 (94), and laboratory-scale tests in Halifax, Canada (95) were followed by further tests on a scale of 40 kg of crude oil. At that time, 50% omega-3 ethyl esters was considered a good possible result and doubling the omega-3 content made the product more acceptable on a retail basis by 1989 (79, 85).

A flow chart, courtesy of H. Breivik of Norway, is provided as Figure 12, the work of Norsk Hydro, Porsgrunn, Norway, and dating to 1990. The molecular weight of ethyl myristate (14:0, 256), palmitate (16:0, 284), palmitoleate (16:1, 282), and even oleate (18:1, 310) are sufficiently different from those of the ethyl esters of C20 and C22 polyunsaturated acids (330 and 356) to allow removal of most of the shorter chain fatty acids by short-path distillation. From Figures 5 and 6, it can be seen that 30–50% of the ethyl esters in question are available to be distilled off, with 18:4n-3 as the omega-3 fatty acid of interest that may be lost. By eliminating these fatty acids as a first step with the simple operation of short-path distillation, the subsequent urea complexing step is much more economically carried out, which led to the very successful Provnova Biocare product of 85% EPA + DHA as ethyl esters. Under the trade name EPAX, a variety of triacylglycerol and ethyl ester products are now offered by this firm, with different proportions of EPA and DHA, which is evidence of further process development since 1990.

At about the same time, the Charleston Laboratory of the U.S. National Marine Fisheries Service prepared menhaden oil omega-3 fatty acid ethyl ester concentrates on a large scale for participants in projects funded by the U.S. National Institutes of Health. Their flow chart, Figure 13, combined development work also carried out at the Seattle and Gloucester Laboratories, much of it recorded in the book by M.E. Stansby (26). The final process adopted includes urea complexing of ethyl esters in a total of seven stages, viz:

- I. Vacuum deodorization
- II. Transesterification

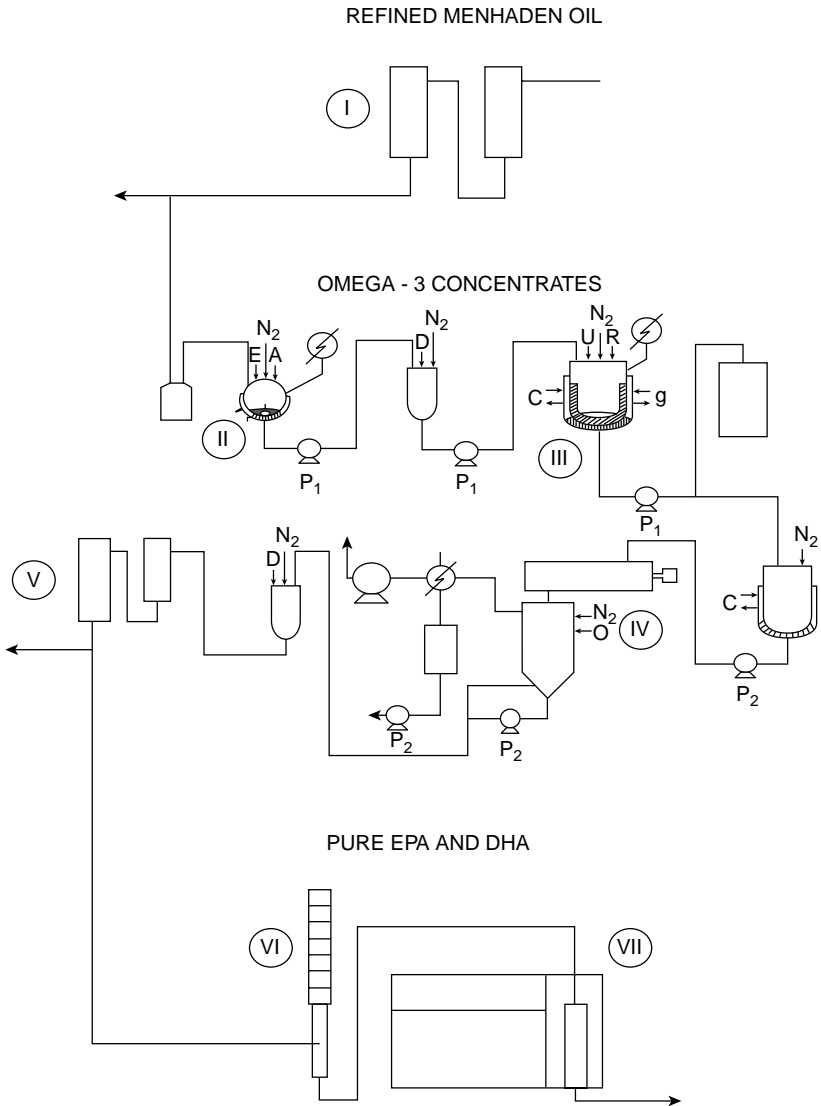
**MASS BALANCE**  
PART OF EARLY SCALE-UP EXPERIMENT  
PRIOR TO LATER MODIFICATIONS



**Figure 12.** Development stage of Norsk Hydro Research Center Porsgrun scheme for production of ethyl ester concentrates of marine oil fatty acids, ca. 1990. Courtesy of H. Breivik.

- III. Urea adduction
- IV. Film evaporation
- V. Short-path distillation
- VI. Supercritical fluid fractionation
- VII. High-performance liquid chromatography

Although subsequently closed down only because of the cessation of the joint project, the demonstration of what could be done, even if uneconomic, created wide



**Figure 13.** Production of biomedical test materials in the Charleston Laboratory of the U.S. National Marine Fisheries Service for a joint NMFS-NIH project. Code numbers for steps are explained in text. Small print identities are  $N_2$  = nitrogen atmosphere, A = alkali, E = ethanol, D = distill, U = urea, R = reflux, C = cold water, and S = steam. From (96).

interest in employing concentrates in clinical trials of omega-3 fatty acids. The product standards that were set were, for the time, remarkably high (Table 8).

Investigations carried out for the NMFS Charleston process indicated that chain length (i.e., molecular size) was a dominant factor observed in research on supercritical  $CO_2$  separations of ethyl esters of marine oil fatty acids (93). The initial

**TABLE 8. Quality Specifications for Fish Oil Derived n-3 Ethyl Esters to be Shipped from Charleston Laboratory.**

Analysis Type	Test Material		
	n-3 Conc	EPA	DHA
Esters, %	>90	>95	>95
EPA, mg/g	>400	>900	<50
DHA, mg/g	>200	<50	>900
Total n-3, mg/g	>700	>950	>950
Free Fatty Acids, %	<0.2	<0.2	<0.2
Trans Acids, %	<5	<5	<5
Cholesterol, mg/g	<5.0	<0.1	<0.1
Peroxide Value, meq/kg	<10.0	<5.0	<5.0
Iodine Value, g l/100g	>320	*	*
Anisidine Value	<80	*	*
Antioxidant Content			
$\alpha$ -tocopherol, mg/g	0.5–5.0	**	**
g-tocopherol, mg/g	0.5–5.0	**	**
TBHQ, mg/g	0.1–0.2	**	**
Moisture, ug/g	<500	<500	<500
Residual urea, ug/g	<20	<20	<20
PCB, ug/g	<0.5	<0.5	<0.5
Total DDT, ug/g	<0.5	<0.5	<0.5
Trace Metals, ug/g			
Arsenic	<1.0	<1.0	<1.0
Cadmium	<1.0	<1.0	<1.0
Lead	<1.0	<1.0	<1.0
Mercury	<1.0	<1.0	<1.0
Selenium	<1.0	<1.0	<1.0
Sensory Attributes:			
Odor (TIO)	<6.0	*	*
Flavor (TIF)	<6.0	*	*
Other:			
Specific Gravity	0.89	**	**
Solidification Range	***	***	***

\*not applicable.

\*\*not enough material to conduct these analyses routinely.

\*\*\*Esters are a liquid at 5°C or higher.

Reproduced from (96).

promise of supercritical fluid extraction (SFE) for actual recovery of lipids from natural samples is a separate issue from oil fractionation, but a few references establish the difficulties faced with use of cosolvents, water removal, and other factors not applicable to oil or ester fractionation (97–99). Thanks to many pioneers and recent commercial stimulus, supercritical fluid fractionation has the potential for concentrating ethyl esters of fish oil fatty acids.

Gradually, health benefits have attracted financial interest and a market role for omega-3 concentrates was seen in U.S. foods. A leader in this field was Roche Vitamins, Inc., of Parsippany, NJ, soon to be acquired by the DSM company of the

**TABLE 9. Product Data for ROPUFA '75' n-3 EE.**

Product identification for refined ethyl esters of fish oil, containing eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester

<b>Specifications</b>	
<i>Appearance:</i> yellowish liquid	<i>EPA content (gas chromatography):</i> min 42%
<i>Acid value:</i> max. 3 KOH/g	<i>EPA content (weight as ethyl ester):</i> min 380 mg/g
<i>Peroxide value:</i> max. 10.0 mEq/kg	<i>DHA content (gas chromatography):</i> min 22%
<i>Anisidine value:</i> max. 20	<i>DHA content (weight as ethyl ester):</i> min 200 mg/g
<i>Oligomers:</i> max. 2%	<i>Total content of n-3 PUFAs (gas chromatography):</i> min 75%
<i>Conjugated dienes:</i> max. 1.5%	<i>Total content of n-3 PUFAs (weight as ethyl ester):</i> min. 720 mg/g
<i>Iron:</i> max. 1 ppm	
<i>Copper:</i> max. 0.1 ppm	
<i>Arsenic:</i> max. 0.1 ppm	

Courtesy of Roche Vitamins Inc., Parsippany, NJ.

Netherlands. The basic product was “ROPUFA ‘75’ n-3 EE” with specifications as set forth in Table 9. Information on this ethyl ester product clearly shows a GLC profile with traces of C16 fatty acids, traces of 18:0 and 18:1, a little of each of 20:4n-6 and of 20:1 and 22:1, and the ubiquitous 21:5n-3 and 22:5n-3.

Many students have received degrees in recent years for exploring enrichment of marine oils (or other oils) by selective hydrolysis of triacylglycerols, or ester interchanges between esters and natural oils by enzymes. These explorations tend to be somewhat theoretical (100), but they can be effective, although impractical, for example, a 100-hour reaction time (101). Having a starting material rich in the desired product fatty acid (DHA) helped in one case (102), but the complexity of these proposed processes requires a separate article.

There is no real reason for concentrations of mixed omega-3 fatty acid ethyl esters to be provided when the estimated need in oral supplement form is approximately 1 g per day for adults. This supplement can be provided by several capsules of any suitable oil taken with meals. However, the food industry requirement is for oils that are microencapsulated powders. These powders have to be provided with stable, yet digestible, shells and in a suitable powder format to be unnoticed in the food and yet carry a significant proportion of mass as the marine oil or omega-3 concentrates. A “capacity” of about 50–50% oil is acceptable, and a target of 70–80% content is being sought. Eventually, concentrates will boost delivery of EPA and DHA.

## 8. THE OTHER OILS

Aside from cod liver oil, no mention has been made of other fish liver oils, although at one time, there was production of vitamins A and D from the liver oil of a Pacific dogfish *Squalus acanthius*. This industry collapsed when synthetic vitamins were introduced. The Atlantic spiny dogfish is essentially the same fish, and the liver oils from both coasts have been compared (103). The distinguishing feature of

**TABLE 10. Liver Oil and Squalene Analysis of Dogfish from the Continental Slope of New Zealand.**

Species	Sex	No. of Samples	Total No. of Dogfish Sampled	Oil Yield (g/100g Liver)		Squalene Yield (g/100g Oil)	
				Mean	Range	Mean	Range
Shovelnose Dogfish	Male	4	40	87.7	86–89	65.7	64–69
Shovelnose Dogfish	Female	11	110	86.4	83–90	52.7	45–59
Shovelnose Dogfish	Juvenile	3	30	89.2	86–90	58.4	57–60
Baxter's Dogfish	All	2	20	77.7	–	48.9	44–54
Seal Shark	All	2	15	84.5	81–88	72.6	72–73
Leafscale Gulper Shark	All	3	22	81.5	74–86	62.9	61–64
Plunket's Shark	All	1	2	84.2	–	1.4	–
Owston's Dogfish	Male	2	20	80.6	–	67.7	66–70
Owston's Dogfish	Female	2	16	–	–	44.2	66–70
Portugese Dogfish	All	1	5	81.4	–	44	41–47

From G. Summers et al. (105).

this shark liver oil is the content of diacyl glyceryl ethers (DAGE, Figure 1). The limited survey showed that the Pacific oil was the richer in DAGE (41% vs. 18% in Atlantic liver oil), but the C20 and C22 omega-3 fatty acids quantitatively were similar (103). In a further examination of the liver oil from Atlantic dogfish, the ability of Atlantic salmon to digest was excellent (104). The oil, therefore, has value for aquaculture feeds.

Squalene (Figure 4) is also sold in capsules in health supplement stores. Shark (and related elasmobranch) livers do not necessarily have this hydrocarbon in any more traces than those found in other species of fish. It depends on the exact species. A survey of New Zealand shark resources (Table 10) shows what may be expected (105). This report also explores liver oil recovery and processing. Unfortunately, local fishing in underdeveloped countries, often for shark fins only, has destroyed a large part of the resource. Squalene can exaggerate the iodine value of such oils (106), but it is easily measured by GLC after hydrogenation of methyl esters carefully prepared from the whole liver oil (107). Curiously, a small anadromous fish, ooligan or eulachon, spawning in the Fraser and other Pacific coast rivers has considerable ( $\geq 19\%$ ) squalene in its body fats (108, 109). The eulachon *Thaleichthys pacificus* was long recognized by local aborigines as a source of inedible but useful oil, but although the fish is fatty, it is edible and is fished to some extent for that reason. As olive oil is a potential source of squalene if it is needed, the slaughter of sharks for this hydrocarbon for any purported health benefits is to be deplored.

Wax esters are another useful marine lipid class, which are now historical when derived from the heads of sperm whales. Although various marine invertebrates contain wax esters (110), there is an unexploited resource in relatively small fish called myctophids. These fish can be caught by modern fishery technology as was shown in South Africa some decades ago, but the use of any oil and meal produced would have to be carefully considered. The biosynthesis of their wax esters has recently been resolved (111) and reviews most questions on that topic that were

left unanswered. Analysis of the whole lipids (18%) of a North Atlantic fish, the barracudina *Paralepis notolepis rissoi krøyeri*, showed this to contain 85% wax esters (112). The fatty acids of the wax esters differed from those of sperm body or head wax esters, but the alcohols from barracudina showed a remarkable similarity to those of sperm head wax.

Algae in theory and in practice can produce oils that are triacylglycerols (39, 113). The DHA-rich oils commercially produced by the Martek Biosciences Corp. are approved for use in infant formulas. They have also been shown to possess the favorable clinical attributes of fish oil DHA in healthy adults, whether alone (114) or combined with arachidonic acid (20:4n-6) of fungal origin (115). Products of these two sources of refined fatty acids show no particular resistance to oxidation, compared with fish oils, when finally purified of natural components that might not be allowed in products for human consumption (116). A competing source of DHA (Nutrinova Inc., Somerset, NJ) advertises its products as "from vegetarian source." As noted earlier, algae can also be good sources of EPA (117), which reopens the question posed earlier. Are the EPA and DHA of fish oils all or mostly from the fatty acids originally supplied by phytoplankton?

## 9. CONCLUSION

In a decade, the "advanced" lipid analytical technology that defines DHA as a natural fatty acid of marine oils from a deep-sea shark described around 1994 (118) has been surpassed by nondestructive NMR measurement of DHA in situ (Table 7). Thus, advanced analytical technology is supporting with new developments the benefits from marine oil omega-3 fatty acids in our daily lives.

## APPENDIX 1. CODEX ALIMENTARIUS EXPLANATORY MATERIALS RELATING TO FISH OIL QUALITY TERMINOLOGY

### Quality Guidelines and Potential Problem Areas or Disadvantages of Various Parameters.

Quality Unit	Disadvantage or Potential Problem Area	Codex Specif. CAC/RS 19-1981 Rev. 1 1989.
Color	Dark-colored oils may be crude and contain contaminants normally removed by refining or the color might indicate overheating during refining.	No Standard
Iodine Value	IV varies with the species of fish. In general, IV is a measure of the unsaturation in oils. High IV oils are generally more susceptible to oxidation.	No Standard
Acid Value <sup>1</sup>	High acid value crude oils might indicate that poor-quality fish were processed or the oil deteriorated in storage.	0.6 mg KOH/g fat max refined oils  4 mg KOH/g fat max virgin oils 4 mg KOH/g fat max cold pressed oils

**Quality Guidelines and Potential Problem Areas or Disadvantages of Various Parameters.**

Quality Unit	Disadvantage or Potential Problem Area	Codex Specif. CAC/RS 19-1981 Rev. 1 1989.
Peroxide Value	Peroxide value is the primary measure of rancidity (Oxidation) in an oil or fat. It reflects recent oxidation.	10 meq/kg fat max virgin and cold pressed oils 5 meq/kg fat max other oils
p Anisidine Number	The Anisidine Number also measures products of oxidation; however, it reflects oxidation that has taken place in the past.	No Standard
Totox Value	A relationship between peroxide value and anisidine number that is used to measure the rancidity level of fats and oils. It is defined as $(2 \times PV) + AN$ . It reflects total oxidation to date.	No Standard
Moisture	Considered an impurity. High levels of moisture in an oil can lead to deterioration in storage.	0.20% max
Soap	Soap can be formed when moisture is present in the crude oil and reacts with the free fatty acids and a catalyst (alkali ion), or it can result from incomplete removal of soap from washed refined oil.	0.005% max
Insoluble Impurities	These are substances including traces of protein, dirt, rust, and other materials that tend to precipitate out of the oil during storage. Depending on the substance, they can reduce the stability of the oil.	0.05% max
Unsaponif. Matter	These are composed of sterols, hydrocarbons, glyceryl ethers, and fatty alcohols. There may also be traces of pigments, vitamins, and oxidized oil. Unsaponifiables vary with the species of fish.	No Standard
Organochlorine, Organophosphorous Pesticides and other Chlorinated Hydrocarbons	Numerous compounds come under this group. Generally, the pesticide content of the oil reflects the environmental conditions in the area where the fish are caught. The level of these compounds in the oil must be within the regulatory limits of the locality involved.	No Standard
Total Cholesterol	Cholesterol is a major part of the unsaponifiable fraction of fish oils. Generally it is not removed except by vacuum stripping of the oil.	No Standard
Iron	Iron is considered a pro-oxidant in fish oil and is removed by degumming and refining.	1.5 mg/kg max refined oil 5 mg/kg max virgin oil 5 mg/kg max cold pressed oil
Copper	Copper is considered a pro-oxidant in fish oil and is removed by degumming and refining.	0.1 mg/kg max refined oil 0.4 mg/kg max virgin oil 0.4 mg/kg max cold pressed oil



**Quality Guidelines and Potential Problem Areas or Disadvantages of Various Parameters.**

Quality Unit	Disadvantage or Potential Problem Area	Codex Specif. CAC/RS 19-1981 Rev. 1 1989.
Arsenic	A heavy metal, naturally occurring in sea water. It is removed by the refining process.	0.1 mg/kg mx
Lead	A heavy metal removed by the refining process.	0.1 mg/kg max
Mercury	A heavy metal removed by the refining process.	No Standard
Selenium	A heavy metal removed by the refining process.	No Standard
Cadmium	A heavy metal removed by the refining process.	No Standard
Hygiene	Microbiological contamination by enterobacteria, salmonella, coliforms, or <i>E. coli</i> would be an indication of the sanitary conditions under which the oil was manufactured.	CAC/RCP 1-1969, Rev. 2 1985 limits.
Oil Soluble Vitamins	Normally part of the unsaponifiable fraction of the oil. High Vitamin A and/or D would indicate that the oil is a liver oil rather than a body oil.	No Standard

<sup>1</sup>Acid value is defined as two times the free fatty acid content of the oil. CAC defines edible fats and oils as foodstuffs composed of glycerides of fatty acids. They are of vegetable, animal, or marine origin. They may contain small amounts of other lipids such as phosphatides, unsaponifiables, or free fatty acids naturally present in the fat or oil. CAC defines virgin fats and oils as edible fats and oils obtained without altering the oil, by mechanical procedures, and the application of heat only. They may be purified by washing with water, settling, filtering, and centrifuging only. CAC defines cold-pressed fats and oils as edible vegetable fats and oils obtained, without altering the oil, by mechanical procedures without the application of heat. They may have been purified by washing with water, settling, filtering, and centrifuging only.

**REFERENCES**

1. S. M. Barlow and M. E. Stansby, eds., *Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oil*, Academic Press, London, 1982.
2. R. G. Ackman, *Fatty Acids in Newer Fats and Oils*, in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, 5th ed., Vol. 1, Edible Oil and Fat Products: General Applications, Wiley, New York, 1996, p. 427.
3. J-L. Sebedio, M. F. Langman, C. A. Eaton, and R. G. Ackman, *J. Amer. Oil Chem. Soc.*, **58**, 41 (1981).
4. J-L. Sebedio and W. W. Christie, eds., *Trans Fatty Acids in Human Nutrition*, The Oily Press, Dundee, (1998).
5. T. Leth, A. Bysted, K. Hansen, and L. Ovesen, *J. Amer. Oil Chem. Soc.*, **80**, 475 (2003).
6. R. G. Ackman, *Natural Trans Fatty Acids*, in Ref. 4, pp. 303–311.
7. J. Dyerberg and H. O. Bang, *Lancet*, (2), 433 (1979).
8. D. Kromhaut, E. B. Bosschieter, and C. De Lezenne Coulander, *New Eng. J. Med.*, **312**, 1205 (1985).
9. Committee on Medical Aspects of Food Policy, Department of Health, "Nutritional Aspects of Cardiovascular Disease. Report of the Cardiovascular Review Group," No. 46, HMSO, London, (1994).

10. P. M. Kris-Etherton, W. S. Harris, and L. J. Appel, for the Nutrition Committee, AHA Scientific Statement, *Circulation*, **106**, 2747 (2002).
11. R. G. Ackman, *Food Revs. Intern.*, **6**, 617 (1990).
12. R. G. Ackman, Fatty Acids in Fish and Shellfish, in C. K. Chow, ed., *Fatty Acids in Foods and their Health Implications*, 2nd ed., Marcel Dekker, New York, 2000, pp. 169–184.
13. W. E. Connor and A. Bendich, eds., *Amer. J. Clin. Nutr.*, **71**(suppl) (1), (2000).
14. A. J. Sinclair, N. M. Attar-Bashi, and D. Li, *Lipids*, **37** 1113, (2002).
15. S. C. Cunnane, *Can. J. Physiol. Pharmacol.*, **74**, 629 (1996).
16. F. Thies, E. A. Miles, G. Nebe-von-Caron, J. R. Powell, T. L. Hurst, E. A. Newsholme, and P. C. Calder, *Lipids*, **36**, 1183 (2001).
17. R. Marchioli, F. Barzi, E. Bomba, et al., *Circulation*, **105**, 1897 (2002).
18. A. Leaf, *Circulation*, **105**, 1874–1875 (2002).
19. S. A. Reed, *J. Sci. Food Agric.*, **15**, 399 (1964).
20. F. V. K. Young, The Production and Use of Fish Oils, in Ref. 1, pp. 1–23.
21. A. P. Bimbo and J. B. Crowther, *J. Amer. Oil Chem. Soc.*, **69**, 221 (1992).
22. J. R. Burt, R. Hardy, and K. J. Whittle, eds., *Pelagic Fish. The Resource and its Exploitation*. Fishing News Books, Blackwell Sci. Pubs., Oxford, United Kingdom, 1992.
23. A. P. Bimbo, *Fish Meal and Oil*, in R. E. Martin, E. P. Carter, G. J. Flick, Jr., and L. M. Davis, eds., *Marine and Freshwater Products Handbook*, Technomic Pub. Co. Inc., Lancaster, Pennsylvania, 2000, pp. 541–581.
24. R. C. Ackman, ed., *Marine Biogenic Lipids*, Vols. 1 and 2, CRC Press, Boca Raton, Florida, 1989.
25. J. K. McElvoy, A techno-economic model for designing large factory vessels and its application to the Antarctic krill-fishery fishery, in Ref. 22, pp. 94–114.
26. M. E. Stansby, ed., *Fish Oils in Nutrition*, An avi Book, Van Nostrand Reinhold, New York, 1990.
27. R. G. Ackman, W. M. N. Ratnayake, and B. Olsson, *J. Amer. Oil. Chem. Soc.*, **65**, 136 (1988).
28. R. G. Ackman, J-L. Sebedio, and M. I. P. Kovacs, *Mar. Chem.*, **9**, 157 (1980).
29. W. N. Ratnayake and R. G. Ackman, *Lipids*, **14**, 795 (1979).
30. W. N. Ratnayake and R. G. Ackman, *Lipids*, **14**, 804 (1979).
31. R. F. Lee and J. S. Patton, Alcohol and Waxes, in Ref. 24, Vol. 1, pp. 73–102.
32. D. Menoyo, C. J. Lopez-Bote, J. M. Bautista, et al., *Aquat. Liv. Resour.*, **15**, 217 (2002).
33. R. N. Lemaitre, I. B. King, and T. E. Raghunathan, et al., *Circulation*, **105**, 697 (2002).
34. A. M. Katz, *Circulation*, **105**, 669 (2002).
35. R. G. Ackman, Fatty Acids, in Ref. 24, Vol. 1, pp. 103–137.
36. Y. Ando, T. Ota, and T. Takagi, *Yukagaku*, Proc. Vol. 2, Sess. Lect. Sci. Pres. ISF-JOCS World Congress, 1989, pp. 1263–1268.
37. R. G. Ackman, *Anal. Chim. Acta*, **465**, 175 (2002).
38. R. G. Ackman, *INFORM*, **5**, 1119 (1994).
39. Y. Itabashi, N. Harada, T. Ota, and K. Matsunaga., *Yukagaku*, **45**, 271 (1996).
40. P. Mayzaud, C. A. Eaton, and R. G. Ackman, *Lipids*, **11**, 858 (1976).

41. P. Mayzaud and R. G. Ackman, *Lipids*, **13**, 24 (1978).
42. J. D. Joseph and R. G. Ackman, *J. Assoc. Off. Anal. Chem. Int.*, **75**, 488 (1992).
43. R. G. Ackman and S. N. Hooper, *Comp. Biochem. Physiol.*, **46B**, 153 (1973).
44. H. E. Worne and L. W. Smith, *Amer. J. Med. Sci.*, 710–721 (1959).
45. C. M. Harlow, L. Steeves, and A. Myrden, *Nova Scotia, Med. Bull.*, **37**, 127 (1958).
46. E. J. Schaefer, *Amer. J. Clin. Nutr.*, **75**, 191–212 (2002).
47. P. Cullen, *Amer. J. Cardiol.*, **86**, 943 (2000).
48. K. H. Bønaa, K. S. Bjerve, B. Straume, I. T. Gram, and D. Thelle, *New Eng. J. Med.*, **322**, 795 (1990).
49. T. Kanayasu-Toyoda, I. Morita, and S. Murota, *Prostaglandins Leukotri. Essent. Fatty Acids*, **54**, 319 (1996).
50. C. Benistant, F. Achard, S. Ben Slama, and M. Lagarde, *Prostaglandins Leukotri. Essent. Fatty Acids*, **55**, 287 (1996).
51. W. S. Harris, *J. Lipid Res.*, **30**, 785 (1989).
52. F. Achard, C. Bénistant, and M. Lagarde, *Biochim. Biophys. Acta.*, **1255**, 260 (1995).
53. J. D. Carver, *Amer. J. Clin. Nutr.*, **77**(suppl.), 1550S (2003).
54. S. M. Innis and S. L. Elias, *Amer. J. Clin. Nutr.*, **77**, 473 (2003).
55. A. Leaf, J. X. Kang, Y-F. Xiao, and G. E. Billman, *Circulation*, **107**, 2646 (2003).
56. J. S. Charnock, *Prog. Lip. Res.*, **33**, 355 (1994).
57. T. Helm, *Dangerous Sea Creatures*, Funk and Wagnall, New York, 1976.
58. W. M. Indrasena and T. A. Gill. *J. Food Sci.*, **65**, 948 (2000).
59. P. Pohl and H. Wagner, *Z. Naturforsch.*, **27**, 53 (1972).
60. K. Hayashi, S. Kida, K. Kato, and M. Yamada, *Bull. Jap. Soc. Sci. Fish.*, **40**, 609 (1974).
61. R. G. Ackman, C. S. Tocher, and J. McLachlan, *J. Fish. Res. Bd. Can.*, **25**, 1603 (1968).
62. S. M. Budge, C. C. Parrish, and C. H. McKenzie, *Mar. Chem.*, **76**, 285 (2001).
63. M. V. Bell, J. R. Dick, and A. E. A. Porter, *Lipids*, **36**, 1153 (2001).
64. M. V. Bell, J. R. Dick, and A. E. A. Porter, *Lipids*, **38**, 39 (2003).
65. C. Ghioni, J. G. Ben, and J. R. Sargent, *Comp. Biochem. Physiol.*, **1143**, 161 (1996).
66. T. Ota and M. Yamada, *Bull. Fac. Fish. Hokkaido University*, **26**, 277 (1975).
67. R. G. Ackman, C. A. Eaton, E. G. Bligh, and A. W. Lantz., *J. Fish Res. Bd. Can.*, **24**, 1219 (1967).
68. W. Schmidtsdorff, Fish meal and fish oil—not only by-products, in A. Ruiter, ed., *Fish and Fishery Products*, CAB International, Wallingford, 1995, pp. 346–376.
69. S. Zhou, R. G. Ackman, and C. Morrison, *Can. J. Fish. Aquat. Sci.*, **53**, 326 (1996).
70. A. P. Bimbo. *INFORM*, **9**, 473 (1998).
71. E. Kulas, E. Olsen, and R. G. Ackman, *Eur. J. Lipid. Sci. Technol.*, **104**, 520 (2002).
72. K. Berger, *INFORM*, **14**, 307 (2003).
73. E. Kulas, R. G. Ackman, *J. Amer. Oil Chem. Soc.*, **78**, 361 (2001).
74. S. Wada, *J. Oleo Sci.*, **50**, 329 (2001).
75. A. P. Bimbo, Production of Fish Oil, in Ref. 26, pp. 181–225.
76. R. G. Ackman, S. N. Hooper, and D. L. Hooper, *J. Amer. Oil Chem. Soc.*, **51**, 42 (1974).

77. R. C. Wijesundera, W. M. N. Ratnayake, and R. G. Ackman, *J. Amer. Oil Chem. Soc.*, **66**, 1822 (1989).
78. K. Julshamn, L. Karlsen, and O. R. Braekkan, Fiskeridirektoratets Skrifter, *Ser. Tek. Undersøkelser*, Vol. 5, No. 15, Directorate of Fisheries Bergen, 1973.
79. R. G. Ackman, *Chemistry and Industry*, **7**, 139 (1988).
80. M. A. Dessi, M. Deiana, B. W. Day, A. Rosa, S. Banni, and F. P. Corongiu., *Eur. J. Lipid. Sci. Technol.*, **104**, 506 (2002).
81. G. Hølmer, Triglycerides, in Ref. 24, Vol. 1, pp. 139–173.
82. R. Ackman, V. R. Orozco, and W. M. N. Ratnayake, *Fat. Sci. Technol.*, **93**, 447 (1991).
83. T. Igarishi, M. Ausand, Y. Hirata, I. S. Gribbestad, S. Wada, and M. Nonaka, *J. Amer. Oil Chem. Soc.*, **77**, 737 (2000).
84. M. Cansell, F. Nacka, and N. Combe, *Lipids*, **38**, 554 (2002).
85. R. G. Ackman, W. M. N. Ratnayake, and E. J. Macpherson, *J. Amer. Oil Chem. Soc.*, **66**, 1162 (1989).
86. V. K. S. Shukla and E. G. Perkins, *INFORM*, **9**, 955 (1998).
87. W. W. Christie, Silver-ion high-performance liquid chromatography, in J. L. Sebedio and E. G. Perkins, eds., *New Trends in Lipid and Lipoprotein Analyses*, AOCS Press, Campaign, Illinois, 1995, pp. 59–80.
88. J. L. Guil-Guerrero and E-H. Belarbi, *J. Amer. Oil Chem. Soc.*, **78**, 477 (2001).
89. J-L. Sebedio and R. G. Ackman, *Lipids*, **16**, 461 (1981).
90. H. J. Passino, *Ind. Eng. Chem.*, **41**, 280 (1949).
91. V. J. Krukonis, Processing with supercritical fluids, overview and applications, in B. A. Charpentier and M. R. Sevenants, eds., *Supercritical Fluid Extraction and Chromatography, Techniques and Applications*, Amer. Chem. Soc., Washington, D. C., 1988, pp. 26–43.
92. S. S. H. Rizvi, R. R. Chao, and Y. J. Liaw, Concentration of omega-3 fatty acids from fish oil using supercritical carbon dioxide, in B. A. Charpentier and M. R. Sevenants, eds., *Supercritical Fluid Extraction*, 1988, pp. 89–108.
93. V. F. Stout, W. B. Nilsson, J. Krzynowek, and H. Schlenk, Fractionation of fish oils and their fatty acids, in Ref. 26, pp. 73–119.
94. R. G. Ackman, R. D. Burgher, and P. M. Jangaard, *Can. J. Biochem. Physiol.*, **41**, 1627 (1963).
95. W. M. N. Ratnayake, B. Olsson, D. Matthews, and R. G. Ackman, *Fat. Sci. Technol.*, **90**, 381 (1988).
96. Jeanne D. Joseph, Ed. 1989. Biomedical Test Materials Program: Production Methods and Safety Manual. NOAA Technical Memorandum NMFS-SEFC-234, 120 p.
97. J. Via, L. T. Taylor, and F. K. Schweighardt, *Anal. Chem.*, **66**, 1459 (1994).
98. S. L. Taylor, F. J. Eller, and J. W. King, *Food Res. Intern.*, **30**, 365 (1997).
99. T. H. Walker, H. D. Cochran, and G. J. Hulbert, *J. Amer. Oil Chem. Soc.*, **76**, 595 (1999).
100. M. Linder, J. Fanni, and M. Parmentier, *OCL*, **8**, 73 (2001).
101. G. P. McNeill, R. G. Ackman, and S. R. Moore, *J. Amer. Oil Chem. Soc.*, **73**, 1403 (1996).
102. Y. Shimada, K. Maruyama, A. Sugihara, S. Moriyama, and Y. Tominaga, *J. Amer. Oil Chem. Soc.*, **74**, 1441 (1997).

103. S-J. Kang, M. C. A. Timmins, and R. G. Ackman, *J. Amer. Oil. Chem. Soc.*, **75**, 1667 (1998).
104. S-J. Kang, S. P. Lall, and R. G. Ackman, *Lipids*, **32**, 19 (1997).
105. G. Summers, R. Wong, and L. Eyres, "Handling and processing shark livers for the recovery of squalene and diacyl glyceryl ethers," DSIR Crop Research Report No. 1, Christchurch, New Zealand, 1990.
106. R. G. Ackman, E. Macpherson, and A. Timmins. *J. Amer. Oil. Chem. Soc.*, **75**, 1223 (1998).
107. R. G. Ackman, E. J. Macpherson, and A. Timmins, *J. Amer. Oil. Chem. Soc.*, **77**, 831 (2000).
108. R. G. Ackman, R. F. Addison, and C. A. Eaton, *Nature*, **220**, 1033 (1968).
109. H. V. Kuhnlein, F. Yeboah, M. Sedgemore, S. Sedgemore, and M. Chan, *J. Food Compos. Anal.*, **9**, 18 (1996).
110. J. Joseph, Distribution and composition of lipids in marine invertebrates, in Ref. 24, Vol. 2, pp. 49-143.
111. H-S. Seo, Y. Endo, M. Moku, K. Kawaguchi, and K. Fujimoto, *Lipids*, **36**, 389 (2001).
112. R. Ackman, S. N. Hooper, S. Epstein, and M. Kelleher, *J. Amer. Oil Chem. Soc.*, **49**, 378 (1972).
113. P. W. Behrens and D. J. Kyle, *J. Food Lipids*, **3**, 259 (1996).
114. J. A. Conquer and B. J. Holub, *J. Nutr.*, **126**, 3032 (1996).
115. S. M. Innis and J. W. Hansen, *Amer. J. Clin. Nutr.*, **64**, 159 (1996).
116. E. N. Frankel, T. Satue-Gracia, A. S. Meyer, and J. B. German, *J. Agric. Food Chem.*, **50**, 2094 (2002).
117. L. Poisson, M. Devos, G. Pancreac'h, and F. Ergan, *OCL*, **9**, 92 (2002).
118. C. G. Bordier, N. Sellier, A. P. Faucault, and F. Le Goffic, *Chromatographia*, **39**, 329 (1994).

# 12

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## *Minor Components of Fats and Oils*

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### **1. INTRODUCTION**

Lipids from natural sources consist mainly of fatty acids esterified to glycerol, predominantly in the form of triacylglycerols (95–98%). Soluble in these lipids are two types of minor compounds: glycerolipids and nonglycerolipids (Table 1). Upon reaction with alkali, the unsaponifiable fractions of vegetable oils (i.e., nonglycerolipids) do not form soap and can be extracted from the saponified mixtures with lipophilic solvents such as diethyl ether, hexane, or cyclohexane. Over the past decades, these unsaponifiable materials have interested researchers for different reasons. In the first instance, the interest in the unsaponifiable fraction stemmed from the observation that many components, especially tocopherols but also phenolic and other compounds, have antioxidant properties of utmost importance for the oxidative stability of these oils. Constituents of the unsaponifiable fraction were also found to provide good markers for the authentication of some oils and fats. Moreover, much research is currently focusing on the biological and physiological activities of various unsaponifiable constituents and their possible contribution to improved human health. Apart from the carotenoids of palm oil, vitamin E-active compounds and phytosterols are perhaps the most important nutraceuticals present

**TABLE 1. Important Minor Constituents of Vegetable Oils.**

Glycerolipids	Non-Glycerolipids
Diacylglycerols	Sterols, triterpene alcohols, and their esters
Monoacylglycerols	Tocopherols/tocotrienols
Phospholipids	Hydrocarbons
Galactolipids	Waxes
Sulfolipids	Free fatty acids
	Lipid-soluble vitamins
	Pigments
	Phenolic compounds
	Metals and Metalloproteins

in vegetable oils. Tocopherols/tocotrienols are purified from the deodorizer distillates to be used as vitamin E supplements in capsules or as food additives, and sterols are recovered to be used as cholesterol-lowering agents in innovative foods, such as commercialized margarines and other products (1, 2). Phospholipids, glycolipids, and other complex lipids are generally present as minor lipid components in crude oils but are mostly removed by degumming during refining. These lipids have interesting properties as emulsifiers, as discussed in more detail in other chapters.

As shown in Table (2), vegetable oils contain variable levels of unsaponifiable matter (3) of variable composition and characteristics (4). The refining process, which is necessary to remove undesirable pigments and fatty acid oxidation products such as peroxides and their degradation products from vegetable oils to make them suitable for consumption, unfortunately, brings about loss of valuable nutrients and natural antioxidants (5–7). The technical and nutritional importance

**TABLE 2. The Content and Special Composition Characteristics of the Unsaponifiable Fraction of Selected Vegetable Oils.**

Oil	Unsaponifiable Matter (%)	
	Schwartz (3)	Special Composition Characteristics
Coconut	0.2	Not reported
Cottonseed	0.7	Not reported
Linseed	0.9	Plastochromanol-8
Olive	0.7	Squalene, special phenolic compounds
Palm	0.1	Red carotenoids
Peanut	0.4	Not reported
Rapeseed	1.0	Brassicasterol, plastochromanol-8
Sesame	1.4	Sesamin, sesamol and derivatives
Soybean	0.6	$\delta$ -tocopherol
Sunflower	0.7	Not reported
Maize	1.5	Not reported
Wheat germ	4.8	Not reported
Rice bran	4.2–5.2	$\gamma$ -oryzanol (cinnamic acid esters of sterols)

of many compounds in the unsaponifiable fraction is increasingly recognized, and extraction and refining technologies are being developed to produce vegetable oils with maximized functional and nutritional properties. This chapter reviews the chemistry and the importance of a wide range of natural minor components occurring in vegetable oils.

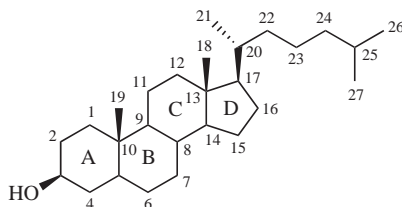
## 2. THE CHEMISTRY OF MINOR LIPID COMPONENTS

### 2.1. Sterols

The sterols (Table 3) are generally the major components of the unsaponifiable fractions of vegetable oils. Their structures are based on a steroidal alcohol framework comparable with that of cholesterol (Figure 1). The molecules are planar and are based on a tetracyclic cyclopentaphenanthrene system with four fused rings (A, B, C, and D). The hydroxyl group at C-3, side chain at C-17, and two methyl groups at C-18 and C-19 are all angular to the ring structure and have  $\beta$ -stereochemistry (i.e., above plane configuration) (8).

Plant sterols, or phytosterols, are generally the predominant compounds in the unsaponifiable fractions of vegetable oils that generally account for about 1% of the oils. The main sterols belong to the 4-desmethylsterols family, but 4-methylsterols and 4,4-dimethylsterols (also called triterpene alcohols) are present as minor components in most oils (Figure 2). Apart from some exceptions, the desmethylsterol,  $\beta$ -sitosterol, is generally the most abundant and is usually accompanied by variable levels of campesterol, stigmasterol,  $\Delta^5$ -avenasterol, and other sterols (Table 4). Some sterols are characteristic for certain oils; e.g., brassicasterol is characteristic for rapeseed/canola oil and can be used to detect the presence of this oil in foods. Recently, it was reported that camolina oil is special because of its high content of cholesterol (188 ppm) besides brassicasterol (133 ppm), campesterol (893 ppm), stigmasterol (103 ppm), sitosterol (1884 ppm), and  $\Delta^5$ -avenasterol (393 ppm) (12). Black cumin (*Nigella sativa* L.) is characterized by high levels of  $\beta$ -sitosterol (1135–1182 ppm),  $\Delta^5$ -avenasterol (925–1025 ppm), and  $\Delta^7$ -avenasterol (615–809 ppm), and small amounts of stigmasterol and campesterol (13).

Sterols occur in vegetable oils in the free and esterified forms in relative levels that are dependent on the type of oil. In the sterol esters of vegetable oils, the hydrogen



**Figure 1.** Structure of cholesterol (CAS # 57-88-5) and numbering of the sterol skeleton and side chain.



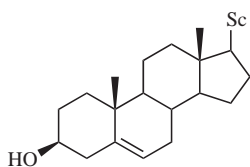
**TABLE 3. Structures, Trivial and IUPAC Numbers of Common Sterols and Triterpene Alcohols in Vegetable Oils.**

Trivial Name	IUPAC Name	CAS Number <sup>a</sup>	Structure <sup>b</sup>
<b>4-desmethylsterols</b>			
Brassicasterol	24 $\beta$ -methyl cholest-5,22E-dien-3 $\beta$ -ol	474-67-9	Ac
Campesterol	24 $\alpha$ -methyl cholest-5-en-3 $\beta$ -ol	474-62-4	Ab
Stigmasterol	24 $\alpha$ -ethyl cholest-5,22E-dien-3 $\beta$ -ol	83-48-7	Ag
Sitosterol	24 $\alpha$ -ethyl cholest-5-en-3 $\beta$ -ol	83-46-5	Af
$\Delta^5$ -Avenasterol	24E-ethylidene cholest-5-en-3 $\beta$ -ol	18472-36-1	Ah
Stigmasterol	24 $\alpha$ -ethyl cholest-7-en-3 $\beta$ -ol	481-18-4	Bf
$\Delta^7$ -Avenasterol	24E-ethylidene cholest-7-en-3 $\beta$ -ol	23290-26-8	Bh
<b>4-methylsterols</b>			
24-ethyl lophenol	4 $\alpha$ -methyl-24 $\alpha$ -ethyl cholest-7-en-3 $\beta$ -ol	36735-29-2	Cf
Gramisterol	4 $\alpha$ -methyl-24-methylene cholest-7-en-3 $\beta$ -ol	1176-52-9	Cd
Citrostadienol	4 $\alpha$ -methyl-24E-ethylidene cholest-7-en-3 $\beta$ -ol	474-40-8	Ch
Obtusifoliol	4 $\alpha$ ,14 $\alpha$ -dimethyl-24-methylene-cholest-8-en-3 $\beta$ -ol	—	Dd
Cycloeucalenol	9,19-cyclo-4 $\alpha$ ,14 $\alpha$ -dimethyl-24-methylene-cholest-8-en-3 $\beta$ -ol	469-39-6	Ed
<b>4,4-dimethylsterols</b>			
Cycloartenol	9,19-cyclo-4,4,14 $\alpha$ -trimethyl-cholest-24-en-3 $\beta$ -ol	469-38-5	Fa
24-Methylenecycloartanol	9,19-cyclo-4,4,14 $\alpha$ -trimethyl-24-methylene-cholestan-3 $\beta$ -ol	1449-09-8	Fd
Cyclobranol	9,19-cyclo-4,4,14 $\alpha$ ,24-tetramethyl-cholest-24-en-3 $\beta$ -ol	—	Fe
$\alpha$ -Amyrin	5 $\alpha$ -urs-12-en-3 $\beta$ -ol	638-95-9	—
$\beta$ -Amyrin	5 $\alpha$ -olean-12-en-3 $\beta$ -ol	559-70-6	—

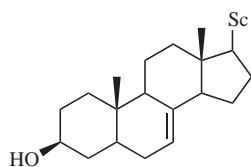
<sup>a</sup>CAS numbers from LIPIDAT, Lipid Molecular Structure Database of Ohio State University ([http://www.lipidat.chemistry.ohio-state.edu/cis888/rehner/searchLMSD\\_2\\_4.asp](http://www.lipidat.chemistry.ohio-state.edu/cis888/rehner/searchLMSD_2_4.asp)).

<sup>b</sup>See Figure 2.

Sterol skeletons  
Desmethyl Sterols

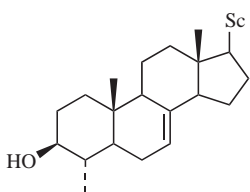


(A)

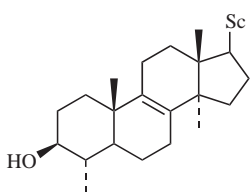


(B)

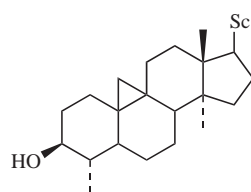
4-Methyl Sterols



(C)

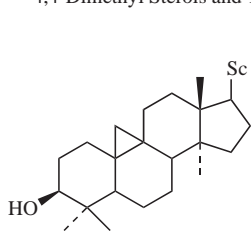


(D)

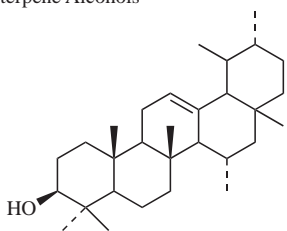
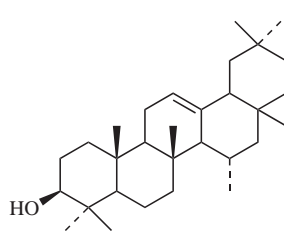


(E)

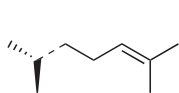
4,4-Dimethyl Sterols and Triterpene Alcohols



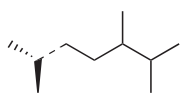
(F)

 $\alpha$ -Amyrin $\beta$ -amyrin

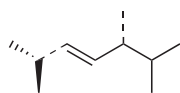
Side Chains (Sc)



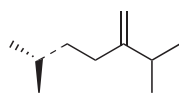
(a)



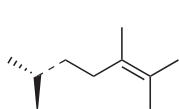
(b)



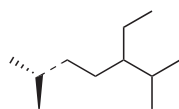
(c)



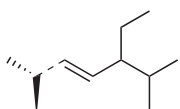
(d)



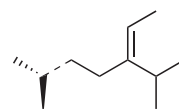
(e)



(f)



(g)



(h)

**Figure 2.** Sterol skeletons and side chains of the major sterols in vegetable oils (for names and structures, see Table 1).

**TABLE 4. Sterol Content (mg/100g) of Selected Vegetable Oils (9–11).**

Sterol	Coconut	Corn	Cotton Seed	Linseed	Olive	Pam	Palm Kernel	Peanut	Rape Seed	Rice Bran	Saf- flower	Sesame	Shea Butter	Soy Bean	Sun flower	Wheat Germ
<b>Desmethylsterols</b>																
Cholesterol	2	—	—	4	—	3	4	—	—	—	—	—	—	—	—	—
Campesterol	2	269	17	122	2	36	12	36	153	505	45	117	—	74	31	570
Stigmasterol	30	70	4	38	1	20	14	216	—	271	31	62	—	72	31	—
β-Sitosterol	132	772	396	193	131	189	92	154	355	885	181	382	—	191	235	1734
Δ <sup>5</sup> -Avenasterol	32	47	8	55	3	5	8	19	12	90	3	43	—	11	16	155
Δ <sup>7</sup> -Stigmasterol	14	12	—	8	6	2	1	7	31	18	70	12	94	11	59	78
Δ <sup>7</sup> -Avenasterol	—	—	—	—	—	—	—	2	—	36	10	—	28	4	16	52
Others	—	—	—	—	—	—	—	—	61 <sup>a</sup>	—	7	—	13	—	4	—
<b>4-Monomethylsterols</b>																
Obtusifoliol	2	4	11	22	2	6	5	4	8	29	10	81	22	5	34	7
Gramisterol	6	4	8	108	4	24	8	4	6	25	5	142	4	16	22	47
Citrostadienol	4	4	17	6	6	3	13	4	2	—	3	126	—	35	35	53
Others <sup>b</sup>	4	—	5	1	2	2	10	2	12	114	6	57	7	8	20	8
<b>4,4-Dimethylsterols</b>																
Cycloartanol	1	tr.	—	1	—	1	—	1	2	106	1	4	—	—	—	7
β-Amyrin	—	tr.	2	12	22	1	3	2	—	—	7	4	306	13	5	40
Butyrospermol	—	5	10	—	10	—	6	—	—	—	—	—	994	14	—	—
Cycloartenol	50	—	—	106	13	—	29	12	32	482	34	62	—	17	29	38
α-Amyrin	—	—	—	—	—	19	21	—	—	—	12	—	1759	—	—	18
24-Methylene- cycloartenol	—	—	19	—	30	—	9	—	—	—	—	—	612	23	12	—
Cyclobranol	12	7	16	34	47	—	3	16	16	494	7	107	—	8	16	99
Cycloaudenol	—	—	—	—	14	10	—	3	—	—	2	—	38	—	6	4
Others <sup>b</sup>	4	—	1	—	—	—	—	—	—	—	—	—	115	—	—	—
<b>Total</b>	<b>286</b>	<b>1194</b>	<b>514</b>	<b>710</b>	<b>293</b>	<b>321</b>	<b>230</b>	<b>482</b>	<b>690</b>	<b>3055</b>	<b>428</b>	<b>1199</b>	<b>3992</b>	<b>502</b>	<b>571</b>	<b>2910</b>

<sup>a</sup>Brassicasterol.

<sup>b</sup>Unknown compounds.

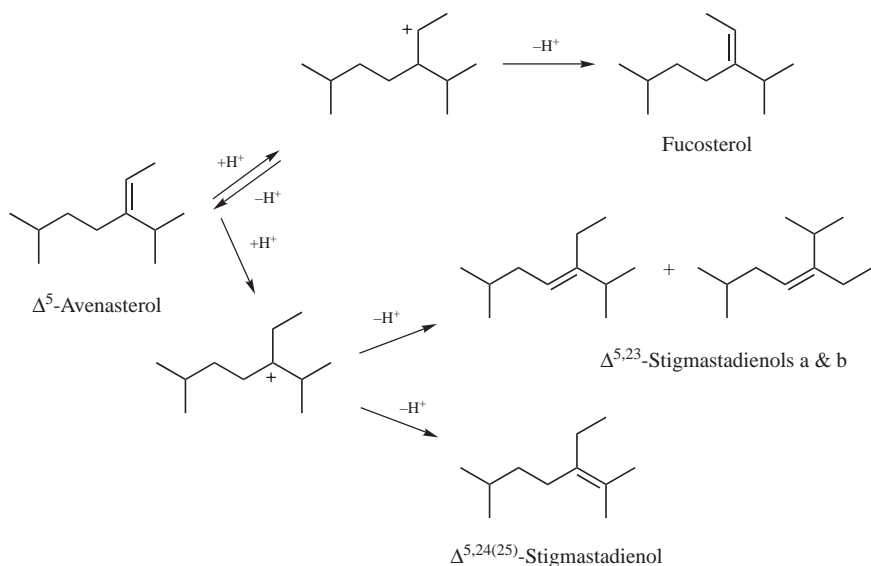
**TABLE 5. Content of Free and Acyl Sterol Esters and Relative Distribution of Sterols in Selected Crude Vegetable Oils.<sup>a</sup>**

Oil	Free Sterols					Acyl Sterol Esters				
	<b>Total (mg/100 g)</b>	Sitosterol (%)	Campesterol (%)	Sigmasterol (%)	$\Delta^5$ -Avenasterol (%)	<b>Total (mg/100 g)</b>	Sitosterol (%)	Campesterol (%)	Sigmasterol (%)	$\Delta^5$ -Avenasterol (%)
Coconut	<b>26</b>	71.9	11.0	17.1	—	<b>41</b>	70.4	11.1	18.5	—
Palm	<b>16</b>	59.5	22.8	11.0	6.7	<b>49</b>	66.0	30.0	14.0	—
Olive	<b>30</b>	65.0	4.6	—	30.4	<b>151</b>	76.9	—	—	23.1
Rapeseed	<b>475</b>	54.0	40.6	—	5.4	<b>336</b>	50.7	29.0	—	20.3
Corn	<b>423</b>	71.6	18.0	7.9	2.5	<b>485</b>	70.2	23.2	6.6	—

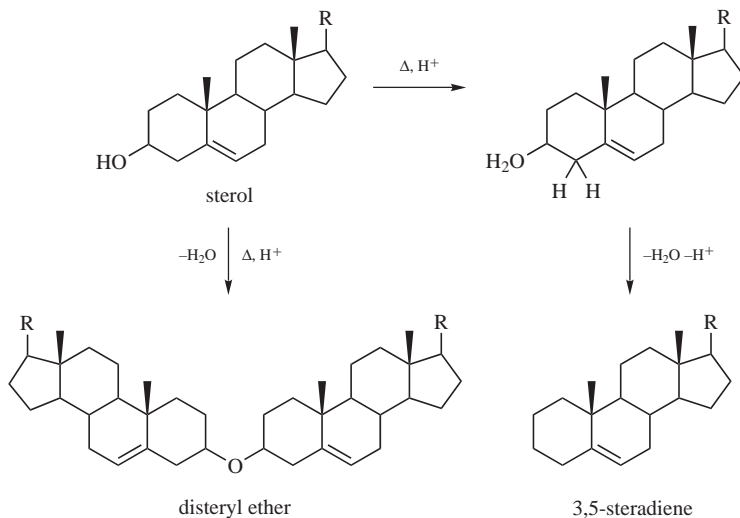
<sup>a</sup>Modified from Verleyen et al. (15).

of the hydroxyl group at C-3 is substituted with a fatty acyl or with ferulic acid as in  $\gamma$ -oryzanol. Oils of corn, cottonseed, and rapeseed are especially rich in sterol esters (14). According to this report, there are no differences in the composition of the free and acylated sterol fractions, which suggests nonspecific esterification (Table 5). Rice bran oil is currently recognized for its high content (about 2–5%) of  $\gamma$ -oryzanol, i.e., ferulic acid esters of mainly cycloartenol [CAS # 21238-33-5] and 24-methylene cycloartanol, but also cyclobranol, cycloeucaenol, sitosterol, and campesterol (16). Two-thirds of the total sterols of wheat germ oil, 2000–4000 mg/100 g, are present in ester form (17).

Refining affects the sterols of vegetable oils in various ways and is responsible for sterol losses in the range of 10–70% (18). Sterols are partially washed with the soap stock after chemical neutralization (5, 19). The use of acid clay bleaching agents and elevated temperatures catalyzes different isomerization, dehydration, and esterification reactions. Tisconia and Bertini (20) observed a very remarkable change in the content of  $\Delta^5$ -avenasterol in olive oil during bleaching depending on the level of bleaching earth used, and Touche et al. (21) made a similar observation for bleached coconut oil. Kesselmeier et al. (22) later suggested that  $\Delta^5$ - and  $\Delta^7$ -avenasterols present in oat lipids are destroyed by acid hydrolysis. Kamal-Eldin et al. (23) explained the mechanism of this transformation as involving secondary and tertiary carbonium ions (Figure 3). Bleaching effects on phytosterols are generally minor and mainly limited to the formation of some nonpolar dehydration products (18, 24) and partial hydrolysis of sterol esters (25). Steradienes and



**Figure 3.** Acid-catalyzed isomerization of  $\Delta^5$ -avenasterol [modified from Kamal-Eldin et al. (23)].



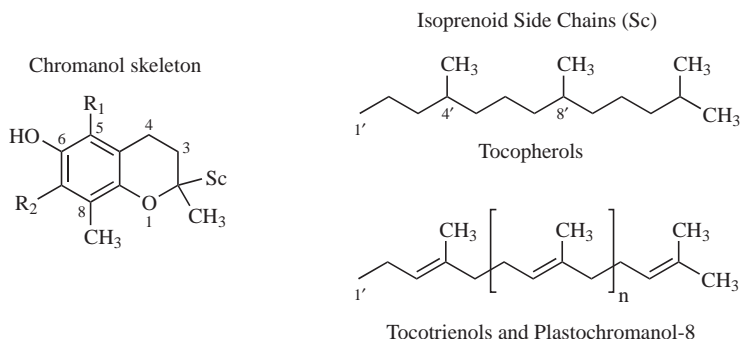
**Figure 4.** Transformation of sterols to steradienes and disteryl esters catalyzed by heat and acid in anhydrous conditions.

disteryl ether dehydration products (Figure 4) are formed during bleaching as well as deodorization (14). Dehydration products of triterpene alcohols were also isolated from stearin fractions of refined shea butter (26). Deodorization, on the other hand, causes a significant reduction in the total sterol because of distillation (5, 19, 27) and esterification of free sterols (14, 25). Whenever applied, hydrogenation has a tremendous effect on sterol structures, including hydrogenation of double bonds, opening of cyclopropane rings, and positional isomerization of side chain unsaturation (28–33).

Phytosterols are industrially isolated from the distillates, resulting from the deodorization of vegetable oils (1, 34–36). Phytosterols are sometimes hydrogenated to produce phytosterols (37). As the solubility of sterols and stanols is very low (<1% at 25°C), it limits their application in food products. Esterification of sterol and stanols is, therefore, performed to make them fat-soluble and easy to incorporate in food products (37, 38). Two margarines containing 8–9% sterols (Becel Proactiv of Unilever) or stanols (Benecol of Raisio), in the form of esters, are now available in the markets in Europe and the United States.

## 2.2. Tocopherols and Tocotrienols

The tocopherols and tocotrienols are generally not the major components of vegetable oil, but their presence is vital for stabilizing the unsaturated fatty acids of these oils against oxidative deterioration (39). Their structures are based on a chromanol head with two rings, one phenolic and one heterocyclic, and a phytyl tails isoprenoid side chain at C-2. The phytyl tail is saturated in the case of



Tocopherol	Tocotrienol	R <sub>1</sub>	R <sub>2</sub>
α-tocopherol	α-tocotrienol	CH <sub>3</sub>	CH <sub>3</sub>
β-tocopherol	β-tocotrienol	CH <sub>3</sub>	H
γ-tocopherol	γ-tocotrienol	H	CH <sub>3</sub>
δ-tocopherol	δ-tocotrienol	H	H

**Figure 5.** Chromanol ring and isoprenoid side chains in tocopherols, tocotrienols ( $n = 3$ ), and plastochromanol-8 ( $n = 8$ ,  $R_1 = H$ ,  $R_2 = CH_3$ ) in vegetable oils (for systematic names, see Table 6).

**TABLE 6.** Structures, Trivial, and IUPAC Numbers of Tocopherols, Tocotrienols, and Related Compounds Present in Vegetable Oils.

Trivial Name	IUPAC Name	CAS Number
<b>Tocopherols</b>		
α-tocopherol	3,4-Dihydro-2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl)-benzopyran-6-ol	59-02-9
β-tocopherol	3,4-Dihydro-2,5,8-trimethyl-2-(4',8',12'-trimethyltridecyl)-benzopyran-6-ol	16698-35-4
γ-tocopherol	3,4-Dihydro-2,7,8-trimethyl-2-(4',8',12'-trimethyltridecyl)-benzopyran-6-ol	54-28-4
δ-tocopherol	3,4-Dihydro-2,8-dimethyl-2-(4',8',12'-trimethyltridecyl)-benzopyran-6-ol	119-13-1
<b>Tocotrienols</b>		
α-tocotrienol	3,4-Dihydro-2,5,7,8-tetramethyl-2-(4',8',12'-trimethyl-3',7',11'-tridecatrienyl)-2H-1-benzopyran-6-ol	1721-51-3
β-tocotrienol	3,4-Dihydro-2,5,8-trimethyl-2-(4',8',12'-trimethyl-3',7',11'-tridecatrienyl)-2H-1-benzopyran-6-ol	490-23-3
γ-tocotrienol	3,4-Dihydro-2,7,8-trimethyl-2-(4',8',12'-trimethyl-3',7',11'-tridecatrienyl)-2H-1-benzopyran-6-ol	91-86-1
δ-tocotrienol	3,4-Dihydro-2,8-dimethyl-2-(4',8',12'-trimethyl-3',7',11'-tridecatrienyl)-2H-1-benzopyran-6-ol	—
Plastochromanol-8	3,4-Dihydro-2,7,8-trimethyl-2-(4',8',12',16',20',24',28',32'-octamethyl-3',7',11',15',19',23',27',31'-octadecaoctenyl)-2H-1-benzopyran-6-ol	—

tocopherols and unsaturated in the case of tocotrienols and other derivatives such as plastochromanol (Figure 5, Table 6). The four members of each subfamily, i.e.,  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -, differ from each other in the number and position of methyl groups on the chromanol ring. Naturally, tocopherols occur only as free alcohols, but tocotrienols were mentioned to also occur in esterified forms (40). The tocopherol molecule has three chiral centers in its phytyl tail, giving the possibility of eight total stereoisomeric forms. Naturally occurring tocopherols have the same molecular configurations (2R, 4'R, 8'R, RRR, or d-) in their phytyl groups, and tocopherols obtained by synthesis (*all-rac*-tocopherols) represent a mixture of approximately equal amounts of the eight possible stereoisomers [2D,4'D,8'D (RRR), 2L,4'D,8'D (SRR), 2D,4'D,8'L (RRS), 2L,4'D,8'L (SRS), 2D,4'L,8'D (RSR), 2L,4'L,8'D (SSR), 2D,4'L,8'L (RSS), and 2L,4'L,8'L (SSS)] (41). The tocotrienols only have one chiral center at position 2, so they are either 2D or 2L stereoisomers. The presence of the double bonds at positions 3' and 7' of the phytyl tails of tocotrienols allows for the existence of four *cis/trans* geometrical isomers, i.e., a total of eight isomers [2D,3'*cis*,7'*cis* (R,*cis-cis*); 2D,3'*cis*,7'*trans* (R,*cis-trans*); 2D,3'*trans*,7'*cis* (R,*trans-cis*); 2D,3'*trans*,7'*trans* (R,*trans-trans*); 2L,3'*cis*,7'*cis* (S,*cis-cis*); 2L,3'*cis*,7'*trans* (S,*cis-trans*); 2L,3'*trans*,7'*cis* (S,*trans-cis*); 2L,3'*trans*,7'*trans* (S,*trans-trans*)] per tocotrienol (42). It is, therefore, easy to detect the presence of synthetic tocopherols/tocotrienols in lipids.

The tocopherol and tocotrienol contents of selected vegetable oils are shown in Table (7). Seed oils are mostly dominated by  $\gamma$ - or  $\alpha$ -tocopherol, but high levels of  $\delta$ -tocopherol especially characterize soybean oil, which is the richest and commercially the most used source of tocopherols (46). Rice bran and palm oils, on the other hand, represent the important sources of tocotrienols (45, 47). Rapeseed

**TABLE 7. Levels (mg/100 g) of Tocopherol, Tocotrienols, and Related Compounds in Selected Vegetable Oils.**

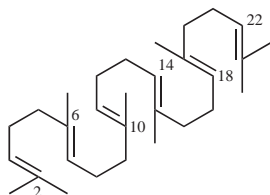
Oil	$\alpha$ -T	$\beta$ -T	$\gamma$ -T	$\delta$ -T	$\alpha$ -T3	$\beta$ -T3	$\gamma$ -T3	$\delta$ -T3	Total
Corn	26	1	75	3	1	—	2	—	108
Cottonseed	40	—	38	tr.	—	1	—	—	79
Linseed <sup>a</sup>	tr.	—	57	1	—	—	—	—	58 <sup>a</sup>
Olive	12	—	1	—	—	—	—	—	13
Palm	38	—	—	—	5	—	13	—	56
Peanut	14	2	13	1	—	—	—	—	30
Rapeseed <sup>b</sup>	19	—	49	1	—	—	—	—	69 <sup>b</sup>
Safflower	45	1	3	1	—	—	—	—	50
Sesame	—	—	58	1	—	—	—	—	59
Soybean	9	1	69	24	—	—	—	—	103
Sunflower	62	2	3	—	—	—	—	—	67
Maize	22	—	57	2	5	1	6	—	93
Rice bran <sup>c</sup>	59	2	6	tr.	45	—	44	3	159
Wheat germ	151	31	53	—	4	—	2	—	241

<sup>a</sup>Linseed oil also contains ca 20 mg/100 g of plastochromanol-8 (43).

<sup>b</sup>Rapeseed oil may also contain 55–80 mg/100 g of plastochromanol-8 (44).

<sup>c</sup>Data from (45).





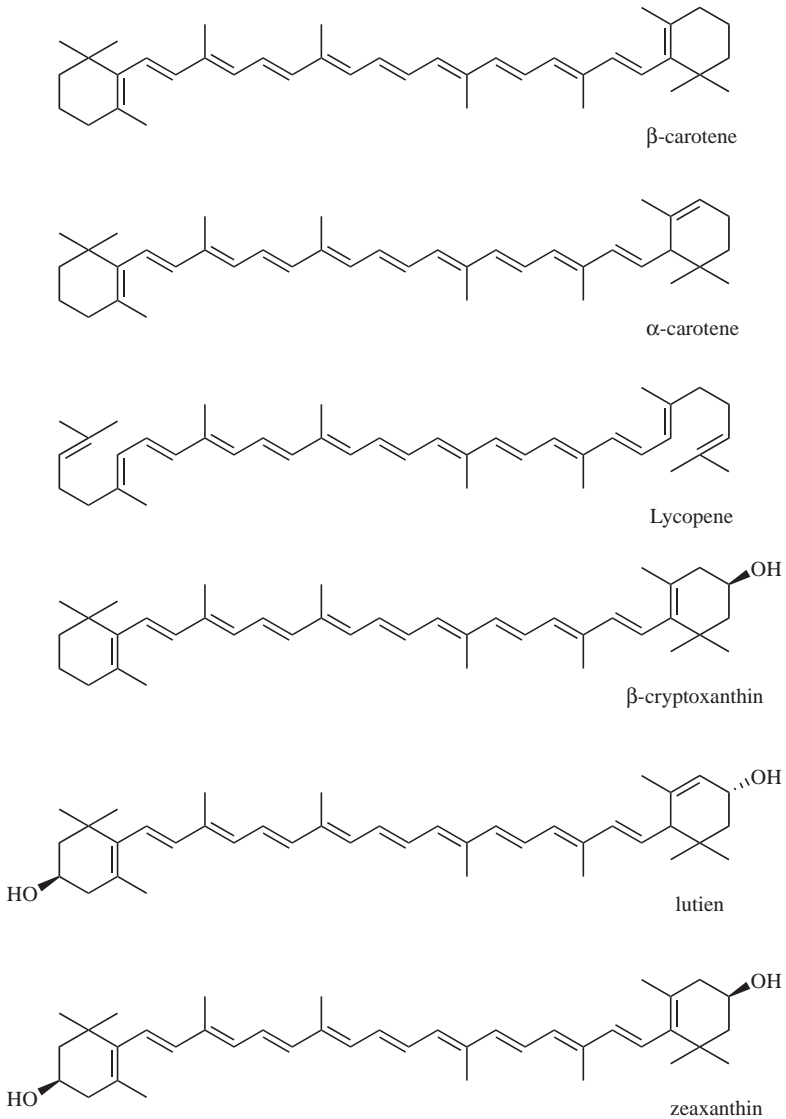
**Figure 6.** Structure of squalene (*all-trans*-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, CAS # 111-02-4), a major hydrocarbon in olive oil.

and linseed oils contain the special, tocotrienol-like, plastochromanol-8 (46–48). Cereal lipids, namely, wheat germ, maize, and rice bran oils, generally contain very high levels of tocopherols and tocotrienols. Of nut oils, almonds and hazlenut oils are rich in  $\alpha$ -tocopherol and pecans and walnuts are rich in  $\gamma$ -tocopherol (49).

### 2.3. Squalene, Carotenoids, and Other Hydrocarbons

Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,20-tetracosahexaene), also known as spinacene, is a naturally occurring 30-carbon chain triterpenoid hydrocarbon (Figure 6). Shark liver oil is the richest source of squalene, about 50% by weight (50, 51). Squalene is present at levels of 0.1–1.2% in olive oils together with low levels of other hydrocarbons (52). Squalene is found in smaller quantities, <30 mg/100 g, in other vegetable oils (53). Squalene is used in health foods, but it is hydrogenated to squalane for use, as a moisturizing agent, in cosmetics because of its high susceptibility to oxidation (54, 55). The double bonds of squalene undergo acid-catalyzed isomerization during vegetable oil refining to produce a large number of positional isomers (56). Other hydrocarbons representing a homologous series of saturated C15–C33 chains are also present in vegetable oils. For example, olive oil was reported to contain odd-numbered n-alkanes (C23–C29, 30–180 ppm), a series of C22–C27 n- $\Delta^9$ -alkenes, 8-heptadecene 6,10-dimethyl-1-undecene (0.5–2 ppm), as well as about 31 sesquiterpenes (2–37 ppm), the most abundant of which were  $\alpha$ -farnesene,  $\alpha$ -copaene, eremophyllene, and  $\alpha$ -muurolene (57).

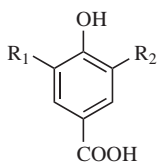
Carotenoids are the yellow-red pigments present in most crude oils, although their presence is often masked by the green color of chlorophyll. They are composed of a long-chain, 40-carbon skeleton, of eight isoprenoid units joined head-to-tail to give a completely conjugated system with alternated double bonds (Figure 7). Biosynthetically, carotenoids are derived from this acyclic long-chain conjugated C<sub>40</sub>H<sub>56</sub> structure of lycopene by hydrogenation, dehydrogenation, cyclization, and oxidation, or a combination of these processes (58). The carotenoids can be classified into (1) nonpolar unsaturated carotenes with the basic structure of lycopene, and (2) more polar carotenoids, or xanthophylls, with oxygen function at one or both end groups. The rules for semisystematic nomenclature of carotenoids have been published by the IUPAC-IUB Commission on Biochemical Nomenclature (59).



**Figure 7.** Structure of some major carotenoids present in vegetable oils, mainly red palm oil and olive oil.

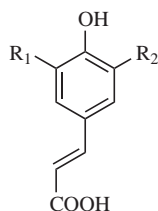
Red palm oil is the richest source of readily available carotenoids and is, therefore, very useful as a pro-Vitamin A supplement. Crude palm oil contains 500–800 ppm of carotenoids, of which  $\beta$ -carotene and  $\alpha$ -carotene account for about 90% (approximately 2:1 w/w), and lycopene, phytoene, and zeacarotenes are *inter alia* the remaining carotenoids (60). Other vegetable oils contain much lower levels of carotenoids (<100 ppm), but these are removed during the bleaching step in

refining. Olive oil contains variable, but low, levels of carotenoids mainly as  $\beta$ -carotene (6–17% of total pigments) and lutein (18–38% of total pigments), but xanthophylls such as neoxanthin, violaxanthin, luteoxanthin, antheraxanthin, mutatoxanthin, and  $\beta$ -cryptoxanthin also occur at low levels (61–63). Marine oils, *inter alia* cupelin and salmon oils, also contain large amounts of carotenoids, mainly xanthophylls (64).



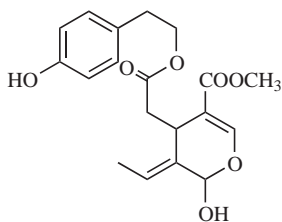
Hydroxybenzoic acid derivatives

*p*-hydroxy benzoic acid ( $R_1$  &  $R_2$  = H)  
 procatechuic acid ( $R_1$  =  $\text{OH}_3$ ,  $R_2$  = H)  
 vanilic acid ( $R_1$  =  $\text{OCH}_3$ ,  $R_2$  = H)  
 gallic acid ( $R_1$  =  $\text{OH}_3$ ,  $R_2$  = OH)  
 syringic acid ( $R_1$  =  $\text{OCH}_3$ ,  $R_2$  =  $\text{OCH}_3$ )

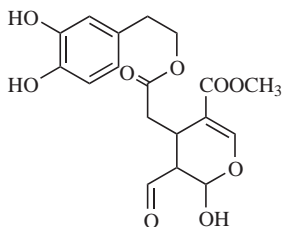


Hydroxycinnamic acid derivatives

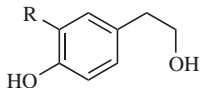
*p*-coumaric acid ( $R_1$  &  $R_2$  = H)  
 caffeic acid ( $R_1$  = OH,  $R_2$  = H)  
 ferulic acid ( $R_1$  =  $\text{OCH}_3$ ,  $R_2$  = H)



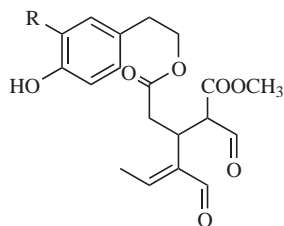
Ligstroside



Oleuropein isomer



*p*-hydroxyphenyl ethanol (*p*-HPEA,  $R$  = H)  
 3,4-dihydroxyphenyl ethanol (3,4-DHPEA,  
 $R$  = OH)



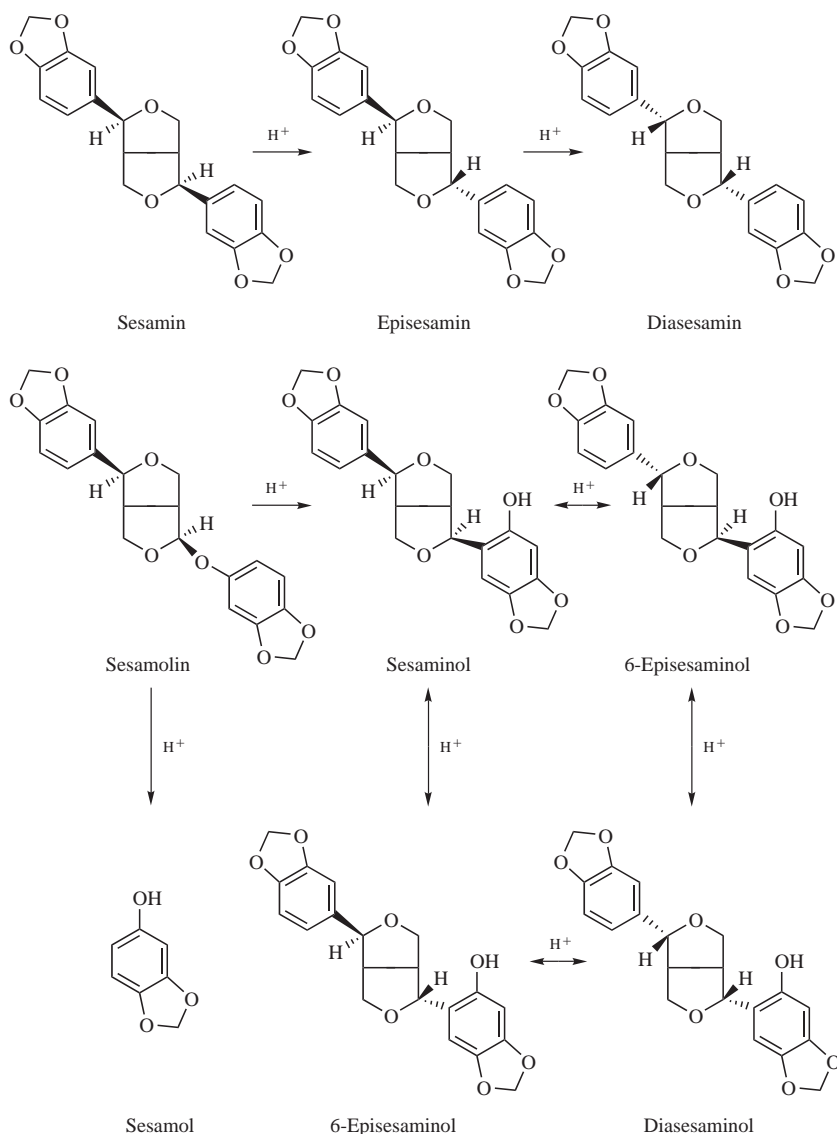
*p*-Hydroxyphenyl ethanol-  
 Eleanoldialdehyde (*p*-HPEA-EDA,  
 $R$  = H)

3,4-Dihydroxyphenyl ethanol-  
 Eleanoldialdehyde (3,4-DHPEA-EDA,  
 $R$  = OH)

**Figure 8.** Chemical structures of secoiridoid derivatives and phenolic alcohols in virgin olive oils.

## 2.4. Phenolic Compounds

Low levels of a wide range of phenolic compounds have been reported to be present in all vegetable oils, which is very important for the oxidative stability of the polyunsaturated fatty acids of these oils. The mostly studied phenolic constituents are perhaps those of olive oil (Figure 8). The level of phenolic compounds in olive oil



**Figure 9.** Sesame seed lignans (sesamin and sesaminol) and their acid-catalyzed transformation products generated during bleaching.

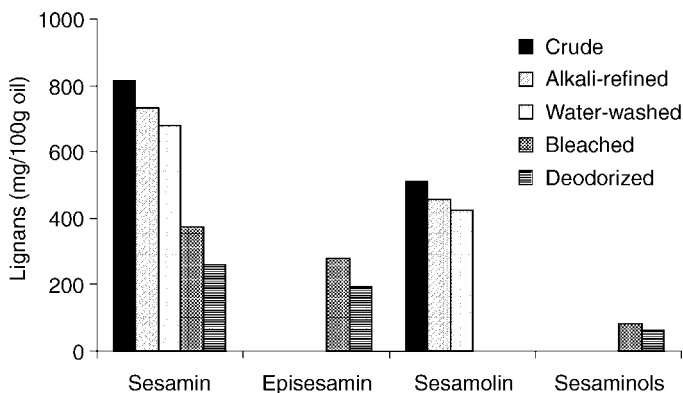


Figure 10. Changes in sesame oil lignans induced by refining [data from Fukuda et al. (75)].

was reported to vary between 50 and 1000 ppm depending on agronomic and environmental factors, ripeness, processing, and storage conditions of the oil (65), but they mostly lie in the range of 200–500 ppm (66). The phenolic compounds of olive oil include the secoiridoid aglycons of oleuropein and ligstroside and the lignans 1-acetoxypinoresinol and pinoresinol (67–72). Tyrosol, hydroxytyrosol, and their esters are also major phenols of olive oils (63) and their levels were found to increase during storage of virgin olive oil (73) because of acid-catalyzed hydrolysis of secoiridoid aglycons (69).

Crude sesame oil is characterized by the presence of two unique lignans: sesamin [2,6-(3,4-methylenedioxyphenyl)-cis-2,7-dioxabicyclo-[3.3.0]-octane] and sesamol [2-(3,4-methylene-dioxyphenoxy)-6-(3,4-methylenedioxyphenyl)-cis-2,7-dioxabicyclo-[3.3.0]-octane] with interesting physiological effects (Figure 9). Sesamin and sesamol are present in the range of 0.1–0.9% and tr.–0.7%, respectively (74). Fukuda et al. (75) showed that when sesame oil is refined, sesamin is partly isomerized into episesamin and sesamol is hydrolyzed to small amounts of sesamol but is mainly lost and isomerized into three sesaminol isomers (Figure 10). These transformations are catalyzed by the acidic residues in the bleaching earth, and they can be used *inter alia* to differentiate crude from refined sesame oils.

## 2.5. Other Minor Compounds

High aliphatic alcohols and wax esters in which aliphatic alcohols or sterols are esterified to fatty or phenolic acids are also present in crude vegetable oils at low levels and are partially removed in the winterization process during oil refining. Waxes, mainly esters of long-chain saturated fatty acids and a monounsaturated alcohol, especially eicosenoic alcohol, are found in crude vegetable oils such as olive, sunflower, soybean or peanut but are absent from corn or rice bran oils

(76, 77). Diterpenic, phytol, and geranylgeranyl esters were reported in olive and sunflower oils, but benzyl esters were only detected in olive oils (78). The wax esters of sunflower oil were found to have carbon atom numbers between 36 and 48, with a high concentration in the C-40–C-42 fraction (79). Waxes in dewaxed and refined sunflower oils mainly contained <42 carbon atoms indicating selective retention after refining.

Vegetable oils, especially in crude forms, contain variable levels of chlorophyll pigments. The maturity of the oilseed and the method of oil extraction determine the content of chlorophyll pigments in the oil. For example, unripe rapeseed contains chlorophyll A, chlorophyll B, pheophytin A, and pheophytin B as major pigments and pheophorbide A, methylpheophorbide A, and pyropheophytin A as minor pigments, whereas ripe seeds are characterized by chlorophylls A and B (80). Industrial extraction and refining of chlorophyll pigments shows selectivity in its effects on chlorophylls and pheophytins so that latter prevail in solvent extracted and bleached oils (80, 81). Pheophytin A is the major pigment in olive oil (44–58% of total pigments) that co-occur with small amounts of pheophytin B and sometimes chlorophylls A and B (61, 62).

Free fatty acids and partial glycerides (mono- and di-acylglycerols) occur in different levels in crude oils but are removed by refining from most oils, except brands of olive, sesame, and nut oils. Phospholipids, or phosphatides, are important minor lipid components because of their profound surfactant effects with wide applications in, *inter alia*, the baking industry. Lecithin (phosphatidyl choline) and other phospholipids (phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, phosphatidic acid, etc.) are important byproducts of vegetable oil refining, especially soybean oil. The presence of phospholipids and other polar lipids is, however, not desirable in refined vegetable oils. Currently, methods are being developed for removal of phospholipids from seed oil by physical refining (82), membrane filtration (83, 84), or enzymatic degumming by phospholipase (85, 86).

Cereal lipids are special because of their content of unusually high amounts of waxes, free fatty acids, unsaponifiable matter, sterol glucosides, diacylglycerols, phospholipids, and glycolipids (87, 88), which cause difficulties in the refining process. The main problem seems to be because of the high content of glycolipids and phosphorus-containing glycolipids (up to 5%), which cause high losses during alkali refining because of their high surface activity. A sample of crude wheat germ oil contained 1428-ppm phosphorus, 15.7% free fatty acids, and 2682-ppm tocopherols. The oil was refined by conventional degumming, neutralization, bleaching, and continuous tray deodorization, and the neutralization step was found to significantly remove the free fatty acids and most of the phospholipids (89). A special physical refining procedure based on simultaneous dewaxing and degumming was recently developed for rice bran oil (90).

Gourmet oils (e.g., olive oil, sesame oil, avocado oil, and nut oils) that are consumed as virgin oils usually have characteristic flavors that distinguish them from other edible vegetal oils. In olive oil, these compounds included, among a wide range of compounds, hexane, heptane, octane, ethanol, isobutanol, pentenol,

hexenol, octanol, pentanal, hexanal, 2-propenal, (E)-2-pentenal, (E)-2-hexenal, (E)-2-heptenal, octanal, (E)-2-octenal, nonanal, (E)-2-nonenal, isovaleraldehyde, decanal, (E)-2-hexen-1-ol, 2-propanone, 3-pentanone, acetic acid, formic acid, hexanoic acid, heptanoic acid, and limonene (91). Hazelnut oil is characterized by the strong flavor of 5-methyl-(E)-2-hepten-4-one (92). More than 30 volatile compounds, including pyrazines, thiazoles, pyridines, furfurals, and oxazoles, were identified as flavor compounds in sesame oils obtained from roasted seeds (93–96). Pyrazines are the major compounds and are responsible for the roasted flavor, and furfurals are responsible for the sweet candy-like flavor (97).

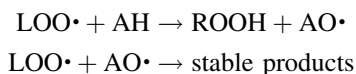
### 3. SIGNIFICANCE OF MINOR LIPID COMPONENTS

#### 3.1. Technical Quality of Vegetable Oils

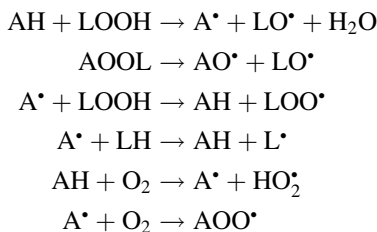
The unsaponifiable matter (e.g., sterols, tocopherols, lipophilic pigments, and hydrocarbons) generally can make up to 2.5% of vegetable oils. When free fatty acids are present at critically high concentrations, they may contribute an undesirable taste to oils that are used in crude forms, e.g., olive oil, sesame oil, and nut oils. This effect markedly results if free short-chain acids (C6–C10) are present in coconut, palm-kernel, and butter oils. Free fatty acids also negatively affect the foaming properties and stability attributes of these oils (98). As another example, olive oil polyphenols have been associated with the bitter taste of the oil but longer shelf life (99). On the other hand, minor lipids (such as diacylglycerols, monoacylglycerols, cholesterol, and phospholipids) are known to significantly affect the interfacial properties and crystallization of fats even at the low concentrations at which they are naturally present in most oils (100, 101). They were found to positively influence crystallization of cocoa butter by affording spherical crystals of regular shape (102).

Vegetable oils are variably susceptible to free radical autoxidation depending on the degree of unsaturation of their fatty acids and their contents of prooxidant and antioxidant species. The unsaponifiable fractions of wheat germ, corn, or olive oil are known to retard oxidation in vegetable oils (103). Trace amounts of metals and metalloproteins occurring in vegetable oils catalyze the oxidation by enhancing initiation and re-initiation by decomposing lipid hydroperoxides. The antioxidant activity of tocopherols is well documented, albeit considerable controversy in connection with the relative antioxidant potency of different tocopherols exists (39). The commencement of exponential oxidation of vegetable oils is associated with critical minimal concentrations of antioxidants (104). For example, a significant increase in the rate of oxidation was observed when the concentration of  $\alpha$ -tocopherol in olive oil reaches a “threshold value” of 60–70 mg/kg (105). The main reaction responsible for the antioxidant action of tocopherols is hydrogen atom donation (39). The antioxidant (AH) tocopherols and tocotrienols scavenge lipid peroxy radical (LOO•) to form a relatively stable lipid hydroperoxide (LOOH). Then the tocopheroxyl or tocotrienoxyl antioxidant radicals (A•), formed by this

reaction, scavenge another peroxy radical through radical-radical coupling to form a variety of stable secondary oxidation products.



Besides scavenging peroxy radicals, tocopherols and tocotrienols are also excellent quenchers for singlet oxygen and peroxy nitrile (39). It has been reported that  $\alpha$ -tocopherol shows a pro-oxidant activity when present at high concentrations (106–108). It was later found that tocopherols do not show a pro-oxidant effect if they are compared with controls devoid of them and other lipid-related antioxidants (109, 110). However, tocopherols lose their antioxidant efficacy at high concentrations because they participate in a number of reactions that lead to production of alkoxy radicals that consume tocopherols and initiate new reaction chains because of their low selectivity (111, 112). Using a kinetic approach, a number of reactions were found to contribute to the loss of tocopherol antioxidant activity that is greater for  $\alpha$ -tocopherol than for  $\gamma$ -tocopherol (113).



$\gamma$ -Tocopherol is generally a better antioxidant for vegetable oils than  $\alpha$ -tocopherol, both at low-temperature storage and under thermo-oxidation conditions (114). It was found that  $\gamma$ -tocopherol degrades at a much slower rate and, thereby, is able to protect the oil longer compared with  $\alpha$ -tocopherol (110, 111).

Photo-oxidation plays a significant role in the oxidation of oils rich in chlorophyll and other photosensitizers. However, tocopherols, carotenoids, and polyphenols present in these oils provide considerable protection against light-induced photo-oxidation. These different antioxidants work synergistically and have different protection abilities. The best example for this situation is olive oil (115). Pheophytin A and  $\beta$ -carotene were found to be sensitive to oxygen, light, and temperature, and  $\alpha$ -tocopherol and polyphenols were more stable toward high temperatures. The chlorophyll pigments function as photosensitizers and  $\beta$ -carotene as an efficient quencher both for the excited chlorophylls and singlet oxygen (116). However,  $\alpha$ -tocopherol and polyphenols are needed to protect  $\beta$ -carotene against destruction caused by heat and excessive oxygen (117–119). During photo-oxidation, squalene was found to protect  $\alpha$ -tocopherol (120).

Paradoxically, chlorophyll was found to synergize the antioxidant effect of  $\alpha$ -tocopherol in the dark (121), but chlorophyll degradation was found to contribute



negatively to the stability of vegetable oils (122–127). High chlorophyll levels in rapeseed oil were also found to decrease oil stability (126) and to increase the rate of tocopherol decomposition and formation of polymers when the oil was heated at 180°C (114). Chlorophyll pigments can be removed from vegetable oil *inter alia* by bleaching using montmorillonite (128, 129) or membrane processing (130). Besides affecting the oxidative stability of vegetable oils, chlorophyll pigments also affect their bleachability during refining and add to refining costs (81).

$\beta$ -Carotene and other carotenoids are known to possess antioxidant activity under low temperatures and oxygen pressure conditions (131). The main mechanism by which carotenoids exert their antioxidant effects is by quenching reactive singlet oxygen and excited photosensitizers (132–143). The second mechanism is by scavenging peroxy and other reactive free radicals (131, 144–150). Exposure of  $\beta$ -carotene to free radical initiators caused the chain reactions to differ from the zero-order reaction kinetics characterizing the induction period of autoxidation reactions (151–154). Although the mechanisms for singlet oxygen quenching by the carotenoids are fairly well understood and defined, mechanisms for their reactions with free radicals are not yet clarified. These reactions seem to be influenced in a very complex manner by a number of factors such as oxygen tension, temperature, and presence of enzymes, among others (153–157). High concentrations of  $\beta$ -carotene were reported to lack antioxidant activity and to cause an increase in peroxide levels in rat plasma and liver (158) and to increase the thiobarbituric acid reactive substances (TBARS) and 15-lipoxygenase activity in rat testis (159).  $\beta$ -Carotene was also found to act as a prooxidant promoting lipid peroxidation in some systems under high oxygen partial pressure (131, 147, 155). The high reactivity of carotenoids with oxygen, especially at high temperatures, poses a great limitation on their use as antioxidants. However, when tocopherols are present or scavenge radical species formed from the reaction of carotenoids with oxygen, a significant synergistic antioxidant effect can be achieved (143, 160–162).

Several lipid minor components are known to synergize the protective effect of tocopherols and protect the unsaturated fatty acids from being rancid. These include phospholipids (163) and unsaponifiable components such as sterols, carotenoids, and squalene (114, 164–166). The phenolic compounds of olive oil have particularly received much attention with respect to their effects on the oxidative stability of the oil (63, 72, 117, 167–171). A study based on virgin olive oil, from Cornicabra variety obtained from three successive crop seasons (94/95 to 96/97), showed a clear influence of total polyphenols on the oil stability and a much lower contribution of  $\alpha$ -tocopherol (172). Univariate analysis revealed a significant correlation between Rancimat<sup>®</sup> stability and the following compositional parameters: phenols ( $R = 0.87$ ), *ortho*-diphenols ( $R = 0.77$ ), oleic/linoleic ratio ( $R = 0.71$ ), total tocopherols ( $R = 0.65$ ), chlorophylls ( $R = 0.68$ ), and carotenoids ( $R = 0.59$ ) (165). When a stepwise linear regression analysis was employed, a high multicollinearity was found for total phenols, oleic/linoleic ratio, and tocopherols as having together the maximum correlation with stability. The regression equation for adjusted- $R^2 = 0.91$  was  $-18.52 + 0.13 \cdot \text{total phenols} + 3.03 \cdot \text{oleic/linoleic ratio} + 0.07 \cdot \text{total tocopherols}$  (165).

### 3.2. Nutritional Value of Minor Substances in Vegetable Oils

During the early 1950s, it was reported that phytosterols lower serum cholesterol (173–175). This effect was appreciated as a possible protection strategy against cardiovascular disease risk after the results of several convincing animal and human studies (176–184). Studies have shown that a daily intake of 2-g phytosterol or phytostanol causes 40–50% reduction in the dietary cholesterol absorption, 6–10% reduction in total serum cholesterol, and 8–14% reduction in the serum low-density lipoprotein cholesterol (37, 185–187).

There is some evidence that  $\gamma$ -oryzanol present in rice bran oil lowers serum total- and LDL cholesterol as well (16, 185, 188). Butter from the Shea tree, *Butyrospermum parkii*, contains a very high level of 4,4-dimethylsterols (about 8%) mostly as esters of cinnamic acid (oryzanols). Apparent absorption of these sterols, as estimated by their disappearance from feces, was found to be 27–52% in Wistar rat and 13–49% in humans (189). It was found that the cinnamic acid esters of the 4-desmethylsterols of rice bran oils, but not those of the 4,4'-dimethylsterols of shea nut oil possess hypolipidemic activity (190).

The mechanism for the inhibition of cholesterol absorption is thought to involve competitive transfer to the micellar phase during absorption from the intestinal lumen. Phytosterols in the micellar phase may also act as emulsifying agents that selectively inhibit the transfer of cholesterol and other lipids (e.g., carotenoids and vitamins) and, thereby, limit their absorption. The exact kinetics governing the sterol competition for transfer are not known, but dietary sterols are absorbed differently in the digestive tract: 40–50% for cholesterol, 12–16% for campesterol, 4–5% sitosterol, and <0.5% for phytosterols (37). Before absorption, esterified sterols are hydrolysed effectively in the upper intestine (191). Absorbed phytosterols are excreted by the liver into the bile but are hardly converted to bile acids (192). Numerous studies in animals and humans approved the safety of phytosterols and phytosterols (37).

Tocopherols are well known for vitamin E activity since the early work of Evans and Bishop (193). Vitamin E compounds (tocopherols and tocotrienols) are well known for their strong inhibitory effects against lipid oxidation in foods and biological systems (41, 194–205). As Vitamin E is only synthesized by plants, humans and animals have to satisfy their nutritional needs by eating plant sources rich in this vitamin (206). The richest sources of tocopherols are by far vegetable oils followed by other sources such as nuts, cereal grains, green vegetables, and milk, among others (207–209).

A number of epidemiological studies suggest that Vitamin E has a moderate protective effect against the progression of cardiovascular diseases (210–215). The antioxidant hypothesis suggests that the inhibition of low-density lipoprotein oxidation is the main mechanism by which Vitamin E exerts this protective effect (187, 216) by scavenging the chain-carrying lipid peroxy radicals (217–220). Other biochemical mechanisms, distinctive of Vitamin E's antioxidant properties, have also been proposed to explain the inhibitory effects on cardiovascular disease. One hypothesis stated that larger dosages of Vitamin E (>400 IU/day) inhibit platelet

adherence to proteins such as fibrinogen, fibronectin, and collagen (221). Another hypothesis states that Vitamin E controls smooth muscle cell proliferation by acting as a sensor and information transducer of the cell's oxidation state (222). At optimum physiological concentrations,  $\alpha$ -tocopherol is thought to bind to a "receptor protein" resulting in the activation of transcription factor AP-1, which leads to the expression of a protein phosphatase that dephosphorylates protein kinase C, forcing its own inhibition and resulting in the inhibition of smooth muscle cell proliferation. In a highly oxidative environments,  $\alpha$ -tocopherol diminishes because of its action as a radical scavenger, and as a result of this diminution, less  $\alpha$ -tocopherol binds to its "receptor protein" resulting in a greater activity of protein kinase C and, thereby, a higher amount of smooth muscle cell proliferation (222).  $\alpha$ -Tocopherol was also reported to inhibit the secretion of interleukin-1 $\beta$  and the adhesion monocyte to endothelium (223).

Currently, much attention is paid to  $\gamma$ -tocopherol, the major tocopherol supplied by most vegetable oils. The amount of  $\gamma$ -tocopherol in the diet of most humans is approximately twice that of  $\alpha$ -tocopherol (224), but its plasma levels are only about 10–20% of those of  $\alpha$ -tocopherol (225). This difference was explained by biodiscrimination by a specific tocopherol-binding protein with special preference for the  $\alpha$ -homologue (226). Upon feeding equal amounts of the two vitamers to experimental rats, it was found that  $\alpha$ -tocopherol is preferentially bound to the cellular membranes of the liver and to the transporting proteins while  $\gamma$ -tocopherol is excreted through the bile because of very low incorporation into the transporting protein (227).  $\gamma$ -Tocopherol may have some specific biological activities that may complement those of  $\alpha$ -tocopherol (228). For example,  $\gamma$ -tocopherol was found to inhibit nitrogen dioxide ( $\cdot\text{NO}_2$ )-mediated nitrosation of morpholine much more significantly than  $\alpha$ -tocopherol (229–231). Nitrogen dioxide, one major constituent of cigarette smoke, is a mutagenic substance reactive toward lipids, DNA, and other biological molecules (232). Relevant to this perhaps is the finding that smoking, which is a recognized risk factor for cancer and cardiovascular disease, causes a decrease in  $\gamma$ -tocopherol levels in LDL and HDL particles (233). Recently, 5-nitro- $\gamma$ -tocopherol was found to increase in Alzheimer brain, which suggests a major role of  $\gamma$ -tocopherol as a scavenger of nitrogen reactive species (234). A study from Loma Linda University showed that a human endogenous natriuretic factor, LLU- $\alpha$ , possibly a product from the *in vivo* oxidative metabolism of  $\gamma$ -tocopherol, inhibited the 70-ps  $\text{K}^+$  channel in the apical membrane of the thick ascending limb of the kidney (235). The effect of this natriuretic factor on the control of the body pool of extracellular fluids, and its implications on hypertension, congestive heart failure, and cirrhosis remains to be investigated. Swedish patients suffering from coronary heart disease were found to have significantly lower serum  $\gamma$ -tocopherol concentrations compared with healthy controls, and  $\alpha$ -tocopherol levels were not significantly different (236). Similar findings were reported by other authors, which suggests a possible role of  $\gamma$ -tocopherol as a marker of atherosclerosis (237–239).

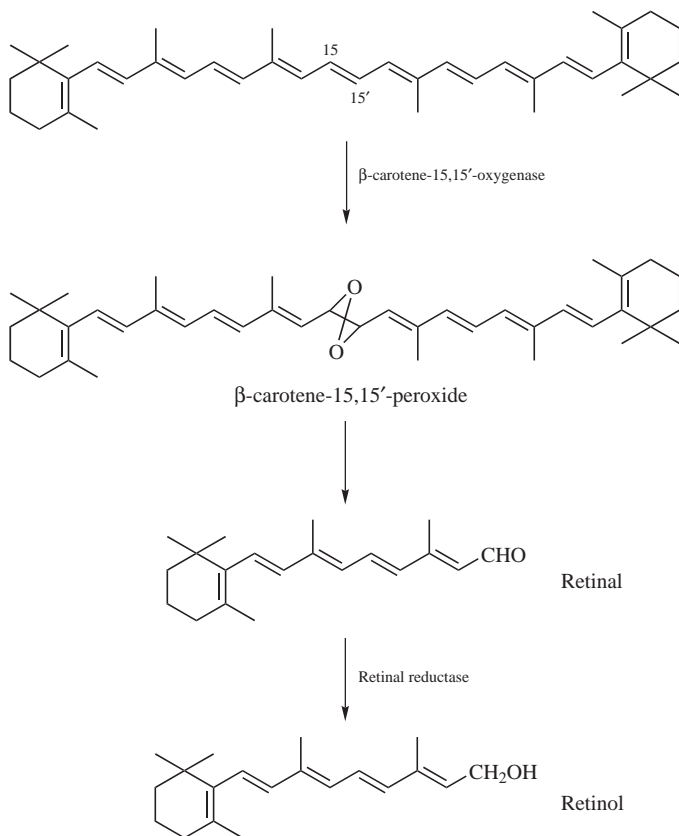
Many studies suggest that  $\alpha$ - and  $\gamma$ -tocopherols influence each other's post-absorption transport and metabolism, and some studies suggested that  $\gamma$ -tocopherol

might transform into  $\alpha$ -tocopherol *in vivo*. For example, supplementation with all racemic- $\alpha$ -tocopheryl acetate was found to cause a significant decrease in  $\gamma$ -tocopherol levels and a significant increase in the  $\alpha$ -/ $\gamma$ -tocopherol ratio in human tissues and adipose tissue. This effect was found to disappear when the supplementation was stopped (240, 241). On the contrary,  $\gamma$ -tocopherol supplements were shown to induce a marked increase in  $\alpha$ -tocopherol concentrations in serum and tissues of Vitamin E-deficient rats (242). A national health survey of 18,000 Germans, age 25–69 years, showed that  $\gamma$ -tocopherol levels were greatly reduced in persons taking supplements containing >50-mg  $\alpha$ -tocopherol/day (243). Based on the results, these authors pointed out that mixed tocopherols should be considered when tocopherol supplemental medication is considered necessary. When  $\gamma$ -tocopherol was supplied continuously in the diet, it accumulated significantly in rat tissues but to a much lesser extent than  $\alpha$ -tocopherol (244). Furthermore,  $\gamma$ -tocopherol was found to accumulate in the plasma in relatively very high concentrations in cases of  $\alpha$ -tocopherol deficiency (245, 246). The studies by Elmadfa et al. (247) show that rats fed high doses of  $\gamma$ -tocopherol were able to survive for four generations because of an *in vivo* conversion of  $\gamma$ -tocopherol to  $\alpha$ -tocopherol. Yamashita et al. (248, 249) showed that a combination of  $\gamma$ -tocopherol and sesamin is able to produce Vitamin E activity equivalent to that of  $\alpha$ -tocopherol in rats.

Together with  $\gamma$ -oryzanol, tocotrienols are responsible for the cholesterol-lowering effect of rice bran oil (250). Tocotrienol concentrates have been shown to have a hypocholesterolemic effect in animals and humans (251–257) possibly because of inhibition of hepatic 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) (251, 258, 259). The presence of  $\alpha$ -tocopherol at concentrations >20% in tocotrienol concentrates was, however, found to attenuate the inhibitory effect of tocotrienols on HMG-CoA reductase, thereby weakening their cholesterol-lowering activities (254). Tocotrienols were especially found to synergize the cholesterol-lowering effect of lovastatin (254). In addition, tocotrienols have been shown to influence certain hemostatic parameters and to reduce the occurrence of chemical-induced tumors in the rat (253).

Carotenoids have long been known as food colorants and as precursors of the Vitamin A retinoids in animals (260, 261). Red palm oil is the richest source of biologically available carotenes, which are efficient in restoring Vitamin A activity in malnourished preschool children (262). The importance of carotenoids and retinoids for vision is well documented (263, 264). The carotenoids are now receiving considerable interest because they are believed to have potential in delaying or preventing degenerative diseases such as atherosclerosis (146, 265, 266) and some types of cancer (266–271), in modulating immune response (272–274) and in providing other beneficial effects such as ulcer inhibition and life extension (275–277).

$\beta$ -Carotene and other hydrocarbon carotenoids are poorly-to-moderately absorbed from the gastrointestinal tract (5–50%), but highly polar carotenoids are poorly absorbed (278). Ingested pro-Vitamin A carotenoids ( $\beta$ -carotene and other carotenoids containing  $\beta$ - or 3-retinylidene end groups) are partly cleaved in the intestinal mucosa to retinoids through the oxidative cleavage of the C-15, C-15' double bond, mainly under the action of the  $\beta$ -carotene-15, 15'-oxygenase



**Figure 11.** Conversion of  $\beta$ -carotene to Vitamin A retinoids.

enzyme (279, 280). The process of carotenoids cleavage to retinol (Figure 11) is relatively inefficient and self-limiting because the conversion rate decreases with increased body Vitamin A status. Originally only about 6% of all carotenoids, those with  $\beta$ -retinylidene end groups, were considered as Vitamin A precursors (provitamin A).

The metabolism and pharmacokinetics of the carotenoids are not well understood, and their bioavailability is associated with much interindividual and intra-individual variation (281).  $\beta$ -Carotene,  $\alpha$ -carotene, cryptoxanthin, lycopene, and lutein are the major carotenoids in human serum, and lycopene and  $\beta$ -carotene are the major carotenoids in other human tissues (282). Small amounts of zeaxanthin, phytofluene, and phytoene are also found in various organs. Various carotenoids tend to be present in similar ratios in human plasma and tissues (282, 283). The carotenoids are safe and even long-term intake of 180 mg of  $\beta$ -carotene per day did not lead to hypervitaminosis. When large amounts of carotenoids are stored in the adipose and other lipid-rich tissues, they may cause reversible yellowing of the

skin (carotenemia). Carotenemia is a benign condition, and Vitamin A poisoning does not occur despite massive doses of carotene because the conversion of carotene to Vitamin A is slow (284).

Recently, olive oil polyphenols have gained much more attention because of their potential beneficial health effects (65, 285–287). For example, olive oil was found to improve lipid metabolism and antioxidant protection in rats fed cholesterol-rich diets (288, 289). Hydroxytyrosol was especially effective in lowering the levels of hydroperoxides, DNA damage, and mRNA levels of the antioxidant enzyme, glutathione peroxidase (290).

The lignans of sesame oil, exemplified in sesamin, are especially interesting from a physiological point of view. Yamashita et al. (291) published a note suggesting that some antioxidants, other than tocopherols, in sesame exert some suppressive effect on the manifestation of senescence in mice. Sesamin was then found to lower cholesterol absorption as well as to inhibit its synthesis by downregulating the activity of 3-hydroxy-3-methyl-glutaryl-CoA reductase, the key enzyme in the biosynthetic pathway (292). Yamashita et al. (248) showed that sesamin caused a significant increase in  $\gamma$ -tocopherol levels in the plasma and liver, an effect that was later confirmed (293, 294). It was recently found that sesamin competitively inhibits certain cytochrome P450 3A isozymes that are involved in  $\gamma$ -tocopherol metabolism (295–297). In addition, sesamin was found to inhibit  $\Delta^5$ -desaturation of n-6 PUFA in rats resulting in accumulation of dihomo- $\gamma$ -linolenic acid at the expense of arachidonic acid (298, 299), consequently decreasing the formation of proinflammatory 2-series prostaglandins (300). This effect might be associated with effects on sterol-binding proteins involved in fatty acid biosynthesis (301). The kinetics of sesamin and episesamin metabolism has shown that they exhibit a peak concentration value in plasma and most tissues by 6 hours after administration and that they are cleared from the body by 24 hours (302). It is worthwhile studying whether the reported physiological effects of sesame lignans can be translated into measurable health benefits in humans.

### 3.3. Authentication of Vegetable Oils

Expensive oils, such as olive oil and cocoa butter, are sometimes mixed with other cheaper oils to provide oils of reasonable price (303–307). Methods for authentication of oil blends and characterization of constituent oils are, therefore, important from an economical point of view (308). The few fatty acyl moieties involved as major lipid components of most oils provide limited structural identity for most vegetable oils especially when mixed together. Thus, lipid scientists were primarily interested to use the knowledge about the minor lipid components as a way to authenticate vegetable oils, to detect adulteration, and to identify the effects of refining on their composition (309, 310). This mission can ideally be achieved by general and specific authentication protocols taking into consideration the combination of characteristics with respect to fatty acid composition, triacylglycerol types, and unsaponifiable constituents. However, these theoretically possible protocols are complex and costly in comparison with alternative protocols based on the

selection of a minimum number of markers. However, caution should always be exercised as the minor markers of vegetable oils can still be removed by refining and might not be detectable in the blends.

The analysis of sterols and sterol esters has been proposed as one way to identify oils in blends (311, 312). Johansson and Croon (313) discussed the use of 4-desmethyl-, 4-monomethyl-, and 4,4-dimethylsterols in characterizing different vegetable oils, and the results are summarized in Table (8). The levels of total sterols and sterol classes as well as the relative distribution of the individual sterol members vary between oils. The presence of steradienes can also be used as a marker for the presence of refined oils (314, 315). High oleic acid oils can easily be used to adulterate olive oil. The presence of rapeseed oil in other oils can be detected by the analysis of brassicasterol and its dehydration product, campestatriene. The presence

**TABLE 8. The Use of Sterol Composition for the Authentication of Vegetable Oils (% in Respective Fraction).**

Vegetable Oil	Desmethyl Sterols	4-Methyl Sterols	4,4-Dimethyl Sterols
Coconut	Total 102 mg/100 g campesterol (8%) stigmasterol (13%) sitosterol (47%) <b><math>\Delta^5</math>-avenasterol (26%)</b>	Total 7 mg/100 g obtusifoliol (9%) <b>cycloeucalenol (36%)</b> citrostadienol (33%)	Total 20 mg/100 g $\alpha$ -amyirin (7%) $\beta$ -amyirin (5%) cycloartenol (55%) 24-methylene- cycloartanol (22%)
Cottonseed	Total 510 mg/100 g campesterol (7%) <b>sitosterol (86%)</b>	Total 12 mg/100 g obtusifoliol (8%) gramisterol (11%) cycloeucalenol (5%) citrostadienol (42%)	Total 17 mg/100 g $\beta$ -amyirin (7%) cycloartenol (12%) 24-methylene- cycloartanol (21%)
Linseed	Total 471 mg/100 g campesterol (27%) stigmasterol (8%) sitosterol (42%) <b><math>\Delta^5</math>-avenasterol (13%)</b>	Total 39 mg/100 g obtusifoliol (35%) gramisterol (16%) cycloeucalenol (10%) citrostadienol (11%)	Total 246 mg/100 g <b>cycloartenol (66%)</b> 24-methylene- cycloartanol (27%)
Olive	Total 150 mg/100 g <b>sitosterol (82%)</b>	Total 68 mg/100 g cycloeucalenol (14%) citrostadienol (22%)	<b>Total 292 mg/100 g</b> cycloartenol (18%) 24-methylene- cycloartanol (31%) <b>cyclobranol (10%)</b>
Palm kernel	Total 140 mg/100 g campesterol (10%) stigmasterol (13%) sitosterol (69%) $\Delta^5$ -avenasterol (7%) <b><math>\Delta^7</math>-avenasterol absent</b>	Total 3 mg/100 g obtusifoliol (9%) cycloeucalenol (33%) citrostadienol (19%)	Total 22 mg/100 g <b>cycloartenol (80%)</b> 24-methylene- cycloartanol (18%)
Peanut	Total 321 mg/100 g campesterol (10%) stigmasterol (7%) sitosterol (69%) $\Delta^5$ -avenasterol (5%)	Total 18 mg/100 g obtusifoliol (19%) gramisterol (16%) cycloeucalenol (11%) citrostadienol (23%)	Total 17 mg/100 g $\beta$ -amyirin (7%) $\beta$ -amyirin (7%) cycloartenol (30%) 24-methylene- cycloartanol (35%)

TABLE 8 (Continued)

Vegetable Oil	Desmethyl Sterols	4-Methyl Sterols	4,4-Dimethyl Sterols
Rapeseed	Total 954 mg/100 g <b>brassicasterol (10%)</b> <i>campesterol (33%)</i> sitosterol (48%)	Total 7 mg/100 g obtusifoliol (26%) gramisterol (21%) cycloeucalenol (17%) citrostadienol (16%)	Total 18 mg/100 g cycloartenol (49%) 24-methylene- cycloartanol (37%)
Sesame	Total 331 mg/100 g campesterol (19%) stigmasterol (6%) sitosterol (57%) $\Delta^5$ -avenasterol (6%)	Total 47 mg/100 g obtusifoliol (17%) gramisterol (15%) cycloeucalenol (12%) citrostadienol (15%)	Total 20 mg/100 g $\alpha$ -amyrin (6%) cycloartenol (51%) 24-methylene- cycloartanol (16%) <b>cyclobranol (5%)</b>
Soybean	Total 394 mg/100 g campesterol (20%) <b>stigmasterol (18%)</b> sitosterol (51%)	Total 25 mg/100 g obtusifoliol (6%) gramisterol (9%) cycloeucalenol (10%) <b>citrostadienol (44%)</b>	Total 40 mg/100 g $\alpha$ -amyrin (5%) $\beta$ -amyrin (9%) cycloartenol (26%) 24-methylene- cycloartanol (13%)
Sunflower	Total 494 mg/100 g campesterol (7%) stigmasterol (7%) sitosterol (59%) $\Delta^5$ -avenasterol (8%)  $\Delta^7$ - <i>stigmasterol (6%)</i> $\Delta^7$ -avenasterol (5%)	Total 78 mg/100 g obtusifoliol (26%) gramisterol (15%) citrostadienol (38%)	Total 33 mg/100 g $\alpha$ -amyrin (11%) $\beta$ -amyrin (5%) cycloartenol (19%) <b>24-methylene- cycloartanol (48%)</b>
Maize	Total 1441 mg/100 g campesterol (17%) stigmasterol (6%) sitosterol (60%) $\Delta^5$ -avenasterol (10%)	Total 62 mg/100 g obtusifoliol (21%) <i>gramisterol (26%)</i> cycloeucalenol (6%) citrostadienol (29%)	Total 54 mg/100 g $\alpha$ -amyrin (7%) $\beta$ -amyrin (12%) cycloartenol (43%) 24-methylene- cycloartanol (40%)
Wheat germ	Total 1425 mg/100 g campesterol (19%) sitosterol (60%) $\Delta^5$ -avenasterol (7%)	Total 59 mg/100 g obtusifoliol (14%) gramisterol (25%) cycloeucalenol (6%) citrostadienol (30%)	Total 59 mg/100 g $\alpha$ -amyrin (7%) $\beta$ -amyrin (12%) cycloartenol (25%) 24-methylene- cycloartanol (33%)
Rice bran	Total 1055 mg/Kg campesterol (24%) stigmasterol (11%) sitosterol (52%) $\Delta^5$ -avenasterol (8%)	3 main unknown compounds in addition to obtusifoliol and gramisterol.	cycloartenol and 24-methylene- cycloartanol present as $\gamma$ - <b>oryzanol</b> (see text).

of high oleic sunflower oil can be detected by analyzing  $\Delta 8(14)$  and  $\Delta 14$ -sterols, which result from the isomerization of  $\Delta^7$ -stigmasterol. Hazelnut oil in olive oil can be detected by analyzing its biomarkers: filbertone (E-5-methylhept-2-en-4-one) and  $\delta$ -tocopherol (306, 316, 317). Sesame oil can be detected in blends by the Bauhin test of its characteristic phenol, sesamol (318).



Squalene was suggested as a biomarker for olive oil, but its use as such was difficult because of the high variability for its occurrence in the oil. Moreover, squalene isolated from rich oil sources can be added to oils to make them more olive-like. For this reason, it was a mandate to add anthranilic acid as a marker for isolated squalene (306). A combination of carotenoid and chlorophyll composition may also be used for the authentication of virgin olive oil. The presence of carotenoid and chlorophyll degradation products can be used as an index for “virgin” olive oil quality. Virgin oil is characterized by a ratio of chlorophyll/carotenoid  $\approx 1$  and a ratio of minor carotenoids/lutein  $\approx 0.5$  (319). The fact that markers might also be present in the adulterant, although in a different ratio, necessitate the use of sophisticated mathematics, e.g., pattern recognition analysis (320).

#### 4. ANALYSIS OF MINOR LIPID COMPONENTS

The principle for the saponification of fats and oils is based on the reaction of the fatty acid moieties of the oil by boiling under reflux with an ethanolic potassium hydroxide solution. Saponification transforms the glyceridic compounds into polar soaps making possible the extraction of the unsaponifiable matter with hexane or diethyl ether. The solvent is evaporated, and then the residue is dried and weighed. Schwartz (3) reported that dry homogenization of oils and alkali in a mortar provides an improved method for obtaining and quantifying the unsaponifiable matter of fats and oils. Saponification is not applicable for glyceridic lipids, such as wax esters, sterol esters, minor glyceridic compounds, and phospholipids, and it is not suitable for the analysis of alkali-sensitive phenolic compounds and pigments. Historically, the content of sterol in vegetable oils was determined gravimetrically after precipitation of its complexes with digitonin (321).

Gas chromatographic analyses for total aliphatic alcohols, sterols, and tocopherols in vegetable oils are usually performed, with or without silylation, using columns with low-polarity or polar stationary phases (322) and flame ionization detectors. Silylation is often preferred because it provides sharp peaks. Analyses of compound classes are sometimes performed separately after fractionation of the respective classes by thin-layer or column chromatography (305). This fractionation is, however, often not necessary, and many unsaponifiable constituents can be quantified directly by gas chromatography (GC) of the unsaponifiable fraction after silylation (323, 324). Thus, a gas chromatogram of the unsaponifiable fractions from different oils can present a fingerprint for oil identity. Besides gas chromatography and gas chromatography–mass spectrometry, methods such as Fourier Transform Raman spectra can be used for the interpretation of these differences (325).

When sterol analysis is performed after saponification of ester bonds, a total value is obtained for the level of each sterol by GC analysis. Free sterols can be separated from sterol esters before saponification by means of thin-layer chromatography (326) or solid phase extraction (327–330) using hexane to elute the sterol esters and diethyl ether or a mixture of diethyl ether and hexane to elute the sterols. Once separated, sterol esters need be saponified to release sterols before gas

chromatography. Sterols and other compounds containing hydroxy or phenolic functional groups are generally converted to silyl, or sometimes acetyl, derivatives to increase their volatility to obtain sharp chromatographic peaks.

Tocopherols and tocotrienols can be analyzed by GC as well as by high-performance liquid chromatography (331). For GC analysis, the elimination of interfering substances, mainly acyl lipids, by saponification is necessary. This preparation step is not needed in HPLC analysis where diluted oil samples can be injected directly into normal- or reversed-phase columns. Normal-column HPLC is preferred because it is able to separate the  $\beta$ - and  $\gamma$ -tocopherol and tocotrienol isomeric pairs, not separable by reversed-phase HPLC, and because it operates with organic solvents and tolerates high loads of lipids that are easily washed-out by nonpolar solvents. Recent developments in the manufacture of normal-phase columns enables reproducible separations (331). Tocopherol/tocotrienol peaks are detected with ultraviolet (290 nm) and evaporative light scattering detectors, but fluorescence and electrochemical detectors provide higher sensitivity and specificity (331, 332).

Carotenoids can be coanalyzed with tocopherols and other fat-soluble vitamins by HPLC using normal-phase (333), or reversed-phase C18 (334) or C30 columns (335) using multiple-channel Ultraviolet or diode array detectors to monitor carotenoids and tocopherols at 295 nm and 450 nm, respectively. Normal-phase chromatography provides the advantage of eliminating the need for saponification to remove nonpolar lipids and ensures long column life. Reversed-phase chromatography columns are affected by the lipid load but have the advantage of providing excellent separation for different carotenoids and their isomers. The C30 reversed-phase columns are currently several times more expensive than C18 and normal-phase columns. The usual methods for determination of chlorophylls in vegetable oils are absorption spectrophotometry, fluorometry, and liquid chromatography using fluorescence detectors (336). Chlorophyll pigments can also be separated and analyzed by HPLC using Ultraviolet-visible detection (337, 338).

Pre-extraction using normal solid-phase extraction columns to remove the neutral lipids by nonpolar solvents followed by extraction with polar solvents (methanol, acetonitrile, etc.) is suitable for the isolation of phenols, sterols, polycyclic aromatic hydrocarbons, and chlorophylls from vegetable oils (52, 339–341). The extracts can then be analyzed by an HPLC-diode array detector to separate individual compounds (342). Using this methodology, the main phenolic compounds in virgin olive oil were identified as a dialdehydic form of elenolic acid linked to tyrosol and dialdehydic form of elenolic acid linked to hydroxytyrosol as well as oleuropein aglycone. Detectors other than diode-array, e.g., electrochemical coularray, can also be used (70, 343).

## REFERENCES

1. S. Ghosh and D. K. Bhattacharyya, *J. Amer. Oil Chem. Soc.*, **73**, 1271–1274 (1996).
2. M. E. M. Almeida, E. C. Gusman, P. R. N. Carvalho, and O. Rusig, *Arq. Biol. Technol.*, **37**, 1003–1011 (1994).

3. D. P. Schwartz, *J. Amer. Oil Chem. Soc.*, **62**, 246–251 (1988).
4. B. S. Narasinga-Rao, *Food Nutr. Bull.*, **22**, 81–86 (2001).
5. T. Gutfinger and A. Letan, *J. Sci. Food. Agric.*, **25**, 1143–1147 (1974).
6. A. Johansson, *J. Amer. Oil Chem. Soc.*, **56**, 886–889 (1979).
7. A. Lanzon, T. Albi, A. Cert, and J. Gracian, *J. Amer. Oil Chem. Soc.*, **71**, 285–291 (1994).
8. IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN), *Eur. J. Biochem.*, **186**, 429–458 (1989). Available: <http://www.chem.qmw.ac.uk/iupac/steroid>.
9. T. Itoh, T. Tamura, and T. Matsumoto, *J. Amer. Oil Chem. Soc.*, **50**, 122–125 (1973).
10. T. Itoh, T. Tamura, and T. Matsumoto, *J. Amer. Oil Chem. Soc.*, **50**, 300–303 (1973).
11. T. M. Jeong, T. Itoh, T. Tamura, and T. Matsumoto, *Lipids*, **9**, 921–927 (1974).
12. V. K. S. Shukla, P. C. Dutta, and W. E. Artz, *J. Amer. Oil Chem. Soc.*, **79**, 965–969 (2002).
13. M. F. Ramadan and J. T. Moersel, *Eur. Food Res. Technol.*, **214**, 202–206 (2002).
14. T. Verleyen, Doctoral dissertation, University of Gent, Belgium, 2002.
15. T. Verleyen, M. Focardes, R. Verhe, K. Dewettink, A. Huyghebaert, and W. de Greyt, *J. Amer. Oil Chem. Soc.*, **79**, 117–122 (2002).
16. E. J. Rogers, S. M. Rice, R. J. Nicolosi, D. R. Carpenter, C. A. McClelland, and L. J. Romanczyk, *J. Amer. Oil Chem. Soc.*, **70**, 301–307 (1993).
17. B. Kiosseoglou and D. Boskou, *Oleagineux*, **42**, 169–170 (1987).
18. S. P. Kochhar, *Prog. Lipid Res.*, **22**, 161–188 (1983).
19. A. Serani and D. Piacenti, *Riv. Ital. Sost. Gras.*, **69**, 311–315 (1992).
20. E. Tisconia and G. C. Bertini, *Riv. Ital. Sost. Gras.*, **52**, 101–108 (1975).
21. J. Touche, M. Derbesy, J. Castang, M. Olle, and J. Estienne, *Ann. Falsif. Expert. Chim.*, **70**, 263–270 (1977).
22. J. Kesselmier, W. Eichenberger, and B. Urban, *Plant Cell Physiol.*, **26**, 463–471 (1985).
23. A. Kamal-Eldin, K. Määttä, J. Toivo, A. M. Lampi, and V. Piironen, *Lipids*, **33**, 1073–1077 (1998).
24. R. A. Ferrari, E. Schulte, W. Esteves, L. Bruhl, and K. D. Mukherjee, *J. Amer. Oil Chem. Soc.*, **73**, 587–592 (1996).
25. E. Homberg and B. Bielefeld, *Fette Seifen Anstrichm.*, **84**, 141–146 (1982).
26. C. Crews, R. Calvet-Sarret, and P. Breton, *J. Chromatogr. A*, **847**, 179–185 (1999).
27. I. M. Jawad, S. P. Kochhar, and B. J. F. Hudson, *Lebensm. Wiss. u. Technol.*, **17**, 155–159 (1984).
28. A. Strocchi and G. Guerzoni, *Riv. Ital. Della Sci. Aliment.*, **14**, 125–133 (1985).
29. A. Strocchi, *Riv. Ital. Sost. Gras.*, **64**, 401–409 (1987).
30. A. Strocchi, *Rev. Fr. Corps Gras.*, **4**, 163–168 (1988).
31. A. Strocchi, *La Riv. Ital. Sci. Aliment.*, **17**, 41–50 (1988).
32. A. Strocchi and G. Marascio, *Riv. Ital. Della Sost. Gras.*, **70** 7–9 (1993).
33. A. Strocchi and G. Marascio, *Fat Sci. Technol.*, **95**, 293–299 (1993).
34. S. Ramamurthi and A. R. Mccurdy, *J. Amer. Oil Chem. Soc.*, **70**, 287–295 (1993).
35. I. Walsh, L. Winters, and R. Gonzalez, *Inform*, **9**, 78–83 (1998).
36. C. Watkins, *Inform*, **11**, 580–588 (2000).
37. I. Wester, *Eur. J. Lipid Sci. Technol.*, **102**, 37–44 (2000).

38. V. Piironen, D. G. Lindsay, T. A. Miettinen, J. Toivo, and A. M. Lampi, *J. Sci. Food Agric.* **80**, 939–966 (2000).
39. A. Kamal-Eldin and L. Å. Appelqvist, *Lipids*, **31**, 671–701 (1996).
40. G. F. Combs, Jr., *The Vitamins: Fundamental Aspects in Nutrition & Health*, Academic Press Inc., San Diego, California, 1992, pp. 179–203.
41. G. W. Burton and M. G. Traber, *Ann. Rev. Nutr.*, **10**, 357–382 (1990).
42. A. T. Diplock, in A. T. Diplock, ed., *Fat Soluble Vitamins*, Technomic Pub., Lancaster, Pennsylvania, 1985, pp. 154–224.
43. D. Olejnik, M. Gogolwski, and M. Nogala-Kalucka, *Nahrung*, **41**, 101–104 (1997).
44. F. D. Goffman and C. Möllers, *J. Agric. Food Chem.*, **48**, 1605–1609 (2000).
45. S.-N. Ko, C.-J. Kim, C.-T. Kim, H. Kim, S.-H. Chung, S.-M. Lee, H.-H. Yoon, and I.-H. Kim, *Eur. J. Lipid Sci. Technol.*, **105**, 225–228 (2003).
46. A. Lampi, A., Kamal-Eldin, and V. Piironen, in M. Le Maquer and J. Shi, eds., *Functional Foods*, Vol. 2, CRC Press, Boca Raton, Florida, 2002, pp. 1–38.
47. S. L. Abidi, *J. Amer. Oil Chem. Soc.*, **80**, 327–333 (2003).
48. L. Velasco and F. D. Goffman, *Plant Syst. Evol.*, **221**, 77–88 (2000).
49. J. Sabate, H. E. T. Bell, and G. E. Fraser, in G. A. Spiller, ed., *CRC Handbook of Lipids in Human Nutrition*, CRC Press, Boca Raton, Florida, 1996, pp. 145–151.
50. O. J. Catchpole, J. C. vonKamp, and J. B. Grey, *Ind. Eng. Chem. Res.*, **36**, 4318–4324 (1997).
51. O. J. Catchpole and J. C. vonKamp, *Ind. Eng. Chem. Res.*, **36**, 3762–3768 (1997).
52. A. Cert, W. Moreda, and M. C. Pérez-Camino, *J. Chromatogr. A*, **881**, 131–148 (2000).
53. M. W. Formo, E. Jungermann, F. A. Norris, and N. O. Sonntag, in D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 1, 4th ed., Wiley, New York, 1979, pp. 1–98.
54. R. Celades, *Rev. Franc. Corps Gras*, **44**, 105–112 (1993).
55. T. Kawakami, T. Kawai, N. Takei, T. Kise, S. Eda, and M. R. Urist, *Clin. Orthopaed. & Related Res.*, **337**, 261–266 (1997).
56. K. Grob, A. Artho, and C. Mariani, *Fat Sci. Technol.*, **94**, 394–399 (1992).
57. R. Bortolomeazzi, P. Berno, L. Pizzale, and L. S. Conte, *J. Agric. Food Chem.*, **49**, 3278–3283 (2001).
58. H. Pfander, *Methods Enzymol.*, **213**, 3–13 (1992).
59. IUPAC-IUB Commission on Biochemical Nomenclature, *Pure Appl. Chem.*, **41**, 407–431 (1975).
60. N. L. Puspitasari-Nienaber, M. G. Ferruzzi, and S. J. Schwartz, *J. Amer. Oil Chem. Soc.*, **79**, 633–640 (2002).
61. B. Gandul-Rojas and M. I. Minguez-Mosquera, *J. Sci. Food Agric.*, **72**, 31–39 (1996).
62. E. Psomiadou and M. Tsimidou, *J. Sci. Food Agric.*, **81**, 640–647 (2001).
63. J. Velasco and C. Dobarganes, *Eur. J. Lipid Sci. Technol.*, **104**, 661–676 (2002).
64. T. Matsuno, *Fisheries Sci.*, **67**, 771–783 (2001).
65. C. Galli, A. Petroni, and F. Visioli, *Eur. J. Pharm. Sci.*, **2**, 67–68 (1994).
66. D. Boskou, *Olive Oil Chemistry and Technology*, AOCS Press, Champaign, Illinois, 1996, pp. 52–83.

67. G. F. Montedoro, M. Servili, M. Baldioli, and E. Miniati, *J. Agric. Food Chem.*, **40**, 1571–1576 (1992).
68. G. F. Montedoro, M. Servili, M. Baldioli, R. Selvaggini, E. Miniati, and A. Macchioni, *J. Agric. Food Chem.*, **41**, 2228–2234 (1993).
69. M. Brenes, F. J. Hidalgo, A. García, J. J. Ríos, P. García, R. Zamora, and A. Garrido, *J. Amer. Oil Chem. Soc.*, **77**, 715–720 (2000).
70. M. Brenes, A. García, P. García, and A. Garrido, *J. Agric. Food Chem.*, **48**, 5178–5183 (2000).
71. R. W. Owen, W. Mier, A. Giacosa, W. E. Hull, B. Spiegelhalder, and H. Bartsch, *Food Chem. Toxicol.*, **38**, 647–659 (2000).
72. M. Servili and G. F. Montedoro, *Eur. J. Lipid Sci. Technol.*, **104**, 602–613 (2002).
73. L. Cinquanta, M. Esti, and E. La Notte, *J. Amer. Oil Chem. Soc.*, **74**, 1259–1264 (1997).
74. A. Kamal-Eldin and L. Å. Appelqvist, *J. Amer. Oil Chem. Soc.*, **71**, 149–156 (1994).
75. Y. Fukuda, M. Nagata, T. Osawa, and M. Namiki, *J. Amer. Oil Chem. Soc.*, **83**, 1027–1031 (1986).
76. G. Henon, K. Recseg, and K. Kovari, *J. Amer. Oil Chem. Soc.*, **78**, 401–410 (2001).
77. C. Mariani and S. Venturini, *Riv. Ital. Sost. Gras.*, **79**, 49–57 (2002).
78. B. Reiter and E. Lorbeer, *J. Amer. Oil Chem. Soc.*, **78**, 881–888 (2001).
79. A. A. Carelli, L. M. Frizzera, P. R. Forbito, and G. H. Crapiste, *J. Amer. Oil Chem. Soc.*, **79**, 763–768 (2002).
80. K. Ward, R. Scarth, J. K. Daun, and C. T. Thorsteinson, *J. Amer. Oil Chem. Soc.*, **71**, 1327–1331 (1994).
81. K. Suzuki and A. Nishioka, *J. Amer. Oil Chem. Soc.*, **70**, 837–841 (1993).
82. J. Cmolik and J. Pokorny, *Eur. J. Lipid Sci. Technol.*, **102**, 472–486 (2000).
83. R. Subramanian, M. Nakajima, A. Yasui, H. Nabetani, T. Kimura, and T. Maekawa, *J. Amer. Oil Chem. Soc.*, **76**, 1247–1253 (1999).
84. A. Koris and G. Vatai, *Desalination*, **148**, 149–153 (2002).
85. L. G. Pan, A. Campana, M. C. Tomas, and M. C. Anon, *J. Amer. Oil Chem. Soc.*, **77**, 1273–1276 (2000).
86. K. Clausen, *Eur. J. Lipid Sci. Technol.*, **103**, 333–340 (2001).
87. W. R. Morrison, in P. J. Barnes, ed., *Lipids in Cereal Technology*, Academic Press, London, U.K., 1983, pp. 11–32.
88. V. L. Youngs, M. Pueskuelcue, and R. R. Smith, *Cereal Chem.*, **54**, 803–812 (1977).
89. T. Wang and L. A. Johnson, *J. Amer. Oil Chem. Soc.*, **78**, 71–76 (2001).
90. K. T. N. Balagopala, V. S. Ramjan, R. B. V. S. Koppeswara, C. P. Prasad, V. Penumarthy, K. Vijay, R. K. N. Prasanna, R. Ongole, B. P. Satya, and R. T. Chandrasekhara, *Eur. J. Lipid Sci. Technol.*, **104**, 203–211 (2002).
91. S. Vichia, A. I. Castelloteb, L. Pizzalea, L. S. Contea, S. Buxaderasb, and E. López-Tamames, *J. Chromatogr. A*, **983**, 19–33 (2003).
92. P. Pfnuer, T. Matsui, W. Grosch, H. Guth, T. Hofmann, and P. Schieberle, *J. Agric. Food Chem.*, **47**, 2044–2047 (1999).
93. S. H. Lee and K. J. Joo, *Korean J. Food Sci. Technol.*, **30**, 260–265 (1998).
94. S. Nakamura, O. Nishimura, H. Masuda, and S. Mihara, *J. Agric. Biol. Chem.*, **53**, 1891–1899 (1989).

95. A. A. Elsaywy, M. M. Soliman, and H. M. Fadel, *Grasas y Aceites*, **39**, 160–162 (1988).
96. M. M. Soliman, A. A. Elsaywy, H. M. Fadel, and F. Osman, *Acta Aliment.*, **15**, 251–263 (1986).
97. Y. G. Lee, S. U. Lim, and J. O. Kim, *J. Korean Agric. Chem. Soc.*, **36**, 407–415 (1993).
98. N. Frega, M. Mozzon, and G. Lercker, *J. Amer. Oil Chem. Soc.*, **76**, 325–329 (1999).
99. F. Gutiérrez, M. A. Albi, R. Palma, J. J. Ríos, and J. M. Olías, *J. Food Sci.*, **54**, 68–70 (1989).
100. M. H. Gordon and I. A. Rahman, *J. Amer. Oil Chem. Soc.*, **68**, 577–579 (1991).
101. C. C. Ho and M. C. Chow, *J. Amer. Oil Chem. Soc.*, **77**, 191–199 (2000).
102. R. A. Tietz and R. W. Hartel, *J. Amer. Oil Chem. Soc.*, **77**, 763–771 (2000).
103. R. J. Sims, J. A. Fioriti, and M. J. Kanuk, *J. Amer. Oil Chem. Soc.*, **49**, 298–301 (1972).
104. G. Blekas, M. Tsimidou, and D. Boskou, *Food Chem.*, **52**, 289–294 (1995).
105. M. Deiana, A. Rosa, C. F. Q. Cao, F. M. Pirisi, G. Bandino, and M. A. Dessi, *J. Agric. Food Chem.*, **50**, 4342–4346 (2002).
106. J. Cillard and P. Cillard, *J. Amer. Oil Chem. Soc.*, **57**, 39–42 (1980).
107. J. Cillard, P. Cillard, M. Cormier, and L. Girre, *J. Amer. Oil Chem. Soc.*, **57**, 252–255 (1980).
108. J. Cillard, P. Cillard, and M. Cormier, *J. Amer. Oil Chem. Soc.*, **57**, 255–261 (1980).
109. M. D. Fuster, A. M. Lampi, A. Hopia, and A. Kamal-Eldin, *Lipids*, **33**, 715–722 (1998).
110. A. M. Lampi, L. Kataja, A. Kamal-Eldin, and V. Piironen, *J. Amer. Oil Chem. Soc.*, **76**, 749–755 (1999).
111. M. Mäkinen, A. Kamal-Eldin, A. M. Lampi, and A. Hopia, *Eur. J. Lipid Sci. Technol.*, **103**, 286–291 (2001).
112. A. Kamal-Eldin, M. Mäkinen, A. M. Lampi, and A. Hopia, *Eur. Food Res. Technol.*, **214**, 52–57 (2002).
113. N. V. Yanishlieva, A. Kamal-Eldin, E. M. Marinova, and A. G. Toneva, *Eur. J. Lipid Sci. Technol.*, **104**, 262–270 (2002).
114. D. Boskou, *Grasas y Aceites*, **49**, 326–330 (1998).
115. J. L. Perrin, *Rev. Fran. Corps Gras.*, **39**, 25–32 (1992).
116. F. Gutierrezrosales, J. Garridofernandez, L. Gallardoguerrero, B. Gandulrojas, and M. I. Mínguezmosquera, *J. Amer. Oil Chem. Soc.*, **69**, 866–871 (1992).
117. M. Rahmani and A. S. Csallany, *J. Amer. Oil Chem. Soc.*, **75**, 837–843 (1998).
118. B. H. Chen and J. H. Huang, *Food Chem.* **62**, 299–307 (1998).
119. O. T. Kasaikina, V. V. Vedutenko, A. M. Kashkay, V. D. Kortenska, M. I. Boneva, and N. V. Yanishlieva, *Oxid. Commun.*, **25**, 232–243 (2002).
120. E. Psomiadou and M. Tsimidou, *J. Agric. Food Chem.*, **50**, 722–727 (2002).
121. B. Le Tutour, C. Brunel, and F. Quemeneur, *New J. Chem.*, **20**, 707–721 (1996).
122. Y. Endo, R. Usuki, and T. Kaneda, *Agric. Biol. Chem.*, **48**, 985–989 (1984).
123. Y. Endo, R. Usuki, and T. Kaneda, *J. Amer. Oil Chem. Soc.*, **61**, 771–784 (1984).
124. R. Usuki, T. Suzuki, Y. Endo, and T. Kaneda, *J. Amer. Oil Chem. Soc.*, **61**, 785–788 (1984).
125. C. L. Tautorius and N. H. Low, *J. Amer. Oil Chem. Soc.*, **70**, 843–847 (1993).
126. C. L. Tautorius and N. H. Low, *J. Amer. Oil Chem. Soc.*, **71**, 1123–1128 (1994).

127. S. Ramamurthi and N. H. Low, *J. Agric. Food Chem.* **43**, 1479–1483 (1995).
128. T. Pagano, M. Sergio, L. Glisenti, W. Diano, and M. A. Grompone, *Ingenieria Química*, **19**, 11–19 (2001).
129. P. Falaras, F. Lezou, G. Seiragakis, and D. Petrakis, *Clays Clay Minerals*, **48**, 549–556 (2000).
130. K. K. Reddy, R. Subramanian, T. Kawakatsu, and M. Nakajima, *Eur. Food Res. Technol.*, **213**, 212–218 (2001).
131. G. W. Burton and K. U. Ingold, *Science*, **224**, 569–573 (1984).
132. C. S. Foote, in W. A. Pryor, ed., *Free Radicals in Biology*, Vol. 2, Academic Press, New York, 1976, pp. 85–133.
133. C. S. Foote, in H. H. Wasserman, and R. W. Murray, eds., *Singlet Oxygen*, Academic Press, New York, 1979, pp. 139–171.
134. E. C. Lee and D. B. Min, *J. Agric. Food Chem.*, **38**, 1630–1634 (1990).
135. M. M. Jung and D. B. Min, *J. Amer. Oil Chem. Soc.*, **68**, 653–658 (1991).
136. D. Bellus, in B. Ranby and J. F. Rabek, eds., *Singlet Oxygen Reactions with Organic Compounds and Polymers*, Wiley, Chichester, U.K., 1978, pp. 61–110.
137. J. Terao, R. Yamauchi, H. Murkami, and S. Matsushita, *J. Food Process Preserv.*, **4**, 79–93 (1980).
138. P. Di Masico, S. Kaiser, and H. Sies, *Arch. Biochem. Biophys.*, **274**, 532–538 (1989).
139. N. I. Krinsky, in H. H. Wasserman and R. W. Murray, eds., *Singlet Oxygen*, Academic Press, New York, 1979, pp. 597–641.
140. N. I. Krinsky, *Free Radical Biol. Med.*, **7**, 617–635 (1989).
141. M. M. Mathews-Roth, *Photochem. Photobiol.*, **43**, 91–93 (1986).
142. M. M. Mathews-Roth, *Fed. Proc.*, **46**, 1890–1893 (1987).
143. P. Palozza and N. I. Krinsky, *Arch. Biochem. Biophys.*, **297**, 184–187 (1992).
144. N. I. Krinsky and S. M. Deneke, *J. Natl. Cancer Inst.*, **69**, 205–210 (1982).
145. J. Terao, *Lipids*, **24**, 659–661 (1989).
146. I. Jialal, E. P. Norkus, L. Cristol, and S. M. Grundy, *Biochim. Biophys. Acta*, **1086**, 134–138 (1991).
147. T. A. Kennedy and D. C. Liebler, *J. Biol. Chem.*, **267**, 4658–4663 (1992).
148. B. P. Lim, A. Nagao, J. Terao, K. Tanaka, T. Suzuki, and K. Takama, *Biochim. Biophys. Acta*, **1126**, 178–184 (1992).
149. D. C. Liebler, *Ann. N. Y. Acad. Sci.*, **691**, 20–31 (1993).
150. S. A. Everet, S. C. Kundu, S. Maddix, and R. L. Willson, *Biochem. Soc. Trans.*, **23**, 230s (1995).
151. F. J. Petrcek and L. Zechmeister, *J. Amer. Chem. Soc.*, **78**, 1427–1434 (1956).
152. M. Elahi and E. R. Cole, *Nature*, **203**, 186–187 (1964).
153. A. H. El-Tinay and C. O. Chichester, *J. Org. Chem.*, **35**, 2290–2293 (1970).
154. W. Boguth, R. Patzelt, and R. Repges, *Int. J. Vitam. Res.*, **41**, 21–32 (1971).
155. T. A. Kennedy and D. C. Liebler, *Chem. Res. Toxicol.*, **4**, 290–295 (1991).
156. G. J. Handelman, F. J. G. M. Van Kuijk, A. Chatterjee, and N. I. Krinsky, *Free Rad. Biol. Med.*, **10**, 427–437 (1991).

157. R. C. Mordi, J. C. Walton, G. W. Burton, L. Hughes, K. U. Ingold, and D. A. Lindsay, *Tetrahedron Lett.*, **32**, 4203–4206 (1991).
158. S. Q. Alam and B. S. Alam, *J. Nutr.*, **113**, 2608–2614 (1983).
159. L. Lomnitski, M. Bergman, Schon, and S. Grossman, *Biochim. Biophys. Acta*, **1082**, 101–107 (1991).
160. N. V. Yanishlieva, V. G. Raneva, and E. M. Marinova, *Grasas y Aceites*, **52**, 10–16 (2001).
161. T. Toyosaki, *Int. J. Food Sci. Nutr.*, **53**, 419–423 (2002).
162. Z. L. Li, J. Cui, X. Y. Hong, Y. C. Liu, and X. L. Liu, *J. Phys. Org. Chem.*, **15**, 314–318 (2002).
163. N. M. Bandarra, R. M. Campos, I. Batista, M. L. Nunes, and J. M. Empis, *J. Amer. Oil Chem. Soc.*, **76**, 905–913 (1999).
164. M. Servili, M. Baldioli, E. Miniati, and G. F. Montedoro, *Riv. Ital. Sost. Gras.*, **73**, 55–59 (1996).
165. R. Aparicio, L. Roda, M. A. Albi, and F. Gutierrez, *J. Agric. Food Chem.*, **47**, 4150–4155 (1999).
166. M. A. Dessi, M. Deiana, B. W. Day, A. Rosa, S. Banni, and F. P. Corongiu, *Eur. J. Lipid Sci. Technol.*, **104**, 506–512 (2002).
167. M. T. Satue, S. W. Huang, and E. N. Frankel, *J. Amer. Oil Chem. Soc.*, **72**, 1131–1137 (1995).
168. M. Baldioli, M. Servili, G. Perretti, and G. F. Montedoro, *J. Amer. Oil Chem. Soc.*, **73**, 1589–1593 (1996).
169. M. Tsimidou, *Ital. J. Food Sci.*, **10**, 99–116 (1998).
170. V. Fogliano, A. Ritieni, S. M. Monti, M. Gallo, D. Della Medaglia, M. L. Ambrosino, and R. Sacchi, *J. Sci. Food Agric.*, **79**, 1803–1808 (1999).
171. E. Pagliarini, B. Zanoni, and G. Giovanelli, *J. Agric. Food Chem.*, **48**, 1345–1351 (2000).
172. M. D. Salvador, F. Aranda, and G. Fregapane, *J. Amer. Oil Chem. Soc.*, **76**, 427–432 (1999).
173. D. W. Peterson, *Proc. Soc. Exp. Biol. Med.*, **78**, 143–147 (1951).
174. O. J. Pollak, *Circulation*, **6**, 459–460 (1952).
175. O. J. Pollak, *Circulation*, **7**, 702–706 (1953).
176. O. J. Pollak and D. Kritchevsky, in T. B. Clarkson, D. Kritchevsky, and O. J. Pollak, eds., *Monographs on Atherosclerosis*, Karger, Basle, Vol. 10, 1981, pp. 1–219.
177. K. Uchida, H. Takese, Y. Nomura, K. Takeda, N. Takeuchi, and Y. Ishikawa, *J. Lipid Res.*, **25**, 236–244 (1984).
178. R. S. Tilvis, M. D. Tatu, and M. D. Miettinen, *Amer. J. Clin. Nutr.*, **43**, 92–97 (1986).
179. L. Laraki, X. Pelletier, and G. Derby, *Ann. Nutr. Metab.*, **35**, 221–225 (1991).
180. L. Laraki, X. Pelletier, J. Mouro, and G. Debry, *Ann. Nutr. Metab.*, **37**, 129–133 (1993).
181. X. Pelletier, S. Belbraouet, D. Mirabel, F. Mordret, J. Perrin, X. Pages, and G. Debry, *Ann. Nutr. Metab.*, **39**, 291–295 (1995).
182. P. J. H. Jones, D. E. Macdougall, F. Ntatnios, and C. A. Vanstone, *Can. J. Physiol. Pharmacol.*, **75**, 217–227 (1997).
183. T. J. Howell, E. D. MacDougall, and P. J. H. Jones, *J. Lipid Res.*, **39**, 892–900 (1998).
184. G. W. Meijer and J. A. Weststrate, *Eur. J. Clin. Nutr.*, **52**, 334–343 (1998).



185. J. A. Weststrate and G. W. Meijer, *Eur. J. Clin. Nutr.* **52**, 334–343 (1998).
186. J. Plat, E. N. M. van Onselen, and R. P. Mensink, *Eur. Heart J. Suppl. 1*, s58–s63 (1999).
187. C. H. Hennekens and J. M. Gaziano, *Clin. Cardiol.*, **16**, 110–115 (1993).
188. N. Rong, L. M. Ausman, and R. J. Nicolosi, *Lipids*, **32**, 303–309 (1997).
189. L. K. Earl, P. Baldrick, and P. A. Hepburn, *Int. J. Toxicol.*, **21**, 353–359 (2002).
190. M. N. Vissers, P. L. Zock, G. W. Meijer, and M. B. Katan, *Amer. J. Clin. Nutr.*, **72**, 1510–1515 (2000).
191. H. Gylling and T. A. Miettinen, *Metabolism*, **48**, 575–580 (1999).
192. T. E. Strandberg, R. S. Tilvis, and T. A. Miettinen, *Lipids*, **24**, 705–708 (1989).
193. H. M. Evans and K. S. Bishop, *Science*, **56**, 650–651 (1922).
194. P. B. McCay, *Ann. Rev. Nutr.*, **5**, 323–340 (1985).
195. F. A. Oski, *New Eng. J. Med.*, **303**, 454–455 (1980).
196. L. J. Machlin, *Vitamin E: A comprehensive Treatise*, Marcel Dekker, New York, 1980.
197. L. J. Machlin, in L. J. Machlin, ed., *Handbook of Vitamins: Nutritional, Biochemical and Clinical Aspects*, Marcel Dekker, New York and Basel, 1984, pp. 99–145.
198. G. W. Burton, A. Joyce, and K. U. Ingold, *Lancet*, **2**, 327 (1982).
199. G. W. Burton, A. Joyce, and K. U. Ingold, *Arch. Biochem. Biophys.*, **221**, 281–290 (1983).
200. G. W. Burton, K. H. Cheesman, D. Doba, K. U. Ingold, and T. F. Slater, in R. Porter and J. Whelan, eds., *Biology of Vitamin E*, Ciba Foundation Symposium 101, Pitman, London, U.K., 1983, pp. 4–18.
201. R. S. Parker, *J. Nutr.*, **119**, 101–104 (1989).
202. H. Sies, *Angew. Chem.*, **98**, 1061–1076 (1986).
203. H. Sies and M. E. Murphy, *J. Photochem. Photobiol.: B. Biol.*, **8**, 211–224 (1991).
204. H. Sies, W. Stahl, and A. R. Sundqvist, in E. Sauberlich and L. J. Machlin, eds., *Beyond Deficiency*, New York Acta, *Ann. N. Y. Acad. Sci.*, **669**, 7–20 (1992).
205. S. A. B. E. van Acker, L. M. H. Koymans, and A. Bast, *Free Rad. Biol. Med.*, **15**, 311–328 (1993).
206. J. L. Hess, in R. G. Alscher, and J. L. Hess, eds., *Antioxidants in Higher Plants*, CRC Press, Boca Raton, Florida, 1993.
207. J. C. Bauernfeind and I. D. Desai, *CRC Crit. Rev. Food Sci. Nutr.*, **8**, 337–382 (1977).
208. J. C. Bauernfeind, in L. J. Machlin, ed., *Vitamin E: A Comprehensive Treatise*, Marcel Dekker, New York, 1980, pp. 99–167.
209. P. J. McLaughlin and J. L. Weihrach, *J. Amer. Dietetic Assoc.*, **75**, 647–665 (1979).
210. K. F. Gey and P. Puska, *Ann. N. Y. Acad. Sci.*, **570**, 268–286 (1989).
211. K. F. Gey, P. Puska, P. Jordan, and U. K. Moser, *Amer. J. Clin. Nutr.*, **53**, 326s–334s (1991).
212. M. J. Stampfer, C. H. Hennekens, J. E. Manson, G. A. Colditz, B. Rosner, and W. C. Willett, *N. Engl. J. Med.*, **328**, 1444–1449 (1993).
213. E. B. Rimm, M. J. Stampfer, A. Ascherio, E. Giovannucci, G. A. Colditz, and W. C. Willett, *N. Engl. J. Med.*, **328**, 1450–1456 (1993).
214. P. Knekt, A. Reunanen, R. Järvinen, R. Seppänen, M. Heliövaara, and A. Aroma, *Am. J. Epidemiol.*, **139**, 1180–1189 (1994).

215. M. Torun, N. Avci, and S. Yardim, *J. Clin. Pharmacol. Ther.*, **20**, 335–340 (1995).
216. J. M. Gaziano, J. E. Manson, J. E. Buring, and C. H. Hennekens, in S. He and M. L. J., eds., *Beyond Deficiency: New Views on The Function and Health Effects of Vitamins*, *Ann. N. Y. Acad. Sci.*, **669**, 249–259 (1992).
217. H. Esterbauer, M. Dieber-Rotheneder, G. Striegl, and G. Waeg, *Amer. J. Clin. Nutr.*, **53**, 314s–321s (1991).
218. I. Jialal and S. M. Grundy, *J. Lipid Res.*, **33**, 899–906 (1992).
219. P. D. Reaven, A. Khouw, W. F. Beltz, S. Parthasarathy, and J. L. Witztum, *Arterioscler. Thromb.*, **13**, 590–600 (1993).
220. J. D. Belcher, J. Balla, G. Balla, D. R. Jacobs, M. Gross, H. S. Jacob, and G. M. Vercellotti, *Arterioscler. Thromb.*, **12**, 1779–1789 (1993).
221. M. Steiner, *Clin. Cardiol.*, **16**, 116–118 (1993).
222. A. Azzi, D. Boscoboinik, D. Marilley, N. K. Ozer, B. Stauble, and A. Tassinato, *Amer. J. Clin. Nutr.*, **62**, 1337s–1346s (1995).
223. S. Devaraj, D. Li, and I. Jialal, *J. Clin. Invest.*, **98**, 756–763 (1996).
224. J. G. Bieri and R. P. Evarts, *Amer. J. Clin. Nutr.*, **27**, 980–986 (1974).
225. M. G. Traber and H. J. Kayden, *Amer. J. Clin. Nutr.*, **49**, 517–526 (1989).
226. M. G. Traber, G. W. Burton, L. Hughes, K. U. Ingold, H. Hidaka, M. Malloy, J. Kane, J. Hyams, and H. J. Kayden, *J. Lipid Res.*, **33**, 1171–1182 (1992).
227. H. J. Kayden and M. G. Traber, *J. Lipid Res.*, **34**, 343–358 (1993).
228. Q. Jiang, S. Christen, M. K. Shigenaga, and B. Ames, *Amer. J. Clin. Nutr.*, **74**, 714–722 (2001).
229. R. V. Cooney, A. A. Franke, P. J. Harwood, V. Hatch-Pigott, L. J. Custer, and L. J. Mordan, *Proc. Natl. Acad. Sci. USA*, **90**, 1771–1775 (1993).
230. R. V. Cooney, P. J. Harwood, A. A. Franke, K. Narala, A. K. Sundstrom, P. O. Berggren, and L. J. Mordan, *Free Radic. Biol. Med.*, **19**, 259–269 (1995).
231. S. Christen, A. A. Woodall, M. K. Shigenaga, P. T. Southwell-Keely, M. W. Duncan, and B. N. Ames, *Proc. Natl. Acad. Sci.*, **49**, 3217–3222 (1997).
232. S. Christen, P. Gee, and B. N. Ames, *Methods Enzymol.*, **269**, 267–278 (1996).
233. A. J. Brown, *J. Nutr. Biochem.*, **7**, 29–39 (1996).
234. K. S. Williamson, S. P. Gabbita, S. Mou, M. West, Q. N. Pye, W. R. Markesbery, R. V. Cooney, P. Grammas, U. Reimann-Philipp, R. A. Floyd, and K. Hensley, *Nitric Oxide: Biol. Chem.*, **6**, 221–227 (2002).
235. W. J. Wechter, D. Kantoci, E. D. Murray Jr., D. C. D’Amico, and M. E. Jung, *Proc. Natl. Acad. Sci.*, **93**, 6002–6007 (1996).
236. M. Öhrvall, S. Tengblad, and B. Vessby, *Eur. J. Clin. Nutr.*, **48**, 212–218 (1994).
237. A. Kontush, T. Spranger, A. Reich, K. Baum, and U. Beisiegel, *Atherosclerosis*, **144**, 117–122 (1999).
238. M. Kristenson, B. Ziedén, Z. Kucinskienė, L. Schäfer-Elinder, B. Bergdahl, B. Elwing, A. Abaravicius, L. Razinkovienė, H. Calkauskas, and A. G. Olsson, *Brit. Med. J.*, **314**, 629–633 (1997).
239. T. Messner, U. B. Erksam, I. B. Gustavsson, S. B. Nilsson, and B. Vessby, *Int. J. Circumpolar Health*, **56**, 21–29 (1997).

240. S. R. Farwer, B. C. der Boer, E. Haddeman, G. A. Kivits, A. Wiersma, and B. H. Danse, *British J. Nutr.*, **72**, 127–145 (1994).
241. G. J. Handelman, W. L. Epstein, J. Peerson, D. Spiegelman, L. J. Machlin, and E. A. Dratz, *Amer. J. Clin. Nutr.*, **59**, 1025–1032 (1994).
242. M. Clement and J. M. Bourre, *Biochim. Biophys. Acta.*, **1334**, 173–181 (1997).
243. H. U. Melchert and E. Pabel, *J. Amer. Oil Chem. Soc.*, **75**, 213–216 (1998).
244. M. Clement, L. Dinh, and J. M. Bourre, *Biochim. Biophys. Acta.*, **1256**, 175–180 (1995).
245. W. A. Behrens and R. Madere, *Nutr. Res.*, **3**, 891–897 (1983).
246. W. A. Behrens and R. Madere, *J. Nutr.*, **117**, 1562–1569 (1987).
247. I. Elmadfa, S. W. Kim, M. Reutlinger, and R. Siewert, *Z. Ernährungswiss.*, **28**, 36–48 (1989).
248. K. Yamashita, Y. Nohara, K. Katayama, and M. Namiki, *J. Nutr.*, **122**, 2440–2446 (1992).
249. K. Yamashita, Y. Iizuka, T. Imai, and M. Namiki, *Lipids*, **30**, 1019–1028 (1995).
250. M. Sugano, K. Koba, and E. Tsuji, *Anticancer Res.*, **19**, 3651–3657 (1999).
251. A. A. Qureshi, W. C. Burger, D. M. Peterson, and C. E. Elson, *J. Biol. Chem.*, **261**, 544–550 (1986).
252. A. A. Qureshi, N. Qureshi, J. J. K. Wright, Z. Shen, G. Kramer, A. Gapor, Y. H. Chong, G. Dewitt, A. S. H Ong, D. M. Peterson, and B. A. Bradlow, *Amer. J. Clin. Nutr.*, **53**, s1021–s1026 (1991).
253. M. N. Gould J. D. Haag, W. S. Kennan, M. A. Tanner, and C. E. Elson, *Amer. J. Clin. Nutr.*, **53**, s1068–s1070 (1991).
254. A. A. Qureshi, S. A. Sami, W. A. Salser, and F. A. Khan, *J. Nutr. Biochem.*, **12**, 318–329 (2001).
255. A. A. Qureshi, S. A. Sami, W. A. Salser, and F. A. Khan, *Atherosclerosis*, **161**, 199–207 (2002).
256. D. T. Tan, H. T. Khor, W. H. S. Low, A. Ali, and A. Gapor, *Amer. J. Clin. Nutr.*, **53**, s1027–s1030 (1991).
257. D. Raederstorff, V. Elste, C. Aebischer, and P. Weber, *Ann. Nutr. Metabol.*, **46**, 17–23 (2002).
258. R. A. Parker, B. C. Pearce, R. W. Clark, D. A. Gordon, and J. J. K. Wright, *J. Biol. Chem.*, **268**, 11230–11238 (1993).
259. H. T. Khor and T. T. Ng, *Int. J. Food Sci. Nutr.*, **51**, s3–s11 (2000).
260. J. C. Bauernfeind, *Carotenoids as Colorants and Vitamin A Precursors: Technical and Nutritional Applications*, Academic Press, New York, 1981.
261. K. L. Simpson, *Proc. Nutr. Soc.*, **42**, 7–17 (1983).
262. C. S. You, R. S. Parker, and J. E. Swanson, *Asia Pacific J. Clin. Nutr.*, **11**, s438–s442 (2000).
263. A. Kassoff, et al., *Arch. Ophthalmol. Chic.*, **119**, 1417–1436 (2001).
264. A. Kassoff et al., *Arch. Ophthalmol. Chic.*, **119**, 1439–1452 (2001).
265. H. Esterbauer, G. Striegel, H. Puhl, S. Oberreither, M. Rotheneder, M. El-Saadani, and G. Jürgens, *Ann. N. Y. Acad. Sci.*, **570**, 254–267 (1989).
266. M. K. Biyani and D. S. Sheorey, *J. Assoc. Physicians India.*, **42**, 899–903 (1994).
267. G. Mozsik, T. Javor, G. Toth, T. Zsoldos, and A. Tigyi, *Acta Phys. Hung.*, **64**, 315–319 (1984).

268. A. M. Y. Nomura, G. N. Stemmermann, L. K. Heilbrun, R. M. Salkeld, and J. P. Vuilleumier, *Cancer Res.*, **45**, 2369–2372 (1985).
269. M. S. Menkes, G. W. Comstock, J. P. Vuilleumier, K. J. Helsing, A. A. Rider, and R. Brookmeyer, *N. Engl. J. Med.*, **315**, 1250–1254 (1986).
270. K. F. Gey, G. B. Brubacher, and H. B. Stahelin, *Amer. J. Clin. Nutr.*, **45**, 1368–1377 (1987).
271. H. Nishino, *J. Cell Biochem.*, **22**, 231–235 (1995).
272. A. Bendich, *Ann. N. Y. Acad. Sci.*, **691**, 61–67 (1993).
273. R. H. Prabhala, H. S. Garewal, F. L. Meyskens, and R. R. Watson, *Nutr. Res.*, **10**, 1473–1486 (1990).
274. R. H. Prabhala, M. Braune, H. S. Garewal, and R. R. Watson, *Ann. N. Y. Acad. Sci.*, **691**, 262–263 (1993).
275. R. G. Cutler, *Proc. Natl. Acad. Sci. USA*, **87**, 7627–7631 (1984).
276. M. M. Mathews-Roth, *Pure Appl. Chem.*, **63**, 147–156 (1991).
277. J. A. Olson and N. I. Krinsky, *FASEB J.*, **9**, 1547–1550 (1995).
278. K. J. Yeum and R. M. Russell, *Ann. Rev. Nutr.*, **22**, 483–504 (2002).
279. W. Miki, *Pure Appl. Chem.*, **63**, 141–146 (1991).
280. J. A. Olson, in M. E. Shils, J. A. Olson, and M. Shike, eds., *Modern Nutrition in Health and disease*, 8th ed., Lea and Febiger, Philadelphia, Pennsylvania, 1993, pp. 287–307.
281. N. V. Dimitrov and D. E. Ullrey, in N. I. Krinsky, M. M. Mathews-Roth, and R. F. Taylor, eds., *Carotenoids: Chemistry and Biology*, Plenum Press, New York, 1989, pp. 269–277.
282. R. S. Parker, in J. E. Kinsella, ed., *Advances in Food and Nutrition Research*, Vol. 33, Academic Press, New York, 1989, pp. 157–232.
283. A. Bendich and J. A. Olson, *FASEB J.*, **3**, 1927–1932 (1989).
284. A. D. Lascari, *Clin. Pediat.*, **20**, 25–29 (1981).
285. F. Visioli and C. Galli, *J. Agric. Food Chem.*, **46**, 4292–4296 (1998).
286. F. Visioli and C. Galli, *Crit. Rev. Food Sci. Nut.*, **42**, 209–221 (2002).
287. F. Visioli, D. Caruso, C. Galli, S. Viappiani, G. Galli, and A. Sala, *Biochem. Biophys. Commun.*, **278**, 797–799 (2000).
288. S. Gorinstein, H. Leontowicz, A. Lojek, M. Leontowicz, M. Ciz, R. Krzeminski, M. Gralak, J. Czerwinski, Z. Jastrzebski, S. Trakhtenberg, M. N. Grigelmo, R. Soliva-Fortuny, and O. Martin-Belloso, *J. Agric. Food Chem.*, **50**, 6102–6108 (2002).
289. M. Fito, E. Gimeno, M. I. Covas, E. Miro, M. C. Lopez-Sabater, M. Farre, R. de la Torre, and J. Marrugat, *Lipids*, **37**, 245–251 (2002).
290. J. L. Quiles, A. J. Farquharson, D. K. Simpson, I. Grant, and K. W. J. Wahle, *British J. Nutr.*, **88**, 225–234 (2002).
291. K. Yamashita, Y. Kawagoe, Y. Nohara, M. Namiki, T. Osawa, and S. Kawagishi, *J. Japan. Soc. Nutr. Food Sci.*, **43**, 445–446 (1990).
292. N. Hirose, T. Inoue, K. Nishihara, M. Sugano, K. Akimoto, S. Shimizu, and H. Yamada, *J. Lipid Res.*, **32**, 629–638 (1991).
293. A. Kamal-Eldin, D. Pettersson, and L. Å. Appelqvist, *Lipids*, **30**, 499–505 (1995).
294. A. Kamal-Eldin, J. Frank, A. Razdan, S. Tengblad, S. Basu, and B. Vessby, *Lipids*, **35**, 427–435 (2000).

295. R. S. Parker, T. J. Sontag, and J. E. Swanson, *Biochem. Biophys. Res. Commun.*, **277**, 531–534 (2000).
296. T. J. Sontag and R. S. Parker, *J. Biol. Chem.*, **277**, 25290–25296 (2002).
297. S. Ikeda, T. Tohyama, and K. Yamashita, *J. Nutr.*, **132**, 961–966 (2002).
298. M. Sugano, T. Inoue, K. Koba, K. Yoshida, N. Hirose, Y. Shinmen, K. Akimoto, and T. Amachi, *Agric. Biol. Chem.*, **54**, 2669–2673 (1990).
299. S. Shimizu, K. Akimoto, Y. Shinmen, H. Kawashima, M. Sugano, and H. Yamada, *Lipids*, **26**, 512–516 (1991).
300. T. Utsunomiya, S. R. Chavali, W. W. Zhong, and R. A. Forse, *Amer. J. Clin. Nutr.*, **72**, 804–808 (2000).
301. T. Ide, L. Ashakumary, Y. Takahashi, M. Kushiro, N. Fukuda, and M. Sugano, *Biochim. Biophys. Acta*, **1534**, 1–13 (2001).
302. R. Umeda-Sawada, M. Ogawa, and O. Igarashi, *Lipids*, **34**, 633–637 (1999).
303. U. Bracco, W. Rostagno, and R. H. Egli, *Int. Chocolate Rev.*, **25**, 41–48 (1970).
304. V. V. S. Mani and G. Lakshminarayana, *J. Oil Technol. Assoc. India*, **5**, 184–193 (1976).
305. A. M. Abu-Hadeed and A. R. Kotb, *J. Amer. Oil Chem. Soc.*, **65**, 1922–1926 (1988).
306. D. Firestone, *J. Assoc. Off. Anal. Chem. Intern.*, **84**, 176–180 (2001).
307. F. Ulberth and M. Buchgraber, *Eur. J. Lipid Sci. Technol.*, **105**, 32–42 (2003).
308. J. B. Rossell, *Fat Sci. Technol.*, **93**, 526–531 (1991).
309. M. H. El-Mallah, T. Murui, and S. El-Shami, *Grasas y Aceites*, **50**, 7–15 (1999).
310. G. Morchio, A. Pellegrino, C. Mariani, and G. Bellan, *Rivista Ital. Sostanze Grasse*, **76**, 115–128 (1999).
311. M. H. Gordon and R. E. Griffith, *Food Chem.*, **43**, 71–78 (1992).
312. K. Grob, A. M. Giuffré, U. Leuzzi, and B. Mincione, *Fat Sci. Technol.*, **96**, 286–290 (1994b).
313. A. Johansson and L. B. Croon, *Lipids*, **16**, 306–314 (1981).
314. E. Schulte, *Fat Sci. Technol.*, **96**, 124–128 (1994).
315. K. Grob, A. M. Giuffré, M. Biedermann, and M. Bronz, *Fat Sci. Technol.*, **96**, 341–345 (1994).
316. C. Mariani, G. Bellan, G. Morchio, and A. Pellegrino, *Riv. Ital. Sost. Gras.*, **76**, 59–67 (1999).
317. C. Mariani, G. Bellan, G. Morchio, and A. Pellegrino, *Riv. Ital. Sost. Gras.*, **76**, 297–305 (1999).
318. R. K. Chevan, V. S. Ghole, and M. Dikshit, *J. Food Sci. Technol. India*, **31**, 433–434 (1994).
319. B. Gandul-Rojas, M. R. L. Cepero, and M. I. Minguez-Mosquera, *J. Amer. Oil Chem. Soc.*, **77**, 853–858 (2000).
320. F. Ulberth and M. Buchgraber, *Eur. J. Lipid Sci. Technol.*, **102**, 687–694 (2000).
321. M. Kates, *Techniques in Lipidology*, 2nd ed., Elsevier Publ. Comp., Amsterdam, The Netherlands.
322. N. Frega, F. Bocci, and G. Lercker, *J. Amer. Oil Chem. Soc.*, **69**, 447–450 (1992).
323. N. Frega, F. Bocci, G. Giovannoni, and G. Lercker, *Chromatographia*, **36**, 215–217 (1993).

324. J. Giacometti, *Analyst*, **126**, 472–475 (2001).
325. V. Baeten, P. Dardenne, and R. Aparicio, *J. Agric. Food Chem.*, **49**, 5098–5107 (2001).
326. A. Kamal-Eldin, L. Å. Appelqvist, G. Yousif, and G. M. Iskander, *J. Sci. Food Agric.*, **59**, 327–334 (1992).
327. P. Hostmann and A. Montag, *Fette Seifen Anstrichm.*, **88**, 262–264 (1986).
328. A. C. Bello, *J. Amer. Oil Chem. Soc.*, **75**, 1120–1123 (1992).
329. B. Ham, B. Butler, and P. Thionville, *LC/GC*, **18**, 1174–1179 (2000).
330. P. Breinholder, L. Mosca, and W. Lindner, *J. Chromatogr.*, **B777**, 67–82 (2002).
331. A. Kamal-Eldin, S. Görgen, J. Pettersson, and A. M. Lampi, *J. Chromatogr. A*, **881**, 217–227 (2000).
332. R. Yamauchi, H. Noro, M. Shimoyamada, and K. Kato, *Lipids*, **37**, 515–522 (2002).
333. M. F. Ramadan and J. T. Morsel, *Eur. Food Res. Technol.*, **214**, 521–527 (2002).
334. E. Gimeno, E. Calero, A. Castellote, R. M. Lamuela-Raventos, M. C. de la Torre, and M. C. Lopez-Sabater, *J. Chromatogr. A*, **881**, 255–259 (2000).
335. D. Darnoko, M. Cheryan, E. Moros, J. Jerrel, and E. G. Perkins, *J. Liquid Chromatogr. Rel. Technol.*, **23**, 1873–1885 (2000).
336. Y. A. Tan, K. S. Low, and C. L. Chong, *J. Sci. Food Agric.*, **66**, 479–484 (1994).
337. K. Ward, R. Scarth, J. K. Daun, and C. T. Thorsteinson, *J. Amer. Oil Chem. Soc.*, **71**, 931–934 (1994).
338. K. Ward, R. Scarth, J. K. Daun, and C. T. Thorsteinson, *J. Amer. Oil Chem. Soc.*, **71**, 811–815 (1994).
339. G. Lercker and M. T. Rodriguez-Estrada, *J. Chromatogr. A*, **881**, 105–129 (2000).
340. P. M. Panagiotopoulou and M. Tsimidou, *Grasas y Aceites*, **53**, 84–95 (2002).
341. A. Bendini, M. Bonoli, L. Cerretani, B. Biguzzi, G. Lercker, and T. G. Toschi, *J. Chromatogr. A*, **985**, 425–433 (2003).
342. S. Gomez-Alonso, M. D. Salvador, and G. Fregapane, *J. Agric. Food Chem.*, **50**, 6812–6817 (2002).
343. C. Capannesi, I. Palchetti, M. Mascini, and A. Parenti, *Food Chem.*, **71**, 553–562 (2000).

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## *Lecithins*

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### **1. INTRODUCTION**

Phospholipids are lipids containing a phosphoric acid residue; they are nature's principal surface-active agents. They are found in all living cells, whether of animal or plant origin. In humans and in animals, the phospholipids are concentrated in the vital organs, such as the brain, liver, and kidney; in vegetables, they are highest in the seeds, nuts, and grains. As constituents of cell membranes, and active participants in metabolic processes, they are essential to life (1–8).

The commercial term “lecithin” is very general, and it describes a composition of lipid constituents and surface-active compounds present in the product rather than in the chemical entity: phosphatidylcholine (PC). Thus, in general usage, lecithin refers to a complex, naturally occurring mixture of polar lipids obtained by water-degumming crude vegetable oils and separating and drying the hydrated gums (8). It is, however, the phospholipid portion of lecithin that is mainly responsible for giving form and function to commercial lecithin (6).

Commercial lecithin is an important coproduct of edible oil processing because of its dietary significance and multifaceted functionality in food systems and industrial applications. Unless indicated otherwise, the term “lecithin” will denote the commercial designation throughout the text in this chapter.

Lecithin has a long history of use in foods, dating back more than 60 years (9). The 1930s brought widespread use of commercial solvent extraction techniques for

vegetable oil production, and because “degumming” of crude vegetable oil became necessary for shipping stability, a large supply of crude lecithin “gums” was produced. These gums were obtained in sufficient quantity to necessitate their becoming an item of commerce (9). In the ensuing years, there was extensive research into developing new lecithin applications, as well as product refinements and modifications. Lecithin ingredients are now recognized as valuable products that have both nutritional value and commercial, i.e., food/feed/industrial utility (2, 5).

Two of the earliest edible applications of lecithin, viscosity reduction in chocolate and confectionery products, and emulsification/antispatter properties in margarine, still enjoy wide popularity and represent outlets for large volumes of lecithin products. In addition, other early uses such as in bakery goods, pasta, textiles, insecticides, and paints, among others, are still active today.

## 2. SOURCES OF PHOSPHOLIPIDS

### 2.1. Human/Animal Tissues

Almost all body cells contain phospholipids. The common animal phospholipids are made of sphingomyelin, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and other glycerol phospholipids of complex fatty acid composition. PC, formerly referred to as “lecithin,” PE, formerly referred to as “cephalin,” and PS are by far the most predominant phospholipids from most animal sources. As constituents of cell walls and active participants in metabolic processes, they appear to be essential to life (8).

The exact composition of human/animal phospholipids depends on the source and the method of extraction and purification. The central nervous system especially has a high phospholipid content. The liver is the site for their biosynthesis, and the lipids of the mitochondria, which are the regulators of cell metabolism and energy production in the body, consist of up to 90% phospholipids (10).

A survey of improved fractionation and analytical methods for elucidating the molecular species of these complex animal phospholipids, and the phospholipids present in primary and processed foods, has been published by Kuksis (11). This survey also includes information on the quantitative analysis of phospholipids, peroxidation products of phospholipids, and the composition of selected animal phospholipids.

Only egg yolk, milk, and brain have served as major animal sources of commercial lecithin. In some instances, isolated and purified lecithins have been developed for clinical nutritional uses. Weihrauch and Son (12) present a concise review of phospholipid composition in various foods.

The following brief discussion covers phospholipids from animal sources that have some commercial significance.

*Egg phospholipids.* At one time, eggs, which possess a relatively high phospholipid content, served as a commercial source until soybean technology made it uneconomical to produce. The phospholipids in eggs are mainly in the yolk, where at least a portion of them are combined with protein and carbohydrates.



**TABLE 1. Composition of Soy and Egg Lecithin (14).**

Polar Lipids	Soy	Egg
Phosphatidylcholine	20–22	68–72
Phosphatidylethanolamine	21–23	12–16
Phosphatidylinositol	18–20	0–2
Phosphatidic acid	4–8	—
Sphingomyelin	—	2–4
Other phospholipids	15	10
Glycolipids	9–12	—

Compositional data on commercial egg products and various lipid extracts from egg yolk have been compiled by Gornall and Kuksis (13), Kuksis (11), Schneider (14), and Satirhos et al. (15). Tables 1 and 2 (14, 16) compare the composition of soy and egg lecithins and their fatty acids, and Table 3 (16–21) shows the distribution of phospholipid classes in egg yolk.

Besides phospholipid composition, the main difference between plant/legume lecithin (e.g., soy) and lecithin in egg yolk is that the former has a higher unsaturated fatty acid content and no cholesterol. Egg lecithin as a commercial ingredient, with the exception of some medical feeding programs, is too expensive for routine use in food (10). In some infant formulas, egg yolk lipids and egg lecithin are used (22).

*Milk phospholipids.* Milk has a phospholipid content of about 0.035%, which is associated with the fat by virtue of being part of a colloidal membrane that surrounds the fat globule. Wittcoff (4), Morrison et al. (23), and Privett et al. (24) have reported the results of TLC analysis of the polar lipids of various milk fractions. Recently, Jensen (25) published extensive high performance liquid chromatography (HPLC) results of the phospholipid composition of cow's milk during lactation, and typical data on phospholipid, sphingolipid, and glycosphingolipid classes in bovine milk. The feeding regime of fatty acids in the diet affects the fatty acid composition of the milk lipids to a certain extent. It might be expected that the fatty acid composition of the phospholipids will be affected as well.

**TABLE 2. Fatty Acid Composition (%) (14).**

Type of Acid	Soy	Egg
Saturated		
Palmitic	15–18	27–29
Stearic	3–6	14–17
Unsaturated		
Oleic	9–11	35–38
Linoleic	56–60	15–18
Linolenic	6–9	0–1
Arachidonic	0	3–5

TABLE 3. Distribution of Phospholipid Classes in Egg Yolk (16).

Phospholipid Classes	Analyses					
	a	b	c	d	e,f	e,g
% Total lipid (w/w)				23		
PC	66–76	69.1	77.0	69	82.6	87.1
PE	15–24	23.9	18.0	24	9.1	7.8
PS	1	2.7		Trace		
CL	1	3.2		Trace		
SPH	3–6	1.0	2.3	3	1.8	2.5
LPC + LPE	3–6		2.5	3	6.5	2.5
Unidentified						

<sup>a</sup>Privett et al. (17).<sup>b</sup>Noble and Moore (18).<sup>c</sup>Cook and Martin (19).<sup>d</sup>Gornall and Kuksis (20).<sup>e</sup>Connelly and Kuksis (21).<sup>f</sup>Commercial sample.<sup>g</sup>Intralipid.

Skim milk and milk serum have the highest portion of polar lipids as percent of the total lipids, and whole milk and cream have the least. Of the polar lipids, phosphatidylethanolamine constitutes the largest component, with phosphatidylcholine and sphingomyelin (being present in about equal proportions) at a significantly lower level (Table 4) (16, 23, 24, 26).

*Brain phospholipids.* The brain is a rich source of phospholipids, and together with the spinal cord, it probably possesses the highest phospholipid content of any of the organs. There are many different types of phospholipids in the central nervous system. As they bypass the blood-brain barrier, adequate nutrition (biosynthesis) of the nerve cells is assured with these substances. Special

TABLE 4. Distribution of Phospholipid Classes in Various Milk Fractions (weight %) (16, 24).

Lipid Classes	Whole Milk	Plastic Skim	Nonfat Skim	Serum	Cream	Milk <sup>a</sup>	Serum <sup>b</sup>
% Total lipid	2.0	19.0	32.0	22.0	1.0–2.0	—	—
Ceramide monohexoside	Trace	19.5	14.1	7.8	17.9	3.0	—
Ceramide dihexoside	Trace	10.1	—	—	—	3.0	—
Phosphatidylethanolamine	36.4	27.5	45.1	32.4	25.3	30.0	26.9
Plasmalogen	—	—	—	—	—	1.0	—
Phosphatidylcholine	27.0	7.3	16.4	23.2	19.9	28.0	29.3
Plasmalogen	—	—	—	—	—	3.0	—
Sphingomyelin	29.0	18.2	14.5	26.9	21.6	19.0	31.3
Phosphatidylserine	Trace	1.9	7.6	3.5	Trace	8.0	5.0
Phosphatidylinositol	Trace	Trace	Trace	1.1	Trace	5.0	5.9
Ganglioside + (unknown)	7.6	15.5 <sup>4</sup>	2.3 <sup>3</sup>	5.1 <sup>4</sup>	15.3 <sup>4</sup>	0.0	1.0

<sup>a</sup>Morrison et al. (23).<sup>b</sup>Santha and Narayanan (26).

**TABLE 5. Distribution of Phospholipid Classes in Brain of Different Animal Species (weight %) (16).**

Lipid Classes	Animal Species							
	Human <sup>a,b</sup>	Bovine <sup>a,b</sup>	Bovine <sup>b,c</sup>	Human <sup>c,d</sup>	Bovine <sup>c,e</sup>	Human <sup>c,e</sup>	Sheep <sup>f</sup>	Rat <sup>g</sup>
% Total lipid								
PC	21.8	18.4	32.4	33.2	48.2	47.6	37.3	36.8
Plasmalogen	—	—	—	—	—	—	0.9	—
PE	35.4	36.1	23.5	25.2	24.2	17.8	7.7	36.4
Plasmalogen	—	—	—	—	—	—	16.5	—
PS	18.8	18.0	11.0	10.7	6.7	9.3	9.2	11.8
PI	1.8	1.8	4.3	4.8	7.1	5.0	2.1	3.1
PA	1.1	1.7	0.9	0.3	1.3	1.2	2.6	1.2
CL	—	—	1.2	1.0	ND <sup>h</sup>	0.3	2.0	2.2
PG	—	—	0.9	ND <sup>h</sup>	0.4	0.6	—	—
LPC (LPE)	2.0	0.2	0.9	1.0	1.0	2.5	—	—
LbisPC	1.0	0.4	2.1	0.2	0.7	0.2	—	—
SPH	16.3	15.0	20.4	17.0	4.9	10.7	12.9	5.7
Unidentified								

<sup>a</sup>Siakotos et al. (28).<sup>b</sup>Myelin.<sup>c</sup>Siakotos et al. (27).<sup>d</sup>Endothelial cells.<sup>e</sup>Nuclei.<sup>f</sup>Scott et al. (29).<sup>g</sup>Wuthier (30).<sup>h</sup>ND = not detected.

enzyme systems see to it that the most efficient functioning is accomplished at all times (4).

The composition of brain phospholipids has been extensively investigated by adsorption column- and thin-layer chromatography (TLC). Table 5 lists the major classes of brain phospholipids from different animal species, as compiled by Kuksis (16, 27–30). At the end of the 1990s, the fear of mad cow disease (BSE) may have addressed the purity criteria for the applications of brain phospholipids from cows.

*Phospholipids in liver, kidney, muscle, and other tissues.* Organ meats such as liver, kidney, and muscles are a major source of dietary phospholipids. The reader is referred to Kuksis (16) for the distribution of various phospholipid classes in the liver, kidney, muscles (heart and skeletal), spleen, lung, blood cells, bile, and adipose tissue of different animal species. Compositional data of fatty acids for these tissues and fluids are also given.

In blood, phosphatidylcholine is quantitatively the most important phospholipid. Sphingomyelin is present in varying amounts in perhaps all of the animal organs, most of it in the soft organs, and to a lesser degree in skeletal muscles and eggs (4). Total blood contains about 0.2% to 0.3% phospholipids. In plasma and serum, phosphatidylcholine predominates, whereas in corpuscles, phosphatidylethanolamine and sphingomyelin constitute the bulk of phospholipids. Most workers have found

that the phospholipid content is greater in red blood cells than in plasma, and it constitutes 60% to 65% of the total lipids in these cells (4).

## 2.2. Soybean

Although the highest concentrations of phospholipids occur in animal products, i.e., meat, poultry, fish, eggs, and milk/cheese, the major commercial source is the soybean, which contains 0.3% to 0.6%. Nevertheless, phospholipids from other vegetable oilseeds, i.e., corn, cottonseed, linseed, peanut, rapeseed, safflower, and sunflower, and plants have also been studied and used (5).

Standard-grade, commercial lecithin from the soybean is a complex mixture. It comprises phospholipids, triglycerides, and minor amounts of other constituents (i.e., phytoglycolipids, phytosterols, tocopherols, and fatty acids). The composition and molecular arrangement of this heterogeneous mixture of compounds defines a product that is low in apparent polarity and has a strong tendency to promote water-in-oil (w/o)-type emulsions (31).

A wide range of data has been published showing the variability in the composition of phospholipids and fatty acids in soybean lecithin (Tables 6 and 7) (32). Older data were often determined by qualitative TLC, whereas today <sup>31</sup>P-NMR, quantitative Li-Sc HPLC, and HPTLC methods have been developed.

Soy lecithin is a coproduct of oil processing. As a result, purification steps used to produce quality oil may affect the lecithin components. Also, soybeans exposed to frost damage, or subjected to prolonged storage, have reduced lecithin yields (33). Phospholipases, which produce phosphatidic acid, are active during storage and may reduce the yield of lecithin (34). During the maturation process, the major phospholipids (PC, PE, and PI) increase, and others decrease or remain constant (32).

A change in the relative concentration of any of these components, or an alteration of their chemical structures, may cause some change in the physical or chemical properties of commercial lecithins. Lecithins can exist as liquids, plastics,

**TABLE 6. Components (%) of Soybean Lecithin (32).**

Component	Range of Composition		
	Low	Intermediate	High
Phosphatidylcholine	12.0–21.0	29.0–39.0	41.0–46.0
Phosphatidylethanolamine	8.0–9.5	20.0–26.3	31.0–34.0
Phosphatidylinositol	1.7–7.0	13.0–17.5	19.0–21.0
Phosphatidic acid	0.2–1.5	5.0–9.0	14.0
Phosphatidylserine	0.2	5.9–6.3	—
Lysophosphatidylcholine	1.5	8.5	—
Lysophosphatidylinositol	0.4–1.8	—	—
Lysophosphatidylserine	1.0	—	—
Lysophosphatidic acid	1.0	—	—
Phytoglycolipids	—	14.3–15.4	29.6

**TABLE 7. Fatty Acids (%) of Soybean Lecithin (32).**

Fatty Acid	Range of Composition		
	Low	Intermediate	High
Myristic (C14:0)	0.3–1.9	—	—
Palmitic (C16:0)	11.7–18.9	21.5–26.7	42.7
Palmitoleic (C16:1)	7.0–8.6	—	—
Stearic (C18:0)	3.7–4.3	9.3–11.7	—
Oleic (C18:1)	6.8–9.8	17.0–25.1	39.4
Linoleic (C18:2)	17.1–20.0	37.0–40.0	55.0–60.8
Linolenic (C18:3)	1.6	4.0–6.2	9.2
Arachidic (C20:0)	1.4–2.3	—	—

or free-flowing solids. Their color, solvent solubility, surfactant properties, and chemical reactivity all can be modified. These modifications, in turn, will alter the functional properties of the lecithin in a given application (31).

The ensuing section is a brief review of phospholipid plant sources other than those from the soybean, which have current or potential commercial applications. Soybean lecithin then will be discussed in more detail later throughout the subsequent sections in this chapter.

### 2.3. Corn

Weeks and Walters (35) have found that 2.5% to 4.5% of the phosphorus in corn is in the form of phospholipids, depending on the variety involved.

The first detailed analysis of commercial corn phospholipids was published by Scholfield et al. (36). Unlike the phenomenal growth in demand for corn sweeteners

**TABLE 8. Distribution (%) of Polar Lipids in Corn and Soybean Lecithin (32).**

Polar Lipid	Corn	Soybean
Sterylglycoside ester	15.0	4.3
Monogalactosyldiglyceride	1.8	0.8
Digalactosyldiglyceride	3.7	3.0
Other glycolipids	9.8	6.4
<i>N</i> -Acyl phosphatidylethanolamine	2.6	2.2
<i>N</i> -Acyl lysophosphatidylethanolamine	3.7	10.4
Phosphatidylethanolamine	3.2	14.1
Phosphatidylglycerol	1.4	1.0
Phosphatidylcholine	30.4	33.0
Phosphatidylinositol	16.3	16.8
Phosphatidic acid	9.4	6.4
Phosphatidylserine	1.0	0.4
Lysophosphatidylethanolamine	Trace	0.2
Lysophosphatidylcholine	1.7	0.9

**TABLE 9. Fatty Acid Composition (%) of Corn and Soybean Lecithin (32).**

Fatty Acid	Composition	
	Corn	Soybean
Palmitic (C16:0)	17.7	17.4
Stearic (C18:0)	1.8	4.0
Oleic (C18:1)	25.3	17.7
Linoleic (C18:2)	54.2	54.0
Linolenic (C18:3)	1.0	6.8

and other products of the corn-refining industry, the commercial exploitation of the coproduct corn lecithin has not taken place in large quantities. Tables 8 and 9 illustrate the distribution of polar lipids and fatty acids in corn lecithin compared with those in soybean lecithin (32).

Similar compositions were noted for corn and soy PC and PI. Phosphatidic acid and glycolipids represent a higher proportion of polar lipids in corn than in soybean lecithin. Cherry (37) and Cherry and Kramer (32) also stated that the percentage of minor components in corn, steryl-glycoside ester, and other glycolipids are more than twice that found in soybean. The physical properties, particularly the emulsifying properties of corn lecithin, differ from those of soybean lecithin because of the higher proportion of glycolipids in the corn lecithin.

Both the glycolipids and the phospholipids of corn have lower percentages of linolenic acid (18:3) and are more saturated than those in the soybean. In general, crude corn and soybean lecithins are equal in linoleic acid (18:2) content, but linoleic acid in corn varies from 42% to 70% depending on the variety of corn. Phytic acid, 88% of which is in the corn germ, is extracted as part of the lecithin fraction (32, 37). Elimination of phytic acid in corn is desirable because it binds zinc, magnesium, and calcium.

## 2.4. Cottonseed

The phospholipids in cottonseed are similar in many respects to those of soybeans, with the exception of their lower level of linolenic and higher level of saturated fatty acid content (38). Cherry and Kramer (32) compiled Table 10 to show the composition of cottonseed lecithin.

Lecithin can be fractionated from cottonseed as phospholipids and glycolipids. Cottonseed lecithin shows flavor and color deterioration when blended with other vegetable oils. The saturated/unsaturated fatty acid ratio of cottonseed phospholipids is approximately 1:2 (39). Palmitic acid constitutes 90% of the total saturated fatty acids (36%), and linoleic acid is approximately 80% of the total unsaturated fatty acids (64%). Gossypol binds to lecithin during oil extraction from glanded cottonseed (approximately 9% in crude phospholipids). This economically negates its

**TABLE 10. Composition (%) of Cottonseed Lecithin (32).**

Component	Extract	Phosphatidylcholine	Phosphatidylethanolamine	Phosphatidylserine
Phospholipids	1.8–2.2	34.9–35.9	13.7–20.1	7.0–26.0
Fatty acids				
Myristic (C14:0)	0.4	0.3	0.4	0.6
Palmitic (C16:0)	32.9	31.1	33.7	33.3
Palmitoleic (C16:1)	0.5	0.3	0.3	0.6
Stearic (C18:0)	2.7	2.8	2.2	0.3
Oleic (C18:1)	13.6	11.5	11.5	14.4
Linoleic (C18:2)	50.0	54.0	49.0	50.4
Total gossypol	9.13	2.34	22.43	19.90
Free gossypol	0.02	2.24	0.05	0.01

use as a commercial source. Cultivars of glandless or gossypol-free cottonseed may have some potential for providing commercial edible lecithins (37).

The composition of cottonseed lecithin and the composition of the phospholipid fraction from hexane-defatted glandless cottonseed oil are summarized in Tables 10 and 11 (32, 37, 40–42).

As cottonseed lecithin contains only trace amounts of fatty acids with more than two double bonds (linolenic acid), it is more stable to oxidation and rancidity than soybean lecithin. Cottonseed phospholipids are relatively high in phosphatidylcholine, which could provide good emulsifying properties in foods (32, 37).

**TABLE 11. Composition of Phospholipid Fraction from Glandless Cottonseed Oil (37, 40, 41).**

Phospholipid <sup>a</sup>	Composition (% of Total Phosphorus)
Origin	4.12
Lysophosphatidylcholine	2.56
Phosphatidylinositol	13.41
Phosphatidylserine	2.38
Phosphatidic acid	8.76
Phosphatidylcholine	23.16
Phosphatidylethanolamine	13.46
Phosphatidylglycerol	7.62
Lysophosphatidylserine	ND <sup>b</sup>
Lysophosphatidylethanolamine	ND
Unknown (sum: 6 TLC spots)	25.30

<sup>a</sup>Water (2–4%) was added to hexane-extracted glandless cottonseed oil, the resulting mixture stirred 30 minutes at 70°C, and centrifuged to separate the oil and phospholipid-containing fraction. The phospholipids were separated by two-dimensional thin-layer chromatography (TLC) on Silica gel-60 plates. Dimension I = chloroform: methanol:7N NH<sub>4</sub>OH (65:30:1); Dimension II = chloroform: methanol:acetic acid:water (170:25:25:4). Quantitation of the phospholipids was according to El-Sebaïy et al. (42).

<sup>b</sup>ND = not detected.

## 2.5. Rapeseed

Rewald (43) found approximately 20% phospholipids in rapeseed. Rapeseed lecithin has been reviewed, and an extensive bibliography has been compiled (44). Table 12 (44) shows the composition of rapeseed and soybean gums.

The major phospholipids present in rapeseed lecithin are phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. The relative proportion of these components does not differ significantly from that of soybean lecithin. In lecithin from high erucic oils, the long-chain fatty acids (C20–C22) are present only in small amounts, and in low erucic oil lecithin (including canola), the fatty acid composition is not markedly different from that of the parent oil, except for a somewhat higher content of C16:0, leaving a slightly higher C18:1 and C18:3 fatty acid level in the degummed oil (44).

Solvent-extracted rapeseed oil has been found to contain the highest level of phosphorus. For this reason, it is common practice to degum solvent-extracted oil or the mixed crude oil from pressing and subsequent solvent-extraction. As the double-zero rapeseed varieties such as canola became available, the applications of rapeseed lecithin have developed positively. Where at first rapeseed lecithin was applied as an emulsifier and energy component in animal feed, the recent concerns about GMO soybean varieties in some parts of the world have increased the market value of the softseed lecithins for food applications (45). The phospholipid composition is similar to soybean lecithin with variations due to crop and processing conditions. The rapeseed phospholipid compositions in Table 12 have been confirmed by recent data, whereas the soybean lecithin composition in

**TABLE 12. Composition of Rapeseed and Soybean Gums (%) (44).**

Components	Rapeseed					Soybean
	(9) <sup>a</sup>	(9) <sup>b</sup>	(10) <sup>b</sup>	(11) <sup>b</sup>	(8) <sup>c</sup>	(8) <sup>c</sup>
Water	24	—	—	—	—	—
Nonlipid	9	—	—	—	—	—
Triglycerides or neutral lipids	16	—	38.1	5.6	29	35
Phospholipids (total)	51	—	—	—	—	—
Phosphatidylcholine	—	22	16.2	24.6	20	21
Phosphatidylethanolamine	—	15	17.5	22.1	16	8
Phosphatidylinositol	—	18	7.6	14.7 <sup>d</sup>	8	20
Lysophosphatidylcholine	—	—	—	19.4	1	0
Lysophosphatidylethanolamine	—	—	2.0	—	—	—
Sterol glycosides or glycolipids	—	9 <sup>e</sup>	7.9	13.6	11	0
Unidentified	—	36 <sup>f</sup>	10.7	—	15	11
Unaccounted for	—	—	—	—	—	5

<sup>a</sup>Complete gum sample, including water.

<sup>b</sup>Acetone precipitate from gum sample.

<sup>c</sup>Dried gum sample.

<sup>d</sup>Tentatively identified.

<sup>e</sup>Tentatively: phytoglycolipids.

<sup>f</sup>Tentatively: 16% acidic phospholipids (plus 20% unidentified).



the last column is not representative because of the low phosphatidylethanolamine content.

Sosada (46) determined the optimum conditions for fractionation of rapeseed lecithin with alcohols to improve purified lecithin yield and phosphatidylcholine enrichment.

## 2.6. Sunflower and Peanut

Although sunflower lecithin currently is not used to any great extent, as sunflower oil production is increased, the availability of lecithin from this oil may be a possibility (47). Because of its high phosphatidylcholine content, sunflower lecithin can be used in foods and feedstuffs. Its use in the manufacture of foods and cosmetics can be increased by refining and fractionation and/or modifications (48). Sunflower lecithin has a mild taste and similar emulsifying properties as soybean lecithin. The crude lecithin is pastier than soybean lecithin, because of waxes, but exact adjustment of the oil and acid content in refined sunflower lecithin results in an effective emulsifier with good handling properties. This makes sunflower lecithin interesting for food manufacturers, particularly in Europe, the biggest sunflower seed producing/processing continent.

The percentage of phospholipids in sunflower oil ranges from 0.02% to 1.5%, with an average of around 0.75%. The composition of the phospholipids is similar to soybean lecithin, with a tendency to higher phosphatidylcholine and lower phosphatidylethanolamine ratios, which might be caused by crop varieties and processing conditions. Hollo et al. (49) report positive results of physical and enzymatic modification of sunflower lecithin for improving the emulsifying properties.

Hilditch and Zaky (50) found that phospholipids in peanuts are considerably less unsaturated than those in soybean and cottonseed. Rewald (51) has shown 35.7% phosphatidylcholine and 64.3% phosphatidylethanolamine content in peanut phospholipids.

## 2.7. Other Plants and Micro-Organisms

Table 13 shows the phospholipid composition of selected plant sources (32). Parsons and Price (52) have published compositional data on the phospholipids of barley grain based on thin-layer chromatography.

Other than in animal tissues, egg, and oilseeds, quantitative data on the phospholipids in plants are meager because of the difficulty involved in their isolation. The phospholipids in wheat are about 80% PC and 20% PE (4). Rye, barley, and other grains, vegetables, and fruits all contain small amounts of phospholipids. Micro-organisms, especially those that are acid-fast, and lower plants, also contain large amounts of lipids, including phospholipids; these entities are of interest for clinical research (4). A survey of microbiological sources of phospholipids has been published by Ratledge (53).

**TABLE 13. Phospholipids (%) of Selected Plant Sources (32).**

Source	Phospholipids		
	Phosphatidylcholine	Phosphatidylethanolamine	Phosphatidylinositol
Rapeseed	16.2; 20.0–24.6	15.0–17.5; 22.1	7.6–8.0; 14.7–18.0
Sunflower seed	12.7–26.8; 42.2–64.2	9.9–29.4; 46.6	3.7–21.4; 24.0–36.6
Peanut seed	49.0	16.0	22.0
Cucurbit seed	55.8–74.9	10.5–18.7	13.7–17.2
Rice bran	20.4–23.1	17.8–20.2	5.8–6.6
Barley seed	44.3–44.4	7.6–8.8	1.1–1.3
Olive fruit	47.3–58.9	5.3–8.0	18.0–23.9
Avocado fruit	37.0–44.9	12.0–19.5	12.1–18.0
Palash seed	44.6	14.8	27.0
Jangli badam seed	30.0	23.0	40.6
Papaya seed	28.1	18.7	34.0
Coriander seed	44.0	29.3	23.1
Carrot seed	29.1	35.4	23.1

### 3. NOMENCLATURE, CLASSIFICATION, STRUCTURE AND COMPOSITION, AND CHEMICAL/PHYSICAL PROPERTIES

#### 3.1. Definition

According to Wittcoff (4), three distinct polymeric alcohols provide the basic constituents for the various phospholipids. The first of these is glycerol, and the phospholipids containing it are referred to as glycerophospholipids. Included herein, in addition to PC, PE, and PS, are the acetalphospholipids or plasmalogens (in body fluids, muscles, and egg), the lysophospholipids, and the phosphatidic acids. The second polyhydric alcohol is the amino-dihydroxy compound sphingosine, which is the basis for not only sphingomyelin (in the brain and spinal cord), but also for other glycolipids. All of these compounds based on sphingosine are also referred to as sphingolipids. The third polyhydric alcohol is inositol, which is included in PI.

Phospholipids also form complexes with proteins (e.g., vitellin in egg yolk, animal and plant tissues, lipoproteins in blood serum, and milk), carbohydrates, glycosides, alkaloids, minerals, enzymes, cholesterol, and other substances. Lysophospholipids represent a special class of compounds resulting from the chemical or enzymatic hydrolysis of phospholipids. The role of phospholipases in normal and pathological conditions as well as in cell metabolism is of great biological significance (4).

For the elucidation, synthesis, chemical properties, physical chemistry, composition, and analytical determination of the various individual phospholipid structures in animal and plant sources, the reader is referred to Wittcoff (4). Schneider (14) discusses the nomenclature used for phospholipids in more detail and provides compositional data on commercial lecithins (Table 14).

Because of the commercial significance of soybean lecithin, this chapter will focus primarily on the structure, composition, analytical determination, properties, and applications of this product.

**TABLE 14. Composition of Commercial Lecithins (%) (on Oil-Free Basis) (14).**

Lecithin	Soy	Corn	Sunflower	Rapeseed	Egg	Bovine Brain
Phosphatidylcholine	21	31	14	37	69	18
Phosphatidylethanolamine	22	3	24	29	24	36
Phosphatidylinositol	19	16	13	14	—	2
Phosphatidic acid	10	9	7	—	—	2
Phosphatidylserine	1	1	—	—	3	18
Sphingomyelin	—	—	—	—	1	15
Glycolipids	12	30	—	20	—	—

The U.S. Food Chemical Codex (54) defines lecithin as follows:

Food-grade lecithin is obtained from soybeans and other plant sources. It is a complex mixture of acetone-insoluble phosphatides that consist chiefly of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol, combined with various amounts of other substances such as triglycerides, fatty acids and carbohydrates. Refined grades of lecithin may contain any of these components in varying proportions and combinations depending on the type of fractionation used. In its oil-free form, the preponderance of triglycerides and fatty acids is removed and the product contains 90% or more of phosphatides, representing all or certain fractions of the total phosphatide complex. The consistency of both natural grades and refined grades of lecithin may vary from plastic to fluid, depending on the free fatty acid and oil content, and upon the presence or absence of other diluents. . .

### 3.2. Classification of Commercial Soybean Lecithin Products

The simplest method for modifying natural (crude) lecithin is the addition of a non-reactive substance. Plastic lecithins are converted to fluid forms by adding 2% to 5% fatty acids and/or carriers such as soybean oil. If the additives react with the lecithin to alter the chemical structure of one or more of the phospholipid components, the resulting product is referred to as a chemically modified lecithin. Modification can also be achieved by subjecting lecithin to partial controlled enzymatic hydrolysis. Finally, refined lecithin products can be obtained by fractionating the various phospholipid components.

A method for classifying lecithin to include modified and refined forms has been proposed by Cowell et al. (55). This classification distinguishes between natural (crude) lecithins and those modified by either custom blending or chemical/enzymatic treatment, e.g., hydroxylation, hydrogenation, acetylation, or refining by acetone or alcohol fractionation. These latter products reflect the state of the art regarding the availability of the various lecithin products on the market and have enhanced properties for specific uses. A listing of soybean lecithin classifications follows (56).

- I. Crude commercial lecithin
  - A. Plastic
    - 1. Unbleached
    - 2. Single bleached
    - 3. Double bleached
  - B. Fluid
    - 1. Unbleached
    - 2. Single bleached
    - 3. Double bleached
- II. Compounded
- III. Chemically modified
- IV. Refined
  - A. Deoiled
    - 1. As is
    - 2. Custom blended
  - B. Fractionated
    - 1. Alcohol soluble
      - a. As is
      - b. Custom blended
    - 2. Alcohol insoluble
      - a. As is
      - b. Custom blended
  - C. Purified phosphatides

*Natural (crude) lecithins.* Specifications as defined by the *National Soybean Processors Association* (1986–1987) for natural (crude) lecithins is presented in Table 15 (57). Specifications have also been published by the Food Chemicals Codex (1996) (54).

Phospholipid content is specified in terms of acetone-insolubles (AI); product clarity, and purity in terms of hexane-insolubles (HI). The lecithins are classified as plastic or fluid in consistency, and they are further subdivided on the basis of manufacturing procedure as natural color, bleached, or double bleached. Acidity of phospholipids plus acidity of the carrier (i.e., the oil and fatty acids) is given by the acid value (AV), i.e., milligram of potassium hydroxide required to neutralize the acids in 1 g of the lecithin sample. Crude lecithin can be filtered for utmost purity and clarity. Filtration removes hexane-insoluble (HI) matter. Such products are in demand for encapsulated nutritional supplements, for pharmaceutical grades, and for advanced technology industrial uses requiring a high level of purity.

*Compounded lecithins.* Compounded lecithins are blended products. Lecithin combined with selected additives can exhibit modified properties and functionalities. Lecithin may have a synergistic action with some additives or, simply, be

TABLE 15. Soybean Lecithin Specifications (57).

Analysis	Grade					
	Fluid Unbleached Lecithin	Fluid Bleached Lecithin	Fluid Double-Bleached Lecithin	Plastic Natural Lecithin	Plastic Bleached Lecithin	Plastic Double-Bleached Lecithin
Acetone insoluble, min.	62%	62%	62%	65%	65%	65%
Moisture, max. <sup>a</sup>	1%	1%	1%	1%	1%	1%
Hexane insoluble, max.	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
Acid value, (mg KOH/g), max.	32	32	32	30	30	30
Color, Gardner, max. <sup>b</sup>	18	14	12	18	14	12
Viscosity, centipoise, at 25°C, max. <sup>c</sup>	15,000	15,000	15,000	—	—	—
Penetration, max. <sup>d</sup>	—	—	—	22 mm	22 mm	22 mm

<sup>a</sup>By Karl-Fischer Titration (AOCS Method Tb 2-64).

<sup>b</sup>Undiluted basis.

<sup>c</sup>By any appropriate conventional viscosimeter, or by AOCS Bubble Time Method Tq 1A-64, assuming density to be unity. Fluid lecithin having a viscosity less than 7,500 centipoises may be considered a premium grade.

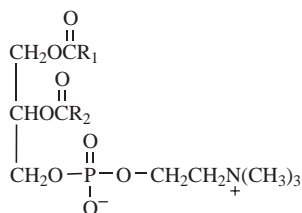
<sup>d</sup>Using Precision cone 73525, Penetrometer 73510; sample conditioned 24 hours at 25°C.

compounded with ingredients for making it more compatible in a particular system. Common additives include special oils, polysorbates, monoglycerides and modified monoglycerides, lanolin derivatives, solvents, plasticizers, or other surfactants. These are added either to the wet gums prior to drying or are blended with dry fluid lecithin at elevated temperatures (7, 58).

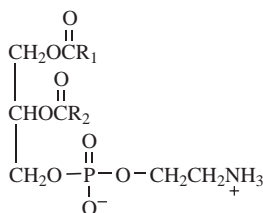
*Modified lecithins.* Lecithins may be modified chemically, e.g., hydrogenation, hydroxylation, acetylation, and by enzymatic hydrolysis, to produce products with improved heat resistance, emulsifying properties, and increased dispersibility in aqueous systems (7, 58, 59). One of the more important products is hydroxylated lecithin, which is easily and quickly dispersed in water and, in many instances, has fat-emulsifying properties superior to the natural product. Hydroxylated lecithin is approved for food applications under Title 21 of the Code of Federal Regulations 172.814 (1998) (60).

*Fractionated and oil-free lecithins.* When crude lecithin is further refined by various fractionation methods to selectively separate its components, acetone and ethanol are the most common solvents used. Fractionation of crude lecithin yielding phosphatidylcholine of greater than 90% purity is done commercially.

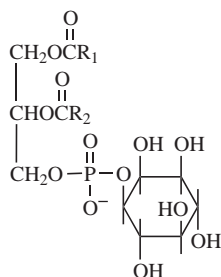
A commercial, nearly oil-free lecithin is prepared by acetone extraction of natural lecithin, which removes all but 2-4% oil and free fatty acids. Then an optional alcohol fractionation step can separate the oil-free lecithin into an alcohol-soluble lecithin enriched in phosphatidylcholine and an alcohol-insoluble fraction enriched in phosphatidylinositol. The choline fraction is an excellent emulsifier for oil-in-water (o/w) emulsions and the inositol fraction for water-in-oil (w/o) emulsions.



Phosphatidylcholine



Phosphatidylethanolamine



Phosphatidylinositol

R<sub>1</sub> and R<sub>2</sub> = C<sub>15</sub>-C<sub>17</sub>Hydrocarbon  
chains**Figure 1.** Three principal components of soybean lecithin (7).

### 3.3. Structure of Phospholipids in Commercial Lecithins

Chemical structures for the most commonly occurring phospholipids in commercial soybean lecithin are shown in Figure 1 (7). PC and PE are cationic and anionic at the same time; that is, they are zwitterions, and thus they can have some buffering action for both bases and acids. PI, however, is a relatively strong acid and, therefore, is anionic. The classes of compounds in commercial lecithin are as follows (31):

#### *Phospholipids*

Anionic

Zwitterionic

#### *Glycolipids*

Steryl glucosides

Esterified steryl  
glucosides

Galactosyl glycerides

The reader is referred to Horrocks (61) for more specific discussion on the nomenclature and structure of phospholipids.

### 3.4. Composition

Specification ranges, chemical and fatty acid compositions for commercial natural lecithins, along with approximate compositional data for commercially refined lecithin fractions are given in Tables 16–19 (8, 62–65), respectively.

**TABLE 16. Specifications for Commercial Soybean Lecithin (62, 63).**

Analysis	Grade					
	Fluid Natural Color Lecithin	Fluid Bleached Lecithin	Fluid Double-Bleached Lecithin	Plastic Natural Color Lecithin	Plastic Bleached Lecithin	Plastic Double-Bleached Lecithin
Acetone insoluble, % min.	62	62	62	65	65	65
Moisture, % max. <sup>a</sup>	1	1	1	1	1	1
Benzene insolubles, % max.	0.3	0.3	0.3	0.3	0.3	0.3
Acid value, max.	32	32	32	30	30	30
Color, Gardner, max. <sup>b</sup>	10	7	4	10	7	4
Viscosity, poises, at 25°C, max.	150	150	150	—	—	—
Penetration, max., in mm <sup>c</sup>	—	—	—	22	22	22

<sup>a</sup>By toluene distillation for 2 hr or less.

<sup>b</sup>As a 5% solution in colorless mineral oil.

<sup>c</sup>By specified cone penetrometer test.

Soybean oil contains 1.5–3.0% phospholipids (71). Crude soybean lecithin has an oil content of about 30%. PC is present at a level of about 16%, PE about 14%, and inositol phospholipids about 12% (7). As can be seen in Table 18 (8), the fatty acid compositions of soybean phospholipids are rich in polyunsaturated fatty acids. Miscellaneous low-level constituents include water, phosphatidic acid, pigments, galactosyl glycerides, various glycolipids, phosphatidylserine, carbohydrates, sterols, and tocopherols. Phosphorus content of crude soybean oil extracted from flours can vary depending on extraction temperature and flour moisture (72).

### 3.5. Chemical/Physical Properties

In practice, commercial lecithin products are not marketed by phospholipid content, but rather by a set of unique chemical and physical properties. These properties, as indicated by product specifications, must be understood because they are used to characterize specific lecithin types.

**TABLE 17. Approximate Chemical Composition of Natural Commercial Soybean Lecithin (64).**

Fraction	%
Soybean oil	35
Phosphatidylcholine	16
Phosphatidylethanolamine	14
Phosphatidylinositol	10
Phytoglycolipids and other minor phosphatides and constituents	17
Carbohydrates	7
Moisture	1

**TABLE 18. Composition Fatty Acids of Soybean Lecithin (%) (8).**

Reference	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20–22
										Unsaturated
Hilditch and Zaky (50)	—	—	11.7	8.6	4.0	9.8	55.0	4.0	1.4	5.5
Rzhekhin et al. <sup>a</sup> (66)	—	—	18.1	—	3.7	22.4	40.0	5.0	2.3	6.2
Vijayalakshmi and Rao <sup>b</sup> (67)	—	—	42.7	7.0	11.7	17.0	20.0	1.6	—	—
Daga (68)	1.9	Trace	26.7	—	9.3	25.1	37.0	—	—	—
Daga (69)	0.3	1.2	25.5	—	10.3	39.4	17.1	6.2	—	—
Rydhag and Wilton <sup>c</sup> (70)	—	—	21.5	—	4.3	7.2	60.9	6.1	—	—
Rydhag and Wilton <sup>d</sup> (70)	—	—	18.9	—	4.1	6.8	60.8	9.2	—	—

<sup>a</sup>Also 2.3% unidentified.<sup>b</sup>CHCl<sub>3</sub>/CH<sub>3</sub>OH extraction.<sup>c</sup>Acetone-precipitated.<sup>d</sup>Granulated.

Commercial soybean lecithin, being a complex mixture of polar lipids, performs as a wetting and emulsifying agent. Stanley (1) states that in heterogeneous systems such as oil and water, the phospholipid molecules arrange themselves in monomolecular layers with the fatty acid portion facing the oil surface and the phosphoric acid portion facing the water surface. The arrangement lowers the interfacial tension of the oil–water boundaries with resultant benefits such as rapid wetting, lowering of viscosity, and better and more stable emulsions or dispersions.

Soybean lecithin is soluble in aliphatic and aromatic hydrocarbon solvents, partially soluble in ethyl alcohol (principally the inositol fraction), and practically insoluble in acetone (less than 0.003% weight/volume at 5°C) and in water (73). When mixed with water, soybean lecithin hydrates to a thick emulsion that can be thinned with water to almost any desired dilution. Acetone does dissolve readily in lecithin and will form a thin, uniform imbibition as long as the quantity of acetone is insufficient to precipitate the phospholipids. Lecithin is soluble in

**TABLE 19. Approximate Composition of Commercially Refined Lecithin Fractions (%) (65).**

Fraction	Oil-Free Lecithin	Alcohol-Soluble Lecithin	Alcohol-Insoluble Lecithin
Phosphatidylcholine	29	60	4
Cephalin	29	30	29
Inositol and other phosphatides, including glycolipids	32	2	55
Soybean oil	3	4	4
Other constituents <sup>a</sup>	7	4	8
Emulsion type favored	Either oil-in-water or water-in-oil	Oil-in-water	Water-in-oil

<sup>a</sup>Includes sucrose, raffinose, stachyose, and about 1% moisture.



mineral oils and fatty acids, and practically insoluble in cold vegetable and animal oils, but it will dissolve in hot oils.

Soybean lecithin has a brown to light yellow color, depending on the conditions used in its manufacture and the degree of bleaching.

*Identification and characterization of phospholipids.* For additional information on various techniques and methods used in the identification and characterization of phospholipids in general, the reader is referred to Kramer et al. (74).

*Fractionation and purification of lecithin.* Because of space limitations, it is not possible to discuss fractionation and purification processes for all vegetable and animal lecithins in this chapter. The reader is referred to Schneider (14) who described the fractionation and purification of various vegetable lecithins and those from egg in considerable detail. Small-scale fractionation processes may include separation of neutral oil and polar lipids (deoiling) including the use of acetone; the adsorption of a hexane solution of lecithin on a silica column; separating neutral and polar lipids from a hexane solution with the aid of membranes; treatment of lipid mixtures with supercritical gases or gas mixtures, e.g., carbon dioxide or propane-carbon dioxide; fractionation of neutral oil containing lecithins by solvent treatment, e.g., aqueous methanol, ethanol, and propanol; fractionation of de-oiled lecithins by solvent treatment, e.g., ethanol; solvent treatment after chemical modification, e.g., acylation prior to acetone de-oiling; precipitation methods, e.g., salt; ultrafiltration methods; and many chromatographic processes, mainly for polar lipid separation but also for separation focused on the degree of unsaturation. The commercial manufacture of fractionated soybean lecithins will be covered later in this chapter.

*Synthesis and modification of phospholipids.* For an excellent review of the synthesis and modification of phospholipids, the reader is referred to Ghyczy (75). According to the review, depending on the starting material used, there are two ways to synthesize phospholipids. In the partial synthesis, phospholipids are isolated from natural sources and the individual constituents, fatty acids, and head groups are exchanged to obtain a certain phospholipid. In the total synthesis, phospholipids are produced from fully synthesized, available, basic molecules that were not obtained from phospholipids. Both methods are of importance today because each manufacturing process has certain advantages with regard to definite products and fields of application.

The partial synthesis may involve several synthetic steps, depending on the basic phospholipid used, the enzyme, the final product desired, and the type and position of the phospholipid constituents to be exchanged. For example, the partial synthesis may avail of the reacylation of 3-*sn*-glycerophosphorylcholine (GPC). Alternatively, by a deacylation step, GPC can be obtained from PC in soybean lecithin (75).

Partial synthesis may include synthesizing PC with mixed fatty acids from GPC as the starting material. Other types of phospholipids yield compounds, after deacylation, which have certain functional groups, e.g., amino groups from PE. PI can be manufactured by using the enzyme PL-D, using phospholipids from soy lecithin (75).

The most suitable starting materials, for the total synthesis of phospholipids, are optically active derivatives from glycerol, called “chiral” C<sub>3</sub> building blocks. In addition to proper configuration, an early differentiation of the hydroxyl groups is also necessary to shorten the process of synthesis (75).

Transesterification has also been investigated as a means for preparing polyunsaturated phospholipids from soy phospholipids (76).

*Specifications for soybean lecithin.* The following methods are routinely used for determining whether the specifications for given products are met:

*AI.* The amount of acetone-insoluble matter (%AI) is a measure of the polar material found in lecithin. In soybean lecithin, the acetone-insolubles typically contain 70–75% phospholipids, with the remaining portion consisting of glycolipids, carbohydrates, and a small amount of residual triglyceride oil. The amount of acetone-insoluble matter is determined by the AOCS Official Method Ja-4-46 (77).

*AV.* The AV is the number of milligrams of potassium hydroxide necessary to neutralize the acids in 1 g of lecithin (62). A product’s AV is representative of the acidity contributed by both the phospholipids and any free fatty acids that are present. The AV is usually not indicative of pH, as the chemical nature of the phospholipid imparts buffering qualities to most systems. Lecithins typically exhibit a neutral pH value in aqueous media. An AV above 36 may indicate degradation of the lecithin because of improper processing or substandard quality soybeans. AV should not be confused with free fatty acid content, pH, or mineral acids. The correct method to assay for free fatty acids is to titrate only the acetone-soluble portion of the lecithin, whereby any contribution from the phospholipids in the acetone-insoluble portion is eliminated. AV is determined by the AOCS Official Method Ja 6-55 (77).

*Moisture.* The water content of lecithin products is usually less than 1.0%. As a consequence of lecithin’s essentially moisture-free state, lecithin products have very low water activity and do not adversely contribute to the microbiological profile of most food systems. Most lecithin products are preserved well in storage. Higher moisture levels usually indicate a greater potential for spoilage or chemical degradation. Moisture is determined by AOCS Official Method Ja 2b-87 (77). A less accurate moisture level can also be determined by azeotropic toluene distillation (AOCS Official Method Ja 2-46) (77). One cannot determine lecithin moisture by vacuum oven methods. These methods are known to degrade lecithin products and yield false moisture levels.

*HI.* The level of HI matter is one measure of the purity of lecithin products. HI matter usually consists of residual fiber, but also particulate contaminants that may be introduced during processing (e.g., filter aids). The level of HI matter in crude lecithin should never exceed 0.3% and rarely exceeds 0.1%. HI matter in lecithin is detrimental to clarity and use in specific applications. HI is measured by an official Food Chemicals Codex (FCC) (1996) method (54) or by AOCS Official Method Ja 3-87 (77).

*Color.* Commercial liquid lecithins vary in color from light honey to dark brown (62). De-oiled lecithins are typically a shade of yellow. Historically, lecithins have been color graded as unbleached, single-bleached, and double-bleached. The color of lecithin is commonly determined with the use of a Gardner-Hellige Varnish Comparator, or simply Gardner Liquid Color Standards. The color of various lecithin products is generally in the range of Gardner 9–18 in an undiluted form (AOCS Official Method Ja 9-87) (77).

*Other physical/chemical properties and quality criteria.*

*Consistency.* Lecithins are available in both fluid and plastic (solid) forms. Fluid lecithins generally follow Newtonian flow characteristics. The viscosity profile of lecithins is a complex function of acetone-insoluble content, moisture, mineral content, acid value, and the combined effects of assorted additives such as vegetable oils and surfactants. Generally, higher AI and/or moisture content yields higher viscosity, whereas an increased AV often decreases viscosity. Certain divalent minerals, such as calcium and others, can also adjust the viscosity level.

*Clarity.* In some soy processing plants, high levels of HI may partition with the lecithin gums on separation from the oil. This lipid-insoluble material can cause haziness in fluid lecithins. With modern miscella and oil filtration techniques, lecithins with very low HI contents can be produced. Consequently, modern lecithins are clear. Additionally, moisture can also contribute to lack of clarity. Generally, moisture levels over 1% can cause haziness. Besides being an aesthetic problem, if haziness is caused by HI material, it can result in sediment over time; solid particles may appear on the bottom of an otherwise clear liquid product containing lecithin.

For a more detailed review of industrial methods of analysis, the reader is referred to Lantz (62). A review of traditional and novel approaches to the analysis of plant phospholipids has been prepared by Marmer (78). Ackman (79) has reviewed the early developments and practical applications of GLC analysis.

Modern phospholipid analysis is typically accomplished by high-performance liquid chromatography (HPLC) methods (80) or by nuclear magnetic resonance (NMR) techniques (81).

*Chemistry and reactivity of phospholipids.* The chemistry of the phospholipids is generally that of their ester linkages, unsaturated fatty acids, and other reactive groups. Most of the applicable reactions of organic chemistry have been employed in their study (82). Baer and Kates (83), Brockerhoff (84), Hanahan (85), Pryde (86), Scholfield (8, 82), Strickland (87), Verheij (88), and Wittcoff (4) provide major reviews of phospholipid chemistry and reactivity under various conditions. The latter covers hydrolysis, hydrogenolysis, acetolysis, hydroxylation, thermal decomposition, hydrogenation, autoxidation, browning reaction, and other reactions (e.g., bromination and complexing with various substances).

*Lecithin interaction with other food ingredients.* Food systems are usually heterogeneous mixes of components, in which the interaction of ingredient classes

**TABLE 20. Alteration of Lecithin Form/Function (31).**

Action	Technique	Utility
Decrease viscosity	Add special diluents	Sprayable
Increase hydrophilic properties	Solvent fractionation	Easier handling
	Chemical modification	o/w emulsifiers
	Enzyme modification	Wetting agents
	Compounding	
Reduce color	Process controls	Light-colored foods
	Oxidative bleaching	
Convert to powder form	De-oil	Dry blendable
	Mix with carrier	

(e.g., proteins, starches, fats, surfactants) can be important to finished product quality, shelf life, and nutritional value.

The most common modifications of lecithin and the intended physical/functional alterations are shown in Table 20 (31). The range of physical/functional properties available in commercial lecithins is listed in Table 21 (31). These changes in lecithin allow for the basic lecithin obtained from soybean oil to be converted to various emulsifier products having a wide variety of food, feed, and industrial applications. Reviews describing chemical reactions for phospholipid modifications intended to obtain specific functionalities include those of Eichberg (89), Hawthorn and Kemp (90), Kuksis (91), Pryde (86), Snyder (92), Strickland (87), and Van Deenen and DeHaas (93).

Model studies have given some insight into the mechanism of protein/phospholipid interactions. The interactions of soy globulins and phosphatidylcholine were reported by Kanamoto et al. (94). The results of these studies suggested that high-energy input is necessary to the formation of stable phospholipid/protein complexes. Interacting PC vesicles with 7S and 11S soy globulins, Beckwith (95) demonstrated that the extent of protein/phospholipid interaction was dependent on both the ratio of the reactants and the specific globulin.

**TABLE 21. Physical/Functional Properties of Commercial Lecithin (31).**

Property	Commercial Range of Values
Viscosity	100 centipoise to plastic
Color	Light honey to dark amber
HLB	2-12
pH	5-8
Flavor/odor	Slightly nutty to moderate bitter, pungent
Solubility	
Nonpolar	Soluble
Lower alcohols	Partially soluble to soluble
Glycerine	Partially soluble to soluble
Water	Insoluble to dispersible

Chen and Soucie (96) showed that treatment of soy protein isolate with hydroxylated lecithin lowered the isoelectric point, increased electrophoretic mobility, and significantly increased protein dispersibility and suspension stability. Nielsen (97) investigated the interaction of peroxidized phospholipids with several proteins under  $N_2$ . His findings demonstrated a covalent attachment of phospholipids to proteins whose molecular size is increased.

The interaction of lecithin with starch can also have great functional significance in food systems. Not surprisingly, the structure of the lecithins involved determines their reactivity and hence functionality. Hydrolyzed lecithins have been shown to complex with starch, retarding starch crystallization, and thus slowing staling in yeast-raised baked goods (98, 99).

The absorption isotherms of several emulsifiers to fat and sugar crystals dispersed in oils have been examined (100). Unsaturated monoglycerides and phospholipids cause a decrease in adhesion for all concentrations examined. Phospholipids reduce the adhesion between sugar crystals, resulting in much denser sediments.

The influence of soybean lecithins on the spontaneous solidification of different model fats has been studied in the presence and absence of water (101). Lecithins added to dry fat do not affect crystallization, but in the presence of water, they clearly delay it.

*Lecithins as antioxidants.* The literature is replete with references to the antioxidant properties of lecithins. For example, Pokorny (102) claimed that the addition of soybean phospholipids reduced the rate of autoxidation of sunflower oil and prolonged the induction period. Hudson and Ghavani (103) published data showing that the addition of 0.3% dipalmitoyl phosphatidylethanolamine (DPE) to refined soybean oil increased the induction time during Rancimat analysis from 8.8 hours to 19.3 hours. Hildebrand et al. (104), and Jung et al. (105), also published data demonstrating the antioxidant properties of various phospholipids and commercial lecithins.

Although lecithins may act as antioxidants in some systems, they also have a strong synergistic effect in combination with other antioxidants. Hudson and Mahgoub (106) found that although 98% PC and 98% PE acted as pro-oxidants in lard model systems, in combination with D-alpha-tocopherol and/or quercetin, they acted as powerful synergists for antioxidant activity. Hudson and Lewis (107) confirmed that PE and PC alone have negligible activity as antioxidants in lard, but they showed that PE is a very effective synergist when used in combination with polyhydroxy flavonoids at levels of 0.1% or more. Hamilton et al. (108) stated that ascorbyl palmitate/lecithin and lecithin/tocopherol binary mixtures were strongly synergistic in delaying peroxidation in fish oils, with ternary blends of ascorbyl palmitate, lecithin, and tocopherols providing the greatest protection against autoxidation.

Various mechanisms have been proposed for the mode of action of phospholipids as antioxidants or antioxidant synergists, including chelation of pro-oxidant metals (106, 108), the release of protons that bring about the rapid decomposition of hydroperoxides without generating free radicals (107), and the regeneration of

the primary antioxidant (107, 108). Saito and Ishihara (109) provide evidence for the mechanism of action behind the antioxidant activity of PE and PC in purified sardine oil. They attributed the antioxidant activity to the basicity of the amino portion of these molecules. The base donates a pair of electrons to an oxygen of a hydroperoxide molecule, binds to this oxygen, and degrades the hydroperoxide to an alcohol.

McLean et al. (110) have examined the role of lipid structure in the activation of phospholipase A<sub>2</sub> by peroxidized phospholipids. Results showed that the increase in rate of hydrolysis of peroxidized phospholipid substrates catalyzed by phospholipase A<sub>2</sub> is largely because of a preference for peroxidized phospholipid molecules as substrates, and that peroxidation of the host lipid does not significantly increase the rate of hydrolysis of nonoxidized lipids.

The reader is referred to Pryde (86) for a more thorough discussion on the kinetics of autoxidation of phospholipids; their forming metal ion, iodine, and other complexes; halogen addition; and their behavior during hydration, hydrogenation (with heterogeneous and homogeneous catalysts), hydrolysis and alcoholysis, hydroxylation, oxidation, radical, and other reactions.

## 4. MANUFACTURE, FRACTIONATION, AND PURIFICATION OF LECITHINS

### 4.1. Manufacture of Crude Lecithin

Commercial soybean lecithin is obtained in the traditional manner by hexane extraction of the crude oil from the soybean flake and then water degumming the oil to yield a viscous fluid product.

The degumming of soybean oil is not an industry-wide practice. Brian (111) estimated that only about one-third of the soybean oil produced in the United States needs to be degummed to meet the U.S. needs for soybean lecithin production.

Removal of all phospholipids and gums is a necessary part of the steam-refining process. However, this process has not yet been developed to the point where it can produce refined soybean oil that meets U.S. competitive requirements. Studies have been carried out to fulfill this objective (112, 113).

Based on a series of samples obtained from commercial processors, degumming of the oil from undamaged soybeans removed 79% to 98% of the phosphorus. Phosphorus content of the oil was lowered from 500 to 900 ppm in the crude to 12 to 170 ppm in the degummed oil (112). Water-degummed oil from damaged beans may have an abnormally high phosphorus level, and degumming these oils poses a difficult problem.

*Oil degumming.* All stages of oilseed processing affect the quality of soy lecithin. Prior to degumming, the seed quality, cleaning of the beans, extraction of the oil, and the handling of the miscella and crude oil all have an important role in making a good quality lecithin.

Hexane extraction removes about 50% of phospholipids from the meal (114). The presence of fines in the miscella is undesirable for making lecithin and should

be kept to a minimum (0.2% or less), and the crude miscella coming from the extractor should be filtered. Several miscella filtration methods are available. The crude oil also can be filtered to produce lecithin.

The method of hexane removal from the miscella is also very important in that dark colors in lecithin are believed to be caused by an aldehyde-amine reaction largely formed by heating the oil during the solvent stripping operation. Most U.S. processors employ a dual-stage evaporator followed by a low-pressure stripping system (115).

Hydration causes most of the phospholipids and gums present in a crude oil to become insoluble in the oil. Such hydration can come about from water added to the oil in the degumming step or from moisture picked up from the air by the oil during storage (116).

*Current commercial practices.* If the oil is recovered by solvent (hexane) extraction, some mills allow a portion of the steam blown through the oil for removal of last traces of solvent to condense and thus hydrate the gums. Sometimes operators find it difficult to closely control the moisture addition with direct steam and prefer to add hot water in controlled amounts.

Crude oil from which the lecithin is to be recovered is usually filtered prior to degumming to remove residual meal fines and seed fragments. Although more difficult to accomplish, dry lecithin can also be filtered. Careful filtration results in a highly clarified lecithin with little or no residual hexane insoluble matter (33).

Brian (111) describes two methods of miscella filtration, one with and one without filter aids, but both result in a lecithin that still remains somewhat cloudy. Highly clarified lecithin products can be obtained only by filtering the crude oil, usually with the aid of vertical leaf or plate and frame filters, wherein the dry oil is heated to 82°C and 0.1% filter aid is added (33).

Two principal degumming methods are employed: batch and continuous. The batch degumming process is shown in Figure 2 (115). A flow sheet for the continuous degumming of soybean oil and production of soybean lecithin is shown in

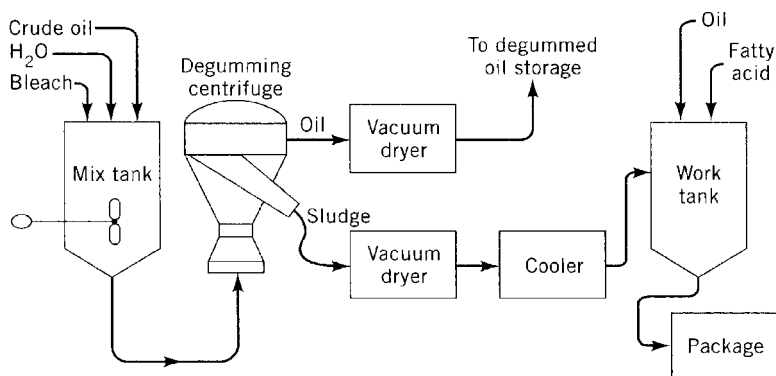


Figure 2. Batch degumming system for lecithin production (115).

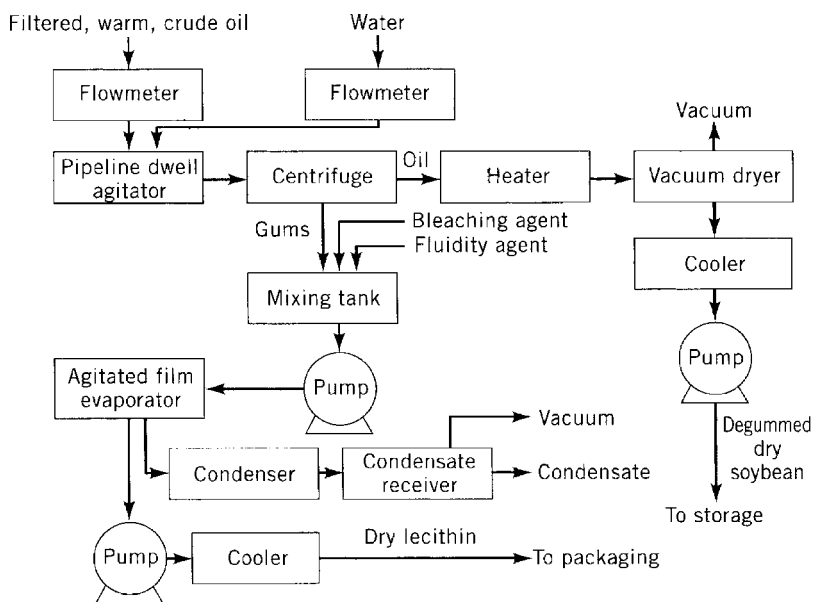


Figure 3. Flowsheet for degumming soybean oil and crude lecithin production (111).

Figure 3 (111). Recommended conditions for degumming of crude vegetable oils can vary greatly as shown in Table 22 (111, 117–124).

In a batch degumming system, crude soybean oil is typically heated to about 70°C in a large tank fitted with an agitator. Water is added (about 2% by volume), and the hydrated oil is agitated for up to one hour. The hydration of soybean

TABLE 22. Degumming Conditions from the Literature (8, 118).

Parameter	Quantity	Reference
Water	75% wt of gums	Crauer (119)
	1–2.5%	Brian (111)
	2–3%	Van Niewenhuyzen (59)
	3%	Bernardini (121)
	1%	Norris (122)
	2%	Carr (123)
	Equal to wt gums	Braae (117)
	2–5%	Andersen (124)
Temperature	32–49°C	Norris (122)
	50–70°C	Van Niewenhuyzen (59)
	65–75°C	Bernardini (121)
	70°C	Carr (123)
	95°C	Andersen (124)
Agitation	Vigorous	Bernardini (121)
	Mechanical agitation	Carr (123)
Time	30–60 min.	Carr (123)
	10–15 min.	Braae (117)



phospholipids proceeds rapidly, and for all practical purposes, 15 minutes is adequate for batch systems. The hydrated “gums” or “lecithin emulsion” are then removed by continuous centrifugation. This step is then followed by drying in a batch or film dryer. The gums are usually dried to a moisture content of less than 1%, typically 0.3–0.75% (3, 33).

In continuous systems, preheated crude oil (80°C) and water are metered into an in-dwell pipeline agitator, or a large agitated tank, and held only for a short period. In both systems, the oil is then pumped to a centrifuge for separation of the lecithin sludge from the oil (33, 118, 125–126). Water with a low concentration of calcium and magnesium is preferred (115).

In commercial processes, the amount of degumming water required (1.5–2.0%) is roughly equivalent to the phospholipid content of the crude oil (118). Too little water will result in a dark, viscous gums phase and hazy degummed oil that contains unhydrated phospholipids. Too much water will result in a three-phase system consisting of free water, a fluid yellowish-brown gums phase, and a hazy degummed oil phase after centrifugal separation (33, 118, 119, 126).

Flider (33) points out that the AI content of the gums is enhanced by raising the temperature of degumming. For example, degumming at 40°C yielded crude lecithin containing 63–65% AI, whereas at 60°C, the yield increased to 68–75%. Although above 60°C some darkening of the lecithin may occur, a higher temperature (e.g., 70–80°C) produces a more consistent lecithin AI on a day-to-day basis.

Agitation is an important factor in batch degumming. The AI content of the crude gums increases with agitation, presumably because at low agitation rates, more oil is entrained in the gums (118).

Three types of centrifuges are in common use for oil–lecithin separations as described by Podbielniak et al. (127) and Sullivan (128): tubular bowl, disk bowl, and concentric plate. All of these centrifuges can be hermetically sealed, thereby protecting the process streams from the harmful effects of air (115). The newer disk-type centrifuges have a solid ejecting feature that allows the discharge of solid impurities on a regular basis. These centrifuges are also equipped with a discharge control valve that can be adjusted to vary the AI content of the gum or sludge phase. Because the hermetic centrifuges are capable of delivering sludge of lower oil content than the conventional open bowl-types, more neutral oil is available for refining, and lecithin with higher AI contents can be obtained (115).

The efficiency of commercial degumming operations is summarized in Table 23 (112). Removal of phospholipids in commercial operation ranges from 75% to 96%, with an average of 87% (112).

For more specific information on the parameters of the degumming operation, the reader is referred to Brekke (129), Flider (33), List (115), List and Erickson (130), and List et al. (118).

*Novel degumming approaches.* List et al. (131) reported on a hexane-extracted crude soybean oil that had been degummed in a reactor by countercurrently contacting the oil with supercritical CO<sub>2</sub> at 10,000 psi at 60°C.

The phosphorus content of the crude oil was reduced from 620 ppm to less than 2 ppm. Degummed feedstocks were fed, without further processing (i.e., bleaching),

**TABLE 23. Removal of Phosphorus by Commercial Degumming of Crude Soybean Oil<sup>a</sup> (112).**

Processor	Phosphorus (ppm)		Phosphorus Removed (%)	Mean (%)
	Crude	Degummed		
A	733	167	77.2	82.8
	683	80	88.3	
B	867	53	93.8	92.3
	684	63	90.7	
C	711	89	87.5	84.8
	588	105	82.1	
D	615	40	93.4	95.9
	713	12	98.4	
E	623	102	83.6	79.7
	580	141	75.8	

<sup>a</sup>Plants located in Illinois, Iowa, Minnesota, Arkansas, and North Carolina. Two samples from each plant separated by at least 2 weeks.

directly to a batch physical refining step consisting of simultaneous deacidification–deodorization (1 h at 260°C, 1–3 mm Hg) with and without 100 ppm citric acid. Flavor evaluation showed that the supercritical CO<sub>2</sub>- processed oil had the same flavor scores, both initially and after 60 days of aging and light exposure tests, as the commercially refined–bleached soybean oil control, deodorized under the same conditions. These results would indicate that bleaching with adsorbent clays may be eliminated by a supercritical CO<sub>2</sub> countercurrent processing step. As a result of the considerable heat-bleaching that takes place during deacidification–deodorization, colors of salad oils produced under the above conditions typically ran 3Y > 0.1R.

A degumming process has been described by Dijkstra (132), wherein the wash-water is recycled to the oil feed and used to dilute concentrated alkali. This process does not generate an aqueous effluent and can be used for both acid and alkali refining, thus allowing refiners to change gradually from alkali refining to physical refining.

A novel degumming process has been described by Jirjis et al. (133), wherein vegetable oil miscella is fed to a conditioned polymeric microfiltration membrane to produce a permeate stream containing decreased weight percentages of phospholipids, and a retentate stream containing increased weight percentages of phospholipids. The membrane is specifically conditioned for the removal of phospholipids by treating with intermediate solvents, and it has an average pore size of about 0.1 to about 2 microns. Both product streams are then desolventized using traditional methods. The resulting vegetable oil stream is claimed to have a phosphorus level of less than 30 ppm.

*Bleaching.* The color of soybean lecithin can be attributed to many factors: carotenoids, melanoids, and porphyrins (125, 134) in the product, age, quality of the source material, pretreatment prior to crushing of soybeans, thickness of flakes and

temperature during extraction, conditions during degumming, and lecithin processing conditions (33).

Natural lecithin often has a brown color, although with advanced soybean processing technology, the color may approximate that of unbleached soybean oil (65).

Traditionally, one referred to an unbleached product as one that has not been treated with bleach. A single-bleached product was treated with only one type of bleach (usually hydrogen peroxide), whereas a double-bleached product usually was treated with two types of bleach (i.e., hydrogen peroxide and benzoyl peroxide). Although these grades continue to exist by name, the bleaching methods used to manufacture them are no longer uniform. High-quality, relatively light-colored, unbleached lecithins are now available through modern manufacturing practices. Additionally, today's double-bleached product may have been treated with only a small quantity of one type of bleaching agent. Products are presently bleached to a color specification only, regardless of bleaching techniques or quantity (7, 33).

Lecithin may also be bleached by replacing a portion of the degumming water with peroxide and carrying out the bleaching and degumming simultaneously. This method is less efficient, however, than bleaching the gums directly (33).

Although the specifications by the National Oilseed Processors Association (NOPA) *Year Book and Trading Rules, 1998-1999* (135) recognizes color grades based on unbleached, bleached, and double-bleached lecithins, this nomenclature is technically incorrect as it is more descriptive of the process rather than of the product.

From a regulatory point, bleached products are traditionally grouped with the unbleached forms of crude lecithin. No distinction is made between the bleached and unbleached forms as far as Generally Recognized as Safe (GRAS) status is concerned (136).

Laboratory studies by List et al. (118) have shown that with 1% hydrogen peroxide, complete bleaching occurs in 30 minutes at 60°C; in commercial operations, where less efficient agitation occurs, up to 1 hour is required (33).

Bleaching with peroxides involves oxidation of the carotenes and the other color bodies within the lecithin. There is no evidence that bleaching with either hydrogen peroxide or benzoyl peroxide functionally modifies lecithins. Bleaching seems only to affect the pigments, which are not functional constituents. Scholfield and Dutton (134) reported that although hydrogen peroxide destroys all color bodies to some extent, its greatest effect is on lutein, the principle pigment (75%) found in soybean lecithin.

The color of most lecithin products will darken on prolonged heating. Color stability can be achieved, however, by avoiding exposure of lecithin to temperatures over 60°C. There are now heat-resistant lecithins on the market that maintain their light color for extended periods even at elevated temperatures (7).

*Drying.* After centrifugal separation and bleaching, the gums (containing 25–50% moisture) are dried (to 0.3–0.75%) as soon as possible to prevent microbial activity.

The drying operation serves not only to remove moisture, but also to lower the peroxide value. Peroxide destruction is rapid at or near temperatures of 100°C.

Two types of dryers are commonly used throughout the industry. The sludge can be dried in batch dryers operating under vacuum (20–60 mm Hg) and equipped with rotating, ball-shaped coils through which warm water is circulated to maintain the lecithin at 60–70°C (140–158°F). Although these dryers require longer residence times (3–5 hrs), they are popular among European processors because less charring is apt to occur (137). In domestic lecithin processing plants, continuous, agitated film evaporators are the standard equipment. Evaporators operating on a vertical or horizontal axis are available. Film evaporators operate at temperatures ranging from 80 to 105°C, with vacuum of 25–300 mm Hg. Residence times are very short, usually 1 to 2 minutes.

Dry lecithin is highly viscous, and the viscosity increases drastically and then falls off as the moisture content increases. Comparative conditions used for drying lecithin in the two types of drying apparatus are given in Table 24 (59, 130).

Because of the sensitivity of lecithin to heat, drying conditions are critical and the product should be cooled to 55–60°C before additional processing, and/or to 35–50°C before storage and packing (33). Shelf life of dried lecithin products in suitable containers is more than 1 year at 21°C (3).

*Fluidizing.* Fluidizing additives such as soybean oil, fatty acids, or calcium chloride can be added to adjust the viscosity. The viscosity of dried crude lecithin can also be decreased by warming it to a maximum of 60°C. The dried crude lecithin product (unbleached or bleached) can also be used to prepare a variety of grades of lecithin by removing the oil to increase the phospholipid content, or by separating the oil-free lecithin into alcohol-soluble and alcohol-insoluble fractions.

Besides calcium chloride, the viscosity of lecithin products may also be modified by the addition of other mono- and divalent ions, phosphoric acid, or acetic anhydride. Monovalent ions, such as sodium or potassium, are attracted to the negatively charged base groups, which tend to increase the crystalline order, thereby increasing viscosity. On the other hand, divalent calcium and magnesium reduce the crystalline order and thus reduce viscosity. These techniques are used to produce fluid lecithins containing 66–70% AI without the addition of fatty acids (33). In commercial practice, fluidized lecithins usually are made by calcium chloride addition to the gums, by the inclusion of fatty acids or vegetable oil, or with the aid of special proprietary diluents.

**TABLE 24. Average Process Conditions for Drying Lecithin Sludge<sup>a</sup> (59, 129).**

Process Variable	Batch Dryer, Bollman Type <sup>b</sup>	Continuous, Agitated-Film Evaporator
Temperature		
°C	60–80	80–95
°F	140–176	176–203
Residence time, min.	180–240	1–2
Absolute pressure, mm Hg	20–60	50–300

<sup>a</sup> Starting product: sludge with 50% moisture. End product: lecithin with less than 1% moisture.

<sup>b</sup> Vacuum dryer with rotating, ball-shaped coils heated with warm water.

Fluidization with phosphoric acid is not recommended because darkening of the product and hydrolysis may occur. Degumming with acetic anhydride results in fluidized lecithins possibly because PE is acetylated by the reagent. Nonedible lecithins may be fluidized by the addition of acidulated and dried soapstock.

Plastic lecithins are available in several forms and are typified by high AI, low AV, high moisture, or their content of certain minerals. One, or a combination of these, can produce a plastic lecithin. Oil-free lecithins are plastic, because of the removal of their nascent oil, i.e., residual soybean oil. They are generally powdered or granular in form.

*Nonhydratable phospholipids.* According to Myers (138), about 90% of the phospholipids are removed from the oil by water degumming. Although most of the remaining phospholipids are removed by alkali neutralization, Braae et al. (139) report that soybean oil and several other types of vegetable oils often contain some phospholipids that are not removed by alkali neutralization and washing.

The impact of enzyme activity on the nonhydratable phospholipid content of crude soybean oil was investigated by List et al. (140). Evaluation of flakes subjected to live steam and whole beans treated by microwave heating to inactivate phospholipase D suggests that heat, moisture, and enzyme activity are important factors contributing to the formation of nonhydratable phospholipids in extracted crude oils. Approximately 8–10 minutes of microwave heating is required to completely destroy enzymatic activity.

List et al. (141) later found that four interrelated factors promote nonhydratable phospholipids (NHP): (1) moisture content of beans or flakes entering the extraction plant; (2) phospholipase D activity; (3) heat applied to beans or flakes prior to and during extraction; and (4) disruption of the cellular structure by cracking and/or flaking. Thus, NHP formation can be minimized by control of the moisture of beans and/or flakes entering the extraction process, inactivation of the phospholipase D enzyme, and optimizing temperatures during the conditioning of the cracked beans or flakes (141).

In a subsequent study, List and Mounts (142) indicated that the adverse effects of storage conditions, excessive moisture levels, and elevated temperatures cannot be completely overcome by inactivation of phospholipase D prior to solvent extraction of the flakes.

Zhang et al. (143) reported the effects of an expander process on the phospholipids in soybean oil by comparing the differences in phospholipid compositions of the oils and the lecithins produced from expander and conventional processes by HPLC. The phosphorus content indicated that the expander-processed oil contained more phosphorus (985 ppm) than the conventionally processed oil (840 ppm). However, the phospholipids in the expander-processed oil were more hydratable than those in the conventionally processed oil. After degumming, the phosphorus content in the expander-processed oil and conventionally processed oil were reduced by 93.2% and 78.6%, respectively. The expander-processed lecithin contained 74.3% AI matter, and the conventionally processed lecithin contained 65.8%. There was also more phosphatidylcholine in the expander-processed lecithin (39.8%,

based on AI) than in the conventionally processed lecithin (34.2%), and the phosphatidylethanolamine was lower (12.4% vs. 18.1%) and the phosphatidylinositol contents were almost the same.

Braae (144) believes that the nonhydratable phospholipids are present as calcium and magnesium salts. These phospholipids can have a deleterious effect on oil quality. They can be removed either by treatment of the oil (at 70–90°C) with a small quantity of concentrated phosphoric acid (0.25%) ahead of the neutralization step (145) or by refining the oil with a mixture of lye and sodium carbonate. The phosphoric acid pretreatment apparently also aids in the removal of deleterious iron compounds in the subsequent processing of the oil, i.e., caustic refining, bleaching, and deodorizing of the oil (113). On the other hand, although such pretreatment aids in the lowering of refinery losses and results in low phosphorus and iron content in the degummed oil, the resulting lecithin is dark and low in acetone-insolubles (33).

The use of acetic anhydride as a degumming adjunct has been described by Hayes and Wolff (146–148) and Myers (138). In this process, 0.1 wt % of acetic anhydride is mixed for 15 minutes with crude soybean oil (phosphorus 750 ppm) that has been preheated to 60°C (140°F), followed by stirring the mixture for 30 minutes after the addition of 1.5% water. The reaction is completed within minutes. After centrifugation and water washing, the phosphorus content of the oil ranged from 2 to 5 ppm.

The advantages claimed for this treatment are thought to be that the caustic refining step can be omitted, and thus the loss of neutral oil because of saponification is eliminated, and higher yields are obtained from both finished deodorized oil and lecithin. On the negative side, the disadvantages found were that equipment and piping must be constructed of type 316 stainless steel to handle the corrosive materials; more care is required in deodorization of the oil; and the process will not produce a satisfactory product from highly colored vegetable oil such as corn and cottonseed oils, nor from some lots of soybean oil (130). Also, according to Evans et al. (149), the process removes phosphorus but not iron, one of the metallic pro-oxidants that can give soybean oil a poor flavor. Lecithin produced from this process, however, is claimed to be similar to that prepared in the conventional manner.

Other degumming agents considered include acetic, oxalic, boric, and nitric acids (150) and surfactants (151). However, none of these are currently used in lecithin manufacture. Ringers (152) obtained good results in a two-step degumming process wherein an edible acid, presumably citric, was used. Soybean oil can also be degummed by heat, but this practice is confined to oils going into industrial uses.

Lunde et al. (153) concluded that the sequestering action of fatty oil for metal ions depends at least in part on the oil's phospholipid content and reaches a maximum at 0.1–2 ppm of phosphorus. As a point of information, hexane extracts only about one-half of the phospholipids present in soybeans (1, 114).

For further information on the nonhydratable phospholipids, the reader is referred to Hvolby (154), Letan and Yaron (155), and Nielsen (114, 156).

## 4.2. Manufacture of Refined-Grade Lecithins

*Producing high-clarity lecithins.* Lecithin destined for certain applications may require more rigorous than usual initial refining conditions. Clarified lecithins are carefully filtered in (1) the full miscella, (2) crude oil, and (3) directly as lecithin. As mentioned before, the filtration is carried out on plate and frame or vertical leaf filters with manual or automatic cleaning cycles. Filtration simply removes HI material, producing products of utmost purity and clarity. Such ingredients can be marketed as encapsulated nutritious supplements, as high-purity pharmaceutical adjuncts, and as additives for high-technology applications.

Although the dry lecithin can be filtered, many processors prefer to filter the crude oil before degumming (111). Usually, the crude oil is filtered through large plate-and-frame filter presses. If the degumming operation is conducted at a solvent extraction oil mill, then either the miscella, i.e., oil-solvent mixture, or the oil can be filtered. If all of the fines and filter aid are not removed, the dry lecithin will appear cloudy.

Brian (111) has made suggestions for the type of pumps, centrifuges, filters, heat exchangers, and drying equipment most suitable for lecithin production. He has also provided useful engineering design data on filtration flow rates for the crude oil, miscella, and dry lecithin, quality of filter aid needed, and the overall heat transfer coefficients for agitated-film evaporators and shell-and-tube heat exchangers used for heating and cooling the oil.

*Producing compounded lecithins.* Compounded lecithins are special purpose products made by the direct addition and/or blending of functional additives, emulsifiers, diluents, surface active agents, and so on.

Water-dispersible lecithins may be produced by adding a hydrophilic surfactant (5–20%) such as polysorbate or ethoxylated monoglycerides. A mixture of lecithin and nonionic surfactants (10–20%) has utility in applications where water dispersibility is needed. Blending of partial glycerides and lecithin, followed by spray cooling, results in flaked or powdered products (33).

When lecithins are diluted with soybean oil or fatty acids, they have a tendency to separate. In such cases, substituting a portion or all of the soybean oil with other oils such as peanut, cottonseed, coconut, or partially hydrogenated soybean oil will increase stability (33).

Lecithins may also exhibit synergistic actions with some compounds. Common additives include special oils, polysorbates, mono- or diglycerides, modified monoglycerides, lanolin and lanolin derivatives, solvents, plasticizers, and other surfactants.

*Producing de-oiled lecithins.* De-oiled lecithin represents a special category where high phospholipid content (above 95% AI) is required. When contacted with acetone, phospholipids precipitate as a fine, free-flowing powder. After removing the acetone, de-oiled lecithins are dry powders or granules (33).

Depending on the type and efficiency of the extraction equipment, the acetone/crude lecithin ratio necessary to achieve a 95% phospholipid product is 10–20:1 (v/v). In batch extraction, the tank is charged with acetone prior to crude lecithin

addition. Crude lecithin is then introduced into a crystallizing vessel with agitation until an acetone/crude lecithin volume ratio of ca. 5:1 is achieved. Only the best-quality fluid crude lecithin should be used for the preparation of de-oiled lecithins. The mixture is then agitated for 20–30 min., after which time the phospholipids are allowed to settle. The triglyceride–acetone miscella is then removed and the vessel charged again with fresh acetone for the second extraction. A single batch may be extracted 2–4 times to obtain the desired phospholipid concentration (95% minimum) (33).

In a continuous extraction, crude lecithin and acetone are simultaneously metered into a vessel. Within limits, acetone consumption can be decreased by increasing residence time in the continuous extractor, increasing raw material efficiency (33).

After extraction, the de-oiled lecithin is recovered by filtration as a cake, containing 25–50% acetone. According to Flider (33), the acetone concentration of the cake is critical for optimal granulation. “Too little acetone will result in the formation of a high concentration of fines and powder. Too much acetone will result in a ‘salt and pepper’ effect (i.e., a mixture of coarse and fine particles) caused by agglomeration of the fines and powder during granulation.” The fines and powder output is 5–50% of the total de-oiled material, depending on production conditions (33).

After granulation, the remainder of the acetone is removed by drying, preferably in a moving bed, forced-air dryer. Such dryers are preferred over fluid-bed dryers because less destruction of the lecithin granules occurs. After drying, the acetone content of the product should be well below 50 ppm, preferably below 25 ppm (33). Flider (33) states that mesityl oxide, through an aldol condensation reaction, may be formed if excess acetone is present. When the lecithin is sufficiently dry, however, this is not a problem. The dried de-oiled lecithin is sized by sieving through a series of screens (33).

As the tocopherols are removed from the lecithin during the extraction process, the de-oiled lecithin has less oxidative stability than the crude product. Also, the surface/volume ratio of the de-oiled lecithin contributes to reduced stability. Mixed soy tocopherols are usually added back at a level of 500 ppm to prevent this. A small percent of an anticaking agent may also be added to ensure that the product remains free-flowing. A free-flowing de-oiled lecithin can be easily added to other products (33).

When compared with crude lecithin, oil-free lecithin is more hydrophilic and seems to have better emulsifying activity than its AI alone would suggest. The removal of odor/flavor components with the oil also produces blander lecithins (7).

Refined de-oiled lecithin can also be blended with carriers such as cocoa butter, hard butters, medium-chain triglycerides, or other diluents to obtain products with more functionality and different physical characteristics. Up to 40% phospholipids may be incorporated in these carriers without the use of solvents. These products are usually stabilized against autoxidation by the addition of antioxidants (33). De-oiled lecithin should be packaged as soon as possible to prevent moisture



absorption. For more specific details on various aspects of de-oiled lecithins, the reader is referred to Flider (33).

*Novel de-oiling approaches.* A novel de-oiling process has been described by Hutton and Guymon (157) wherein a mixture of crude phospholipids and hexane is fed to a polyvinylidene membrane to produce a permeate stream containing triglycerides and hexane, and a retentate stream containing phospholipids (35–40%) and hexane (60–65%). The membrane has a molecular weight cutoff of 10,000–50,000 daltons. The permeate stream is then desolventized using traditional methods. Bleaching earth is added to the retentate stream for color removal at a rate of 5–8% of the phospholipid mass portion of the stream. The bleaching earth is then filtered out of the mixture.

Antioxidants in the form of mixed tocopherols are added to the phospholipid/hexane mixture. The phospholipids are then desolventized through the use of a drum desolventizer followed by a fluid bed dryer. Solvent residuals in the dried product are less than 5 ppm. The dried flakes are placed in storage bins. From there, the flakes are ground into powder and then agglomerated into granules. The acetone-insoluble content of the finished product is claimed to be in the range of 90–99.9%.

Another novel de-oiling process has been described by Wendel (158) wherein supercritical gases are used to produce de-oiled lecithin. The crude lecithin is fed into a column where the supercritical gas mixture of propane and carbon dioxide flows at a pressure of 80 bar and temperature between 40% and 55°C. This fluid then goes to a regeneration column where the temperature is increased to 75°C, and the lecithin component precipitates and falls to the bottom of the column. As the lecithin falls, it encounters pure supercritical extraction fluid and the oil component is extracted. Oil-rich solvent leaves the top of the column. Through pressure and temperature changes, the lecithin and the oil are precipitated out of their respective streams and continuously removed from the process flow. The oil-free, lecithin-free solvent is returned to the column for reuse.

*Producing modified lecithins.* The chemistry of lecithin has been reviewed by Pryde (86) and by Wittcoff (4). Schmidt and Orthoefer (58) have discussed the manufacture and use of modified lecithin products. The latter class is represented by chemically or enzymatically modified products that are commercially available in both fluid and de-oiled forms.

The traditional approach to the modification of phospholipid properties is by fractionation, isolation, and purification of a single component. Functions of phospholipid mixtures are also modified by partial chemical or enzymatic hydrolysis, acetylation, hydrogenation, and hydroxylation (5).

Crude lecithin contains a number of functional groups that can be successfully hydrolyzed, hydrogenated, hydroxylated, ethoxylated, halogenated, sulfonated, acylated, succinylated, ozonized, and phosphorylated, to name just a few possibilities (1). The only chemically modified food-grade products produced in significant commercial quantities at the present time are the ones obtained by hydroxylation, acetylation, and enzymatic hydrolysis (58). Hydroxylated or acylated lecithins represent chemical modifications to improve the functionality in water-based systems.

*Acetylated lecithin.* Acetylation occurs primarily on the amino group of phosphatidylethanolamine (146–148). The amino group of phosphatidylethanolamine, when acetylated, receives an acetyl group on the positively charged portion of the phosphatidylethanolamine, which converts it to a negatively charged lecithin with improved solubility and oil-in-water emulsifying properties (159). Lecithin can be acetylated using acetic anhydride either by adding the reagent prior to degumming or adding it to the wet gums. Acetylated lecithin products are made from natural soy lecithin hydrates by treating them with low levels of acetic anhydride (1.5–5.0%). Lecithin hydrates are obtained during the degumming of crude soybean oil. After the reaction with acetic anhydride, the resulting product is neutralized with a food-grade alkali to raise the pH to 6.5 to 8.0, depending on the intended application. The amount of acetic anhydride used in the process depends on the level of phospholipids in the gums, and the intended use of the final product, requiring different degrees of acetylation for optimum functionality. The same is true for the choice of alkali (e.g., sodium hydroxide, calcium hydroxide, etc.) used. The product is then vacuum-dried (film dryer in a commercial operation) to a final moisture of less than 1.0% (160).

The degree of reaction is measured by determining amine nitrogen content in the resulting product (usually by formol titration). Maximum (100%) acetylation would be indicated by a zero amine nitrogen value, whereas a minimally acetylated commercial product has approximately 1.7-mg amine nitrogen/g content. In a typical commercial operation, the amine nitrogen content is usually in the range of 0.7 to 1.7 mg/g.

The total acetone insolubles content of commercial acetylated lecithin products can vary from about 52% to about 97%, the remainder being soybean oil (or another food-grade triglyceride or fatty acid as a natural constituent or added diluent), natural pigments, sterols, and other minor constituents present in crude lecithin from the soybean. The acetylated lecithin meets all the compositional requirements of the U.S. Food Chemicals Codex (54).

Typical specifications for a minimally and maximally acetylated, liquid product are given below.

#### **Minimally Acetylated, Commercial, Liquid Lecithin Specification**

Acetone insolubles (%)	60.0 min.; 64.0 max.
Moisture (%)	0.75 max.
Acid value	24 max.
Viscosity (cP at 25°C)	10,000 max.
Color	17 max.
Peroxide value (meq/kg)	10 max.
Hexane insolubles (%)	0.09 max.
Amino nitrogen (mg/g)	1.65 max.
Divalent metals (%)	0.42 min.; 0.48 max.
pH	6.5 min.; 8.0 max.

#### **Maximally Acetylated, Commercial, Liquid, Lecithin Specification**

Acetone insolubles (%)	53.0 min.; 56.0 max.
Moisture (%)	0.75 max.

Acid value	36 max.
Viscosity (cP at 25°C)	2,900 max.
Color	12+ - 14
Peroxide value (meq/kg)	100 max.
HIM (Hexane insolubles 0.8 $\mu$ -Millipore)	100 ppm max.
Amino nitrogen (mg/g)	1.0 max.
pH	7.0 min.; 7.5 max.
Visual clarity	pass
Heat resistance test (Lovibond red)	8.0 max.
Appearance	Clear and brilliant at 25°C

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Acetylated lecithins have improved fluid properties, improved water dispersibility, and are effective oil-in-water emulsifiers for a wide variety of food formulations (56, 58). Moderately and highly acetylated lecithins are resistant to heat and can be repeatedly heated and cooled without darkening. The intended uses for minimally acetylated products are in infant foods, coffee whiteners, meat sauces, and gravies, and for oil-in-water cosmetic emulsions. Moderately and maximally acetylated products are used in cheese sauces, release agents in pumpable and aerosol formulations, and shortenings.

The following patents have been issued on the topic of making and using acetylated lecithins:

1. U.S. Pat. 3,301,881 "Process of Phosphatide Separation," 1967.
2. U.S. Pat. 3,359,201 "Lecithin Product and Method," 1967.
3. U.S. Pat. 3,499,017 "Alkaline-Hydrolyzed Phosphatides," 1970.
4. U.S. Pat. 3,823,170 "Phosphatides," 1974.
5. U.S. Pat. 3,928,056 "Pan Release Product and Process," 1975.
6. U.S. Pat. 3,962,292 "Phosphatide Preparation Process," 1976.
7. U.S. Pat. 4,479,977 "Method of Preparing Heat-Resistant Lecithin Release Agent," 1984.

*Hydroxylated lecithin.* Hydrogen peroxide, in addition to bleaching, can also hydroxylate lecithin. Hydroxylation imparts hydrophilic properties, improves moisture retention, and contributes to the formation of stable oil-in-water emulsions.

Hydroxylated lecithin is a light-colored product with increased water dispersibility and enhanced oil-in-water emulsifying properties. Hydroxylated lecithin is useful in many applications in which a water-dispersible lecithin is desired. It is especially useful in baking applications where it can improve the dispersion of fats and retard staling.

Hydroxylation of lecithin is carried out by the reaction of crude lecithin with hydrogen peroxide and lactic acid or acetic acid. Active sites for peroxidation appear to be double bonds as measured by IV drop and the isolation of dihydroxystearic acid from the reaction mixtures. Hydroxylation is allowed to proceed until

a 10% reduction in iodine value occurs (115). The ethanolamine group is also modified during hydroxylation (58, 161).

*Hydrolyzed lecithin.* Crude lecithin is readily hydrolyzed in the presence of strong acids or bases. Enzymes can be used for very selective hydrolysis. Prolonged treatment leads to fatty acids, glycerophosphoric acid, or their salts, with mixtures of amino compounds and carbohydrates (4, 115).

In a commercial process, it is desirable to control the reaction so that just one of the fatty acids is cleaved from the phospholipid molecule. As acid or base hydrolysis is nonspecific and very difficult to control, enzymes are usually preferred for most applications (58). A number of phospholipase enzymes are available (i.e., phospholipase A<sub>1</sub> or phospholipase A<sub>2</sub>).

Haas et al. (162) have studied enzymatic phosphatidylcholine hydrolysis in organic solvents by examining selected commercially available lipases. Enzymatic hydrolysis of oat and soy lecithins, and its effect on the functional properties of lecithin, was investigated by Aura et al. (163). The phospholipase used was most effective at low enzyme and substrate concentrations.

Partially hydrolyzed lecithins exhibit enhanced oil-in-water emulsifying properties, particularly in the presence of calcium and magnesium ions. They do not lose their emulsifying action in the presence of calcium and magnesium ions as rapidly as do the unmodified types. Enzymatically modified lecithins have been used in calf milk replacement formulations to improve the emulsification and digestibility of fats (56).

Enzymatic hydrolysis of the polar head group of a phospholipid molecule can be carried out with phospholipase C and phospholipase D. Phospholipase D is used to exchange the amino head group of phosphatidylcholine with serine to form phosphatidylserine (164).

*Transesterification.* Transesterification allows for the incorporation of free fatty acids into lecithin molecules. Unhydrolyzed lecithin contains two fatty acids, and the fatty acid moiety can be different at the two positions on the phospholipid molecule. The fatty acid composition can have an effect on the stability and functionality of the lecithin. Changes in the fatty acid composition can be done through transesterification (165). Transesterification using lipases can be used for the addition of polyunsaturated fatty acids to lecithin to enhance the essential fatty acid profile, or to improve functionality (166).

*Hydrogenated lecithin.* Lecithin can be hydrogenated to a stearin-like solid that has greater oxidative stability and is less hygroscopic than unmodified lecithin, but it has reduced solubility in the usual solvents. Phospholipids are not hydrogenated as readily as soybean oil, which at lower hydrogenation pressures and temperatures, can be selectively hydrogenated (58).

Hydrogenation of lecithin is usually done under conditions to reach iodine values of 10–20 in the presence of a nickel or palladium catalyst and a suitable solvent (e.g., ethyl acetate) at 75–85°C under 70 atmospheres pressure. Bromine or chlorine also readily adds across double bonds yielding products useful in lubricant formulations. Iodine can be added by warming granular lecithin dissolved in acetic acid in the presence of iodine and magnesium or aluminum catalyst (58).

*Producing fractionated lecithins.* Finally, fractionating crude lecithin directly, or after de-oiling, is another way of creating a variety of products with tailor-made functionalities. Alcohol, or mixed solvent fractionation, combined with other techniques, can produce lecithin products that have been greatly enriched in particular phospholipids. Separating the acetone-solubles from crude lecithin increases the amount of phospholipids in the acetone-insoluble fraction by decreasing the amount of triglycerides.

Further fractionation can separate the alcohol-soluble phosphatidylcholine from the alcohol-insoluble phosphatidylinositol. Commercial products that are alcohol-soluble contain concentrated phosphatidylcholine (40–60%) and only low levels of phosphatidylinositol. The alcohol-insoluble products are enriched in phosphatidylinositol (40–60%), whereas their phosphatidylcholine content is greatly reduced. The phosphatidylethanolamine component is approximately equally partitioned between the two fractions. The alcohol-soluble grades tend to be more oil-in-water emulsifiers, whereas the alcohol-insoluble grades are more effective in water-in-oil systems (7).

The PC/PE ratios of alcohol-fractionated lecithins are largely determined by processing variables such as alcohol polarity, concentration, lecithin/alcohol ratio, temperature, and extraction time (33). By extracting natural lecithin with a PC to PE ratio of 1.2:1 with 90% ethanol, an alcohol-soluble fraction with a PC/PE ratio of 8:1 can be obtained (33, 120). The fractions may be blended with other surfactants or carriers to obtain desired functionality.

To obtain individual phospholipids of greater than 50–60% purity, some form of selective adsorption process is usually required. Adsorption and distribution chromatography present these options. Treatment of the alcohol-soluble lecithin with alumina yields a fraction very rich in phosphatidylcholine and free of phosphatidylethanolamine and phosphatidylinositol (167). Although these products are available only in very limited quantities for highly specialized markets, products such as a lecithin containing up to 95% PC can be obtained commercially.

*Storage and handling.* Liquid lecithin can be kept for years provided closed containers are used and the temperature does not exceed 20–25°C. Bleached products require more careful storage and handling. Color reversion will occur rapidly in bleached products, particularly at elevated temperatures. Decomposition of peroxide is thought to contribute to color reversion in bleached products. In order to prevent this phenomenon, low storage temperatures are recommended (115).

Very low temperatures should, however, be avoided when storing liquid lecithin products because physical separation of the phospholipids and oil may occur. Physical separation is more likely to occur in low AI products. When separation does occur, remixing at 40–60°C will redisperse the oil and lecithin phases. In bulk handling of lecithin, storage temperatures of 30–35°C are acceptable. However, prolonged storage at these temperatures may cause darkening (115).

De-oiled granular lecithin can be stored up to 2 years at temperatures below 25°C. If desired, it may be stored in a frozen state at 0°C, but because of its hygroscopic nature, the product should be allowed to come to room temperature before it is exposed to the atmosphere (33).

## 5. FOOD-GRADE LECITHIN PRODUCTS, USES

### 5.1. Functionality

Commercial lecithins are multifunctional food ingredients. The combined hydrophilic and lipophilic properties of phospholipid molecules give them surface-active effects in many applications. As surfactants, they can exhibit a variety of functions common to other surfactants while they also have unique functionalities of their own.

Commercial lecithin products that were sold many decades ago for applications such as chocolate and confectionery products, margarine, bakery goods, pasta products, textiles, insecticides, and paints are still active today because of their emulsifying, wetting, colloidal, antioxidant, and physiological properties. Lecithin's multifunctional properties and its "natural" status make it an ideal food ingredient. The major applications and functional properties of lecithin products are shown in Table 25 (7).

**TABLE 25. Functional Properties (7).**

Ingredient in	Function(s)
Margarine	Emulsifier, antispattering agent
Confections and snack foods	
Chocolate	Crystallization control, viscosity control, anti-sticking
Caramels	
Coatings	
Instant foods	
Cocoa powders	Wetting and dispersing agent, emulsifier
Instant drinks	
Instant cocoa	
Instant coffee	
Protein drinks	
Dietetic drinks	
Coffee whiteners	
Milk replacers	
Cake mixes	
Puddings	
Instant toppings	
Commercial bakery items	
Breads	Crystallization control, emulsifier, wetting agent, release agent (internal and external)
Rolls	
Donuts	
Cookies	
Cakes	
Pasta products	
Pies	
Cheese products	
Pasteurized processed	Emulsifier, release agent
Cheese and cheese food	
Imitation cheese	

TABLE 25 (Continued)

Ingredient in	Function(s)
<b>Meat and poultry processing</b>	
Meat and poultry glazes and basting compounds	Browning agent, phosphate dispersant
Pet foods	Dietary supplement, release agent, emulsifier
Bacon	
<b>Dairy and imitation dairy products</b>	
Infant, milk formulas	Emulsifier, wetting and dispersing agent, anti-spattering agent, release agent
Milk and cream replacers	
Egg replacers	
Imitation eggs	
Whipped toppings	
Ice cream	
Flavored milks	
Flavored butters (garlic, etc.)	
Basting butters	
<b>Miscellaneous products</b>	
Peanut spreads	Crystallization control, emulsifier
Salad products	
Flavor and color solubilization	
<b>Packaging aid</b>	
Polymer package, interior coating	Release agent, sealant
Can interior coating	
Sausage casing coating	
Stocking net	
<b>Processing equipment</b>	
Frying surfaces	Internal (in product) and/or external release agent, lubricant
Extruders	
Conveyors	
Broilers	
Dryers	
Blenders	
Evaporators	

As a variety of methods are available for modifying the emulsifying properties of commercial lecithin, the potential for improved, tailor-made, functional products is unlimited. The main functional properties are emulsification, antispatter, instantizing/wetting/dispersing, release/parting, viscosity modification, and baking applications.

These functional characteristics are primarily derived from the chemical structures of lecithin's major phospholipids (Figure 1) (7). Phospholipid molecules contain two long-chain fatty acids esterified to glycerol, as well as a phosphodiester bonding a choline, inositol, or ethanolamine group. A phospholipid's fatty acid end is nonpolar and thereby lipophilic (or fat loving). Conversely, the phosphodiester, with the above-mentioned constituents, is zwitterionic (or dipolar), which

explains the hydrophilic (or water loving) properties of this portion of the molecule (65).

Because of their charged nature, the phospholipids are susceptible to the ionic environment in which they function. Based on testing in the laboratory, Dashiell (31) suggests that salt concentrations greater than 2%, and pH less than 4, contribute to a detectable loss in lecithin functionality. Similar results have been reported elsewhere (73).

The following commercial lecithin modifications were described in a publication from Central Soya Co., Inc. (168).

1. The use of oil free lecithins as emulsifiers, lubricity enhancing agents, and blending aids.
2. Producing low-viscosity, fluid lecithins as wetting, dispersing, and release agents.
3. Hydroxylated lecithins with enhanced emulsification, dispersing, and wetting properties.
4. Highly filtered lecithins for use in health food applications.
5. Special heat-resistant lecithins for release applications.
6. Lecithin/distilled monoglyceride blend for bakery applications.
7. Enzyme-hydrolyzed lecithin for bakery, and emulsification applications.

Weete et al. (169) have reported on the improvement of lecithin as an emulsifier for water-in-oil emulsions by thermalization. Various forms of lecithins can be heated under certain conditions of time and temperature to greatly improve their properties as emulsifiers for water-in-oil emulsions. Viscosity, discontinuous phase-holding capacity, stability, and water retention were greatly enhanced in emulsions containing thermalized lecithins as the emulsifier compared with those prepared with corresponding amounts of nonthermalized lecithins. The improved emulsification properties of thermalized lecithins appeared to be, at least in part, because of an increase in diglycerides and free fatty acids resulting from the thermal degradation of phospholipids.

*Emulsifying properties.* One of the major functions of commercial lecithins is to emulsify fats. In an oil:water system, the phospholipid components concentrate at the oil:water interface. The polar, hydrophilic parts of the molecules are directed toward the aqueous phase, and the nonpolar, hydrophobic (or lipophilic) parts are directed toward the oil phase. The concentration of phospholipids at the oil:water interface lowers the surface tension and makes it possible for emulsions to form. Once the emulsion is formed, the phospholipid molecules at the surface of the oil or water droplets act as barriers that prevent the droplets from coalescing, thus stabilizing the emulsion (159).

Commercial lecithins are used in both water-in-oil (w/o) and oil-in-water (o/w) emulsions. For w/o emulsions, like margarine or ready-to-use frostings, oil-loving, lipophilic lecithins are typically used. For o/w emulsions, like sauces or infant formulas, water-dispersible, hydrophilic lecithins are typically used (7, 31). The use of



lecithin in oil-in-water emulsions requires the modification of lecithin to increase its apparent hydrophilicity. The techniques available are somewhat limited for food-grade lecithins. The commonly employed methods for producing water-dispersible lecithins include acetylation, hydroxylation, enzyme-hydrolysis, fractionation, de-oiling, or blending with other hydrophilic emulsifiers.

The manner in which lecithin is modified to achieve increased hydrophilicity will greatly affect its emulsification properties. Different modifications will create lecithin products with different "apparent HLB (hydrophile-lipophile balance) values," a term used to convey the approximate degree of water dispersibility (hydrophilicity) of lecithin products (31). The higher its HLB value, the more water dispersible the lecithin product. In o/w emulsions, the type of fat to be emulsified may require a specific type of hydrophilic lecithin for optimum emulsion stability. Dashiell (31) provides a short listing of fat types, and the corresponding class of lecithin found to give the most stable emulsion in model systems of water/fat/emulsifier.

Standard-grade (crude) lecithins are excellent water-in-oil emulsifiers. However, modified lecithins can function to emulsify either water-in-oil or oil-in-water emulsions, depending on the type of lecithin modification and the specific parameters of the system. These system parameters can include pH, types of components, component ratios, solids content, and others. Unlike crude lecithins, hydroxylated lecithins are stable in acid systems ( $\text{pH} \approx 3.5$ ). Fractionated lecithins can be manufactured for specific emulsion types. As lecithin's emulsifying activity is partially dependent on its phospholipid ratio, changing the ratio can alter its emulsifying capabilities (7).

Emulsifier/stabilizer systems are normally used to make stable food emulsions. Thus, lecithin is generally not called on to handle the entire emulsification, but it works in combination with other emulsifiers and stabilizing polymers such as proteins, starches, and gums (31). Lecithin will break up (emulsify) the particles, and a stabilizer (water-soluble polymer, etc.) will hold the particles in a dispersed orientation when a stable emulsion is formed.

Marrs et al. (170), showed that a combination of lecithin and carrageenan produced more stable o/w emulsions with corn oil than either the lecithin or carrageenan alone. The emulsifying properties of the lecithin/carrageenan combination was thought to be caused by interactions in the aqueous phase between the negatively charged sulfate ester groups on the carrageenan with the positively charged amino head groups on the PC and PE of the lecithin. This stabilization occurs when the carrageenan component is of the kappa or iota type, both of which form macromolecular networks in the presence of cations.

The rate of creaming of an emulsion is governed by Stoke's Law (171). Consequently, in an o/w emulsion, fat separation can be delayed by reducing the fat droplet size, by matching the densities of the dispersed and continuous phases, and/or by increasing the viscosity of the continuous phase. The amount of energy applied to the system when the emulsion is created (for example, with a high-pressure homogenizer) determines the initial fat droplet size of the emulsion, and the presence of an emulsifier on the surface of the droplets, prevents the dispersed oil

droplets from coming together and creaming out. The viscosity of the continuous phase can be increased by the addition of gums, starches, or other stabilizers.

Dashiell (31) reported that with high levels of good-quality protein present, the selection of a system-specific lecithin (one giving the best emulsion in an oil/emulsifier/water system) becomes less important. In fat creaming tests, in fat/water/protein/lecithin emulsions, results showed that with limited protein in a whey-stabilized (low-protein) system, more functional lecithins gave a clear advantage. In a casein-stabilized system (containing abundant protein), differences between lecithin products were less dramatic. Agboola et al. (172), showed that the presence of 0.25% of a de-oiled, hydroxylated lecithin, stabilized o/w emulsions formed with whey protein hydrolysate after retorting.

*Solubilization.* Most lecithins can aid in the production of microemulsions, an example being oil-soluble flavors in aqueous systems. Although standard-grade lecithins do not disperse in water, many modified or fractionated lecithins are water-dispersible, and they can be used to produce microemulsions. Standard-grade lecithin can be blended with other surfactants (e.g., ethoxylated monoglycerides) to produce synergistic emulsifier blends that are also effective in producing microemulsions.

*Solid particle dispersions (Sols).* Many lecithin products are still the best and most effective surfactants for dispersing sols. This seems to be because of lecithin's affinity for solids—liquid surface interfaces. Phospholipids seem particularly attracted to particles containing metals and metal salts. Examples of food sols include some liquid chocolates, instant drinks, frosting mixes, pigmented foods, and others. The nonfood applications include paints, inks, and other pigmented coatings.

*Foams.* Refined lecithins have been employed as effective foam control agents. Examples include whipped toppings, ice creams, and many types of candies. Refined lecithin products have also been employed as effective defoaming agents in foams caused by powdered proteins in water. This is an excellent example of the system specificity of lecithin products (7).

*Wetting/instantizing properties.* Lecithin products are effective wetting agents for a wide variety of powdered or granular products. Lecithination of powders for improved wetting, and control of dusting problems, is widely practiced. Instantizing effects can be obtained by including the proper lecithin product in a food formulation. Specific lecithin products that are compatible with the various manufacturing techniques used for instantizing are commercially available.

Lecithin products have been formulated to instantize many types of food powders to achieve rapid wetting and dissolution. As powder compositions can vary greatly (from hydrophilic to lipophilic), proper lecithin selection is done on something of an empirical basis (31). Certain general principles apply, however. If a powder is hydrophobic, or contains a significant amount of surface fat, typically a water-dispersible, hydrophilic lecithin is used to reduce the surface tension between the powder and the water so that the powder wets and disperses easily. If a powder is hydrophilic, like protein concentrates or isolates, typically a lipophilic lecithin is used to control the rate of hydration of the powder so that it wets and disperses without skinning or forming large lumps.

Manufacturing techniques employed in producing instant products include spray-coating dry powders with fluid lecithin products, cospray drying powders with more hydrophilic lecithins such as the oil-free forms, or hydroxylated lecithin, and agglomeration of the powder with an aqueous dispersion of a hydrophilic lecithin. Some types of powders, for example, starches, gums, and chocolate drink mixes, require agglomeration with an aqueous dispersion of lecithin to achieve optimum wettability and dispersibility.

Examples of foods that can be instantized with lecithins include cocoa powders, instant drink mixes, powdered coffee whiteners, milk replacers, cake mixes, powdered instant puddings, and instant soups and sauce mixes.

*Release/parting properties.* Lecithin functions as the active ingredient in a wide variety of food-grade release formulations. Products for institutional and retail use are available in aerosol and nonaerosol forms containing from 0.5% to about 15% lecithin (31). Common ingredients in release formulations are as follows (31) (from a Central Soya Co. market survey, 1986).

- Vegetable oil (all major vegetable oil classes are used)
- Hydrogenated vegetable oil
- Mineral oil
- Lecithin
- Flour
- Amorphous silica
- Artificial flavor
- Artificial color
- Beta-carotene
- Preservatives
- Antioxidants
- Antifoams
- Water

Lecithin usage levels in commercial release formulas are limited by a tendency of the lecithin to separate from some oils. The tendency of ordinary lecithins to darken, polymerize, and foul on heated metal baking surfaces also limits their use level in commercial release formulas. Moderately and highly acetylated lecithins, however, are resistant to heat and can be repeatedly heated and cooled without darkening (173).

Lecithins can be dissolved in oil, dispersed in water, or used as is, in release applications. Oil-free lecithins can be dry blended into breadings, coatings, and spice or seasoning mixes for release of the coated food product from the food-contact surface. In food products that have a high surface area-to-volume ratio, like pancakes or fortune cookies, lecithin can be added directly to the product formulation to achieve release from the cooking/baking surface. Effective release depends on

the presence of lecithin between the food and the food contact surface. If this is achieved, the food product should not stick to the food contact surface.

*Crystallization control.* Lecithin can control crystallization in various food systems. In foods containing sugars or fats, the presence of as little as 0.5% lecithin can produce altered crystal sizes and structures that can have positive effects on product texture and viscosity. This is important in cookie fillings, butter-containing maple syrups, ice cream toppings, and similar products (7).

## 5.2. Specific Food Applications

As mentioned previously, soybean lecithin is used in food because of its emulsifying, wetting, release, and other surfactant qualities. Relatively small amounts of the lecithin are needed, often only 0.1% to 2% in foods. These use levels are more or less consistent with those of chemical surfactants (7). At these low levels of usage, the color, flavor, and odor of the lecithin normally are not noticeable. When lecithin is used in conjunction with synthetic emulsifiers, it sometimes has a synergistic effect, and thus lesser amounts of the synthetic emulsifiers need be used.

General food applications of lecithin include margarine, confections, snack foods, soups, instant foods, bakery products, simulated dairy products, processed meat/poultry/seafood products, and dietary applications. The most widespread uses of crude lecithin products are in confections and margarine (7, 174).

*Margarine/shortenings.* Standard-grade (crude) lecithin is the classic emulsifier in margarine and is added at the 0.1–0.5% level to the fat phase. It is commonly used in conjunction with mono- and diglycerides. The lecithin prevents “weeping” or “bleeding” of the moisture present, reduces spattering, promotes browning during frying, increases the shortening effect when margarine is used in baking, and helps to protect the Vitamin A in fortified margarine from oxidation (174). More complete and uniform blending of shortening occurs when 0.5% to 1.0% lecithin is added (160).

*Confections.* There are three major specific properties for lecithin in confections: emulsification (e.g., caramels), anti-stick/release properties, and viscosity modification (e.g., chocolate) (175). None of these properties stand alone. For example, emulsification in caramels will influence shelf life and texture. In chocolate, viscosity modification will alter production costs and texture of the finished product.

Addition of 0.25–0.35% standard-grade lecithin to the chocolate used in candy-making reduces its viscosity markedly, enables the manufacturer to apply a uniform coating and thus use lesser amounts of expensive cocoa butter, decreases the time for grinding and mixing the various ingredients, and produces a more stable chocolate. Stabilized lecithin-containing chocolate has improved handling characteristics and is more resistant to fat-and-sugar bloom or “graying.” Use of lecithin in other fat-containing candies also prevents graining, streaking, and greasiness.

Studies by Sinram and Schmitt (176) have shown significant improvements in dark and milk chocolates using a fractionated, phosphatidylcholine-enriched soy lecithin as compared with a standard soy lecithin or no lecithin at all. The effect

of reduced viscosity and improved yield value in chocolate depended on the type and dosage of lecithin as well as the fat content of the chocolate.

Addition of 1% to 2% of lecithin to peanut butter gives a smoother, creamier spread. The peanut butter does not separate under wide temperature variations.

*Bakery products.* Lecithin is a useful emulsifier in baked goods such as bread, cakes, sweet goods, biscuits, and crackers. Standard fluid lecithin is not readily dispersible in water, giving it limited functionality in a dough or batter where water is a key component. Modified lecithins, which are water dispersible, provide many benefits to baked goods including improved shelf life, a stronger gluten complex in yeast-leavened dough, reduced dough stickiness, improved tenderness, better release, and reduced checking in products such as crackers and thin bread sticks. Occasionally lecithin is incorporated into shortenings (solid or fluid) that are used in baking, but it is more frequently added as a separate ingredient. It can also be added as part of a dough improver. Gaubert et al. (177) patented a baking improver composition that contained 20% to 30% (by weight) lecithin. It is an easy-to-handle dough improver where the lecithin acts as a binding agent and an emulsifier.

In yeast-leavened dough, the addition of 0.1–0.3% commercial lecithin improves water absorption, ease of handling, fermentation tolerance, shortening value of fat, volume and uniformity, and shelf life (89). If enzyme modified lecithin is used, it extends shelf life by retarding staling or starch retrogradation (99, 178). Lecithin is employed in cake formulations, such as box mixes, so that they will wet rapidly when mixed with water. In biscuits, crackers, pies, and cakes, 1% to 3% lecithin (on shortening basis) promotes fat distribution and shortening action, facilitates mixing, and acts as a release agent (179).

*Bread and rolls.* There is a general consensus about the beneficial effects of lecithin on dough-handling properties [Aberham (180), Kuntze (181), Pomeranz (179), Puchkova et al. (182), Pylar (183), and Zapryagaeva et al. (184)]. Lecithin also has a beneficial effect on baking performance. It is commonly believed that surfactants, such as water-dispersible lecithin, form lamellar-type, ordered structures in the water phase of the dough. Those ordered structures improve the stability of the film surrounding entrapped carbon dioxide (185, 186), resulting in increased volume and improved crumb structure (178). Over 50 years ago, it was confirmed that the addition of lecithin also improved the extensibility, dryness, and machinability of dough, producing bread that has improved symmetry, grain, and texture (187).

Researchers have shown that the native phospholipids in wheat play an important role in the baking quality of flour (188), and the addition of enzyme modified soy lecithin can make further improvements (99). Chung and Pomeranz (189) reported that fractionated lipids, especially phospholipids at 0.2%, provided a significant increase in loaf volume when shortening was added. Johnson et al. (190) reported that adding soy lecithin (PC) to chlorinated, petroleum ether-extracted flour, at 0.2% flour weight, improved volume and grain beyond that obtained with the unextracted flour. Chung et al. (191) found that petroleum-extracted polar lipids were required at a level of 180 mg per 100 g of flour (H.R.W. 12% protein) to

produce bread of desirable volume. Polar lipids were 50 times more functional than protein in improving loaf volume. Cole et al. (192) studied the effect of phosphorus-containing lipids (polar lipids) and soy (PC) lecithin on the quality of cookies baked from defatted flour. They found that although those fractions containing lecithin completely restored cookie quality, a phosphorus-free lipid fraction did not.

Enzyme-modified lecithin has the ability to form a complex with the amylose portion of starch, and the straight portions of amylopectin (193–195). By forming a complex with starch, enzyme-modified lecithin effectively slows starch retrogradation and staling. In a study, two water-dispersible lecithins were evaluated against hydrated monoglycerides as starch complexing agents to prevent staling in white pan bread (196). The staling indices demonstrated that water-dispersible, de-oiled soy lecithin gave no improvement in softening versus the control to which no emulsifier was added. An enzyme-modified lecithin, however, gave a significant softening response. This behavior was attributed to starch complexation by the lysophospholipids.

Many workers have demonstrated the synergistic effects in bread making of lecithin in combination with mono- or diglycerides and other surface-acting agents. According to Hampl and Tvrznik (197), the use of lecithin in combination with monoglycerides (1) improves quality characteristics of the raw materials, (2) optimizes technical processing, (3) reduces shortening requirements, and (4) improves overall quality of the final product, including freshness retention and nutritive value. Haarasilta et al. (198) patented an enzyme product containing de-oiled lecithin, or lecithin spray dried with a carrier, for use as a dough improver for bread.

Pomeranz et al. (199) have also studied the effect of 0.5% commercially available lecithins on the quality of bread made from untreated and petroleum ether-extracted flour, at three different shortening levels (0.0%, 0.5%, and 3.0%). The best results were obtained with alcohol-soluble soy phospholipids containing a 2:1 mixture of PC and PE in both untreated and petroleum ether-extracted flours. When added to petroleum ether-extracted flours, 0.5% alcohol-soluble phospholipids replaced 0.8% extracted free flour lipids and 3.0% shortening. Excellent results were also obtained with hydroxylated lecithin, but only with shortening present. In a separate study, Glabe and Anderson (200) tested carrageenan and hydroxylated lecithin in continuous mix bread. Their results indicated that hydroxylated lecithin increased dough stability and loaf volume when carrageenan was present.

Pylar (183) reports that hydroxylated lecithin improves dough extensibility. It has been suggested (179) that hydroxylated lecithins are particularly valuable in bakery products because of their apparent synergy with mono- and diglycerides in addition to their high dispersibility in water systems in contrast to the oil solubility of most lecithins.

Adler and Pomeranz (201) have shown that the addition of lecithin to soy flour-enriched bread can improve its consumer acceptability in the absence of shortening. Even in the presence of shortening, an improvement was observed with the use of lecithin (202).

Mizrahi et al. (203) described the improving effect of soy lecithin on bread containing soy protein isolate. The use of soy lecithin in conjunction with sucrose

esters exerted an improving effect on bread quality in high-protein breads made with soy flour (204).

There is a patent on the synergistic effect of hydrophilic lecithins (HLB-8 or higher) on lipophilic surfactants such as glycerol monostearate (GMS), used primarily in bread and other bakery foods to retard staling (205). Not only does the use of a hydrophilic lecithin result in improved shelf life, but also in improved dough conditioning as exemplified by increased loaf volume, improved symmetry, grain, and texture. Larsson and Eliasson (186) tested the effect of added lecithin on the dough rheology of flour milling streams. They used liposomes formed with a high phosphatidylcholine lecithin, and they concluded that flours containing less polar lipid would be most improved by lecithin addition.

Shogren et al. (206) have found that lecithin, and other dough-conditioning surfactants, counteracted the deleterious effect of up to 15% wheat bran that was added as a source of fiber in bread.

*Cookies, crackers, waffles.* In the processing of cookies and other baked goods containing significant quantities of fat, lecithin promotes even dispersion of the fat throughout the dough (1).

Lecithin aids in the dispersion of fat in semisweet dough, and it improves the emulsification during the creaming stage of short dough (207). It also extends the fat. Lecithin is easily mixed into cracker and cookie dough to modify the consistency, and to make machining easier by reducing stickiness in the finished product. Greasiness of cookies with high shortening content is often reduced by adding small amounts of lecithin to the dough.

Matz (208) also has reported on the improvements obtained with the use of lecithin in the production of cookies. Cookie dough is drier and more machinable with the use of lecithin. Lecithin improves the dispersion of fat so that it more readily mixes with sugar, flour, and other ingredients. Improved emulsification also reduces mixing times. Overdevelopment of the dough can result in lack of tenderness in the cookie. The release quality of lecithin improves the extrudability and release from the die, improving definition of impression.

Kissel and Yamazaki (209) studied the effects of lipid extracts from wheat flour and soy and safflower lecithins on improving cookie spread when the cookies were made from protein-fortified wheat flour. Soy lecithin was most effective, and it also improved the quality of cookies made from weak flours such as sorghum and millet (210).

Waffles made from dough that contains lecithin show better iron-grid release and easier handling. The waffles are stronger, crisper, do not become soggy, and retain freshness better (211). Pomeranz (179) reported that the use of lecithin in waffle formulations improves release from the grill, provides strength and crispness, and reduces sogginess. He also cited the use of lecithin as a mold release agent (i.e., 0.3%) in low-fat formulations such as ice cream cones.

*Cakes, cake mixes, pancakes, doughnuts, sweet rolls, fillings, etc.* Incorporating lecithin in cake formulations substantially improves the quality of cakes (212). Hydroxylated products that have an intermediate degree of saturation were found to be best. Lecithins have been recommended as emulsifying agents for cake mixes

to assure easy pan-release and to prevent the cakes from falling or dipping in the center (213, 214). They also improve volume, crumb structure, tenderness, and shelf life. When lecithin was incorporated at a rate of 1% to 3% into a powdery foaming agent for cake and fry batters, it homogenized the monoglycerides and provided the foaming property (215). Enzyme-modified lecithin could be used effectively.

Wolf and Sessa (216) have advocated the use of lecithin in cake doughnut formulations at 0.5% to 1.0% (based on mix weight) to accelerate mixing of the batter. Prolonged batter mixing results in less tender crumb in the finished product. Lecithin is also beneficial in white cakes, and others that contain only egg whites, by acting to replace the phospholipids normally coming from egg yolk in the formula.

According to Wolf and Cowan (71), the emulsifying properties of phospholipids find extensive use in cake mixes and instant foods. Adding 0.5% to 1.0% lecithin promotes wetting, thereby speeding up mixing of cake-doughnut mixes. Adding lecithin improved keeping qualities, grain, and texture in sweet-dough products (coffee cakes, sweet rolls, etc.), and produced "shorter" dough in these items.

Incorporating lecithin into pie crusts reduced mixing time, produced flakier dough, enhanced release, contributed to uniform browning, and aided as a moisture barrier to protect the crust (179).

Lecithin acts as an aid for the blending of unlike ingredients. An excellent example is the formulation of cream fillings for sandwich cookies. The use of low levels of lecithin significantly improves the ease of blending and mouthfeel of these products, which consist mostly of low-polarity shortening and high-polarity sugar. The lecithin serves as an intermediary to significantly reduce the stiffness and mixing time of the filling (31).

*Reduced fat baked goods and extrusion.* Lecithin is well known for its lubricating effects. In 1947, Pratt (217) concluded that lecithin increased the effect of shortening in bread. Since then, it has become widely recognized that lecithin imparts a lubricating effect to dough and finished baked goods. Lecithin also has a positive effect on lubricity in extruded products. It contributes to increased throughput and decreased clean-out time. Lecithin improves product flow, and it does not have a negative effect on density. In the production of extruded, fat-free pretzels, down time for cleaning the cutting knives can be significantly reduced with the use of 0.5–0.75% (flour basis) de-oiled lecithin. De-oiled lecithin can also be used to replace monoglycerides in some extruded breakfast cereals and special pastas (218).

De-oiled lecithin is also recommended to increase the lubricating properties of reduced fat dough. It helps reduce stickiness for improved production yields and reduce stress on pumps, belts, and motors.

Lecithin is included in several patents for reduced fat bakery products. In a patent for a low-fat cereal-grain, food composition, lecithin is preferably included when an egg-like substance is used. The lecithin, along with other emulsifier components and gums, presumably functions to incorporate air, which would otherwise be incorporated by fat (219). Another patent describes the production of reduced-fat, low-fat, and no-fat baked goods, in which a substantial portion of the shortening



or fat is replaced with an emulsifier composition (220). The emulsifier composition preferably contains 10% to 25% by weight of at least one type of lecithin. Specific reduced-fat products that can be made with the emulsifier composition include fermented crackers, unfermented crackers, cookies, and brownies.

In a patent obtained by Gauthier and Dyer (221), a fat-sparing composition is described that may be used to extend vegetable fat in cookie filler cremes and other cremes. The patent states that lecithin may be advantageously used in that composition. In a similar patent, Abboud (222) used lecithin in a fat replacement composition for ready-to-spread frosting.

*Instant foods.* Lecithin has been used as a wetting agent and emulsifier in instant foods. Foods including cocoa powder, instant drinks, instant cocoa and flavored coffee, powdered protein drinks, coffee whiteners, instant puddings, cake mixes, and instant toppings are widely employed applications for specific lecithins. The most common method to incorporate lecithin is as an external coating on the powder particles. The particular lecithin to be employed largely depends on the hydrophilicity or lipophilicity of the powder system (7).

In recent years, instant food manufacturers have become interested in oil-free lecithins. These products are granular, but they can be fluidized by the addition of water or fats. Additionally, fluid products are now available that are based on oil-free lecithin and have obvious advantages, because of their blandness and handling properties, for instant food manufacturers (223).

*Pan and food release agents.* Lecithin-based release agents are employed in many applications such as frozen waffle manufacture, bakery products, pizza baking, and pasta products. Most industrial griddle frying fats are formulated with lecithin, solely for its release functionalities (7). The products may be spray- or brush-applied to achieve a thin film capable of promoting easy release of baked items from pans and belts. Bakery release agents may contain 2–6% lecithin in a variety of oil bases, and they may also be formulated with particulate matter to provide an additional mechanical release.

The simplest of all food release agents are found in the category of pan oils or griddle greases. These products contain low levels of lecithin (0.5–1.0%). Most of the release action is provided by the mechanical barrier established by liberally coating the cooking surface.

Retail release agents for home use are marketed as aerosols and occasionally as pump sprays. In this setting, the release agent will be used for everything from simple release tasks like pancakes and fried eggs to more challenging systems like cakes or muffins. Consequently, retail products are formulated with relatively high levels of lecithin to provide extra release for difficult applications.

As stated previously, moderately and highly acetylated lecithins exhibit heat-resistant properties that are very desirable to have in many release agent applications (173). A natural crude lecithin is subject to thermally induced reactions that are responsible for the darkening and formation of insolubles that occur after prolonged heating. There are several viscosity grades of heat-resistant lecithins available, and lecithin viscosity varies with temperature. Low-viscosity lecithins can be easily sprayed without dilution, or prepared as part of a spray release system.

Typical formulas for lecithin-based release systems are pan bread: oil 98%, lecithin 2%; aerosol spray: oil 70%, lecithin 8%, propellant 22%.

Release formulas for cakes, cookies, and other difficult specialty products often include 5–15% lecithin, 1–10% particulates (flour, silica, etc.), and various types of oils (mineral, vegetable, etc.).

Lecithins can be directly applied to the surfaces of griddles, continuous oven conveyers, flame broiling equipment, and other cooking surfaces for better release and ease of cleaning. The thinnest layer possible should be used for surface release. Lecithin prepared according to U.S. Pat. 4,479,977 (173) is a very effective release agent when applied to a surface in a very thin film, or used in spray pan release systems. For use on a grill, grinders, extruders, pans, or skewers, spray coverage should be applied with lower misting rather than with an air sprayer.

Continuous, multipurpose ovens that are used to precook foods may use water-filled dip tanks for cleaning and rinsing the conveyor belt. An aqueous release system, containing a water-dispersible lecithin, is added to the dip tank to facilitate release of the food from the oven belt, as well as promote better rinsing and cleaning during cooking. A 10% aqueous dispersion of lecithin is commonly used for this application (224).

An alternative to the use of dip tanks for continuous band ovens is to blend 2–10% heat-resistant lecithin in liquid oil or melted shortening, and spray-apply the blend to the conveyor belt with an air spray system (224).

Refined fluid lecithins are also used to prevent the sticking of high-moisture sliced and shredded products like cheese. Specialty fluid lecithin products are sprayed or wiped onto sheets of processed cheese prior to slicing and stacking. Effective separation of the cheese product requires an even, very thin distribution of a low-viscosity lecithin applied as a fine mist to the moving sheet of processed cheese. Lecithin also works well in separating the slices of certain natural cheeses where the manufacturing process allows the lecithin to be applied as the cheese is sliced. For separating cheese slices and shredded cheese products, 1 kg for approximately 500 m<sup>2</sup> (equivalent to 45,000 slices) of a low-viscosity, sprayable lecithin is used (224).

*Dairy-type foods.* Another major application for lecithin products is in dairy and imitation dairy products. Bily (225) has shown that the addition of de-oiled lecithin to milk during cheese manufacture resulted in an increase of mozzarella cheese yield by 3.58%, and an increase in cottage cheese yield by 8.90%, primarily by increasing the moisture content of the finished cheeses. Turcot et al. (226), confirmed that as the phospholipid content of buttermilk increased, the moisture content of low-fat cheese increased in spite of cheese manufacturing modifications. Drake et al. (227) showed that de-oiled lecithin improved process cheese texture without negatively affecting flavor or acceptance. Trained sensory panelists determined that reduced-fat cheeses containing lecithin were more similar in texture attributes to full-fat control cheeses than reduced-fat cheeses without lecithin. Sipahioglu et al. (228) demonstrated that yield loss and the increase in hardness of feta cheese associated with fat reduction, was overcome by the water absorption capacity of starch and lecithin. The presence of lecithin in low-fat and reduced-fat

cheeses improved the yield, primarily by increasing moisture, and reduced the hardness, while improving the flavor and texture.

Lecithin is used to improve the wettability and dispersibility of various milk powders including whole milk powder (229, 230) and caseinates (231). Oldfield et al. (232) demonstrated that lecithinated whole milk powder had increased coffee stability, with decreased coffee sediment levels over a water hardness range of 0–308 mg/L.

Enzyme-hydrolyzed lecithin has been shown to improve the heat stability of recombined milk products (233, 234), and almost all infant formulas contain either hydrophilic or de-oiled lecithins as fat emulsifiers (235). Other dairy applications in which lecithins are used include frozen desserts, whipped toppings, and yogurt.

*Processed meats.* De-oiled lecithin is used as a key ingredient for the emulsification of fat in canned or frozen meat-containing products. When properly formulated, the lecithin can dramatically reduce or eliminate “fat-capping” in products such as canned chili, sloppy joes, gravies, Mexican meat fillings, and other meat products containing a sauce or gravy (236). A combination of lecithin, textured soy concentrate, and a starch has been recommended for reducing fat separation in canned meat products that are cooked in the can (237).

Oil-free lecithins, or lecithin/distilled monoglyceride combinations, have been recommended for reducing water purge in frankfurter formulations that contain high levels of water (238, 239). Although the lecithin is functional in low-fat or high water frankfurter formulations, it has failed to function in some full fat-containing emulsion meats (240). It was concluded that the presence of de-oiled lecithin in comminuted pork emulsions contributed to the destabilization of the emulsions. This destabilization of meat emulsions by higher-HLB surfactants was also seen by Cheong and Fischer (241).

U.S. Patent 4,434,187 (242) covers a meat curing composition that contains de-oiled, powdered lecithin. The purpose of lecithin in this application is to prevent separation of the brine solution. Lecithin is also used in release agents for meat casings and nets. Hammer, et al. (243) recommended that an aqueous-based release agent for cellulose sausage casings, which contained 5–12% lecithin, be applied at a rate of 450–800 mg/m<sup>2</sup>, based on the lecithin weight.

*Egg replacers.* Lecithins are used in conjunction with dairy and vegetable proteins in an attempt to functionally mimic the lipoprotein complex of egg yolks. A coagulable egg replacer based on whey protein, polyunsaturated fat, and lecithin has been described (31). Another formulation included soy and wheat flour blended with oil, lecithin, carrageenan, and polysorbate 60 to replace up to 75% dry or liquid eggs in a variety of mixes and prepared foods (31). Dashiell (31) also reported on a lipoprotein complex formed from soy isolate, oil, carbohydrate, and various emulsifiers, which is claimed to be useful for whole or partial replacement of egg yolks in baked goods.

*Nutritional and health-related applications.* Lecithin has long been known in the worldwide nutritional community. Dietary supplement lecithin is generally derived from soybean lecithin. Because of its composition of various phospholipids, vitamins, and fatty acids, lecithin is involved in numerous physiological actions that

**TABLE 26. Basic Physiological Functions of Lecithin/Choline.**

Function	Description
Cell membranes	PC provides structural stability for cell membranes, provides a reserve supply of choline, and acts as a second messenger.
Fat transport	PC is a main constituent of the membrane surrounding the fat transporting molecules called lipoproteins.
Methyl metabolism	Choline is one of a few B Vitamins that participate in methyl group metabolism. Methyl groups (CH <sub>3</sub> ) are components of numerous important biological compounds.
Cholinergic neurotransmission	Choline is a principal component in the synthesis of acetylcholine.

range from the molecular to the organ level. Among the phospholipid composition of lecithin, PC is considered the most nutritionally significant. Indeed, choline phospholipids are involved in a myriad of essential metabolic reactions, are important structural components of cell membranes, and are important mediators and modulators of transmembrane signaling. Table 26 provides an overview of the physiological functions of lecithin/choline.

Lecithin is the main dietary source of choline. The U.S. National Academy of Science's (NAS) Food and Nutrition Board recently underscored the importance of lecithin in human nutrition by assigning choline (the vitamin component of PC) a dietary reference intake (DRI) in 1998 (244). Although it is true that lecithin and choline are present in a variety of foods, the specific amount of lecithin and choline in foods is currently unknown. At this time, no comprehensive analysis of choline in the food supply exists. Such an analysis is, however, underway and it is estimated that the extensive USDA database of foods will be fully analyzed for choline content by 2005. The analysis will include all forms of choline found in food.

It is known that the richest sources of lecithin/choline are high-fat/saturated fat/cholesterol-containing foods such as egg yolks, organ meats (liver, kidney), and whole milk. Analyses from the 1970s estimated choline intake to be 730–1040 mg/day in adults consuming a typical western diet (244). However, since the 1970s, dietary recommendations have strongly advocated reducing intake of foods high in fat, saturated fat, and cholesterol. Although this dietary guideline policy has resulted in lower fat intake for many Americans, it is likely that lecithin intake has also decreased considerably in recent years. Indeed, a number of sources have reported steep declines in consumption of eggs, red meat, and whole milk over the past 25 years (245).

Choline has been shown to be essential to the body. In a landmark study in 1991, Zeisel et al. (246) showed that healthy men with normal folate and Vitamin B12 status fed a diet deficient in choline have diminished plasma choline and PC concentrations and subsequently developed liver damage. In other words, when other nutrients are adequate, the body is not able to produce choline in quantities sufficient to prevent liver damage as assessed by elevated serum levels of alanine-aminotransferase (ALT), a critical liver enzyme. These data served as the supporting

TABLE 27. DRI for Choline (244).

Group	Age	Adequate Intake Level for Males (mg/day)	Adequate Intake Level for Females (mg/day)	Upper Limit (g/day)
Infants	0–6 months	125	125	Not possible to establish
	7–12 months	150	150	Not possible to establish
Children	1–3 years	200	200	1
	4–8 years	250	250	1
	9–13 years	375	375	2
	14–18 years	550	400	3
Adults	19+ years	550	425	3.5
Pregnancy			450	3.5
Lactation			550	3.5

evidence for the establishment of the DRI levels for choline. The adequate intake level for choline was determined as the point at which ALT levels returned to the normal range. The adequate intake level was originally set for adult males and has been calculated/extrapolated for other populations. Tolerable upper limits for choline have been set for various age groups. High doses of choline, in the form of choline salts (choline chloride or choline bitartrate), have been associated with sweating, salivation, a fishy body odor, and hypotension (low blood pressure). Table 27 shows the AI and UI values for various populations (244).

Although healthy individuals eating an omnivorous diet are not likely to be at risk of choline deficiency, some groups such as vegetarians, athletes, dieters, and pregnant and lactating mothers can deplete choline stores. Lowered serum choline concentrations have major consequences including hepatic, renal, vascular, neuronal, and infertility problems (5, 247). Further, there is evidence that supplemental choline (intake levels >DRI levels) may be beneficial to the health of some individuals. The following sections discuss the role of lecithin and choline health and disease prevention.

*Liver health.* As noted above, a biomarker of choline deficiency is elevated serum ALT levels, which is an indication of liver damage. One of the many functions of the liver is its role in fat metabolism. Without PC, the liver is unable to synthesize lipoproteins. Of particular importance in liver is the synthesis of *very low-density lipoproteins* (VLDL). With diminished VLDL production, the liver is not able to export lipid. This results in an accumulation of fat in the liver. Lipid accumulation in the liver leads to various stages of liver disease such as liver cell death, fibrosis, cirrhosis, and liver cancer (248–250). The role of choline in liver disease was underscored in the early 1990s when it was determined that patients on extended total parental nutrition (TPN) treatment developed fatty livers (251). At that time, TPN formulas did not include choline. Adding choline (in the form of lecithin) to TPN formulas reversed fatty buildup in these patients, and a

long-term study strongly suggests choline is an essential nutrient during long-term TPN (252). This finding led to the series of experiments that eventually determined the essentiality of choline, and to the choline DRI values.

Because of the link between lecithin/choline and liver health, these substances are being studied for their therapeutic potential. In humans, the primary cause of fatty liver is overconsumption of alcoholic beverages. Results from several studies indicate that the phospholipids found in lecithin, particularly PC, may significantly reduce liver damage caused by alcohol consumption (253, 254), and this effect was not seen when choline was supplemented in the form of choline salts. Interestingly, an editorial on diet and liver disease stated that the most exciting finding regarding diet and liver health is that supplementation with PC protects the liver from the damage associated with long-term alcohol consumption (255).

Other studies in animal models show that a choline-deficient diet promotes liver carcinogenesis (256–261). In fact, choline is the only known nutrient for which deficiency is directly linked to liver cancer in the absence of any known carcinogen (262, 263). Choline deficiency is therefore considered to have both cancer initiating and cancer promoting activities.

*Effects on blood cholesterol.* Many studies conducted from the 1960s through the 1980s investigated the relationship between lecithin administration and serum cholesterol concentrations. In 1989, Knuiman et al. reviewed 24 of these studies (264) and concluded that most of lecithin's cholesterol-lowering benefits were caused by its content of polyunsaturated fatty acids, primarily linoleic acid. They stated that soy lecithin, therefore, was no better than soybean oil in lowering total serum cholesterol. However, this research group failed to consider the effects in subclasses of lipoproteins, basing their conclusions only on total cholesterol. The analysis did not include the positive effects of lecithin on HDL cholesterol, the lipoprotein fraction that lowers cardiovascular disease (CVD) risk by reverse cholesterol transport in which cholesterol is removed from arteries and other tissues and returned to the liver for resynthesis. In a comprehensive review of risk factors for atherosclerosis, Ridker et al. considered 11 biomarkers thought to be associated with increased or decreased risk of CVD (265). They determined that the strongest predictor of cardiovascular disease is an unfavorable ratio of total cholesterol to HDL cholesterol, meaning that those most likely to develop heart and vascular disease are those with low HDL cholesterol and high total cholesterol. Thus, simply considering effects of lecithin on total serum cholesterol tells only part of the story regarding risk factors for CVD.

O'Brien and Andrews (266) found that, in men, soy lecithin increased HDL levels significantly more than egg lecithin or fatty acids. Wojcicki et al. also showed that lecithin can reduce LDL cholesterol, although this study lacked proper control (267). The ability of soy lecithin to alter blood cholesterol has led to some provocative research in adding lecithin to certain cholesterol-lowering drugs. One of the problems with drugs like clofibrate is that, although it can lower levels of VLDLs, thus lowering CVD risk, it can raise LDL levels, increasing CVD risk. One study found that, when administered with clofibrate, lecithin prevented the increase in LDLs seen with clofibrate alone (268).

The primary mechanism by which lecithin lowers cholesterol is by decreasing the absorption of dietary cholesterol from the intestine to the blood stream (269, 270). There is also evidence that lecithin intake lowers cholesterol by increasing the amount of cholesterol used in the production of bile salts (271). As more cholesterol is used for bile salt synthesis, less is available to reach the blood stream and damage blood vessels. Lecithin also contributes polyunsaturated fatty acids to the diet, which may help control blood cholesterol levels.

*Direct effects on atherosclerosis and artery health.* Data from the 1960s suggest that phospholipids may play a dual anti-atherosclerosis role by mobilizing cholesterol from arteries and by preventing the atherosclerogenic actions of cholesterol (272). Phospholipids administered intravenously have been observed to have an anti-atherosclerotic effect in several experimental animal species (273–279). There are a number of theories on the mechanisms of lecithin's anti-atherosclerotic actions. Older theories attributed these effects to "detergent" effects and simply the lowering of serum cholesterol (278). More recently though, Hsia et al. (279) suggested that PC is taken up by HDL to form PC-rich vesicles that have a greater capacity than native HDL for promoting cholesterol efflux, leading to depletion of cellular cholesterol and increased LDL receptor activity and reduced arterial damage. However, exact mechanisms on endothelial repair and growth factors are yet to be determined.

Finally, lecithin may also affect cardiovascular health by interactions with certain drugs. One such interaction is lecithin and nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs (aspirin, ibuprofen, and naproxen) are currently being studied in a number of trials for their potential effect on the reduction of arterial disease by decreasing the production of certain prostaglandins that cause inflammation. However, because prostaglandins also act to protect the stomach lining, these drugs can also cause upset stomach, gastrointestinal bleeding, and ulcers. The addition of lecithin to NSAIDs not only prevents the stomach irritation associated with these drugs (282), but also may potentiate their effects (283). A study in rats showed that free and lecithin associated-aspirin appear to be equally absorbed, but the latter form has greater antipyretic, anti-inflammatory, and analgesic efficacy. The improved efficacy may be caused by increased uptake by target cells, increased binding to cyclooxygenase (the rate limiting enzyme in the synthesis of pro-inflammatory and other prostaglandins), or prolonged half-life (283).

*Effects on brain development.* In the developing fetus, choline plays a critical role in the development of the hippocampus and septum, brain areas thought to be heavily involved in the formation and retrieval of memories (284, 285). Given the role of choline in the developing brain, it was speculated that choline supplementation may improve cognitive ability. In a series of experiments, Meck and Williams gave pregnant rats supplemental choline at various times during pregnancy (286–289). After the pups were born and weaned, they were tested in a series of maze memory tests. In these maze tests, the rats need to "remember" which arms contain food. Rats with a greater ability to form memory cues make fewer mistakes when recovering the food. It was determined that rats whose mothers were given the supplemental choline in their drinking water during days 12–17

of pregnancy (corresponding to weeks 20–25 in a human pregnancy) had significantly better memories than the control animals and animals provided supplemental choline during other periods of gestation. Interestingly, this critical period for choline supplementation and cognitive enhancement corresponds with progenitor cell differentiation and migration during development of the hippocampus. Additional experiments indicate that numerous aspects of cognitive function are enhanced by supplying additional choline during this gestational period (286–288, 290, 291).

There may also be an additional critical period for choline supplementation soon after birth. Feeding choline to the rat pups, from day 16 to 30 of life, also resulted in better memories, but not as dramatically as did supplementation during the prenatal period (289). Importantly, the cognitive enhancements observed are not simply transient effects in young animals. Rats whose mothers were given supplemental choline during pregnancy consistently performed better in the maze and other tests throughout their lives (292). Further, when tested at a very elderly stage of life, these rats continued to have better memories and less mental deterioration.

Choline plays many critical roles in human fetal development, and pregnant or lactating women have increased needs for choline. Choline is transported across the placenta from the mother to the fetus by a 1:14 ratio (293, 294). This creates a large drain on maternal stores of choline making the mother more vulnerable to the effects of choline deficiency during pregnancy. Similarly, the choline concentration in mother's milk is over 100 times the level in the maternal bloodstream (295). Because of this heightened requirement for choline, it is likely that both pregnant and lactating mothers have lower serum choline concentrations than nonpregnant women. Currently, research is underway to investigate the impact of choline supplementation during human pregnancy, and during the first 6 weeks after birth, on the brain function of babies.

*Effects on cognitive performance and memory in young adults.* Because lecithin supplies added choline and PC, the precursors to the formation of acetylcholine in the hippocampus, lecithin has been studied for its possible memory-enhancing effects (296). Interestingly, it was found that, although improvement in memory was seen in almost all participants, those who were the slowest learners initially showed the greatest improvement with administration of a 25-g dose of lecithin (supplying 3 g of choline). Using a similar memory test, a second group of researchers found that subjects ingesting 7.5 g of choline (as choline chloride) significantly improved/learning performance (297). Again, the memory improvements were most effective in the slowest learners in the study.

*Effects on memory in older adults.* Studies have also shown that choline supplementation improved both immediate memory and logical memory, or free recall, in older adults with memory deficiencies (298, 299).

These studies demonstrate that choline ingested in the form of lecithin or lecithin precursors [diacylglycerol choline phosphotransferase (CDP)-choline] can improve mild memory loss associated with the aging process. Choline chloride, however, appears to be less effective than lecithin for improving memory, likely because of its lower bioavailability (300).



### 5.3. Flavor in Food Systems

The flavor of lecithin products, and the effect of lecithin on other flavoring materials, has received some attention, but much remains to be done. Some factors affecting flavor biogenesis in phospholipids have been reviewed by Sessa (301). The flavor profile of commercial lecithins can vary greatly. The typical odor/flavor profile of commodity grade soybean lecithins is slightly green and beany. Certain additional notes may be present in chemically modified or fractionated lecithins. Much of the green, beany character can be eliminated by removal of the vegetable oil fraction, leaving a slightly nutty-flavored, dry powder or granule. Through gas chromatography and mass spectroscopy, 79 volatile compounds were identified in soybean lecithin (302).

Producing bland fluid lecithins is possible by redispersing the de-oiled lecithin in clean oils that have been properly refined and deodorized. Lecithin flavors are not typically a problem in food applications because lecithins are used in most foods at low levels. Laboratory tests have shown that in finished food formulations, lecithins can either accentuate or reduce the flavor intensity of salt, acids, and some spices. However, lecithin flavors can penetrate bland dairy-based systems. Studies have shown that 0.25% de-oiled lecithin could be detected in cottage cheese (31).

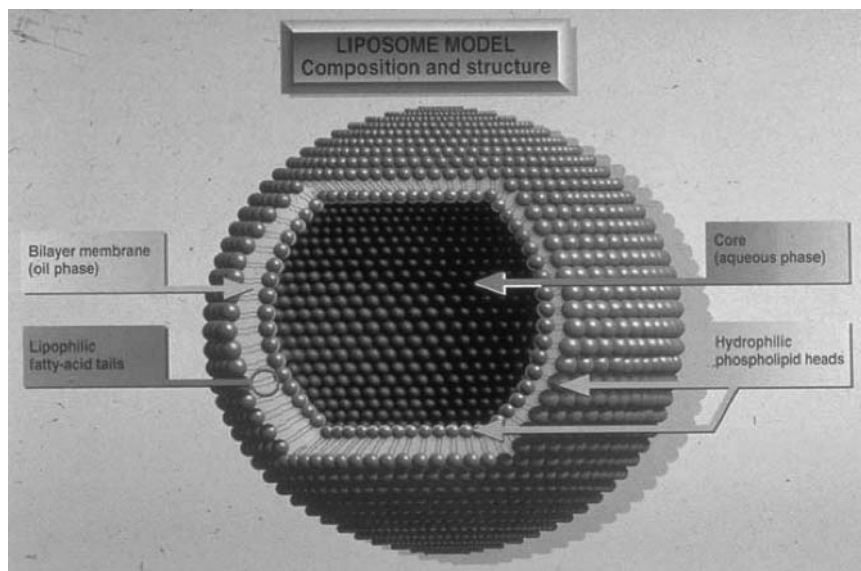
Lecithin is used in some applications for its apparent ability to modify certain flavors. Dashiell (31) has reported on the use of lecithin to modify flavors in chewing gum products, claiming that the lecithin reduced certain bitter flavor principles.

In summary, phospholipids affect the sensory properties of appearance, color, flavor, taste, and texture of foods, the key attributes that determine consumer acceptance. The flavor of phospholipids, and their interaction with both desirable and undesirable flavors, is extremely critical and contributes to the acceptability of foods containing these preparations. As various phospholipids have different physical and chemical properties, they can affect food quality to different degrees. This depends on the phospholipid's nature and content in the oil, the presence of other compounds, and the oil system in which phospholipids exist (301).

Although much progress has been made in reducing flavor, some residual flavors always remain (303). In most foods, they are diluted and masked when the lecithins are incorporated at low levels. Flavor is a particular problem, however, in bland foods, such as dairy products. For more details on the role of lecithin in flavor development in foods, the reader is referred to reviews by Min and Stasinopoulos (304), Sessa (301), and Sipos (305).

### 5.4. Liposomes

Liposomes typically consist of bilayers of phospholipid molecules arranged in a spherical manner. Liposomes can be either unilamellar, consisting of a single bilayer, or multilamellar, consisting of concentrate spheres of bilayers with water separating each bilayer. Liposomes have generated great interest as carriers for various active ingredients, with water-soluble actives encapsulated in the liposome core, and oil-soluble actives encapsulated in the bilayer. Figure 4 depicts a model of a liposome (306).



**Figure 4.** Model of a Liposome (306). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

Liposomes have been used for years as components of drug delivery systems, and as transdermal carriers of active ingredients in the cosmetic industry (307, 308). More recently, liposomes have found use in the food and nutritional supplement industries. Keller (308) lists more than a dozen nutritional products on the market that have been formulated with novel liposome-based delivery systems. In the food area, liposomes have been studied for their ability to encapsulate and provide controlled release of enzymes (309, 310), and liposome-encapsulated enzymes have been used to accelerate the ripening of cheese (311).

U.S. Patent 4,999,208 (312) covers the extrusion baking of cookies having liposome encapsulated ingredients.

## 6. ANIMAL FEEDS, USES

### 6.1. Animal Feeds

Many companies sell crude lecithin gums to the feed industry with little or no further processing. However, the full nutritional and functional advantages of phospholipid use in animal feeds can be obtained through exploitation of the different types of lecithins available (313).

**6.1.1. Functional Uses of Lecithin in Animal Feeds** Lecithins can be employed in animal feeds for their numerous functional properties. These applications result from lecithins' emulsifying, wetting, colloidal, antioxidant, and physiological properties.

*Milk replacer fat emulsion.* One important application of lecithin in animal feeds is as an emulsifier (314). As an example, milk replacer fat is emulsified to improve product wetting and dispersion upon mixing, to help minimize fat separation (i.e., emulsion stability), and to significantly reduce fat particle size, allowing improved fat digestibility. As an emulsifier in milk replacers, lecithin is used at 3–12% of the added fat. The type of lecithin used is affected by factors such as fat type, protein type, and the presence of stabilizers.

*Canned food emulsion.* Another emulsification application for lecithins is in canned pet foods. Lecithin can help maintain homogeneity within the ingredient mixture during pumping to the cans, can reduce fat capping and crystallizing within the can, and can keep gravies and sauces consistent and smooth, without separation while stored in the cans. Lecithin is used at 0.25% to 0.75% of the canned pet food formula to achieve these effects (315).

*Canned food lubricity.* Lecithin provides lubricity in canned pet food applications. This lubricity reduces stickiness and eases the flow of the food through the processing equipment. In this regard, lecithins are superior to fats. For this reason, the use of lecithin can reduce the need for fat in ingredient blends, and a superior machinability is achieved. Lecithin has been used at rates of 0.25–1.0% of the ingredient mixture in canned pet foods for these benefits (315). Lecithin also functions as a release agent. Spraying a thin film of lecithin on the inside surface of cans prior to filling helps to provide cleaner and faster parting of the contents from the can (315).

*Extrusion.* Lecithin is used during the extrusion of pet foods. Specifically, the lubricity property of lecithin has been capitalized on for extrusion of companion animal foods (e.g., dogs, cats, horses). De-oiled lecithin (0.5–1.0%) and fluid lecithin (0.75–1.5%) reduce extruder motor load, allowing an increase in production rate at the same energy cost. At the quoted lecithin usage, production rate increases of 10% to 25% have been observed. Another aspect of lecithin use during extrusion is production stability. Ingredient combinations of some companion animal formulas are resistant to passage through an extruder. This characteristic results in “surging” of production as pressure is built up and released during extrusion. Lower calorie, higher fiber food production is prone to this difficulty. Lecithin reduces this fluctuation of rate through the extruder, which allows more rapid establishment and maintenance of production stability. The natural lubrication properties of lecithin also reduce mechanical wear. Less wearing of parts increases their functional life and reduces production costs. Maintaining particle size and shape during extrusion is a problem with some foods. In some formulas, lecithin smoothes the passage of the extrudate through the die, which leads to improved product uniformity. Additionally, lecithin reduces the amount of clumping that occurs in some products’ particles prior to reaching the dryer. Clumped product is screened away and must be discarded or reworked, which decreases production capacity.

Lecithin use in extruded foods increases bulk density 0–20%. The level of change is related to the amount of lecithin and the ingredient composition of the food. In some cases, the difference in density is so slight that no change in

packaging is indicated. When density is significantly increased, existing packaging is too large without modification. However, changes can be made during production to maintain product density as a constant when lecithin is used. For example, increased steam addition, and/or decreased die area, increase expansion and decrease density. An increase in density is not always a negative, especially with new formulas and low-calorie or “light” foods. Higher density foods require smaller bags and lower storage and transportation volumes. Lecithin benefits water distribution and retention in finished extruded foods. Lecithin reduces moisture migration into and out of products. This influence provides a benefit through a reduction in the development of “staleness” of the food (316, 317).

*Baked pet food.* A variety of baked products (foods, biscuits, cookies, and crackers) are manufactured for dogs. Lecithin has the same functions and provides the same benefits in these products, as described earlier in this chapter for baked goods produced for human consumption. A usage of 1–3% lecithin, on a fat basis, promotes fat distribution in these baked products. Lecithin is also used in reduced-fat baked items for pets, to replace the lubricity that is lost when the fat is removed. Lecithin is used at rates of 0.25–2.0% of the mixture for these benefits. In reference to machinability, lecithins are superior lubricants to fats. For this reason, the use of lecithin can reduce the need for fat in pet product ingredient blends (318).

Lecithin will function as a release agent, providing cleaner and faster pet food or biscuit parting from a stamp or mold. A reduction in the amount of pressure required to create an impression with a stamp is seen when lecithin is used in the mix. The ease of formation of intricate, stamped, surface appearance details, especially with low-fat products, is also improved with lecithins. When dough is being cut, lecithin improves release from the die, especially with rotary cutters. With superior release, foods and biscuits are formed with a better impression, and lower numbers of “cripples” (i.e., improperly formed product). Some pet biscuits are formed from a batter injected into a mold prior to cooking. Lecithin can improve flowability of the batter so that it fills all the mold corners and cavities. After cooking, lecithin improves release from the mold.

Lecithin is known for its ability to create a nonstick surface. Although fats and oils have been used for this purpose, they can add unnecessary calories and leave an oily residue on the product surface. Lecithin functions by modifying the boundary between a pet food or biscuit and the cooking or mold surface. In this manner, lecithin prevents the products from coming into contact with surfaces where sticking can occur. Low-viscosity lecithins can easily be sprayed without dilution. Other lecithins can be mixed with oils, fats, or water, and then sprayed. Still other lecithins are “heat resistant” and can be used in applications where exposure to prolonged heating would normally cause darkening.

Development of checks or cracks, potentially leading to in-package breakage, can be a problem with baked products, particularly those that are brittle. A low-level lecithin addition (0.25–0.50%) helps to reduce this problem by increasing product cohesiveness. A reduction in fines development within the packaging has also been noted.

*Antioxidant.* Lecithin is a natural complement to antioxidants for stabilizing animal fats and vegetable oils. Phospholipids thus help to reduce development of rancidity and to prolong product freshness (319). Lecithin functions particularly well as a synergist with antioxidants.

*Aquatic feeds.* Lecithin has also been found to have functional application in foods for aquaculture. Extruded fish or crustacean diets, which are designed to sink, must possess particular characteristics to satisfy physical and nutritional requirements. These include a high density, which enables the food pellet to sink in extreme open sea conditions; hardness, which allows the pellet to withstand transfer and handling throughout the feed mill and during shipment to the aquaculture farms; and an open cell structure to facilitate high external fat uptake. The use of de-oiled lecithin (e.g., 0.5%) in the aquatic food addresses these needs (320). Lecithins also reduce leaching of water-soluble nutrients (321) and act as an effective binder (322) in fish foods. The latter authors indicate that de-oiled lecithin is more effective than fluid lecithin in reducing dissolution time and leaching of water-soluble nutrients. Lecithin also has application in artificial microparticulate diets for larval fish. Phospholipids can benefit lipid emulsion and lipid-walled microcapsule formation.

**6.1.2. Nutritional Uses of Lecithin in Animal Feeds** The functional aspects of lecithin use as an emulsifier are referenced above. Emulsification of dietary lipid is also nutritionally important for digestion. Immature animals of all species (e.g., calves, dogs, fish, pigs, and poultry) have limited production of digestive emulsifiers (i.e., bile) and enzymes. Providing lecithin as a dietary emulsifier allows improved utilization of feed energy.

*Piglet digestion.* Low feed intake capacity and poor digestive capabilities characterize the early weaned pig. A challenge is to formulate a palatable, nutrient dense, highly digestible diet. In order to increase dietary energy, supplemental fat is used. An approach to improving digestion and pig performance is to use various supplemental dietary lecithins with or without supplemental fats. Pig responses with dietary lecithin have been inconsistent (323). The successes and failures seen in past studies are difficult to compare and critique. Variables that could have influenced the experiments' outcomes include type and level of lecithin, type and level of fat, diet composition, dietary calorie:amino acid ratio, absolute dietary caloric density and amino acid content, age of pigs, and performance potential of the pigs. Overall, the trend is for dietary lecithin addition, at a rate of 5–10% of the fat, to improve piglet performance.

*Calf digestion.* The digestive system of young calves changes dramatically during the first three weeks of life. During this time, calves are severely restricted in the nutrients that they can digest. Milkfat is the most highly digestible lipid source; however, other fats (e.g., tallow, lard, white grease, palm oil, coconut oil) can be used if they are emulsified and homogenized properly. The major factor influencing digestion and absorption of fats by preruminant calves is emulsification of the milk replacer fat to a particle size less than 10 microns (324). Addition of

lecithin (e.g., 10%) to the fat prior to mixing the fat into the milk replacer can influence the fat droplet size in the emulsion.

In ruminants, lecithins have also been shown to control bloat, a digestive disorder typified by distension of the rumen. The distension is caused by the formation of a foam above the rumen liquor, which blocks the escape of gases (325).

*Crustacean digestion.* Initially, lecithins were believed to be required as a surfactant for lipid emulsification and digestion by crustaceans. However, other evidence suggested that dietary phospholipid improved growth and survival by effects other than enhancement of emulsification and absorption of dietary lipid from the digestive tract. The current consensus is that dietary phospholipids influence the mobilization of lipids (e.g., cholesterol and triglycerides) from the midgut gland to the hemolymph and other extra hepatic tissues rather than in the direct digestion and absorption of dietary lipids. A significant dietary interaction between cholesterol and lecithin exists. Neither of these lipid materials can replace the other in the diet of crustaceans. The zwitterionic properties of phospholipids have been shown to enhance the absorption of various drugs, including the drugs' lipid permeability. This fact becomes interesting in terms of the use of carotenoids in shrimp nutrition. Carotenoids play important roles in health, in reproduction (e.g., development of eggs), and during embryonic and postembryonic development stages. A synergy could exist between phospholipids and carotenoids in the nutrition of shrimp. Another possible role of dietary phospholipids could be improved palatability (chemoattraction) resulting in stimulated food consumption. The phospholipid requirement for larval shrimp is in the range of 3.0% to 9.0% from soy lecithin, in order to supply 1–3% phosphatidylcholine plus phosphatidylinositol. The phospholipid need for juvenile shrimp is within the range of 3.75–4.5% soy lecithin (1.2–1.5% phosphatidylcholine plus phosphatidylinositol). The phospholipid requirement for broodstock is at least 2% soy lecithin (0.65% phosphatidylcholine plus phosphatidylinositol) (326).

Most larval fish (e.g., sea bass, salmon, trout, turbot, seabream, flounder, carp, and goldfish) have a requirement for dietary phospholipids. The phospholipid requirement generally decreases with age or developmental stage. The requirements also differ between species (321).

*Companion animal digestion.* Some nutritionists use phospholipids in the foods of puppies and kittens to improve dietary fat digestion. Other reasons exist for improving the nutrient provision of canine and feline foods. A desire to produce nutritionally optimal versus adequate foods has resulted in changes in pet foods. Many of these changes are reflective of alterations in the eating habits of pet owners. A realization is that not all canines and felines have the same nutritional needs throughout their lifetimes. Advantages to companion animal life cycle foods exist. Foods for senior dogs and cats are among these. Objectives of nutritional management for older dogs and cats include enhancing the quality of life, delaying the onset of aging, extending life expectancy, slowing or preventing progressions of disease, eliminating or relieving clinical signs of disease, and maintaining optimal body weight. Lecithin use at a rate of 1.0–5.0% in the diet of older pets can be a nutritionally advantageous source of essential fatty acids, choline, and energy (327).

*Skin and coat health.* Further justification of optimizing the nutrient provision of companion animal foods is an enhanced owner awareness of, and interest in, nutrition. Owners are more understanding of the quality and longevity of their pets'/companions' lives and the role that nutrition plays. The skin is the largest organ in the body. Maintaining the integrity of the skin, its component cells, and the plasma membranes of the cells is critical not only to the appearance of the animal, but also to the animal's health and well-being. Significant scientific information has been reported concerning the role of phospholipids and essential fatty acids (omega 6, omega 3) in the health of skin and other epithelial tissues such as hair and hooves. Lecithin is an excellent source of phospholipids and essential fatty acids. Dietary fortification and/or daily supplementation with lecithin can be part of an optimal nutritional program for companion animals. Phospholipids and essential fatty acids are present in companion animal's foods. However, specific dietary recommendations for use of lecithin are anecdotal. Lecithin use in dog and cat foods has been in the range of 1–3% (e.g., equivalent to 0.5–2.0% phospholipids). As a daily supplement approximately 10 to 20 g lecithin per 100 pounds body weight (45 kg) is a starting point. A teaspoon of granule de-oiled lecithin is about 2 g and a tablespoon is about 7.5 g. The amount of supplemental lecithin to achieve the desired nutritional effect is influenced by breed and individual animal genetics as well as environmental variables such as humidity level, exposure to sun and wind, frequency of bathing, and others (e.g., allergies). Lecithin is also used in complete equine foods. The usage level has been 1–5% (e.g., 0.5–3.0% phospholipids), with a more common usage level in the 1.0–2.5% range. As a daily nutritional supplement, the amount of lecithin fed is related to state of nutrition, general health, weight, age, and others. Lecithin supplementation can range from 15 to 20 g up to 90 to 100 g per 1000 pounds body weight (450 kg). Note: An eight-ounce standard measuring cup holds approximately 100 g of de-oiled lecithin granules (328). Lecithin is also used in the diets of fur-bearing animals (e.g. foxes, mink) to promote healthy, full hair coats. The reason for lecithin's use in this application is the same as for its nutritional use with companion animals (328).

*Equine health.* The lining of the gastrointestinal tract of animals is composed of a specialized epithelial tissue. Equine physiology, in combination with the employment (e.g., racing, trial events) of this companion animal, creates a unique nutritional application of phospholipids. Stomach ulcers represent a significant problem, and well-studied health condition, in foals and adult horses. The incidence of ulcers in young foals may be 50–60%. In adult horses involved in routine training or racing, the incidence of stomach ulcers can be 80–90%. The presence of ulcers in horses is associated with poor condition, irritability, and poor performance. A majority of equine ulcers occur in the nonglandular fundic region of the stomach. Significant research has suggested that surface-active phospholipids can play a role in supporting the defenses of the squamous epithelium in this region of the stomach. In fact, a number of studies suggest that orally supplemented lecithin can help reduce formation of, and contribute to, healing of stomach ulcers. Reviewing the published information, an approximate daily consumption of 90–155 g of lecithin for a treatment period of 2–4 weeks may be indicated. Routine feeding of lecithin at

a lower rate may help maintain gastric tissue health and avoid ulcer formation. Direct study of lecithin in horses is needed to gain more exact information (329).

*Companion animal memory.* As mentioned, the emotional relationship between people and their companion animals continues to grow. Other areas of companion animals' health and quality of life that can be addressed through their food include brain development, learning ability, and memory. Brain development and maintenance is a life-long process beginning in utero and continuing until death. The importance of optimizing the ability of companion animals to learn and remember will add to the quality of life that they and their owners experience. To this end, choline deserves special attention when companion animal foods are formulated. The unique attributes of lecithin (e.g., phosphatidylcholine) as a highly bioavailable source of choline should be recognized. Optimizing choline consumption during pregnancy can stimulate brain and nervous system development and growth in the fetal young, can result in improved ability of young animals to learn and remember, and can benefit memory into old age. Optimizing dietary choline/lecithin for young adults can improve serial learning and selective remembering, as well as benefit behavior (e.g., reduced excitability, anxiety, reactivity, and aggression). Although memory diminishes with age, optimal dietary supplementation with choline/lecithin can reduce brain cell shrinkage and improve short-term memory and learning in geriatric animals (329).

*Companion animal exercise.* In addition to choline/lecithin involvement in neural functions related to learning and memory, they are also involved with muscle function. Cholinergic nerves carry signals to muscle fibers. Research has shown that intense, sustained exercise lowers plasma choline levels. These declines are prevented by choline/lecithin supplements, which in many cases enhance physical performance (330). In humans (e.g., marathon runners, swimmers, skiers), supplementation is reported to decrease the feeling of fatigue, increase the feeling of vigor, and improve race times. Mechanistically, the supplementation can alter the synthesis and release of acetylcholine from nerve endings, enhancing skeletal muscle contraction. Choline supplementation is also associated with increased choline content of skeletal and cardiac muscle cell membranes. This storage benefits acetylcholine formation and release. Further, the supplementation complements membrane growth and repair. Lecithin use can expand the unsaturated fatty acid content of membranes, increase membrane permeability and cell energy metabolism, and improve exercise capability. The observed result can be improved physical capabilities of exercising horses and dogs (331).

*Poultry.* Lecithin has nutritional application in poultry feeds. The caloric value of lecithin (8900 kcal/kg) for broilers was reported to be greater than tallow (5000–7000 kcal/kg) or soybean oil (8800 kcal/kg) (332). This research was complementary to earlier observations that fat utilization and metabolizable energy value were improved when lecithin was also included in chick diets (333, 334). Phospholipids can increase pigment absorption contributing to egg yolk pigmentation (335). Nutrition also plays a key role in the value of meat. Brazilian research found that lecithin in the diets of broilers improved the sensory scores for breast meat (336).



## 7. NONFOOD AND INDUSTRIAL USES

Utilization of lecithins has expanded beyond traditional applications in oil-based paints, chocolate, margarine, and bakery goods. Lecithin is not only used as a release agent for the baking industry, but also in the adhesives and plastics industries, and is used as a dispersing agent in many industrial applications. Lecithin destined for certain industrial applications may require more rigorous refining conditions than conventional lecithin grades. Cross-reacting lecithin also expands the applicability of lecithin for specific applications.

Lecithin serves as a dispersing agent in water-thinned paints, latex paints, and printing inks; as an antisludge additive in motor lubricants; and in combination with phenolic antioxidants, it serves as an antigumming agent in gasoline. It serves as an emulsifier, penetrant, spreading agent, and antioxidant in the textile industry, and as an antioxidant and dispersant in the production of rubber compounds. Lecithin has found a place in pesticide and herbicide formulations, and it is being used in cosmetics and the pharmaceutical industry. It has also been used for many years as a surfactant in magnetic recording media. Miscellaneous functions of lecithins in industrial applications and a listing of their nonfood uses are given in Table 28 (56).

**TABLE 28. Nonfood Applications for Lecithin, Levels of Use, and Functions (56).**

Application	Levels of Use (%) <sup>a</sup>	Miscellaneous Functions
Adhesives		Dispersing agent or mixing aid, plasticizer
Adsorbents		Adhesion aid, coupling agent, flocculant
Catalysts		Catalyst, emulsifier or surfactant, modifier, wetting agent
Ceramics and glass		Dispersing agent or mixing aid, release or nonstick agent, water repellent
Cosmetics and soaps	1–5	Antioxidant, dispersing agent or mixing aid, conditioning agent, emollient or softening agent, emulsifier or surfactant, liposomal encapsulating agent, moisturizer, nutritional supplement or vitamin source, penetrating agent, stabilizer, wetting agent
Detergents		Anticorrosive, emulsifier or surfactant
Dust control		Adhesion aid, antidusting agent
Dyes	0.5–2	Dispersing agent or mixing aid, coupling agent
Explosives		Antidusting agent, emulsifier or surfactant, stabilizer
Fertilizers		Antidusting agent, conditioning agent, spreading aid
Inks	0.5–3	Color intensifier, dispersing agent or mixing aid, emulsifier or surfactant, grinding aid, stabilizer, suspending agent, wetting agent
Leather	1–2 of oil	Conditioning agent, emollient or softening agent, emulsifier or surfactant, lubricant, penetrating agent

TABLE 28 (Continued)

Application	Levels of Use (%) <sup>a</sup>	Miscellaneous Functions
Magnetic tapes	0.5–1.5	Antioxidant, dispersing agent or mixing aid, emulsifier or surfactant, lubricant, wetting agent
Masonry and asphalt products		Anticorrosive, dispersing agent or mixing aid, emulsifier or surfactant, plasticizer, release or antistick agent, strengthening agent, wetting agent
Metal processing		Anticorrosive, antispatter agent, flocculant, lubricant, release or antistick agent
Paints and other coatings	0.5–5 of pigment	Antioxidant, color intensifier, dispersing agent or mixing aid, emulsifier or surfactant, grinding aid, promoter, spreading aid, viscosity modifier, stabilizer, suspending agent, wetting agent
Paper		Dispersing agent or mixing aid, emollient or softening agent
Pesticides	0.1–5	Adhesion aid, antioxidant, biodegradable additive, dispersing agent or mixing aid, emulsifier or surfactant, penetrating agent, spreading aid, stabilizer, synergist, biologically active agent
Petroleum and other fuel products	0.005–2	Adhesion aid, anticorrosive, antioxidant, dispersing agent or mixing aid, emulsifier or surfactant, lubricant, stabilizer
Pharmaceuticals		Antioxidant, emulsifier or surfactant, liposomal encapsulating agent, machining aid, nutritional supplement or vitamin aid, stabilizer, wetting agent, biologically active agent
Polymers, including rubber	0.5–1.5	Antibleed agent (as in fat bloom), antioxidant, emulsifier or surfactant, dispersing agent or mixing aid, modifier, plasticizer, release or antistick agent, stabilizer, strengthening agent
Printing, photocopying, and photography		Color intensifier, dispersing agent or mixing aid, grinding aid, photosensitive agent, wetting agent
Release agents	2–10	Emulsifier or surfactant, lubricant, release or antistick agent
Textiles	0.2–0.5	Antidusting agent, conditioning agent, dispersing agent or mixing aid, emollient or softening agent, emulsifier or surfactant, lubricant, release or antistick agent, water repellent, wetting agent
Waste treatment		Adhesion aid, dispersing agent or mixing aid, flocculant

<sup>a</sup>Usage level is 0.05–1.5% unless indicated.

## 7.1. Catalyst Applications

Lecithin has some catalytic or cocatalytic effects in multiphase systems because of its surface-active properties. Lecithin is reported to be useful as an emulsifier in the curing of aqueous dispersions of unsaturated polyesters (337). The products are more easily removed from their molds and have improved mechanical properties when lecithin is used. In a fermentation application, 1.5% soybean lecithin acts as an inducer in the preparation of cholesterol esterase using a strain of *Pseudomonas* bacteria (338). Aside from its role as a catalyst, initiator, or modifier, lecithin may have ancillary uses in catalyst systems as part of a protective coating (339).

## 7.2. Detergents

A patent describes the use of soybean lecithin to improve the detergency of anionic detergent compounds in the cleaning of dishes containing dried or baked soils (340). The use of soybean lecithin also has been described to improve a fish-cleaning composition containing alkali metal salts and soaps (341). Fractionated lecithin improves the detergency of *N*-acylglutamate salts (342). The addition of lecithin to a detergent for cleaning hard surfaces can leave behind a protective film that protects against further soil deposition (343).

## 7.3. Paper, Printing, and Ink

Lecithin is reported to be useful as a dispersing and softening agent in paper manufacturing (344). Addition of lecithin to a softened cellulose product such as tissue imparts lubricity that is perceived as softness. It can also help achieve the properties of strength and absorbency that are necessary (345). Partially saponified lecithin has been recommended for this use (346). Lecithin has functionality in printing inks and toner formulations for photocopying (347). It also increases the intensity of colors and serves as a grinding and remixing aid. Some patented products containing lecithin include multidetectable ink compositions (348) and magnetic ink (349). Lecithin is also useful in formulations for removing offset and printing inks (350).

Paper and paperboard are often coated to improve properties such as printability and appearance (351). A coating with superior uniformity, flow properties, stability, and brightness is obtained with a blend of fatty acids and lecithin (351). The increased production speed in paper manufacturing today requires improved lubrication and release. For these purposes, lecithin can be included in the papermaking slurry, or sprayed directly on to the drying drums (352). Lecithin significantly improves the rheological properties of aqueous coatings for rapidly moving webs (353). Hydroxylated and acetylated lecithins can eliminate the need for other surfactants in paper coating and improve the printed surface (354). The use of lecithin can permit the use of high amounts of polymeric binders in conventional processes and equipment and improve the strength of the final product (355).

In recent years, there has been an increasing awareness of recycling and the reuse of cellulose products. Often, used paper is contaminated with coatings and

adhesives. Lecithin is useful as a dispersant for removal of wax and similar contaminants in a recycling process (355). Using lecithin in a rewet process enables processing speeds of 50 m/min in continuous operation for more than 24 hours without lowering the quality of the cast paper (356).

Oil-free phospholipids can be used as photosensitive agents for making positive and negative photographic images for lithographic prints, printed circuits, or similar uses (357, 358). An electrosensitive film useful for duplicating designs uses lecithin (359). Incorporating lecithin in the formulation of nitrocellulose-coated polypropylene films, for preparation of plastic printing plates, improves the clarity and integrity of the copy (360). Lecithin can be used as a dispersing aid for oil-soluble photographic additives (361). It can also be used for pressure-sensitive sheets for transferring colors (362–364) or for correcting typewriter errors (365).

#### 7.4. Agriculture

Lecithin can effectively be used as an additive in agricultural formulations for a wide variety of purposes. According to the environmental hazards they may cause, conventional agrochemicals can be classified in two categories. One includes pesticides with high nonselective toxicity, and the other comprises those compounds that, because of their chemical persistence, are easily concentrated in the food chain (366). In addition to the usual functional properties of emulsification, dispersion, and improved suspension, lecithin can improve adhesion, penetration, and performance (367). Efficacy of agricultural pesticides can be enhanced by incorporating the active ingredient into liposomes (368). Liposomes can also reduce, or eliminate, the phytotoxicity of the active ingredient to the recipient plant, with longer duration because of the time release properties obtained with the liposome (368, 369). It has been reported that lecithin can combine with the fatty substances of vegetable leaves, and form a coating that aids in the rejection of plant viruses. Phospholipid vesicles have been successfully used as model membrane systems to study permeability, ion transport, fluidity, and other properties of biological membranes (370).

The interactions among active ingredients, adjuvants, and target pests are very specific (371, 372). Machine oil and phospholipid mixtures have been patented as fungicides (373). Misato et al. (366, 374) have found that soybean lecithin will arrest the development of powdery mildew on cucumbers, eggplants, green peppers, and strawberries. Sodium bicarbonate containing 0.1% lecithin inhibited citrus common green mold, cucumber powdery mildew, and rice blast better than the bicarbonate alone (375). Another environmentally friendly fungicide, containing lecithin and phosphate, is effective against powdery mildew (oidium), peronospora, botrytis, red fire disease, and black spot (376).

Lecithin-containing formulations are active against mites (377, 378) and against worm infestations on apples (379). Recent developmental work on pesticide formulations has focused on targeted biological agents that are disease specific. A suspension of lecithin and nuclear polyhedrosis virus (NPV) was much more effective at infecting and killing the corn earworm (*Heliothis armigera*) than was NPV by itself

(380). The insecticidal activity of *Bacillus thuringiensis* and its toxin is considerably increased when combined with lecithin (381).

Many concerns have been raised regarding the topical application of N,N-diethyltoluamide (DEET), the primary active ingredient in most commercial insect repellants (382). Because of this concern, alternative ingredients are being sought. A recent patent (382) has been filed on the insect repellent characteristics of a blend of lecithin with other plant extracts. Incorporation of lecithin in an insecticidal collar not only facilitates the migration of the insecticide onto the animal's fur, but also provides a softener and protector on the animal's skin where it is most needed (383). Lecithin is reported to be an active agent in mosquito control (384, 385). The exact mechanism of action is unknown, but the lecithin forms a stable film on water surfaces, which may interfere with the breathing mechanism of the mosquito pupae. Phosphatidylcholine is increasingly being used in insecticide formulations to improve performance. In the presence of lecithin, the stability of photolabile insecticides can be significantly improved (386).

Phospholipids formulated with alcohols, glycols, and/or glycol ethers are reported to have antibacterial effects in agrichemical applications (387). Lecithin is a component of coating compositions recommended for postharvest treatment of a variety of fruits, vegetables (388), and fungi (389). Postharvest treatment of apples with aqueous emulsions containing lecithin and various fats and oils can reduce the incidence of soft scald that occurs during cold ( $-1^{\circ}\text{C}$ ) storage (390). Fungicidal preparations containing organic acids and lecithin have been reported to be useful for oranges (391). As with the pesticide applications, efficacy of any treatment may be very specific, not only fruit or vegetable specific, but cultivar specific has been observed with apples (392).

Lecithin may also find a significant home in applications of fertilizers or growth promoters. Lecithin that has been acidified with propionic acid can significantly enhance the performance of some plant growth modifiers (393). With continued emphasis on environmentally friendly agrochemicals, and concern about pesticide residues in the food supply, the potential for lecithin in agriculture appears poised for growth.

## 7.5. Cosmetics and Personal Care Items

Lecithin, being a natural compound and having multifunctional properties, is a logical choice for cosmetic formulations. These preparations include skin creams and lotions, foundations and cleansing creams, sunscreens, soaps, bath oils, shampoos and hair conditioners, shaving creams, preshave and aftershave lotions, nail enamels, face powder, eye color creams, lipstick, and hair sprays. Lecithin can be used as an emulsifier, spreading agent and/or wetting agent, or as a penetration aid, but one of the primary reasons lecithin is chosen for use in cosmetics is its role in providing "skin feel."

When lecithin is used in coatings containing pigments and other particulates (e.g., Kaolin) (394–396), it produces coatings with smoother surfaces, improved

adhesion to the skin, and better color stability (397). This translates to longer-wearing blushers, eye shadow, and face powders. Lecithin can also reduce the undesirable oily feeling in cosmetics containing oils. In the latter, the product also wears longer and reduces the transfer of substances to clothing because of improved film adhesion. Lecithin alters the emulsion break (“rubout”) during the application of day creams, while acting as a moisturizer (398).

Another important reason for using lecithin in cosmetics is the potential for a reduction in skin irritation. Addition of lecithin to cosmetic powders it can reduce irritation of inorganic powders, and it can even form a barrier that prevents these powders from coming into contact with the skin and drying it out (399). The irritation caused to the skin by soap can be significantly reduced, if not eliminated, by incorporation of lecithin (400, 401). In moisturizing cosmetics, lecithin forms highly viscous liquid crystals, the structure of which inhibits the evaporation/transpiration of water from the skin (402). This moisturizing property extends to keratinized appendages as well. Phospholipid incorporation in formulations to treat hair, hooves, horns, claws, and finger and toe nails has proven to be effective at moisturizing and strengthening these same structures (403, 404).

The release properties of lecithin play a function in dental care as well. Lecithin can be used to loosen plaque on tooth surfaces. In saliva, up to 14% of the total lipid content is lecithin (405). Once bacteria are removed, lecithin can be used as a barrier agent to prevent bacteria from sticking to the teeth. The charged head group of lecithin adheres to the enamel surface, and the hydrophobic portion facilitates removal of plaque and bacteria upon brushing (405, 406). Replacement dentures can also benefit from the unique properties of lecithin. At low levels, lecithin can reduce the viscosity of denture pastes and permit higher adhesive loading, which ultimately results in a stronger adhesive that holds longer, and resists deterioration by saliva and moisture (407).

Lecithin, however, does have some disadvantages. It makes products more susceptible to microbial growth and, being multifunctional, its efficiency and ability to accommodate varying HLBs does not allow it to compete with a host of customized synthetics in special situations (398).

## 7.6. Pharmaceutical and Medical

A significant pharmaceutical application for lecithin is the forming of a protective coating on a capsule or tablet. However, this application will be addressed in a subsequent section (see 7.7.2). This section will focus on the incorporation of phospholipids in pharmaceutical formulations. Phospholipids are components of biological membranes in all living systems. They play important roles in biological processes such as cell permeability, the regulation of membrane-bound enzymes, and lipid and cholesterol transport (89, 408). The neurological importance of phospholipids is emphasized by the phospholipid composition of dry brain tissue, which is about 25% (409). Because of this biological activity, there is a great variety of applications for lecithin in the pharmaceutical field. Liposomes have long been known as potential carriers of pharmacologically active ingredients.

The inherent compatibility of lecithin with living membranes can improve the acceptance of the body for medications and implantable devices by modification of their surface chemistry. The poor aqueous solubility of many biologics presents a problem for human administration. Indeed, the delivery of pharmacologically active agents that are inherently insoluble can be seriously impaired if oral delivery is not effective (410). Liposomes have proved a valuable tool as an *in vivo* delivery system for enhancing the efficacy of various pharmacologically active molecules. Animal studies have shown that liposomes can decrease the toxicity of several anti-tumor and antifungal drugs (411). Studies have shown diseases associated with above normal levels of certain lipoproteins. Intravenous administration of liposomes can reduce lipoprotein concentrations in subjects at risk for such diseases (412). Arterial atherosclerotic lesions and plaques have been shown to shrink when treated with liposome infusions (413).

Although liposomes make use of native phospholipids, conjugation of many biologically active agents with phospholipids via a phosphodiester bond will significantly enhance the bioactivity or bioavailability of such agents (414–416). Modifying the phospholipids with a characteristic antibody, a liposome can be created to target specific cells (414). The biocompatibility of materials used in medical devices, or other implantable materials, is improved by covalently attaching PC to the surface of the device (417). Incorporating PC into the filling solution for soft tissue implants improves the stability, biocompatibility, and appearance of the implant (418).

Aside from being an active pharmaceutical agent, or a liposome-type transport agent, lecithin also has a more traditional function as an emulsifier, wetting and dispersing aid, and stabilizing agent, in pharmaceutical applications. Representative formulations incorporating lecithin include  $\beta$ -lactam antibiotics (419), steroids, such as diethylstilbesterol (420), prostaglandins (421), vitamins (422), and antidiarrhea medicine (423). Lecithin has been used as an aid to facilitate microencapsulation of pharmaceuticals with ethyl cellulose (424). Lecithin can also improve the processing properties of medications formed by extrusion (425).

Medical testing can also benefit greatly by the use of phospholipids. The Venereal Disease Research Lab test for syphilis is based on the use of an antigen solution that includes cardiolipin, cholesterol, and lecithin (426).

## 7.7. Paints and Coatings

**7.7.1. *Paints*** Paints and other types of coatings make up a major portion of non-food applications for lecithin products. The broad range of functional properties of lecithin makes it highly suitable for many different coating formulations. These include paints, waxes, polishes, and wood preservatives. Lecithin is also used as a release agent in coating-related manufacture, including applications in cosmetics and magnetic tape coatings.

The literature is replete with information on special lecithin products in coating applications (56). Various formulations have been published, and special lecithin blends patented, for improved functionality in specific areas. Examples of these

functions are antioxidant; color intensifier agent; catalyst; conditioning aid modifier; dispersing aid; emulsifier/surfactant; grinding, mixing, or spreading aid; wetting agent; stabilizer; suspending agent; synergist; and viscosity regulator (427). Miscellaneous applications involving coating principles include cosmetics, mold release agents, magnetic tape coatings, printing and inks, toner formulations, photographic additives, and polyamide coatings (i.e., nylon leather substitutes) (56).

As lecithin products can function as interfacial agents in paints, lacquers, and printing inks, they can serve as wetting, dispersing, suspending, and stabilizing agents in both oil-base and latex/resin emulsion paints. In paints, lacquers, and printing inks, the choice of wetting agents to use depends on the nature of the pigment, the vehicle, and the processing procedure. As a rule, natural grades of lecithin have been recommended up to 1% (on pigment weight basis), and in some formulas containing carbon black or iron blues, as much as 2% may be required. However, comparative tests have shown that in many coatings, refined-grade lecithins are among the best wetting and dispersing agents, and they can compete with other surfactants available on the market (1, 56, 428).

Lecithin products can function in paints to facilitate pigment dispersion and redispersion (429, 430), and to regulate (generally reduce) viscosity. Lecithin serves as an emulsifier and wetting, dispersing, and stabilizing agent in both oil- and latex-based paints. It shortens mixing time, aids brushing, and increases the covering power of the paint. In oil-based paints, lecithin has been traditionally used as a low-cost pigment grinding aid. Lecithin coats the particles of metal oxide pigments rendering them readily dispersible. If more than one pigment is used, lecithin coating helps maintain a uniform mixture (427).

In water-based paint systems, water-dispersible lecithin products are recommended as low-cost emulsifiers, stabilizers, thickening agents, and spreading aids. It has been demonstrated that in latex paints, pigments were dispersed more rapidly in the presence of lecithin. Paints based on rubber-type vehicles may also be formulated with lecithin to improve color uniformity. In addition, the advantages claimed for lecithin in oil-base paints (i.e., aiding pigment dispersion, shortening grinding time, preventing undesirable hard settling of pigments, and eliminating deflocculation while contributing to the overall stability of the emulsion system) are also valid in water-based coatings (427).

Water-dispersible lecithins are made by chemical modification, or by mixing ordinary lecithin with nonionic surfactants. Many of the products recommended in the literature and technical brochures for water-based compositions include such chemically modified, water-dispersible, lecithin compounds (e.g., hydroxylated, acylated, fractionated, and refined grades) (428, 431–433). Usually 0.5% to 1% modified lecithin is recommended in polyvinyl acetate-based paints, acrylic emulsions, and in butadiene—styrene emulsion paints.

In mixed pigmentations, lecithin additives help to maintain a uniform mixture in both alkyd and latex paints, ensuring uniform settling in storage. Latex paint redispersion is also made more effective with a water-dispersible lecithin additive than with a synthetic surfactant. Paint specimens with lecithin additives, remixed after 28 months of storage, maintained a very high level of uniform color stability (434).



Metal salts of lecithin have been patented as paint additives (435). These salts are reported to improve pigment dispersion, and act as drying promoters and inhibit yellowing of the product. The same patent also promotes the use of phosphorylated lecithin as a rust inhibitor in primer paints. Further, if metal oxides are chemically reacted with lecithin by heating, the dispersibility of metal oxides in both aqueous and organic solvents is improved (436).

Not only is lecithin used as a compounding and formulation aid in paints, but it has demonstrated useful properties in the coating. Standard-grade lecithin, as well as certain derivatized and hydrolyzed lecithins, was found to be useful in coatings to inhibit the rusting of steel in salt spray tests (437, 438). These findings are expected, because lecithin functions as an antioxidant in a number of applications. Lecithin is particularly well suited for coatings used on food-grade containers. One example is its use on cardboard to help form a food-grade barrier (439). Lecithin is also used for its release and sealant properties in polymer coatings for interior coatings (440).

Coating organic polymer films with lecithin can improve the release properties of the film from the crimp jaws of the automated packaging machine (441). In analytical equipment, lecithin is used to improve the wettability of the contact surface, which enables the solvent to be presented uniformly for analysis (442). Incorporation of lecithin in a masking application can reduce bubble formation and improve the uniformity of the application (443). And finally, lecithin can be used as a protective coating for a painted surface such as found on automobiles. Once applied, it facilitates the removal of insects and debris. The coating is resistant to rain and washing away (444).

**7.7.2. Coatings** Lecithin is also used in the production of a protective surface in the manufacture of pharmaceuticals. Many medical substances undergo degradation during storage because of contact with moisture absorbed from the atmosphere (445). Frequently, lecithin is used as a detackifier in coatings for tablets, pharmaceuticals, food, confectionary, and seeds. Often it is used in conjunction with maltodextrin, gelatin, and cellulosic polymers (446-448). Lecithin is also used in coating formulations to produce a coating that imparts sheen to the pharmaceutical, thereby improving its visual appeal (449). Some solid drugs are scarcely soluble in water. Because of low solubility, these drugs have a low degree of bioavailability. The coatings on pharmaceuticals also play a vital role of improving the dispersibility, and hence bioavailability, of pharmaceuticals (448, 450).

On a larger scale, lecithin is used in many applications to reduce the viscosity of the coating material. When used with a casting resin, the integrity of the cast is improved by the viscosity reduction (451). When lecithin is incorporated into a rubberized asphalt coating (i.e., roofing composition or floor coating), it reduces the viscosity and acts as a wetting agent, enabling the coating to be sprayed more easily (452, 453).

**7.7.3. Magnetic Recording Media** Much of the magnetic recording media incorporates lecithin as a processing aid. This includes audio and video tapes, as

well as magnetic media for digital applications. Lecithin functions as a dispersing agent, lubricant, wetting agent, and antioxidant in these formulations. De-oiled lecithins used for magnetic tape should be derived from standard-grade lecithin with very low peroxide and hexane-insoluble values.

A typical coating contains iron oxides (sometimes doped with cobalt or chromium), a polymer system that acts as a binder, various additives (including up to 1.5% lecithin on a dry solids basis), and solvents such as methyl ethyl ketone and cyclohexanone (454–458). This material is milled into a dispersion and deposited onto the tape and dried.

Lecithin can also be used in a tape coating containing abrasive cleaning compounds for cleaning tape recorder heads (459). A magneto-optical imaging film can be made by coating a support with a photosensitive resin-containing magnetic material (460). The coating is prepared using lecithin, and the film is especially useful for Chinese character pattern memory.

Chagnon and Ferris (461) reported a study on the effectiveness of soy lecithin as a surfactant in dispersing pigments for use in magnetic tapes (i.e., in recording media). These experiments indicated that oil-free soy lecithin is effective as a surfactant, and at dispersing a wide range of magnetic particle types in a wide range of solvents.

**7.7.4. Adhesives** Adhesives fill a special niche in the coating arena. They are not to protect a surface, but rather perform the function of bonding two surfaces together. In this application, lecithin can demonstrate its diverse nature by functioning either as a release aid or to enhance the surface interactions. When it is applied to the backside of a pressure-sensitive adhesive, lecithin can prevent the films from sticking to themselves (462). In the production of adhesives, and in processes involving the use of adhesives, invariably adhesive material needs to be removed from adjacent surfaces. Lecithin can be applied to these surfaces as a release aid to prevent adhesive from sticking to the equipment and facilities, and enabling easier cleanup (463). In processes with porous material, such as wood, lecithin can act as both a release aid, and a surface-active agent. Adhesive that gets on the clamp platen surface cures on a lecithin film and can easily be removed. Because of the emulsifying action, the adhesive penetrates the substrate better to provide an improved bond (464). Lecithin can also improve the interaction between two materials, such as rubber belts and steel pulleys, to prevent slippage (465).

## 7.8. Lubrication

With the excellent release properties of lecithin, lubrication would seem like a natural area for its use. Indeed, lecithin has been used as an emulsifier to stabilize oil and water metal-cutting fluids (466). Incorporation of lecithin in the lubricant for forming sheet metal products can improve the electrostatic application properties of the lubricant, especially for food contact applications (467). Soaking valve seals in a solution containing lecithin impregnates the rubber and imparts improved lubrication (468). As with other areas discussed, modification of the lecithin for the

specific application improves the desired properties. Hydrogenation of lecithin produces an effective friction modifier for use in lubricating oils, transmission fluids, hydraulic fluids, and the like (469), whereas the pressure, thermal, and lubrication properties of grease can be improved by incorporation of a phospholipid–boron composition (470).

## 7.9. Bioremediation

There are many areas throughout the world where chemicals of varying persistency and toxicity have been disposed of by dumping onto the ground. In addition to presently existing dumps, there are numerous instances at industrial sites such as petroleum tank farms, chemical plants, and elsewhere, where crude or refined hydrocarbons, such as oil, gasoline, or the like, are spilled on the ground or into bodies of water. Such wastes not only spread out and soak through the surface of the ground, but also seep into the ground and disperse into the ground water (471). One method for cleaning up contaminants is by enhancing the degradation of them through the use of micro-organisms. Phospholipids facilitate the cleanup of contaminated soils by a combined effect of enhancing bacterial growth by supplying essential nutrients, while concomitantly modifying the physical state of oil or wastes, thereby making them accessible to interaction with, and biodegradation by, micro-organisms (471). Tests were conducted on soils artificially contaminated with polychlorinated biphenyls (PCBs) using de-oiled lecithin as a natural surfactant. The degradation of PCBs was significantly enhanced at lecithin concentrations in slight excess of the critical micelle concentration. Lecithin favors the degradation by both increasing the bioavailability of the contaminants and supporting the growth of the degradation bacteria (472).

## 7.10. Plastics and Polymers

Lecithin functions as a pigment-dispersing agent and mold-release agent in the preparation of various polymer products. It facilitates dispersion of polymer resins in plasticizers to form plastisols (473). Polymerization of vinyl chloride in the presence of poly(vinyl alcohol) and lecithin gave a poly(vinyl chloride) that was more thermally stable than the polymer prepared without the lecithin (474). A curing agent for polyurethane contains lecithin as a dispersing agent (475). Inclusion of hydroxylated lecithin in the formulation of polyurethane elastomer functions effectively as an internal mold release agent (476). Hydroxylated lecithin and hydrolyzed lecithin are also suitable dispersants for aqueous polymer emulsions. Aqueous polymer emulsions form polymer films on evaporation of the aqueous dispersing media. On this basis, they are widely used as binders in paper coating slips, textile inks, coating material, finishes, and adhesives. They are particularly useful in that they have a small tendency to promote foam formation, and they are biodegradable (477).

More recent work on inclusion of lecithin in plastics has focused on applications in the medical field. Phospholipids can be used as plasticizers or release agents in

thermoplastics or elastomers for use in biomedical applications (478). Biomedical applications of polymers include use as implants, prostheses, blood or tissue contacting devices, and artificial organs. There are many difficulties because of the incompatibility of living tissues with foreign materials inserted into the body (478). The presence of appropriate phospholipids entrapped in the polymer matrix, at its interface with biological fluids or tissue, can modify the interface and provide the appropriate biological effect (478).

### 7.11. Others

An interesting coatings application for lecithin is in producing nonpyrophoric Raney nickel (339). The lecithin in this application creates a lipophilic surface on the metal, along with a wax, fat, or organic polymer.

Polyamide coatings containing lecithin are recommended as a finish coat in nylon leather substitutes (479). Lecithin has been used in sizing for textiles to prevent the sizing from adhering to non-PTFE-coated equipment (480). In leather cleaning and conditioning compounds, lecithin can improve the wetting and penetration of the conditioning compounds into the leather (481).

Lecithin is used as an emulsifier, wetting and dispersing agent, plasticizer and/or release agent in concrete, asphalt and tar shingling, linoleum tiles and surface sealants, and caulking compounds (4, 440, 473, 482–485). Lecithin is reported (at low levels, 0.05–0.1%) to improve the properties of water-glass-containing coatings for foundry cores and molds (486). Phospholipids are naturally occurring emulsifiers in rubber latex. They can be used as a foam stabilizer in foam fire extinguishing agents (487). Lecithin has also been used to improve the combustion of burner fuels. Burner fuels are typically sprayed into the combustion chamber of a boiler to heat water for steam or power generation. Emulsification of water into the burner fuel can improve the combustion efficiency through a secondary atomization of the oil droplet as the emulsified water vaporizes to steam. These much smaller oil droplets burn more completely, resulting in significant emissions reductions (488).

Finally, lecithin-diamine compounds have been used as functional ingredients in multipurpose gasoline additives (489, 490). For additional lecithin uses, the reader is referred to List and Von Kleinsorgen (482), Meshandin et al. (491), Schmidt and Orthoefer (56, 58), and Wittcoff (4).

## 8. AVAILABILITY AND ECONOMICS

Although lecithin may be obtained from other plant seeds and animal sources, soybeans provide the bulk of what is commercially available. Because they are multifunctional ingredients, lecithin products sell at a premium versus soybean oil.

Current world demand is estimated to be 180,000 to 210,000 metric tons (150,000–170,000 MT of standard products and 30,000 to 50,000 MT of value-added

lecithins). Value-added products have been gaining market share because their specialized chemical and physical properties and the inherent limitations of the standard grades (160).

There is no industry-wide reporting of lecithin sales in specific application areas, and so the exact percentage breakdown of uses is difficult to determine. This problem is also complicated by the fact that as much as 30–40% of the domestic market involves hundreds of small volume users who are serviced by distributors (56). Additionally, some customers may have both food and nonfood applications for lecithin.

For the most part, the greatest percentage of lecithin is used in foods. The second highest use is in animal feeds, followed by industrial applications. Paints and other coatings consume a major portion of the nonfood lecithin. Pharmaceuticals, including dietary supplements, and inks and cosmetics, however, also consume significant portions.

## 9. REGULATORY ASPECTS

In the United States, lecithin is affirmed by the FDA as GRAS and meets standards set by the Food Chemicals Codex (54). It is also an approved ingredient in many important foods having a standard of identity.

Hydroxylated lecithin has special FDA approval with limitations for level and use (60).

The U.S. Standards for Identity for Margarine and Bakery Products, and Food Additive Regulations, allow the use of lecithin; hydroxylated lecithin is allowed in breads without level limitations. Similarly, the use of lecithins is permitted in most countries.

In Europe, lecithins are covered under E322 regulations (492).

Advances in biotechnology in the 1990s has led to genetically modified organism (GMO) issues for phospholipids from soybean, corn, and canola. The European community has reacted to consumer pressure and the “right to know” by establishing GMO regulations (493) that clearly label the presence of GMO products. This has forced the ingredient supplier to validate non-GMO products by DNA quantification and identity preserved (IP) testing. No food regulations are anticipated, at this time, for GM in the United States.

The European reaction to traces of GMO DNA in foods has markedly affected the uses of phospholipids in food and health applications. The “consumers right to know,” and ingredient users’ avoidance of GM products, has led to a decrease in soybean and corn lecithin usage, with a shift to the usage of soft-seed lecithins (rapeseed and sunflower) that are non-GMO.

The U.S. Food and Drug Administration has addressed the issue of labeling regarding genetically engineered foods in its policy position published in the May 29, 1992 Federal Register Notice (57 FR 22984). The U.S. policy reflects the science-based consensus that there is nothing inherently unsafe about genetically engineered foods, and consequently, these foods do not warrant labeling

considerations solely because of the application of genetic processes to maintain and improve characteristics.

## 10. FUTURE PROSPECTS

Just as in some food uses, some of the nonfood applications of lecithin are in mature market areas such as paints and inks. Judging from patent activity, however, growth in some areas seems reasonably assured.

In recent years, pharmaceutical patents accounted for almost 25% of the nonfood patent activity. Pharmaceutical applications, particularly those involving liposomes, should require increasing quantities of refined lecithins. An increased demand for lecithin as a dietary supplement is also anticipated, as the result of dietary reference intakes being established for choline. Besides being a multifunctional food ingredient, lecithin has the benefit of being a widely recognized health food.

The major process developments at the present time are in the fractionation of lecithin, and alternative extraction and de-oiling processes. Markets appear to be developing in the pharmaceutical and cosmetic industries, particularly for concentrated forms of PC. De-oiled, granular lecithins have firmly established markets, and new markets are developing that should ensure the growth of these products as emulsifiers. Overall, the availability looks good for lecithin in both food and nonfood uses if good contact between commercial processors and patent developers can be maintained (7, 56).

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## REFERENCES

1. J. Stanley, in K. S. Markley, ed., *Soybeans and Soybean Products*, Vol. 2, Interscience Publishers, Inc., New York, 1951, pp. 593–647.
2. B. F. Szuhaj, ed., *Lecithins: Sources, Manufacture, & Uses*, (AOCS monograph), American Oil Chemists' Society, Champaign, Illinois, 1989.
3. B. F. Szuhaj, *J. Amer. Oil. Chem. Soc.*, **60**, 258A–261A (1983).
4. H. Wittcoff, ed., *The Phosphatides*, American Chemical Society Monograph Series, Reinhold Publishing Corp., New York, 1951.
5. B. F. Szuhaj and G. R. List, eds., *Lecithins*, (AOCS monograph), American Oil Chemists' Society, Champaign, Illinois, 1985.

6. B. F. Szuhaj and E. F. Sipos, in G. Charalambous and G. Doxastakis, eds., *Food Emulsifiers—Chemistry, Technology, Functional Properties and Applications*, Elsevier, Amsterdam, The Netherlands, 1989, pp. 113–186.
7. W. E. Prosize, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 163–182.
8. C. R. Scholfield, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 1–20.
9. A. Wendel, *Inform*, **11**, 885–890, 892, 992–997 (2000).
10. W. Schaefer and V. Wywiol, eds., *Lecithin Der Unvergleichliche Wirkstoff*, Alfred Strothe Press, Frankfurt, Germany, 1986.
11. A. Kuksis, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 32–71.
12. J. L. Weihrauch and Y-S. Son, *J. Amer. Oil Chem. Soc.*, **60**, 1971 (1983).
13. D. A. Gornall and A. Kuksis, *J. Lipid Res.*, **14**, 197 (1973).
14. M. Schneider in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 109–130.
15. N. Satirhos, B. Herslof, and L. Kenne, *J. Lipid Res.*, **27**, 386 (1986).
16. A. Kuksis in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 105–162.
17. O. S. Privett, M. L. Blank, and J. A. Smith, *J. Food Sci.*, **27**, 463 (1962).
18. R. C. Noble and J. H. Moore, *Can. J. Biochem.*, **43**, 1677 (1965).
19. W. H. Cook and W. G. Martin, in E. Tria and A. Scanu, eds., *Structural and Functional Aspects of Lipoproteins in Living Systems*, Academic Press, Inc., New York, 1969, pp. 579–615.
20. D. A. Gornall and A. Kuksis, *Can. J. Biochem.*, **49**, 51 (1971).
21. P. W. Connelly and A. Kuksis, *Can. J. Biochem.*, **61**, 63 (1983).
22. H. Nielsen, *British Poultry Sci.*, 53–56 (1998).
23. W. R. Morrison, E. L. Jack, and L. M. Smith, *J. Amer. Oil Chem. Soc.*, **42**, 1142 (1965).
24. O. S. Privett, L. J. Nutter, and R. A. Gross, in H. F. Brink and D. Kritchevsky, eds., *Dairy Lipids and Lipid Metabolism*, Avi Publishing Co., Westport, Connecticut, pp. 99–115.
25. R. G. Jensen, *J. Dairy Sci.*, **85**, 295–350 (2002).
26. I. M. Santha and K. M. Narayanan, *J. Food Sci. Technol.*, **15**, 24 (1978).
27. A. N. Siakotos and S. Fleischer, *Lipids*, **4**, 234 (1969).
28. A. N. Siakotos, G. Rouser, and S. Fleischer, *Lipids*, **4**, 239 (1969).
29. T. W. Scott, Setchall, and J. M. Bassett, *Biochem. J.*, **104**, 1040 (1967).
30. R. E. Wuthier, *J. Lipid Res.*, **7**, 544 (1966).
31. G. L. Dashiell, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 213–224.
32. J. P. Cherry and W. H. Kramer, in Ref. 2, Chapt. 3, pp. 16–31.
33. F. J. Flider, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 21–38.
34. E. J. Weber, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 39–56.

35. M. E. Weeks and A. Walters, *Soil Sci. Soc. Amer., Proc.*, **11**, 189 (1946).
36. C. R. Scholfield, T. A. McGuire, and H. J. Dutton, *J. Amer. Oil Chem. Soc.*, **27**, 352 (1950).
37. J. P. Cherry, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 57–78.
38. H. S. Olcott, *Science*, **100**, 226 (1944).
39. Y. A. El-Shattory, *Studies on Cottonseed Phospholipids*, Ph.D. dissertation, National Research Center, Cairo.
40. J. P. Cherry, *J. Amer. Oil Chem. Soc.*, **60**, 360 (1983).
41. J. P. Cherry and H. R. Leffler, in R. J. Kohel, and C. F. Lewis, eds., *Cotton Monograph*, Am. Soc. Agron. Crop Sci. Soc. Amer., Madison, Wisconsin, 1984, p. 511.
42. L. A. El-Sebaiy, A. R. El-Mahdy, E. K. Moustafa, and M. S. Mohamed, *Food Chem.*, **5**, 217 (1980).
43. B. Rewald, *J. Soc. Chem. Ind.*, **56**, 403T (1937).
44. F. W. Hougen, V. J. Thompson, and J. K. Daun, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 79–103.
45. W. van Nieuwenhuyzen, *Agro Food Industry Hi-Tech.*, **Jan.-Feb.**, 11–14 (1999).
46. M. Sosada, *J. Amer. Oil Chem. Soc.*, **70**, 405–410 (1993).
47. W. H. Morrison III, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 97–104.
48. J. Hollo, *INFORM*, **3(4)** 483 (1992).
49. J. Hollo, J. Peredi, A. Ruzics, M. Jeranek, and A. Erdelyi, *J. Amer. Oil Chem. Soc.*, **70(10)**, 997–1001 (1993).
50. T. P. Hilditch and Y. A. H. Zaky, *Biochem. J.*, **36**, 815 (1942).
51. B. Rewald, *Biochem J.*, **36**, 822 (1942).
52. J. G. Parsons and P. B. Price, *J. Agric. Food Chem.*, **27**, 913–915 (1980).
53. C. Ratledge, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 72–96.
54. *Food Chemicals Codex*, 4th ed., National Academy Press, Washington, D. C., 1996, pp. 220–221.
55. R. D. Cowell, D. R. Sullivan, and B. F. Szuhaj, in *Surfactant Science Series*, Vol. 12, Marcel Dekker, Inc., New York, 1982, pp. 230–264.
56. J. C. Schmidt and F. T. Orthoefer, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 183–202.
57. National Soybean Processors' Association (NSPA), *Yearbook and Trading Rules (1986–1987)*, The Association, Washington, D.C., 1987.
58. J. C. Schmidt and F. T. Orthoefer, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 203–212.
59. W. Van Nieuwenhuyzen, *J. Amer. Oil Chem. Soc.*, **53**, 425–427 (1976).
60. *Code of Federal Regulations, Title 21:172.814 (Food and Drugs)*, U. S. Government Printing Office, Washington, D. C., 1998.
61. L. A. Horrocks, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 1–6.



62. R. A. Lantz, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 162–173.
63. National Soybean Processors' Association (NSPA), *Yearbook and Trading Rules (1976–1977)*, The Association, Washington, D.C., 1977.
64. B. F. Szuhaj, private communication, Central Soya Co., Inc., Fort Wayne, Indiana, 1978.
65. D. R. Sullivan and B. F. Szuhaj, *J. Amer. Oil Chem. Soc.*, **52**, 125A, abstract no. 39, presented at Amer. Oil Chem. Soc. Meeting, Dallas, Texas, April 1975.
66. V. P. Rzhekhin, N. I. Pogonkina, and I. A. Sloveva, *Maslob.-Zh. Prom.*, **30**, 11 (1965).
67. B. Vijayalakshmi and S. V. Rao, *Lipids*, **9**, 82 (1972).
68. H. G. M. Daga, *Indian Chem. J.*, **11**, 17 (1976).
69. H. G. M. Daga, *Paintindia*, **27**, 21 (1977).
70. L. Rydhag and I. Wilton, *J. Amer. Oil Chem. Soc.*, **83**, 830 (1981).
71. W. J. Wolf and J. C. Cowan, *CRC Crit. Rev. Food Technol.*, **2**, 81 (1971).
72. P. K. Clark and H. E. Snyder, *J. Amer. Oil Chem. Soc.*, **68**, 814–817 (1991).
73. R. Aneja, J. S. Chadha, and R. W. Yoell, *Fette Seifen, Anstrichm.*, **73**, 643–651 (1971).
74. J. K. G. Kramer, F. D. Sauer, and E. R. Farnsworth, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 97–108.
75. M. Ghyczy, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 131–144.
76. Y. Totani and S. Hara, *J. Amer. Oil Chem. Soc.*, **68**, 848–851 (1991).
77. American Oil Chemists' Society (AOCS), *Official Methods and Recommended Practices*, Champaign, Illinois, including Updates and Revisions up to 1993.
78. W. N. Marmar, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 247–288.
79. R. G. Ackman, *INFORM*, **5**, 1119–1128 (1994).
80. International Lecithin and Phospholipid Society (ILPS), Analytical Method AM 101, HPLC Determination of Lecithins (1998).
81. T. Glonek, *J. Amer. Oil Chem. Soc.*, **75**, 569–573 (1998).
82. C. R. Scholfield, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 7–15.
83. E. Baer and M. Kates, *J. Biol. Chem.*, **185**, 615 (1950).
84. H. Brockerhoff, *J. Lipid Res.*, **4**, 96 (1963).
85. D. J. Hanahan, *Lipid Chemistry*, Wiley, New York, 1960.
86. E. H. Pryde, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 213–246.
87. K. P. Strickland in G. B. Ansell, J. N. Hawthorn, and R. M. C. Dawson, eds., *Form and Function of Phospholipids*, Elsevier Publishing Co., Amsterdam, 1973, p. 9.
88. H. M. Verheij, in R. M. Burton and F. C. Guerra, eds., *Fundamentals of Lipid Chemistry*, BI Science Publication Division, Webster Groves, Missouri, 1974, p. 225.
89. J. Eichberg, in M. Grayson, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 14, 3rd ed., Wiley, New York, 1981, pp. 250–262.
90. J. N. Hawthorn and P. Kemp, *Adv. Lipid Res.*, **2**, 127 (1964).
91. A. Kuksis, *Prog. Chem. Fats and Other Lipids*, **12**, 5 (1972).

92. F. Snyder, in H. Busch, ed., *Cancer Research*, Vol. 6, Academic Press, Inc., New York, 1971, pp. 410–419.
93. L. L. M. Van Deenen and D. H. DeHaas, *Adv. Lipid Res.*, **2**, 167 (1964).
94. R. Kanamoto and co-workers, *Agric. Biol. Chem.*, **41**, 2021 (1977).
95. A. Beckwith, *J. Agric. Food Chem.*, **32**, 1397 (1984).
96. W. S. Chen and W. G. Soucie, *J. Amer. Oil Chem. Soc.*, **62**, 1686 (1985).
97. H. Nielsen, *Lipids*, **16**, 215–222 (1981).
98. K. Allen-Seabolt, Central Soya R&D Report. La-05, Central Soya Co., Inc., Ft. Wayne, Indiana, 1997.
99. P. Forssell, S. Shamekh, H. Harkonen, and K. Poutanen, *J. Sci. Food Agric.*, **76**, 31–38 (1998).
100. D. Johansson and B. Bergenstahl, *J. Amer. Oil Chem. Soc.*, **69**, 705–717 (1992).
101. E. Sambuc, Z. Dirik, G. Reymond, and M. Naudet, *J. Amer. Chem. Soc.*, **58**, 645A (1981).
102. J. Pokorny, *Rev. Fr. Corps Gras*, **28**, 151–160 (1981).
103. B. J. F. Hudson and M. Ghavani, *Lebensm.-Wise Technol.*, **17**, 191–194 (1984).
104. D. H. Hildebrand et al., *J. Amer. Oil Chem. Soc.*, **61**, 552–555 (1984).
105. U. Jung, S.-K. Lee, S.-H. Kyung, G.-H. Chang, I.-M. Chang, and C.-H. Lee, *Food Sci. Biotechnol.*, **10**, 354–359 (2001).
106. B. J. F. Hudson and S. E. O. Mahgoub, *J. Sci. Food Agric.*, **32**, 208–210 (1981).
107. B. J. F. Hudson and J. I. Lewis, *Food Chem.*, **10**, 111–120 (1983).
108. R. J. Hamilton, C. Kalu, G. P. McNeill, F. B. Padley, and J. H. Pierce, *J. Amer. Oil Chem. Soc.*, **75**, 813–822 (1998).
109. H. Saito and K. Ishihara, *J. Amer. Oil Chem. Soc.*, **74**, 1531–1536 (1997).
110. L. R. McLean, K. A. Hagaman, and W. S. Davidson, *Lipids*, **28**, 505–509 (1993).
111. R. Brian, *J. Amer. Oil Chem. Soc.*, **53**, 27–29 (1976).
112. G. R. List, C. D. Evans, L. T. Black, and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **55**, 275–276 (1978).
113. G. R. List, T. L. Mounts, K. Warner, and A. J. Heakin, *J. Amer. Oil Chem. Soc.*, **55**, 277–280 (1978a).
114. K. Nielsen, *J. Amer. Oil Chem. Soc.*, **37**, 217–219 (1960).
115. G. R. List, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 145–161.
116. J. P. Burkhalter, *J. Amer. Oil Chem. Soc.*, **53**, 332–333 (1976).
117. B. Braae, *J. Amer. Oil Chem. Soc.*, **53**, 353–357 (1976).
118. G. R. List, J. M. Avellenada, and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **58**, 892 (1981).
119. L. S. Crauer, Paper presented at *Meeting of International Society for Fat Research*, Goteborg, Sweden, The DeLaval Separator Co., Poughkeepsie, New York, 1972.
120. W. Van Nieuwenhuyzen, *J. Amer. Oil Chem. Soc.*, **58**, 886 (1981).
121. E. Bernardini, *The New Oil and Fat Technology*, Vol. 2, rev. ed., Technologie s.r.l., Rome, Part 3, Sec. 5, pp. 505–538, 1973.
122. F. A. Norris, in D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, 3rd ed., Wiley-Interscience, New York, 1964, pp. 719–792.

123. R. A. Carr, *J. Amer. Chem. Soc.*, **53**, 346–352 (1976).
124. A. J. C. Andersen, in P. N. Williams, ed., *Refining of Oils and Fats for Edible Purposes*, rev. ed., Pergamon Press, New York, 1962, pp. 28–42.
125. P. Sartoretto, in E. A. Parolla, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 12, 2nd ed. Wiley, New York, 1967, pp. 343–361.
126. K. Klein and L. S. Crauer, *J. Amer. Oil Chem. Soc.*, **51**, 368–370 (1974).
127. W. J. Podbielniak, A. M. Gavin, and H. R. Kaiser, *J. Amer. Oil Chem. Soc.*, **33**, 24–26 (1956).
128. F. E. Sullivan, *J. Amer. Oil Chem. Soc.*, **32**, 121–123 (1955).
129. O. L. Brekke in D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soy Oil Processing and Utilization*, American Soybean Association, St. Louis, Missouri and American Oil Chemists' Society, Champaign, Illinois, 1980, pp. 71–88.
130. G. R. List and D. R. Erickson, in American Soybean Association, St. Louis, Missouri and American Oil Chemists' Society, Champaign, Illinois, 1980, pp. 267–354.
131. G. R. List, J. W. King, J. H. Johnson, K. Warner, and T. L. Mounts, *INFORM*, **4**, 510 (1993).
132. A. J. Dijkstra, *INFORM*, **4**, 538 (1993).
133. B. Jirjis, H. S. Muralidhara, and D. D. Otten (to Cargill, Inc.), U.S. Patent 6,207,209, March 27, 2001.
134. C. R. Scholfield and H. J. Dutton, *J. Amer. Oil Chem. Soc.*, **31**, 258 (1954).
135. National Oilseed Processors Assoc., Yearbook and Trading Rules 1998–1999.
136. *Fed. Reg.*, **48**, 51149–51150 (1983).
137. R. Leysen, *J. Amer. Oil Chem. Soc.*, **56**, 892A–894A (1979).
138. N. W. Myers, *J. Amer. Oil Chem. Soc.*, **34**, 93–96 (1957).
139. B. Braae, U. Brimberg, and M. Nyman, *J. Amer. Oil Chem. Soc.*, **34**, 293–299 (1957).
140. G. R. List, T. L. Mounts, A. C. Lanser, and R. K. Holloway, *J. Amer. Oil Chem. Soc.*, **67**, 867–871 (1990).
141. G. R. List, T. L. Mounts, and A. C. Lanser, *J. Amer. Oil Chem. Soc.*, **69**, 443–446 (1992).
142. G. R. List and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **70**, 639–641 (1993).
143. F. Zhang, S. S. Koseoglu, and K. C. Rhee, *J. Amer. Oil Chem. Soc.*, **71**, 1145–1148 (1994).
144. B. Braae, *Chem. Ind. (London)*, **36**, 1152–1160 (1958).
145. F. E. Sullivan (to the De Laval Separator Co.), U.S. Patent 2,702,813, 1953.
146. L. P. Hayes and H. Wolff (to A. E. Staley Manufacturing Co.), U.S. Patent 2,881,195, 1959.
147. L. P. Hayes and H. Wolff (to A. E. Staley Manufacturing Co.), U.S. Patent 2,792,411, 1957.
148. L. P. Hayes and H. Wolff (to A. E. Staley Manufacturing Co.), U.S. Patent 2,782,216, 1957.
149. C. D. Evans, G. R. List, R. E. Beal, and L. T. Black, *J. Amer. Oil Chem. Soc.*, **51**, 444–448 (1974).
150. R. Ohlson and C. Svenson, *J. Amer. Oil Chem. Soc.*, **53**, 8–11 (1976).

151. A. M. Nash, E. N. Frankel, and W. F. Kwolek, *J. Amer. Oil Chem. Soc.*, **61**, 921 (1984).
152. H. J. Ringers (to Lever Brothers Co.), U.S. Patent 4,049,686, 1977.
153. G. Lunde, L. H. Landmark, and J. Gether, *J. Amer. Oil Chem. Soc.*, **53**, 207–210 (1976).
154. A. Hvolby, *J. Amer. Oil Chem. Soc.*, **48**, 503–509 (1971).
155. A. Letan and A. Yaron, *J. Amer. Oil Chem. Soc.*, **49**, 702 (1972).
156. K. Nielsen, *Studies on the Nonhydratable Soybean Phosphatides*, Trans. V. Bonde, Maxsons & Co., Ltd., London, 1956.
157. K. J. Hutton and J. S. Guymon (to ADM Co.), U.S. Patent 6,140,519, October 31, 2000.
158. A. Wendel, *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 15, Wiley, New York, 1995, pp. 197–201.
159. W. J. Wolf and D. J. Sessa, in M. S. Peterson and A. H. Johnson, eds., *Encyclopedia of Food Science*, AVI Publishing Co., Westport, Connecticut, 1975, p. 476.
160. Central Soya Co., Inc., unpublished data, Fort Wayne, Indiana, 1994.
161. P. L. Julian, U.S. Patent 2,629,662, 1953.
162. M. J. Haas, K. Scott, W. Jun, and G. Janssen, *J. Amer. Oil Chem. Soc.*, **71**, 483–490 (1994).
163. A. M. Aura, P. Forssell, A. Mustranta, T. Suortti, and K. Poutanen, *J. Amer. Oil Chem. Soc.*, **71**, 887–891 (1994).
164. L. Juneja, T. Kazuoka, N. Koto, T. Yamane, and S. Shimizu, *Biochimica et Biophysica Acta*, **1003**, 277–283 (1989).
165. I. Svensson, P. Adlercreutz, and B. Mattiasson, *Appl. Microbiol. Biotechnol.* **33**, 255–258 (1990).
166. G. G. Haraldsson and A. Thorarensen, *J. Amer. Oil Chem. Soc.*, **76**, 1143–1149 (1999).
167. H. Betzing, German Patent 1,902,607, 1969.
168. *The Lecithin Book*, Central Soya Co., Inc., Fort Wayne, Indiana, 2000.
169. J. D. Weete, S. Betageri, and G. L. Griffith, *J. Amer. Oil Chem. Soc.*, **71**, 731–737 (1994).
170. W. M. Marrs, G. Sworn, and R. J. Hart, The British Food Manufacturing Industries Research Association Research Report 636, Jan. 1989.
171. I. Y. S. Rustom, M. H. Lopez-Leival, and B. M. Nair, *Int. J. Food Sci. Technol.* **30**, 773–781 (1995).
172. S. O. Agboola, H. Singh, P. A. Munro, D. G. Dalgleish, and A. M. Singh, *J. Agric. Food Chem.*, **46**, 1814–1819 (1998).
173. G. L. Dashiell and W. E. Prosis, (to Central Soya Co., Inc.), U.S. Patent 4,479,977, 1984.
174. T. A. M. Fujikawa and M. Hamashima, Jpn. Kokai Tokkyo Koho, JP 83910 (1979).
175. R. C. Appl, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 207–212.
176. R. D. Sinram and H. Schmitt, *INFORM*, **4**, 509 (1993).
177. H. Gaubert, D. Le cren, and J. Perrier, U. S. Patent 5,650,188, 1997.
178. K. Allen Seabolt, Central Soya Co., Inc., unpublished data, Fort Wayne, Indiana, 1997.
179. Y. Pomeranz, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 289–322.
180. H. Aberham, *Deutsche Mueller-Z*, **70**, 228 (1972).

181. R. Kuntze, Proceedings of the 7th International Congress on the Problems of Modern Grain Processing and Cereal Chemistry, Bergholz-Rehbruecke, 1972, pp. 73–82.
182. L. I. Puchkova and co-workers, *Izv. Vysshikh Uchebnykh Zaved. Pishchevaya Technol.*, **2**, 68 (1975).
183. E. J. Pyler, *Baking Science and Technology*, Seibel Publishing Co., Chicago, Illinois, 1973, pp. 464–485.
184. A. V. Zapryagaeva, L. I. Puchkova, G. S. Fedorova, and L. E. Chernenko, *Izv. Vysshikh Uchebnykh Zav. Pischhevaya Technol.*, **1**, 53 (1970).
185. N. Krog, *Cereal Chem.*, **58**, 158–163 (1981).
186. H. Larsson and A.-C. Eliasson, *J. Sci. Food Agric.*, **77**, 552–558 (1998).
187. F. E. Walrod, in *Proceedings of the 23rd Annual Meeting of the American Society of Bakery Engineers*, 1947, p. 76.
188. O. K. Chung, Y. Pomeranz, E. C. Hwang, and E. Dikeman, *Cereal Chem.*, **56**, 220 (1979).
189. O. K. Chung and Y. Pomeranz, *Bakers Dig.*, **51**, 32 (1977).
190. H. C. Johnson, R. C. Hosney, and E. Varriano-Marston, *Cereal Chem.*, **56**, 333 (1979).
191. O. K. Chung, Y. Pomeranz, and K. F. Finney, *Cereal Chem.*, **59**, 14 (1982).
192. E. W. Cole, D. K. Mecham, and J. W. Pence, *Cereal Chem.*, **37**, 109 (1960).
193. M. Kugimiya and J. W. Donovan, *J. Food Sci.*, **46**, 765 (1981).
194. A.-M. Aura, P. Forssell, and A. Mustranta, *J. Amer. Oil Chem. Soc.*, **71**, 887–891 (1994).
195. W. van Nieuwenhuyzen, *Eur. Food Drink Rev.*, 43–47 (1999).
196. S. Onata and co-workers, *Application of Enzymatic Modification of Phospholipids in Breadmaking*, Am. Assoc. Cereal Chem. 69th Annual Meeting.
197. J. Hampl and K. Tvrznik, Proceedings of the 7th International Congress on the Problems of Modern Grain Processing and Cereal Chemistry, Bergholz-Rehbruecke, 1972, pp. 59–64.
198. S. Haarasilta, T. Pullinen, S. Vaissnen, and I. Tammersalo-Karsten, U.S. Patent 4,990,343, 1991.
199. Y. Pomeranz, M. D. Shogren, and K. F. Finney, *Food Technol.*, **22**, 897 (1968).
200. E. F. Glabe and P. W. Anderson, *Cereal Sci. Today*, **9**, 300 (1964).
201. L. Adler and Y. Pomeranz, *J. Sci. Food Agric.*, **8**, 449 (1959).
202. K. F. Finney, Y. Pomeranz, and G. Rubenthaler, *Cereal Sci. Today*, **8**, 166 (1963).
203. S. Mizrahi, Z. Berk, and U. Kogan, *Cereal Sci. Today*, **10**, 45 (1965).
204. Y. Pomeranz, M. D. Shogren, and K. F. Finney, *Cereal Chem.*, **46**, 512 (1969).
205. W. H. Knightly, U.S. Patent 4,684,526, 1987.
206. M. D. Shogren, Y. Pomeranz, and K. F. Finney, *Cereal Chem.*, **58**, 142 (1981).
207. D. J. R. Manley, *Technology of Biscuits, Crackers and Cookies*, Ellis Horwood Ltd., West Sussex, England, 1983. p. 90.
208. S. A. Matz, *Cookie and Cracker Technology*, AVI Publishing Co., Westport, Connecticut, 1968, pp. 54–55 and 121–122.
209. L. T. Kissel and W. T. Yamazaki, *Cereal Chem.*, **52**, 638 (1975).
210. S. M. Badi and R. C. Hosney, *Cereal Chem.*, **53**, 733 (1976).
211. W. Schaefer, *Getreide, Mehl, Brot.*, **27**, 132 (1973).

212. C. C. Elsesser and S. W. Bogyo, U.S. Patent 2,954,297, 1960.
213. Central Soya Co., Inc., technical brochure, Fort Wayne, Indiana, 2002.
214. C. F. Obenauf and C. W. Tatter, U.S. Patent 3,060,030, 1962.
215. H. Sakka, Y. Ikezoe, and T. Fukuda, U.S. Patent 5,196,226, 1993.
216. W. J. Wolf and D. J. Sessa, in M. S. Peterson and A. H. Johnson, eds., *Encyclopedia of Food Technology and Food Sciences*, AVI Publishing Co., Inc., Westport, Connecticut, 1978, pp. 461–467.
217. D. B. Pratt, Jr., *Baker's Helper*, **84**, 38, 64, 66, 99 (1945).
218. Central Soya Co., Inc., technical brochure, Fort Wayne, Indiana, 1998.
219. P. W. Hahn, U.S. Patent 5,262,187, 1993.
220. E. L. Zimmerman, U.S. Patent 5,514,404, 1996.
221. P. M. Gautchier and W. Dyer, U.S. Patent 5,626,903, 1997.
222. A. Abboud, Canadian Patent 2,201,462, 1997.
223. Anonymous, *Cereal Foods World*, **24**, 52 (1979).
224. Central Soya Co., Inc., technical brochure, Fort Wayne, Indiana, 1993.
225. R. R. Bily, U.S. Patent 4,277,503, 1981.
226. S. Turcot, S. L. Turgeon, and D. St.-Gelais, *Lait*, **81**, 429–442 (2001).
227. M. A. Drake, V. D. Truong, and C. R. Daubert, *J. Food Sci.*, **64**, 744–747 (1999).
228. O. Sipahioglu, V. B. Alvarez, and C. Solano-Lopez, *Int. Dairy J.*, **9**, 783–789 (1999).
229. J. Pisecky and V. Westergaard, *Dairy Industries Int.*, **37**, 144–147 (1972).
230. A. J. Baldwin and W. B. Sanderson, *N. Z. J. Dairy Sci. Technol.* **8**, 92–100 (1973).
231. M. J. James, M. Brault, and L. Coucoulas, Leatherhead Food R. A. Research Report 689, 1991.
232. D. J. Oldfield, C. M. Teehan, and P. M. Kelly, *Int. Dairy J.*, **10**, 659–667 (2000).
233. H. Singh, R. Sharma, and R. P. Tokley, *Australian J. Dairy Technol.*, **47**, 33–37 (1992).
234. C. H. McCrae, *Int. Dairy J.*, **9**, 227–231, (1999).
235. E. H. Sander, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 197–206.
236. Central Soya Co., Inc., technical brochure, Fort Wayne, Indiana, 1992.
237. J. Casey, and S. Campano, Central Soya R&D Report, Lb-15, Central Soya Co., Inc., Ft. Wayne, Indiana, 1998.
238. M. Thomas, Central Soya R&D Reports, Central Soya Co., Inc., Ft. Wayne, Indiana, 1996 & 1997.
239. J. Casey, Central Soya R&D Report Lb-15, Central Soya Co., Inc., Ft. Wayne, Indiana, 1999.
240. J. Casey, Central Soya R&D Report, Central Soya Co., Inc., Ft. Wayne, Indiana, 1993.
241. S. H. Cheong and A. Fischer, *Fleischwirtschaft*, **73**, 677–678, 681–683 (1993).
242. W. S. Chandler, W. F. Wilkens, and J. F. Heiss, U.S. Patent 4,434,187, 1984.
243. K.-D. Hammer, L. Mans, and M. Siebrecht, U.S. Patent 5,480,691, 1996.
244. National Academy of Sciences Food and Nutrition Board, *Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline*, National Academy Press, Washington D.C., 1998, pp. 309–422.

245. C. W. Enns, J. D. Goldman, and A. Cook, *Fam. Econ. Nutr. Rev.*, **10**, 1 (1997).
246. S. H. Zeisel and co-workers, *FASEB J.*, **5**, 2093–2098 (1991).
247. R. T. Bartus, R. L. Dean, A. J. Goas, and A. S. Lippas, *Science*, **209**, 301 (1980).
248. K. da Costa, E. F. Cochary, J. K. Blusztajn, S. C. Garner, and S. H. Zeisel, *J. Biol. Chem.*, **268**, 2100 (1993).
249. P. M. Newberne and A. E. Rogers, *Ann. Rev. Nutr.*, **6**, 407 (1986).
250. P. M. Newberne, V. Suphiphath, and M. Locniskar, *Nutr. Cancer*, **14**, 175 (1990).
251. N. F. Sheard, J. A. Tayek, B. R. Bistrrian, G. L. Blackburn, and S. H. Zeisel, *Am. J. Clin. Nutr.*, **43**, 219 (1986).
252. R. L. Buchman, M. Dubin, and D. Jenden, *Gastroenterology*, **102**, 1363 (1992).
253. C. S. Lieber, L. M. DeCarli, K. M. Mak, C. I. Kim, and M. A. Leo, *Hepatology*, **Dec. 12**, 1390 (1990).
254. C. S. Lieber, S. J. Robins, J. Li, L. M. DeCarli, K. M. Mak, J. M. Fasulo, and M. A. Leo., *Gastroenterology*, **106**, 152 (1994).
255. G. A. Macdonald and M. R. Lucey, *J. Clin. Gastroenterol.*, **18**, 274 (1994).
256. D. H. Copeland and W. D. Salmon, *Am. J. Pathol.*, **22**, 1059–1081 (1946).
257. A. K. Goshal and E. Farber, *Carcinogenesis*, **5**, 1367–1370 (1984).
258. J. Locker, T. V. Reddy, and B. Lombardi, *Carcinogenesis*, **7**, 1309–1312 (1986).
259. A. E. Rogers, *Am. J. Clin. Nutr.*, **61**, 659S–665S (1995).
260. W. D. Salmon, D. H. Copeland, and M. J. Burns, *J. Natl. Cancer Inst.*, **15**, 1549–1568 (1955).
261. N. Shivapurkar, M. J. Wilson, K. L. Hoover, Y. B. Mikol, D. Creasia, and L. A. Poirier, *J. Natl. Cancer Inst.*, **77**, 213–217 (1986).
262. J. Locker, T. V. Reddy, and B. Lombardi, *Carcinogenesis*, **7**, 1309–1312 (1986).
263. Y. B. Mikol, K. L. Hoover, D. Creasia, and L. A. Poirier, *Carcinogenesis*, **4**, 1619–1629 (1983).
264. J. T. Knuiman, A. C. Beynen, and M. B. Katan, *Am. J. Clin. Nutr.*, **49**, 266–268 (1989).
265. P. M. Ridker, M. J. Stampfer, and N. Rifai, *JAMA*, **285**, 2481–2485 (2001).
266. B. C. O'Brien and V. G. Andrews, *Lipids*, **28**, 7–12 (1993).
267. J. Wojcicki, A. Pawlik, L. Samochowiec, M. Kaldonska, and Z. Mysliwiec, *Phytotherapy Res.*, **9**, 597–599 (1995).
268. J. Schneider, R. Muller, W. Buberl, H. Kaffarnik, R. Schubotz, L. Hausmann, G. Muhlfellner, and O. Muhlfellner, *Eur. J. Clin. Pharmacol.*, **19**, 15–19 (1979).
269. F. Beil and S. Grundy, *J. Lipid Res.*, **21**, 525–536 (1980).
270. A. Rampone and C. Machida, *J. Lipid Res.*, **22**, 744–752 (1981).
271. I. Mastellone et al., *J. Nutr. Biochem.*, 461–466 (2000).
272. C. W. M. Adams, O. B. Bayliss, M. Z. M. Ibrahim, and M. W. Webster, *J. Path. Bact.*, **86**, 431 (1967).
273. M. Friedman, S. O. Byers, and R. H. Rosenman, *Proc. Soc. Expt. Biol. Med.*, **95**, 586–588 (1957).
274. S. O. Byers and M. Friedman, *J. Lipid Res.*, **1**, 343–348 (1960).
275. W. W. Stanford and C. E. Day, *Artery*, **1**, 106–114 (1975).
276. C. R. Sirtori, *Atherosclerosis Rev.*, **24**, 175–198 (1993).

277. A. N. Howard, J. Patelski, D. E. Bowyer, and G. A. Gresham, *Atherosclerosis*, **14**, 17–29 (1971).
278. C. W. M. Adams, Y. H. Abdulla, O. B. Bayliss, and R. S. Morgan, *J. Path. Bact.*, **94** (1967).
279. S. L. Hsia, J. L. He, Y. Nie, K. Fong, and C. Milikowski, *Artery*, **22**, 1–23 (1996).
280. S. S. Kang, P. W. Wang, and M. R. Manilow, *Annu. Rev. Nutr.*, **12**, 279–298 (1992).
281. C. J. Boushey et al., *JAMA*, **274**, 1049 (1995).
282. L. M. Lichtenberg, A. Wang, J. J. Romero, et al., *Nature Med.*, **1**, 154 (1995).
283. L. M. Lichtenberg, C. Ulloa, A. Vanous, et al., *J. Pharmacol. Exp. Ther.*, **277**, 1221 (1996).
284. C. D. Albright, C. B. Friedrich, E. C. Brown, M. H. Mar, and S. H. Zeisel, *Brain Res. Dev. Brain Res.*, **115**, 123–129 (1999).
285. C. D. Albright, A. Y. Tsai, C. B. Friedrich, M. H. Mar, and S. H. Zeisel, *Brain Res. Dev. Brain Res.*, **113**, 13 (1999).
286. W. Meck and C. Williams, *Neuroreport*, **8**, 3053 (1997).
287. W. Meck and C. Williams, *Neuroreport*, **8**, 3045 (1997).
288. W. H. Meck and C. L. Williams, *Devel. Brain Res.*, **118**, 51 (1999).
289. W. H. Meck, R. A. Smith, and C. L. Williams, *Dev. Psychobiol.*, **21**, 339 (1988).
290. W. Meck and C. Williams, *Neuroreport*, **8**, 2831 (1997).
291. R. Loy, D. Heyer, C. L. Williams, and W. H. Meck, *Adv. Exp. Med. Biol.*, **295**, 373 (1991).
292. G. Pyapali, D. Turner, C. Williams, W. Meck, and H. S. Swartzwelder, *J. Neurophysiol.*, **79**, 1790 (1998).
293. S. S. Kang, P. W. K. Wong, and M. Norusis, *Metabolism*, **5**, 458 (1987).
294. S. H. Zeisel, in M. J. Dowdall and J. N. Hawthorne, eds., *Cellular and Molecular Basis of Cholinergic Function*. Horwood Press, Chichester, England, 1987, pp. 709–719.
295. S. H. Zeisel, D. Char, and N. F. Sheard, *J. Nutr.*, **116**, 50–58 (1986).
296. S. L. Ladd, S. A. Sommer, S. LaBerge, and W. Toscano, *Clin. Neuropharmacol.*, **16**, 40–49 (1993).
297. N. Sitaram, H. Weingartner, and J. C. Gillin, *Science*, **201** (4352), 74–76 (1978).
298. P. Spiers, D. Myers, G. Hochanadel, H. Lieberman, and R. Wurtman, *Arch. Neurol.*, **53**, 441–448 (1996).
299. X. A. Alvarez, M. Laredo, D. Corzo, L. Fernandez-Novoa, R. Mouzo, J. E. Perea, D. Daniele, and R. Cacabelos, *Methods Find Exp. Clin. Pharmacol.*, **19**, 201–210 (1997).
300. R. C. Mohs, K. L. Davis, J. R. Tinklenberg, and L. E. Hollister, *Neurobiol. Aging*, **1**, 21–25 (1980).
301. D. J. Sessa, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 347–374.
302. K. Heasook, C.-T. Ho, and S. S. Chang, *J. Amer. Oil Chem. Soc.*, **61**, 1235–1238 (1984).
303. J. E. Kinsella and S. Damodaran, in G. Charlabous, eds., *The Analysis and Control of Less Desirable Flavors in Foods and Beverages*, Academic Press, Inc., New York, 1980, p. 95.
304. D. B. Min and T. G. Stasinopoulos, in *Lecithins*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 375–384.



305. E. F. Sipos, in D. B. Min and T. H. Smouse, eds., *Flavor Chemistry of Lipid Foods*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 265–289.
306. Stern Lecithin & Soja GmbH & Co. KG, technical brochure, Hamburg, Germany, 1994.
307. S. Siwolop and C. S. Eklund, *Business Week*, (July 6) 87–88 (1987).
308. B. C. Keller, *Trends Food Sci. Technol.*, **12**, 25–31 (2001).
309. K. Koide and M. Karel, *Int. J. Food Sci. Technol.*, **22**, 707–723 (1987).
310. U. R. Pothakamury and G. V. Barbosa-Canovas, *Trends Food Sci. Technol.*, **6**, 397–406 (1995).
311. C. J. Kirby, B. E. Booker, and B. A. Law, *Int. J. Food Sci. Technol.*, **22**, 355–375, (1987).
312. B. V. Lengerich, L. C. Haynes, H. Levine, M. S. Otterburn, P. Mathewson, and J. Finley, U.S. Patent 4,999,208, 1991.
313. D. A. Lindsey, Specialty Products Research Notes LEC-T-05, Central Soya Co., Inc., Ft. Wayne, Indiana, 1997.
314. J. N. Casey, Specialty Products Research Notes LEC-T-37, Central Soya Co., Inc., Ft. Wayne, Indiana, 1997.
315. J. C. Russett, Specialty Products Research Notes LEC-D-42, Central Soya Co., Inc., Ft. Wayne, Indiana, 1998.
316. J. C. Russett, D. A. Lindsey, and A. Konwinski, Specialty Products Research Notes LEC-T-08, Central Soya Co., Inc., Ft. Wayne, Indiana, 1997.
317. J. C. Russett, Specialty Products Research Notes LEC-T-43, Central Soya Co., Inc., Ft. Wayne, Indiana, 1998.
318. J. C. Russett, Specialty Products Research Notes LEC-T-35, Central Soya Co., Inc., Ft. Wayne, Indiana, 1997.
319. J. N. Casey, Specialty Products Research Notes LEC-T-36, Central Soya Co., Inc., Ft. Wayne, Indiana, 1997.
320. J. C. Russett, Specialty Products Research Notes LEC-A-23, Central Soya Co., Inc., Ft. Wayne, Indiana, 1996.
321. P. Coutteau et al., *Aquaculture*, **155**, 149 (1997).
322. F. P. Orthofer, S. U. Gurkin, and J. A. Fisk, *Nutrition and Utilization Technology in Aquaculture*, AOCS Press, Champaign, Illinois, 1995 p. 114.
323. J. C. Russett, Specialty Products Research Notes LEC-S-29, Central Soya Co., Inc., Ft. Wayne, Indiana, 1996.
324. C. L. Davis and J. K. Drackley, *The Development, Nutrition and Management of the Young Calf*, Iowa State University Press, Ames, Iowa, pp. 20, 30, 32, 227, 228.
325. R. F. Holmes and C. K. Kandel, South African Patent ZA7,405,866, 1975.
326. J. C. Russett, Specialty Products Research Notes LEC-A-52, Central Soya Co., Inc., Ft. Wayne, Indiana, 2000.
327. J. C. Russett, Specialty Products Research Notes LEC-D-49, Central Soya Co., Inc., Ft. Wayne, Indiana, 1999.
328. J. C. Russett, Specialty Products Research Notes LEC-D-56, Central Soya Co., Inc., Ft. Wayne, Indiana, 2002.
329. Russett, J. C. Specialty Products Research Notes LEC-H-55, Central Soya Co., Inc., Ft. Wayne, Indiana, 2002.

330. J. C. Russett, Specialty Products Research Notes LEC-D-54, Central Soya Co., Inc., Ft. Wayne, Indiana, 2002.
331. D. Canty, S. Zeisel, and A. Jolitz, Lecithin and Choline: Research Update on Health and Nutrition, Central Soya Company, Inc. Fort Wayne, Indiana, 1996, p. 28.
332. W. R. Cox, S. J. Richie, M. Sifri, B. Bennett, and D. D. Kitts, 98th Annual Poultry Sciences Association Meeting Proceedings, abstract 293, 2000.
333. I. R. Sibbald, S. J. Slinger, and G. C. Ashton, *Poultry Sci.*, **41**, 46 (1962).
334. D. Polin, *Poultry Sci.*, **39**, 1652 (1980).
335. A. Caltron, D. Sanders, and F. Brinkhaus, *Rivista di Avicoltura*, **67**, 36 (1998).
336. M. A. C. Morales, I. S. Schneider, and R. J. J. Forster, *Texture Sci.*, **12**, 63 (1980).
337. T. Otsuki and K. Nagashima, Jpn. Kokai Tokkyo Koho JP 7545086, 1975.
338. K. Beaucamp and co-workers, German Patent DE 2,933,648, 1981.
339. P. Nayler, European Patent Application 20,123, 1980.
340. L. W. Bernardino U.S. Patent 4,297,251, 1981.
341. Jpn. Kokai Tokkyo Koho JP 8128298, 1981.
342. Takisawa and co-workers, Jpn. Kokai Tokkyo Koho, JP 7570410, 1975.
343. C. Fuggini and A. Streit, European Patent EP 0 374 471 A1, 1990.
344. B. F. Szuhaj, in F. T. Frederick, ed., *World Research Conference II: Proceedings*, Westview Press, Boulder, Colorado, 1980, pp. 681–691.
345. R. Evans, S. Morrison, and C. Clayton, PCT WO 97/31153, 1997.
346. M. Foa, French Patent 2,475,053, 1981.
347. N. E. Wolff and J. W. Weigh, in M. Grayson, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 8, Wiley, New York, 1981, pp. 794–826.
348. W. F. McDonough and co-workers, U.S. Patent 3,928,226, 1975.
349. H. Friedman and co-workers, French Patent 1,548,700, 1968.
350. G. Hajduk and co-workers, Polish Patent PL 104,287, 1979.
351. C. White, to Hopton Technologies, Intl., LLC, PCT WO 01/51706 A1, 2001.
352. H. Kinsley, Jr., U.S. Patent 5,328,567, 1994.
353. K. Nikoloff, U.S. Patent 4,766,015, 1988.
354. K. Nikoloff, U.S. Patent 5,858,933, 1999.
355. S. Severston and M. Coffey, U.S. Patent 6,248,793 B1, 2001.
356. K. Nojima, K. Shono, and M. Takahashi, U.S. Patent 4,686,119, 1987.
357. L. P. Hayes, *Canada Chem. Abstr.*, 945800, 1974.
358. L. P. Hayes and co-workers, South African Patent ZA 6906125, 1970.
359. S. Shimomukai and co-workers, German Patent DE 2,916,494, 1979.
360. M. Huang, U.S. Patent 6,010,822, 2000.
361. Jpn. Kokai Tokkyo Koho JP 80 88,045, 1980.
362. P. E. Kinzli and co-workers, German Patent DE 2,117,214, 1971.
363. P. N. Maniar, German Patent DE 2,117,075, 1971.
364. D. A. Newman, German Patent DE 2,116,902, 1971.
365. W. Dabisch, U.S. Patent 3,515,572, 1970.
366. T. Misato, Y. Homma, and K. Ko, *Neth. J. Plant Pathol.*, **83**(Suppl. 1), 395–402 (1977).

367. J. Esposito, U.S. Patent 4,822,407, 1989.
368. C. Milne and P. Shelby, PCT WO 98/21945, 1997.
369. G. Kalocsai and co-workers, *Hung. Teljes*, **18**, 326 (1980).
370. R. L. Glass, *Pesticide Biochem. Phys.*, **32**, 93 (1988).
371. W. Steurbaut, H. S. Megahed, G. Van Roey, T. Melkebeke, and W. Dejonckheere, in C. L. Foy, ed., *Adjuvants for Agrichemicals*, CRC Press, Ann Arbor, pp. 623–635.
372. K. Zobitne, PCT WO 99/52359, 1999.
373. Jpn. Tokkyo Koho JP 8108804, to Kumiai Chemical Industry Co., Ltd., 1981.
374. T. Misato and co-workers, U.S. Patent 3,873,700, 1975.
375. Y. Homma and co-workers, *Nippon Noyaku Gakkaishi*, **6**, 145–153.
376. A. Burkhart and M. Burkhart, U.S. Patent 5,674,536, 1997.
377. Jpn Kokai Tokkyo Koho 81 41383, 1981.
378. Jpn. Kokai Tokkyo Koho JP 81140910, 1981.
379. G. Ritzman and co-workers, German Patent DF 3,010,041, 1981.
380. S. J. Tuan and R. F. Hou, *J. Invert. Pathol.*, **52**, 180–182 (1988).
381. A. Degetto, A. Cappai, and V. Caprioli, European Patent EP 0 448 070 B1, 1991.
382. S. Partelow, U.S. Patent 6,300,324 B1, 2001.
383. M. Rombi, U.S. Patent 4,879,117, 1989.
384. A. I. McMullen, British Patent 1,357,952, 1974.
385. D. P. Paapahadjopoulos and co-workers, German Patent DE 2,907,303, 1979.
386. K. M. S. Sundaram and J. Curry, *J. Environ. Sci. Health*, **B31**, 1041–1060 (1996).
387. M. Okauchi and co-workers, Jpn. Kokai Tokkyo Koho, JP 76104033, 1976.
388. W. L. Shillington and J. J. Liggett, U.S. Patent 3,533,810, 1970.
389. M. Nisperos-Carriedo and E. Baldwin, U.S. Patent 5,198,254, 1993.
390. R. B. H. Wills and co-workers, *J. Sci. Food Agric.*, **31**, 663–666.
391. T. Misato and co-workers, Jpn. Kokai Tokkyo Koho JP 7622825, 1976.
392. C. B. Watkins, J. E. Harman, and G. Hopkirk, *New Zealand J. Exp. Agric.*, **16**, 55–61 (1988).
393. C. F. Green, I. F. Chambers, and S. J. Packe-Drury-Lowe, *Ann. Appl. Biol.*, **113**, 177–188 (1988).
394. Miyoshi Kasei Kogyo, Y. K., Jpn. Kokai Tokkyo Koho, JP 85 190,705, 1985.
395. Miyoshi Kasei Kogyo, Y. K., Jpn. Kokai Tokkyo Koho, JP 85 184,571, 1985.
396. U.S. Patent 4,126,591, 1978.
397. R. Macchio, J. Russ, B. Slobody, and M. Tietjen, U.S. Patent 5,023,075, 1991.
398. C. Baker, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 253–260.
399. N. Avalle, European Patent EP 0 882 443 A2, 1998.
400. E. Pittz and R. Smerbeck, U.S. Patent 4,780,249, 1988.
401. K. Kimura, K. Ofuchi, and K. Oda, U.S. Patent 4,647,394, 1987.
402. K. Oyama, T. Narita, M. Fujisawa, N. Tsuchiya, and M. Tsuji, U.S. Patent 6,416,771 B1, 2002.
403. J. Devos, European Patent EP 0 463 099 B1, 1990.

404. J. Komerska, G. Petit, and B. Rybalka, U.S. Patent 4,943,462, 1990.
405. R. Roydhouse, European Patent Appl. EP 0 180 483 A2, 1985.
406. A. Homola and R. Dunton, U.S. Patent 5,888,480, 1999.
407. W. Prosis, U.S. Patent 5,286,764, 1994.
408. G. B. Ansell and co-workers, *Form and Function of Phospholipids*, Elsevier, New York, 1973.
409. H. J. Devel, Jr., *The Lipids, Their Chemistry and Biochemistry*, Vol. 2, 1955, p. 743.
410. G. Retzinger and A. Deanglis, PCT WO 99/55314, 1999.
411. C. Benz, D. Papahadjopoulos, J. Park, K. Hong, and D. Kirpotin, U.S. Patent 6,214,388, 2001.
412. S. Kurtz, U.S. Patent 6,261,597, 2001.
413. M. Hope and W. Rodriguez, U.S. Patent 6,312,719 B1, 2001.
414. F. Chasalow, U.S. Patent 5,703,063, 1997.
415. F. Chasalow, U.S. Patent 5,888,990, 1999.
416. F. Chasalow, U.S. Patent 6,127,349, 2000.
417. A. Kohler, D. Moordian, and L. Furcht, U.S. Patent 5,711,959, 1998.
418. B. Purkait, U.S. Patent 5,941,909, 1999.
419. German Patent DE 2,756,079, 1978.
420. N. H. Ludwig and W. A. White, U.S. Patent 3,808,338, 1974.
421. M. Gordon and J. A. Wiesbach, *Canadian Chem. Abstr.*, 932324 (1973).
422. R. Yehara and co-workers, Jpn. Kokai Tokkyo Koho JP 8449313, 1980.
423. G. H. Bullous, British Patent 1,182,463, 1970.
424. M. Samejima and co-workers, German Patent DE 3,039,908, 1981.
425. J. Zeidler, J. Rosenberg, J. Neumann, and J. Breitenbach, U.S. Patent 6,187,342 B1, 2001.
426. A. Janoff, J. Rauch, and T. Taraschi, U.S. Patent 6,261,792, 2001.
427. E. F. Sipos, in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 261–276.
428. M. Kronstein, *Mod. Paint Coat.*, **68**, 59–63 (1978).
429. F. Augustin and L. Bartling, U.S. Patent 4,221,601, 1980.
430. M. Kronstein and J. Eichberg, U.S. Patent 4,520,153, 1985.
431. M. Kronstein, *Mod. Paint Coat.*, **Feb.**, 19–27 (1975).
432. M. Kronstein, *Amer. Chem. Soc., Div. Org. Coat. Plast. Chem.*, **34**, 521–526 (1974).
433. M. Kronstein, *Paint Ind. Mag.*, **72**, 6, 10 (1957).
434. M. Kronstein and J. Eichberg, U.S. Patent 4,056,494, 1977.
435. M. Kronstein and J. Eichberg, U.S. Patent 2,997,398, 1961.
436. M. Kronstein and J. Eichberg, U.S. Patent 4,305,853, Dec. 15, 1981.
437. H. Pardun, *Seifen, Oele, Fette, Wasche*, **107**, 409–411 (1981).
438. J. Eichber and K. MacDonald, U.S. Patent 5,120,357, 1992.
439. U. Wissgott, European Patent EP 0 816 561 B1, 1996.
440. R. M. Earidjani and Vanlerberghe, U.S. Patent 4,217,344, 1978.
441. J. Godfrey, U.S. Patent 4,091,170, 1978.

442. J. Wannlund, U.S. Patent 5,063,090, 1991.
443. E. Woodhall and N. Kondrats, U.S. Patent 5,750,190, 1998.
444. M. Bakalar, U.S. Patent 5,753,310, 1998.
445. M. Jordan, U.S. Patent 5,885,617, 1999.
446. S. Grillo, R. Steffenino, D. Kunkle, and K. Saracenni, European Patent EP 0 527 153 B1, 1993.
447. E. Woznicki and S. Grillo, European Patent EP 0 321 464 B1, 1986.
448. G. Amidon and J. Crison, U.S. Patent 5,851,275, 1998.
449. L. Daher and L. Sackman, U.S. Patent 5,122,385, 1992.
450. G. Amidon and J. Crison, U.S. Patent 5,686,133 1997.
451. G. Ittman, M. Krieg, U.S. Patent 4,826,901, 1989.
452. R. Zimmermann, U.S. Patent 4,066,599, 1978.
453. R. Zimmermann, U.S. Patent 4,195,009, 1980.
454. A. Hosaka and co-workers, German Patent DE 3,104,702, 1982.
455. Intermagnetics Corp., German Patent (Demand) FR 2,448,560, 1979.
456. H. Kawakara, German Patent DE 3,114,646, 1982.
457. M. Ohlinger, German Patent DE 2,942,646, 1981.
458. H. Tamura, German Patent DE 3,017,651, 1980.
459. M. Buzniak, Polish Patent PL 117,092, 1982.
460. Jpn. Kokai Tokkyo Koho JP 80 83,041, 1980.
461. M. Chagnon and J. Ferris in *Lecithins: Sources, Manufacture, & Uses*, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 277–283.
462. M. Williams and R. Trahan, Jr., U.S. Patent 5,413,815, 1995.
463. M. Besse, C. Ross, and H. Kleaver, U.S. Patent 5,587,009, 1996.
464. T. Kamikaseda, M. Furudate, and M. Yamamoto, to National Starch and Chemical Corp., U.S. Patent 4,634,727, 1987.
465. M. Lawson, U.S. Patent 5,356,466, 1994.
466. J. Stewart, U.S. Patent 4,948,521, 1990.
467. J. Anglin and D. Smith, U.S. Patent 6,207,286 B1, 2001.
468. D. Greenleaf, P. Howarth, and P. Jinks, PCT WO 93/14005 (1993).
469. S. Brois, F. Femino, H. Deen, and A. Gutierrez, European Patent Application 0 386 923 A1, 1990.
470. J. Vinci, C. Luciani, and S. Abbas Rizvi, The Lubrizol Corp., European Patent Application 0 597 712 A1, 1992.
471. S. Gatt, Y. Barenholz, and H. Bercovier, European Patent EP 0 499 441 B1, 1992.
472. F. Fava and D. Di Gioia, *Biotech. Bioeng.*, **72**, 177–184 (2001).
473. J. Stanley, in M. Grayson, ed., *Kirk-Other Encyclopedia of Chemical Technology*, Vol. 14, 3rd ed., pp. 250–269.
474. A. Heijkoop, German Patent DE 2,213,927, 1972.
475. P. P. Caruso, U.S. Patent 4,282,344, 1981.
476. L. Meyer, U.S. Patent 4,500,442, 1985.
477. H. Leube, U.S. Patent 5,767,180, 1998.

478. G. Valencia, U.S. Patent 5,624,975, 1997.
479. K. Kuriyama and M. Toyama, Jpn. Kokai Tokkyo Koho JP 74 481,167, 1974.
480. M. McAbee, W. Perkins, and D. Shomake, European Patent O 877 837 B1, 1997.
481. J. Vlasblom and V. Dotolo, U.S. Patent 5,415,789, 1995.
482. P. H. List and R. Von Kleinsorgen, German Patent DE 2,928,040, 1980.
483. F. Bove, U.S. Patent 3,819,523, 1974.
484. E. Manandhar and A. Usmani, European Patent Application O 718 373 A1, 1995.
485. E. Manandhar and A. Usmani, U.S. Patent 5,744,524, 1998.
486. R. I. Buhlstein and co-workers, U. S. S. R. Patent SU 624697, 1978.
487. H. Tjujimito and co-workers, U.S. Patent 4,049,556, 1977.
488. D. Krivohlavek, U.S. Patent 5,834,539, 1998.
489. D. Sincroft and E. Sipos, U.S. Patent 2,987,527, 1959.
490. E. Sipos and D. Sincroft, U.S. Patent 2,991,163, 1959.
491. A. G. Meshandin and co-workers, U.S.S.R. Patent SU 905,236, 1982.
492. *Official Journal of the European Communities*, **L339/46** (1996).
493. EC Regulation 1139/98.

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## *Lipid Emulsions*

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### **1. INTRODUCTION**

Many natural and processed foods contain small droplets of oil dispersed in an aqueous medium (e.g., milk, cream, fruit beverages, soups, cake batters, mayonnaise, cream liqueurs, sauces, deserts, salad cream, and ice cream) or small droplets of water dispersed in a lipid medium (e.g., butter and margarine). Despite the considerable diversity of physicochemical and sensory characteristics exhibited by these foods, they can all be considered to fall into a class of material called “emulsions” and their properties can be understood using the concepts and techniques of “emulsion science” (1–4). Emulsion science is a multidisciplinary subject that combines chemistry, physics, and engineering (5–12). The objective of emulsion scientists working within the food industry is to use the principles and techniques of emulsion science to improve the quality of the food supply and the efficiency of food production. The purpose of this chapter is to introduce the basic principles and techniques of emulsion science that are relevant for understanding, characterizing, and manipulating the properties of food products.

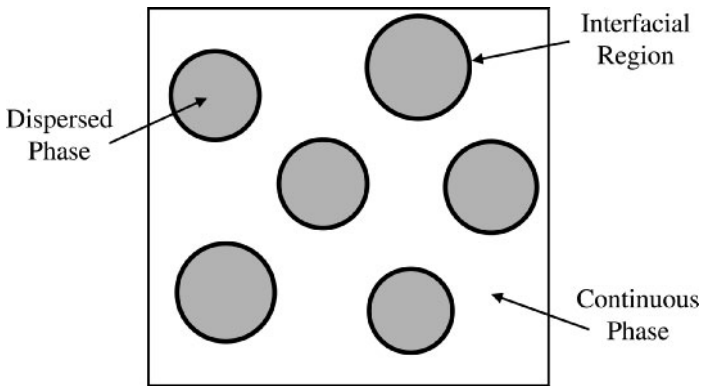
The wide diversity of physicochemical and organoleptic characteristics exhibited by food emulsions is the result of product formulation and processing conditions used to create them. The manufacture of an emulsion-based food product with specific desirable quality attributes depends on the selection of the suitable raw

materials (e.g., water, oil, emulsifiers, thickening agents, minerals, acids, bases, vitamins, flavors, colorants, etc.) and optimization of processing conditions (e.g., mixing, homogenization, pasteurization, sterilization, etc.). The product must be transported and stored under appropriate conditions to maintain its desirable quality attributes prior to consumption (e.g., exposure to temperature variations, light, and mechanical agitation). Historically, the food industry relied largely on craft and tradition for the formulation of food products and the establishment of processing and storage conditions. Today, this approach is becoming increasingly unfeasible for the modern food industry, which must rapidly respond to changes in consumer preferences demanding a greater variety of cheaper, healthier, and more convenient foods (13–15). In addition, large-scale production operations are required for profitability of modern food companies. Large quantities of foods can consequently be produced at relatively low cost. The development of new foods, the improvement of existing foods, and the efficient operation of food processing operations require a more systematic and rigorous approach than was used previously (16). There have been considerable advances in our understanding of the physicochemical basis of the properties of food emulsions during the past decade. These advances are largely because of the availability of inexpensive but powerful personal computers that enable food scientists to carry out complex theoretical predictions and numerical simulations in a reasonably short time. The increasing availability of powerful new analytical instruments for probing the structure, interactions, and physicochemical properties of emulsions and their components further contribute to these advances.

## 2. DEFINITIONS

An emulsion can be defined as a material that consists of small spherical droplets of one liquid dispersed in another liquid in which it is at least partly immiscible (Figure 1). Typically, the diameters of the droplets in food emulsions lie somewhere between 0.1 and 100  $\mu\text{m}$  (1, 2, 17, 18). It is convenient to classify emulsions according to the relative organization of the oil and aqueous phases. A system that consists of oil droplets dispersed in an aqueous phase is called an oil-in-water or o/w emulsion, whereas a system that consists of water droplets dispersed in an oil phase is called a water-in-oil or w/o emulsion. The material within the emulsion droplets is usually referred to as the *dispersed, internal, or discontinuous phase*, whereas the material that makes up the surrounding liquid is usually referred to as the *continuous or external phase*. It is also possible to prepare multiple emulsions, e.g., oil-in-water-in-oil (o/w/o) or water-in-oil-in-water (W/O/W) type (19). For example, a W/O/W emulsion consists of water droplets dispersed within larger oil droplets, which are themselves dispersed in an aqueous continuous phase (20). These multiple emulsions may have advantages over traditional emulsions for certain applications, e.g., fat reduction, controlled ingredient release, or isolation of one ingredient from another (19).





**Figure 1.** Schematic illustration of a dispersed system that consists of two fully or partially immiscible liquids. The dispersed phase is surrounded by molecules of the continuous phase, and the two phases are separated by an interfacial region.

The process of creating an emulsion from two separate immiscible liquids, or of reducing the size of the droplets in a preexisting emulsion, is called *homogenization*. In the food industry, this process is normally carried out using mechanical devices known as *homogenizers*, which subject the liquids to intense mechanical stresses that result in droplet disruption. An emulsion can be formed by homogenizing pure oil and pure water together, but the two phases rapidly separate into a system that consists of a layer of oil (lower density) on top of a layer of water (higher density). Phase separation occurs because droplets tend to merge with each other when they collide. The driving force for the phase-separation process is the fact that the contact between oil and water molecules is thermodynamically unfavorable, because of the hydrophobic effect (21). As a result, emulsions are considered to be *thermodynamically unstable*. However, it is possible to form emulsions that are *kinetically stable* (metastable) for a reasonable period of time (a few days, weeks, months, or years), by including two different classes of substances, *emulsifiers* and *thickening agents*, prior to homogenization. Emulsifiers are *surface-active* molecules that absorb at the surface of droplets that are generated during homogenization. There they form a protective membrane that prevents the hydrophobic water molecules from coming into direct contact with the hydrophilic lipid molecules, thus preventing aggregation. The most commonly used emulsifiers in the food industry are amphiphilic proteins, polysaccharides, small molecule surfactants, and phospholipids (22). The second class of compounds used to improve kinetic stability of emulsions is thickening agents. Thickening agents increase the viscosity of the continuous phase of emulsions, and they are used to modify emulsion texture and to enhance emulsion stability by retarding the movement of droplets. The most common thickening agents used in the food industry are polysaccharides, e.g., xanthan gum, alginate, carageenan, and guar gum (23). The term

*stabilizer* is used to refer to any ingredient that can improve the stability of emulsions, and it may therefore be either an emulsifier or a thickening agent.

### 3. DROPLET CHARACTERISTICS

Many of the unique physicochemical and sensory properties of food emulsions are determined by the presence of the droplets they contain. The most important characteristics of emulsion droplets are therefore discussed below.

#### 3.1. Droplet Concentration

The concentration of droplets in an emulsion is one of the key parameters influencing its appearance, texture, stability, and flavor. For example, opacity, viscosity, and creaming stability of emulsions usually increase as the droplet concentration increases. The droplet concentration is normally expressed in terms of the *disperse-phase volume fraction* ( $\phi$ ), which is equal to the volume of emulsion droplets ( $V_D$ ) divided by the total volume of the emulsion ( $V_E$ ):  $\phi = V_D/V_E$ . Nevertheless, it can also be expressed in terms of the *disperse-phase mass fraction* ( $\phi_m$ ), which is equal to the mass of emulsion droplets ( $M_D$ ) divided by the total mass of the emulsion ( $M_E$ ):  $\phi_m = M_D/M_E$ . The mass fraction can be related to the volume fraction using the following expression:

$$\phi_m = \frac{\phi \rho_2}{\rho_2 \phi + (1 - \phi) \rho_1}, \quad (1)$$

where  $\rho_1$  and  $\rho_2$  are the densities of the continuous and dispersed phases, respectively. If the densities of the two phases are equal, the mass fraction is equivalent to the volume fraction. The droplet concentration may also be presented as a mass ( $\% \phi_m = 100 \phi_m$ ) or as a volume ( $\% \phi = 100 \phi$ ) percentage. In many cases, the droplet concentration of an emulsion is known because the concentration of the ingredients used to prepare it is carefully controlled during emulsion production. Nevertheless, local variations in droplet concentration may occur within the emulsion, for example, when the droplets accumulate at the top or bottom of an emulsion because of gravitational separation. Such an emulsion will have properties that are significantly different from a homogeneous product. In addition, the droplet concentration of an emulsion may vary during the course of a processing operation, e.g., if a mixer or homogenizer is operating inconsistently. These operational inconsistencies may be caused by variations in homogenization pressure and temperature, volume-flow rate, or stirrer speed. It is therefore often important to be able to quantify the droplet concentration of an emulsion. The droplet concentration can be measured using traditional proximate analysis techniques (e.g., drying, solvent extraction, and density measurements) or by using more sophisticated modern analytical techniques (e.g., light scattering, electrical pulse counting, and ultrasonic spectroscopy) (4).

### 3.2. Droplet Size Distribution

The size of the droplets in an emulsion has a strong influence on many of its physicochemical and sensory properties, e.g., shelf life, appearance, texture, and flavor (1, 2, 4). For example, the stability of an emulsion to gravitational separation or droplet aggregation can be greatly improved by decreasing the droplet size. This is because the velocity of sedimentation is proportional to the square of the droplet size. The size of the droplets in an emulsion is largely determined by the emulsifier type and concentration, the physicochemical properties of the component phases, and the homogenization conditions (4). A food manufacturer normally specifies a preestablished desirable droplet size distribution for a particular product. If the product does not meet this specification, it typically must be reprocessed or even discarded.

An emulsion that contains droplets that all have the same size is referred to as being “monodisperse,” whereas an emulsion that contains droplets that have a range of different sizes is referred to as being “polydisperse.” The size of the droplets in a monodisperse emulsion can be completely characterized by a single number, such as the droplet diameter ( $d$ ) or radius ( $r$ ). Monodisperse emulsions, while difficult to produce, are sometimes prepared for use in fundamental studies because the interpretation of experimental measurements is usually much simpler than for polydisperse emulsions. In most industrial applications, food emulsions droplet sizes are always distributed, and so the specification of their droplet size is more complicated than for monodisperse systems. In some situations, it is important to have information about the full particle size distribution of an emulsion, i.e., the fraction of droplets in each specified size range. In most other situations, it is sufficient to simply know the mean size of emulsion droplets and the width of the distribution (9). Polydisperse emulsions can be characterized according to the general shape of the particle size distributions as being “unimodal,” “bimodal,” or “multimodal” depending on whether there are one, two, or more peaks in the distribution.

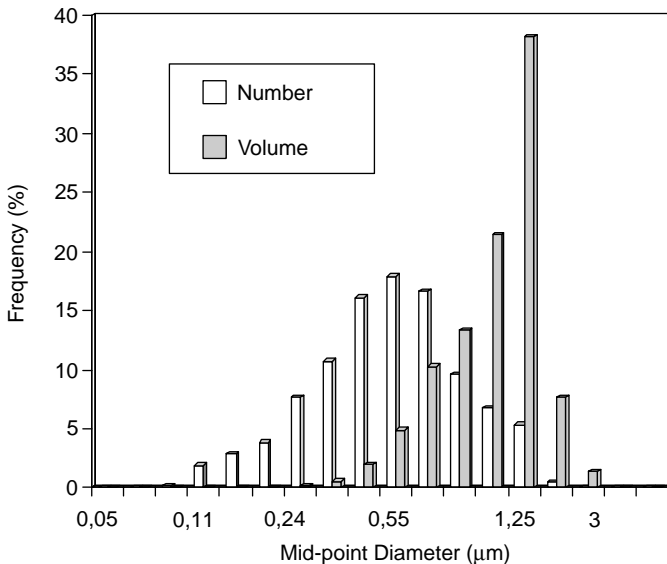
The number of droplets in most emulsions is extremely large, and so their size can vary continuously from some minimum value to some maximum value. When presenting particle size data, it is convenient to divide this size range into a number of discrete size classes and stipulate the number of droplets that fall into each class (9). The resulting data can then be represented in tabular form (Table 1) or plotted as a histogram that shows the number of droplets in each size class (Figure 2). Rather than presenting the number of droplets  $n_i$  in each size class, it is often more informative to present the data as the number frequency,  $f_i = n_i/N$ , where  $N$  is the total number of droplets, or as the volume frequency,  $\phi_i = v_i/V$ , where  $v_i$  is the volume of the droplets in the  $i$ th size class and  $V$  is the total volume of all the droplets in the emulsion. The shape of a particle size distribution changes appreciably depending on whether the fraction of droplets in each size category is presented as a number or a volume frequency (Figure 2). Hence, it is always important to clearly specify which parameter has been used when reporting particle size data. As the volume of a droplet is proportional to  $d^3$ , the volume distribution

**TABLE 1. The Particle Size Distribution of an Emulsion can be Conveniently Represented in Tabular Form. Note that the Volume Frequency is much more Sensitive to Larger Droplets than the Number Frequency.**

Size Class [ $\mu\text{m}$ ]	$d_i$ [ $\mu\text{m}$ ]	$N_i$	$f_i$ [%]	$\phi_i$ [%]	$C(d_i)$ [%]
0.041–0.054	0.048	0	0.0	0.0	0.0
0.054–0.071	0.063	2	0.1	0.0	0.1
0.071–0.094	0.082	4	0.2	0.0	0.3
0.094–0.123	0.108	50	2.5	0.0	2.8
0.123–0.161	0.142	84	4.2	0.1	7.0
0.161–0.211	0.186	152	7.6	0.3	14.6
0.211–0.277	0.244	224	11.2	1.1	25.8
0.277–0.364	0.320	351	17.6	3.9	43.35
0.364–0.477	0.420	470	23.5	11.8	66.85
0.477–0.626	0.551	385	19.2	21.8	86.1
0.626–0.821	0.723	190	9.5	24.3	95.6
0.821–1.077	0.949	64	3.2	18.5	98.8
1.077–1.414	1.245	21	1.0	13.7	99.85
1.414–1.855	1.634	3	0.2	4.4	100
1.855–2.433	2.144	0	0.0	0.0	100

is more representative of larger droplets present in the emulsion. The number distribution is more representative of droplets that may be small and therefore have low-volume fractions but may be present in large numbers.

A particle size distribution can also be represented as a continuous curve, such as the distribution function  $F(d_i)$  or the cumulative function  $C(d_i)$ . The (number)



**Figure 2.** Histogram of the volume- and number-based particle size distribution of an emulsion.

distribution function is constructed so that the area under the curve between two droplet sizes,  $d_i$  and  $d_i + \delta d_i$ , is equal to the number of droplets  $n_i$  in that size range, i.e.,  $n_i = F(d_i)\delta d_i$  (9). This relationship can be used to convert a histogram to a distribution function, or vice versa. The cumulative function represents the percentage of droplets that are smaller than  $d_i$  and can be obtained through integration of the distribution function. The resulting curve has an S-shape that varies from 0% to 100% as the particle size increases. The particle size at which half the droplets are smaller and the other half are larger is known as the median droplet diameter,  $d_m$ . The particle size distribution of an emulsion can also be modeled using mathematical theories (e.g., normal or log-normal distributions), which is convenient because the full data set can be described by a small number of parameters (9). Nevertheless, care should be taken to ensure that the mathematical model used realistically represents the actual data.

The size of droplets in a polydisperse emulsion may be expressed by one or two numbers, rather than stipulating the full particle size distribution (9). The most useful numbers are the mean diameter  $\bar{d}$ , which is a measure of the central tendency of the distribution, and the standard deviation,  $\sigma$ , which is a measure of the width of the distribution:

$$\bar{d} = \frac{\sum n_i d_i}{N}, \quad (2)$$

$$\sigma = \sqrt{\frac{\sum n_i (d_i - \bar{d})^2}{N}}. \quad (3)$$

The above mean is also referred to as the mean length diameter,  $d_L$ , because it represents the sum of the length of the droplets divided by the total number of droplets. It is also possible to express the mean droplet size in a number of other ways (Table 2). Each of these mean sizes has dimensions of length (meters), but stresses a different physical aspect of the distribution, e.g., the average length, surface area, or volume. For example, the volume-surface mean diameter is related to the surface area of droplets exposed to the continuous phase per unit volume of emulsion,  $A_S$ :

$$A_S = \frac{6\phi}{d_{VS}}. \quad (4)$$

**TABLE 2. Different Ways of Expressing the Mean Droplet Diameter of a Polydisperse Emulsion.**

Name of Mean	Symbol	Definition
Length	$\bar{d}$ or $d_L$	$d_L = \sum n_i d_i / \sum n_i$
Surface Area	$d_S$	$d_S = \sqrt{\sum n_i d_i^2 / \sum n_i}$
Volume	$d_V$	$d_V = \sqrt[3]{\sum n_i d_i^3 / \sum n_i}$
Volume-Surface Area	$d_{VS}$ or $d_{32}$	$d_{VS} = \sum n_i d_i^3 / \sum n_i d_i^2$

This relationship is particularly useful as it allows one to calculate the total surface area of droplets in an emulsion, an important parameter that can be used to estimate the emulsifier concentration required to produce a kinetically stable emulsion. An appreciation of the various types of mean droplet diameter is also important because different experimental techniques used to measure droplet sizes are sensitive to different mean values (24). Consequently, it is always important to be clear about which mean diameter has been determined in an experiment when using or quoting droplet size data.

The importance of the particle size distribution in determining the physicochemical properties of food emulsions means that it is important to have analytical techniques to quantify this parameter. The size of the droplets in emulsions can be measured using a variety of different analytical methods, the most common being microscopy, light scattering, electrical pulse counting, sedimentation, and ultrasonic techniques (4). Each of these techniques has its own advantages and disadvantages. For example, some techniques are only suitable for analyzing very dilute emulsions (e.g., light scattering, electrical pulse counting), whereas others can be used to analyze concentrated emulsions *in situ* (e.g., ultrasonic spectrometry, NMR).

### 3.3. Droplet Charge

The electrical charge of emulsion droplets has an important impact on the stability of emulsion droplets and, in turn, influences physicochemical and organoleptic properties of food emulsions (1). The electrical charge on the droplets in food emulsions is usually the result of the adsorption of emulsifier molecules that contain ionized or ionizable groups, e.g., ionic surfactants, phospholipids, proteins, and polysaccharides (4). The magnitude and sign of the electrical charge at the droplet interface largely depends on type and concentration of surface-active molecules present at the interface, as well as pH and ionic composition of the aqueous phase. Droplet charge is a key parameter that determines not only how a droplet interacts with other charged species (e.g., emulsion droplets, polymers, mineral ions) but also how it behaves in the presence of an electrical field (which is the basis of experimental measurements of electrical charge). Charged species of opposite sign are attracted towards each other, whereas those of similar sign are repelled from each other. All of the droplets in an emulsion are usually coated with the same type of emulsifier and, as such, have the same electrical charge (if the emulsifier is ionized). When this charge is sufficiently large, the droplets are prevented from aggregating because of the electrostatic repulsion between them. The properties of emulsions stabilized by ionized emulsifiers are particularly sensitive to the pH and ionic strength of the aqueous phase. If the pH of the aqueous phase is adjusted so that the emulsifier loses its charge, or if salt is added to “screen” the electrostatic interactions between the droplets, the repulsive forces may no longer be strong enough to prevent the droplets from aggregating. Droplet aggregation is often undesirable in food emulsions because it can lead to an increase in emulsion viscosity and a decrease in creaming stability.

Electrostatic interactions also influence the interactions between emulsion droplets and other charged species, such as biopolymers, surfactants, vitamins, antioxidants, flavors, and minerals (2, 25–27). These interactions often have significant implications for the overall quality of an emulsion product. For example, volatility of a flavor may be reduced if flavor molecules are electrostatically attracted to the surface of emulsion droplet thereby altering the flavor profile of food emulsions (26). The susceptibility of oil droplets to lipid oxidation depends on whether the catalyst is electrostatically attracted to the droplet surface (27). In case of a repulsion of oxidation catalysts from the lipid–water interface, the extent of lipid oxidation in emulsions can be reduced. The accumulation of charged species at a droplet surface and the rate at which this accumulation takes place depends on the sign of their charge relative to that of the surface, the strength of the electrostatic interaction, their concentration, and the presence of any other charged species that might compete for the surface.

The electrical charge on an emulsion droplet can be manipulated by choosing emulsifiers with desirable charge characteristics (e.g., sign, magnitude, isoelectric point) and controlling the aqueous phase properties (e.g., pH and ionic strength). Consequently, it is possible to control the bulk physicochemical properties of emulsions by manipulating their electrical charge, e.g., aggregation stability, flavor distribution, and lipid oxidation. A variety of analytical techniques have been developed to measure the magnitude and sign of the charge on emulsion droplets, the most commonly used being particle electrophoresis and electroacoustics (4).

### 3.4. Droplet Crystallinity

Another parameter that influences the overall properties of the bulk emulsion is the physical state of the lipid droplets in an emulsion (17, 19, 28–31). Crystallization of lipid droplets in emulsions can be either beneficial or detrimental to product quality. Margarine and butter, the most common water-in-oil emulsions in the food industry, are prepared by a controlled destabilization of oil-in-water emulsions containing partly crystalline droplets. The stability of dairy cream to mechanical agitation and temperature cycling depends on the nature and extent of crystallization in milkfat globules. It should be noted that because the density of the phases can change as crystallization occurs, the rate at which milkfat droplets cream can be altered as droplets solidify. Emulsion manufacturers should therefore understand which factors influence the crystallization and melting of emulsified substances, and be aware of the effect that droplet phase transitions can have on the properties of emulsions.

The melting and crystallization behavior of emulsified substances can be quite different from that of the same substance in bulk (19). In particular, the degree of supercooling in emulsified materials is usually much greater than in bulk materials because the probability of finding a catalytic site that can promote nucleation is smaller in a particular droplet than in a bulk phase. A variety of experimental techniques are available for providing information about the crystallization and melting behavior of emulsion droplets, including microscopy, differential scanning calorimetry, NMR, and ultrasonics (4).

**TABLE 3. Effect of Particle Size on the Physical Characteristics of 1 g of Oil Dispersed in Water in the Form of Spherical Droplets. Values were Calculated Assuming the Oil had a Density of  $920 \text{ kg m}^{-3}$  and the End-to-End Length of the Oil Molecules was 6 nm.**

Droplet Radius [ $\mu\text{m}$ ]	No. of Droplets Per Gram Oil [ $\text{g}^{-1}$ ]	Droplet Surface Area Per Gram Oil [ $\text{m}^2 \text{g}^{-1}$ ]	Percent Oil Molecules at Droplet Surface [%]
100	$2.6 \times 10^5$	0.03	0.02
10	$2.6 \times 10^8$	0.3	0.2
1	$2.6 \times 10^{11}$	3	1.8
0.1	$2.6 \times 10^{14}$	30	18

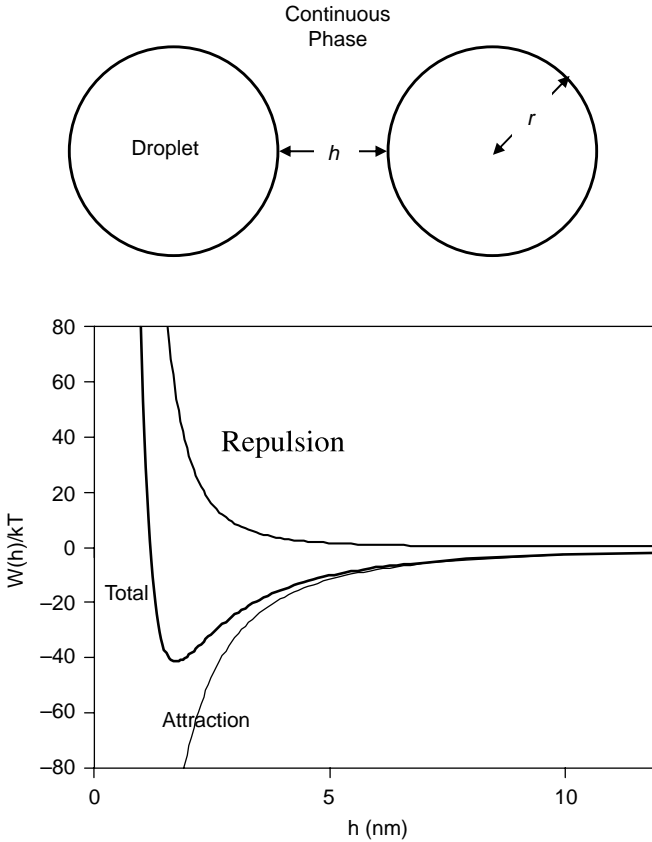
### 3.5. Droplet Interfacial Properties

The droplet interface is comprised of a narrow region (typically 2 nm to 20 nm thick) that surrounds each emulsion droplet and contains a mixture of oil, water, and emulsifier molecules (9, 10). The interfacial region typically does not contribute significantly to the total volume of an emulsion unless the droplet size is smaller than approximately 1  $\mu\text{m}$  (Table 3). Contrary to this, the interfacial membrane does play a major role in determining bulk physicochemical and organoleptic properties of food emulsions. For this reason, food scientists are particularly interested in elucidating the factors that determine the composition, structure, thickness, and rheology of the interfacial region (2, 19, 32–35). The composition and structure of the interfacial region are determined by the type and concentration of surface-active species present, as well as by the events that occur both during and after emulsion formation, e.g., competitive adsorption. The thickness and rheology of the interfacial region influences the stability of emulsions to gravitational separation, coalescence, and flocculation, and determines the rate at which molecules leave or enter the droplets (4, 35). A variety of analytical techniques are available to provide information about the composition, thickness, and rheology of interfacial membranes. Some of these techniques can be directly applied to emulsions, whereas others can only be carried out at interfaces separating planar oil-water interfaces.

### 3.6. Droplet-Droplet Interactions

Colloidal interactions govern whether emulsion droplets aggregate or remain as separate entities thereby impacting the characteristics of any aggregates formed, e.g., their size, shape, porosity, and deformability (2, 19, 36, 37). The rheological properties and creaming stability of many food emulsions depend on the extent of droplet aggregation and the characteristics of any aggregates formed (38, 39). The interactions between two emulsion droplets can be described in terms of an interdroplet pair potential (4). The interdroplet pair potential,  $w(h)$ , is the energy required to bring two emulsion droplets from an infinite distance apart to a surface-to-surface separation of  $h$  (Figure 3). The overall interdroplet pair potential acting between two droplets is the sum of many different types of interactions, including van der Waals, steric, electrostatic, depletion, hydrophobic, and hydration interactions (4). These individual interactions can vary in their sign (attractive or





**Figure 3.** Difference between thermodynamic and kinetic stability. A system will remain in a thermodynamically unstable or metastable state for some time if there is a sufficiently large energy barrier preventing it from reaching the state with the lowest free energy.

repulsive), magnitude (weak to strong), and range (short to long) (Table 4). Each of the individual interactions usually has a simple monotonic dependence on surface-to-surface separation, but the sum of the interactions can exhibit a fairly complex behavior having both minima and maxima at certain separation distances

**TABLE 4. Summary of Major Colloidal Interactions between Emulsion Droplets. Sign: Attractive (A), Repulsive (R); Magnitude: Weak (W), Medium (M), Long (L); Range: Short (S), Medium (M), Long (L).**

Type	Sign	Magnitude	Range	Major Factors Influencing
Van der Waals	A	S	L	Refractive index, dielectric constant
Electrostatic	R or A	W-S	S-L	pH, ionic composition
Steric	R	S	S	Solvent quality, thickness
Depletion	A	W-M	M	Excluded species size and concentration
Hydrophobic	A	S	L	Surface hydrophobicity, temperature

(Figure 3). Generally, droplets tend to aggregate when attractive interactions dominate, but remain as individual entities when repulsive interactions dominate (4).

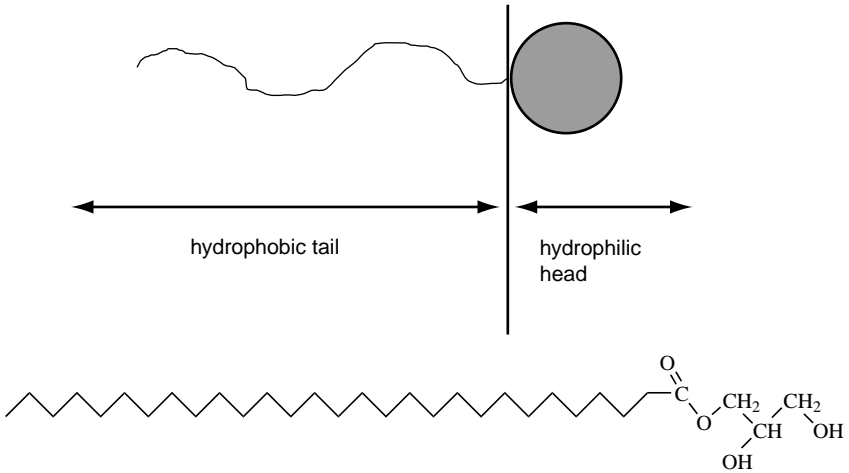
## 4. EMULSION PREPARATION

### 4.1. Introduction

The process of converting two immiscible bulk-phase liquids into an emulsion, or of reducing the size of the droplets in a preexisting emulsion, is known as homogenization. The mechanical device designed to carry out this process is called a homogenizer (40). Homogenization can be separated into two categories depending on the nature of the starting material. The formation of an emulsion directly from two separate bulk liquids is referred to as primary homogenization, whereas the reduction in size of the droplets in an existing emulsion is referred to as secondary homogenization. The creation of a particular type of food emulsion may involve the use of either of these types of homogenization, or a combination of both. In large scale food processing operations, it is often more efficient to prepare an emulsion in two stages (1). First, the separate oil and water phases are converted to a coarse emulsion that contains fairly large droplets using one type of homogenizer (e.g., a high-speed blender). The droplets of the emulsion premix, having a low kinetic stability are further reduced in size using a different type of homogenizer (e.g., a high-pressure valve homogenizer). It should be noted that there is no clear distinction between most of the physical processes that occur during primary and secondary homogenization, e.g., mixing, droplet disruption, and droplet coalescence. Finally, some homogenizers are capable of producing emulsions with small droplet sizes directly from separate oil and water phases, e.g., high-intensity ultrasonicators, microfluidizers, or membrane homogenizers. As previously shown (see 3.2), many of the important characteristics and quality aspects of food emulsions depend on the size of the droplets they contain, including their stability, texture, appearance, and taste. Consequently, the major objective of homogenization is to create an emulsion in which the majority of droplets fall within an optimum size range that yields emulsions with properties specified by food manufacturers. We will therefore briefly discuss the major factors that determine the size of the droplets produced after the homogenization process.

### 4.2. Emulsifiers

**4.2.1. Emulsifier Structure and Emulsifier Chemistry** One of the key factors to successfully produce a stable lipid emulsion is the addition of a suitable emulsifier. Although a detailed discussion of emulsifiers and emulsifier chemistry is beyond the scope of this chapter, a brief introduction to emulsifiers/surfactants seems appropriate. Surfactants are surface-active compounds that can adsorb to appropriate interfaces once dispersed in a solvent (41–48). Emulsifiers are those surfactants that are specifically used to stabilize emulsions. Surfactants are



**Figure 4.** Structure of a simple surfactant.

amphiphilic molecules (Figure 4). They have a hydrophilic and a hydrophobic area. The hydrophilic part is usually referred to as the head group of the surfactant, whereas the hydrophobic group is called the tail. The amphiphilic character of the molecule also causes the molecule to orient at interfaces, a reason for their surface activity. Surface or interfacial films (or layers) are therefore formed with the consequence that the surface tension is reduced. Hence, less energy is needed to disrupt the droplets further. Depending on the ratio of hydrophilic group to hydrophobic group, surfactants can have a higher solubility in either one of the two phases, oil or water. Surfactants are often much more complex than the simple example shown in Figure 4. Proteins, for example, are surface active and are classified as surfactants but they possess a highly complex three-dimensional structure that undergoes structural rearrangements upon adsorption at an interface. Other complex surfactants include modified starches or block copolymers. Specialty surfactants have been designed that contain not only one tail or head group but many to increase the steric stabiliztion effect. There is no single classification scheme available to categorize all types of surfactants. The situation is also complicated by the fact that new surfactants are continuously developed. Nevertheless, there are a few characteristics listed below that can be used to classify the various surfactants:

- Charge of the hydrophilic group (anionic, cationic, nonionic, or zwitterionic)
- Nature of the lipophilic group (n-alkyl, iso-alkyl, (un)saturated alkyl)
- Solubility in various solvents
- Ratio of hydrophilic to hydrophobic group
- Crystalline phases
- Molecular geometry

**TABLE 5. Applications and Corresponding HLB Values.**

HLB Range	Application
<3	Surface films
3–6	Water-in-oil emulsifiers
7–9	Wetting agents
8–15	Oil-in-water emulsifiers
13–15	Detergents
15–18	Solubilizers

One of the most important parameter is the charge of the head group. The charge of the surfactant can have a large impact on the chemical reactivity of the emulsion. For example, rates of lipid oxidation in emulsions that are susceptible to radical driven degradation processes differ dramatically depending on whether the emulsion was stabilized by a nonionic, cationic, or anionic emulsifier. This is because of repulsive or attractive interactions between the droplet interface and metal catalysts that may be present in the system. Another valuable classification tool involves the so-called HLB value. It is a measure of the ratio of the hydrophilic head group to the lipophilic tail. HLB values are very useful in order to select a surfactant for a particular application and are often listed by the surfactant manufacturer. Table 5 illustrates the ranges of HLB values that are most suited for a particular application.

**4.2.2. Nonionic Emulsifiers** Nonionic surfactants are the principal surfactants encountered in food systems. They have several advantages over ionic surfactants. Nonionic surfactants can cover a wide range of HLB values. They are more environmentally friendly because they are easily biodegradable. The traditional source of the hydrophobic part of nonionic surfactants is fatty acid triglycerides, both from animal and plant sources. Primarily the higher members of the series such as palmitic, steric, oleic, and linoleic acid are used. Major utilization of fatty acids in surfactant chemistry involves the following reactions:

- Esterification of fatty acids and polyhydric alcohols: The reaction of fatty acids with polyhydroxy compounds, such as ethylene glycol or glycerol, yields monoglycerides or polyglycerides. Prominent members of the higher polyol series, for example, include sorbitol and mannitol.
- Alkanolamides of fatty acids: The condensation of fatty acids with mono- thanolamines or diethanolamines yields a group of products called alkanolamides. They usually contain a range of surface-active byproducts such as amino esters.
- Oxyethylated surfactants: The multiple condensation of ethylene oxide with a hydrophobe that contains accessible hydrogen atoms yields a polyethyleneoxide with an attached hydrophobic tail group. Polyethyleneoxide surfactants constitute the major portion of nonionic surfactants. The ability to control the

polymeric chain reaction has resulted in a group of surfactants that span a wide variety of HLB values. Often used in food applications are surfactants of the Tween<sup>®</sup> series that are obtained through a reaction of a sugar/fatty acid ester with ethylenoxide.

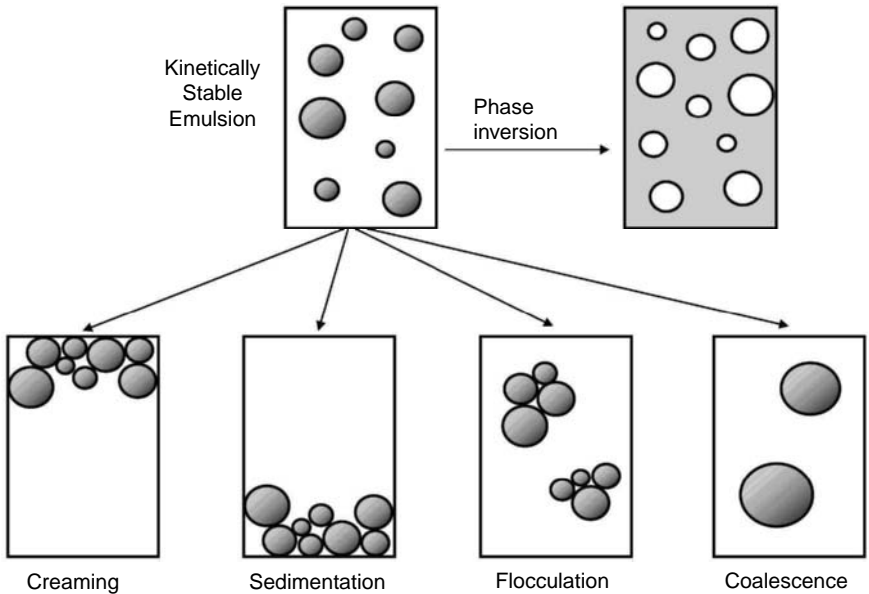
**4.2.3. Anionic Emulsifiers** Anionic surfactants make about 75% of all the consumption of surface-active material. They are rarely encountered in the preparation of an actual food. The toxicity level of anionic surfactants is high and even small doses of anionic surfactants can cause allergic reactions and nausea. However, as they are very strong detergents, they can be used to solubilize components such as proteins. Due to their strong electrostatic repulsion, they are also very effective in stabilizing emulsions and are therefore often applied in technical emulsions such as emulsified lubricants.

The major subgroups of anionic surfactants include the alkali carboxylates (soaps), sulfates, sulfonates, and to a smaller degree, phosphates. The esterification of alcohol with sulfuric acid yields probably the best-studied surfactant, sodium dodecylsulfate or SDS. SDS, a sulfate ester, is an extremely effective emulsifier because of its high-electrostatic repulsion. Other sulfates are, for example, sulfated esters from fatty acids, sulfated ethers, and sulfated fats and oils. Sulfonates stem from the reaction of sulfonic acid with suitable substrates. Members of the class of sulfonates are, for example, sulfonic acid salts or aliphatic sulfonates. Other anionic surfactants include substances such as carboxylated soaps and esters of phosphoric acid.

**4.2.4. Cationic Emulsifiers** Cationic surfactants are primarily recognized because of their strong bacteriostatic properties. While the total market share of cationic surfactants is less than 5%, they continue to play an important role as sanitizing and antiseptic agents, textile softeners, corrosion inhibitors, foam depressants, flotation chemicals, and as components in fungicides and germicides. Typically, cationic surfactants consist of a hydrophobic chain group derived from either fatty acid or petrochemical sources and a positively charged nitrogen atom. The hydrophobic group can be directly attached to the nitrogen or be indirectly linked via a bridging group such as a polyethyleneoxide. Alternatively, the nitrogen can also be part of a heterocyclic ring as is the case in alkyipyridinium salts.

### 4.3. Physical Basis of Homogenization

The physical processes that occur during homogenization can be highlighted by considering the formation of an emulsion from pure oil and pure water. When the two liquids are brought in contact, they tend to adopt the configuration that is thermodynamically most stable and has the lowest free energy, which consists of a layer of oil on top of a layer of water (Figure 5). This arrangement is adopted because it minimizes the unfavorable contact area between the two immiscible liquids, and because oil has a lower density than water. To create an emulsion, energy is needed to disrupt and intermingle oil and water phases. The energy is



**Figure 5.** Food emulsions may become unstable through a variety of physical mechanisms, including creaming, sedimentation, flocculation, coalescence, and phase inversion.

usually supplied in the form of some mechanical agitation (49). The oil droplets formed during the application of the mechanical agitation are constantly moving around and frequently collide and coalesce with neighboring droplets (49). If no more mechanical energy is supplied to the system, the droplets formed during the agitation process will move in the opposite direction of the gravitational field and eventually merge together to form a separate layer, i.e., they will phase separate (Figure 4). This process is enthalpically driven and favors the minimization of the contact area between the oil and water while the kinetics of the phase separation depends on the strength of the gravitational field and the nature of the two liquids.

To form an emulsion that is (kinetically) stable for a reasonable period of time, one must prevent the droplets from merging together after they have been formed (49, 50). This is achieved by having a sufficiently high concentration of emulsifier present during the homogenization process. Emulsifier molecules adsorb at the oil-water interface during homogenization to form a protective membrane that prevents droplets from coming into close contact required for coalescence. The size of droplets produced during homogenization, therefore, is a balance of two opposing physical processes: *droplet disruption* and *droplet coalescence*. The efficiency of the emulsification process increases if (1) the initial droplet size can be kept small and (2) the droplets are rapidly stabilized against coalescence once they are formed (Figure 5).

**4.3.1. Droplet Disruption** The initial stages of primary homogenization involve the break-up and intermingling of the bulk oil and bulk aqueous phases so that

fairly large droplets of one of the liquids become dispersed throughout the other liquid (49, 50). Later stages of primary homogenization, as well as the entire secondary homogenization, involves the disruption of larger droplets into smaller ones. Ultimately, the disruption of a droplet depends on a balance between interfacial forces that oppose enlargements of the interfacial area and disruptive forces generated within the homogenizer (49, 50). A thermodynamic consideration of a system with constant pressure, temperature, and composition but with varying interfacial areas yields the following expression for the free energy change (51):

$$\Delta G = \frac{\partial G}{\partial A} \Delta A. \quad (5)$$

Equation 5 illustrates that the overall free energy change of the system is a function of increases in the interfacial area and a system specific thermodynamic parameter,  $\partial G/\partial A$ , also known as the interfacial tension  $\gamma$ . Equation (5) also indicates that the work required to deform and disrupt a droplet during homogenization must be significantly larger than  $\gamma\Delta A$  (17, 50). This relationship also explains why emulsifiers, capable of readily adsorbing to the interfaces of emulsion droplets during homogenization and reducing their interfacial tension, will decrease the amount of work required for droplet disruption, thus improving homogenization efficiency.

The nature of the disruptive forces that act on droplets during the homogenization process depend on the flow conditions they experience (i.e., laminar or turbulent), and therefore on the type of homogenizer used (49, 52). For a droplet to be broken up during homogenization, the magnitude of the disruptive forces must exceed that of the interfacial forces and their duration must exceed the time required to deform and disrupt the droplet (53, 54). The susceptibility of emulsion droplets to disruption can be characterized by the Weber Number ( $We$ ), which is the ratio of the disruptive forces to the interfacial forces (50). Above a characteristic critical Weber number, droplets are disrupted, below this Weber number they remain intact. The value of the critical Weber number generally depends on the ratio of the viscosity of the dispersed phase to the continuous phase,  $\eta_D/\eta_C$ , and theoretical or semi-empirical expressions for the Weber number have been derived for a number of different flow conditions found in homogenizers, e.g., laminar flow and turbulent flow (54).

**4.3.2. Droplet Coalescence** Droplet-droplet collisions occur frequently during homogenization because of the intense mechanical forces experienced by emulsions inside homogenizers. If emulsion droplets are not covered by a sufficiently strong interfacial membrane, they will tend to coalesce with one another during a collision (49). Immediately after the disruption of an emulsion droplet, the freshly formed interfaces are insufficiently covered by emulsifier molecules and therefore the new droplets are highly susceptible to coalescence when they collide with their neighbors. To prevent coalescence it is necessary to rapidly form a stable membrane of emulsifier molecules that is able to induce repulsive interactions between the droplets. The size of droplets produced in the homogenization process, therefore,

depends not only on process parameters but also on the kinetics of the emulsifier adsorption at droplet interfaces ( $t_{\text{adsorption}}$ ) relative to the rate of droplet-droplet collisions ( $t_{\text{collision}}$ ). Thus, the flow situation in the homogenizer, the bulk physicochemical properties of the oil and aqueous phases, and the nature of the emulsifier used all impact the resulting droplet size (51). Droplet coalescence during homogenization can therefore be reduced by using an emulsifier with rapid adsorption kinetics or by increasing the emulsifier concentration that ensures that  $\tau_{\text{adsorption}}/\tau_{\text{collision}} \ll 1$ . The importance of emulsifier adsorption kinetics on the size of the droplets produced during homogenization has been demonstrated experimentally (55). Under the same homogenization conditions, it has been shown that emulsifiers that adsorb rapidly produce smaller droplet sizes than those that adsorb slowly. Most food emulsifiers do not adsorb rapidly enough to completely prevent droplet coalescence, and so the droplet size achieved during homogenization is greater than that which is theoretically possible (56).

#### 4.4. Homogenization Devices

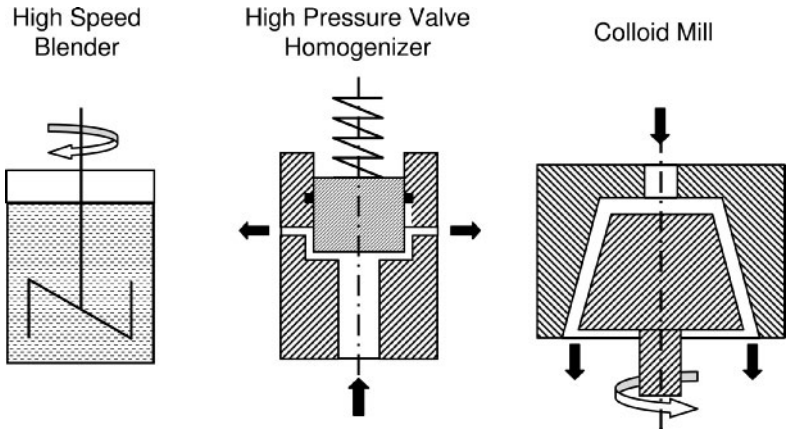
A number of different types of homogenization devices are used to produce food emulsions (Table 6). Each of these devices has its own advantages and disadvantages and is often best suited for a particular type of product. In selecting a homogenizer, one needs to consider volume-flow rates, the nature of the starting materials, the desired droplet size distribution, the required physicochemical properties of the final product, availability of space, power, or pressure requirements, and the cost of purchasing and operating the equipment. The most commonly used homogenizers in the food industry at present are high-speed blenders, high-pressure valve homogenizers, and colloid mills (Figure 6). Ultrasonic homogenizers, while rarely used in industry, have proven to be very useful on a laboratory scale because of the small amounts of sample that can be processed and their low cost.

*High-speed blender:* The disruption of droplets in a blender occurs mainly due to the existence of a turbulent flow situation. The energy input per unit volume is unevenly distributed in the apparatus. This results in a broad droplet size distribution.

**TABLE 6. Comparison of Different Types of Homogenizer Used in the Food Industry.**

Homogenizer	Throughput	Relative Energy Efficiency	Minimum Droplet Size	Sample Viscosity
High-speed blender	Batch	Low	2 $\mu\text{m}$	Low to medium
Colloid mill	Continuous	Intermediate	1 $\mu\text{m}$	Medium to high
High-pressure homogenizer	Continuous	High	0.1 $\mu\text{m}$	Low to medium
Ultrasonic probe	Batch	Low	0.1 $\mu\text{m}$	Low to medium
Ultrasonic jet homogenizer	Continuous	High	1 $\mu\text{m}$	Low to medium
Micro-fluidizer	Continuous	High	<0.1 $\mu\text{m}$	Low to medium
Membrane processing	Batch or Continuous	High	0.3 $\mu\text{m}$	Low to medium





**Figure 6.** Technical schematics of the three most often used homogenization devices: (a) high-speed blender, (b) high-pressure valve homogenizer, and (c) colloid mill.

The major droplet disruption occurs in the immediate vicinity of the rotating blades where shear forces are highest, e.g., due to the presence of Taylor vortices. The effectiveness of droplet disruption depends on the geometry of the mixer and the rotational speed of the blades. Operational parameters include blade and vessel geometries and rotation speed of blades.

*High-pressure valve homogenizer:* Within a high-pressure valve homogenizer, extensive droplet disruption occurs. The homogenization valve can have various geometries with externally adjustable gap sizes. The premix is pumped into a valve with pressures between 10–100 MPa. Within the annulus of the valve, velocities exceed more than 200 m/s. The average residence time of the premix in the valve is less than a few milliseconds. Due to the fast acceleration of the liquid in the annulus, the hydrostatic pressure in the annulus can drop below the vapor pressure of the liquid. As a consequence, steam bubbles are formed. The formation of steam bubbles is transient and bubbles collapse in the rear part of the annulus where pressure and temperature increase again. The collapse of these cavitation bubbles is the primary source of the mechanical energy that causes oil droplet disruption. The effectiveness of the droplet disruption in a high-pressure valve homogenizer can be directly related to the applicable pressure difference.

*Colloid Mill:* Colloid mills are rotor-stator systems that can be used to reduce the particle size distribution of both liquid dispersions (emulsions) and solid dispersions (suspensions). The emulsion or suspension is pumped through a narrow gap that is formed by the rotating inner cone and the stationary outer cone. The width of the annulus can be adjusted by changing the relative position of the two cones. The principal size reduction in colloid mills is due to the high shear forces that are caused by the velocity difference between the rotor and the stator surfaces. To increase wall friction and reduce slip, surfaces are usually not smooth but are roughened or toothed, which, in turn, changes the flow conditions from laminar to turbulent, thereby increasing the shear forces in the annulus.

*High-Intensity Ultrasonicator:* Droplets are disrupted within a field of high-intensity ultrasonic waves. Droplet disruption occurs either due to cavitation or because the frequency of the ultrasonic wave equals the resonance frequencies of the droplets. This causes the droplets to oscillate vigorously. Eventually, the oscillation becomes supercritical and the droplets are disrupted. The effectiveness of sonication, therefore, depends on the nature of the continuous and dispersed phase. The type of oil, as well as the nature of the surfactant, is the limiting factor for the minimal droplet size that can be achieved.

## 4.5. Factors Influencing Droplet Size

From an operational point of view, food manufacturers need to be able to optimize both the composition of an emulsion and the required homogenization process to achieve a product that fulfills predetermined design criteria. As the majority of emulsion characteristics depend primarily on the droplet size distribution, the specific factors that impact droplet size need to be discussed.

**4.5.1. Emulsifier Type and Concentration** For a fixed concentration of oil, water, and emulsifier, there is a maximum interfacial area that can be completely covered by an emulsifier. As homogenization proceeds, the size of the droplets decreases and the interfacial area increases. Once the emulsion interfacial area increases above a certain level, there may be insufficient emulsifier present to completely cover the surface of any newly formed droplets. This will not only increase the energy required for subsequent droplet disruption but also increase the probability for droplet coalescence. The minimum size of stable droplets that can be produced during homogenization is governed by the type and concentration of emulsifier present:

$$r_{\min} = \frac{3 \cdot \Gamma_{\text{sat}} \cdot \phi}{c_S}, \quad (6)$$

where  $\Gamma_{\text{sat}}$  is the excess surface concentration of the emulsifier at saturation (in  $\text{kg m}^{-2}$ ),  $\phi$  is the disperse phase volume fraction, and  $c_S$  is the concentration of emulsifier in the emulsion (in  $\text{kg m}^{-3}$ ). For a polydisperse emulsion, the radius  $r_{\min}$  is the volume to surface mean radius. The minimum droplet size that can be produced during homogenization can be decreased by increasing the emulsifier concentration, decreasing the droplet concentration or using an emulsifier with a smaller  $\Gamma_{\text{sat}}$ . For a 10% oil-in-water emulsion containing 1% of emulsifier, the minimum droplet radius is about 60 nm (assuming  $\Gamma_{\text{sat}} = 2 \times 10^{-6} \text{ kg m}^{-2}$ ). While Equation (5) provides a first estimation to design a homogenization process, it should be noted that the actual mean diameter of emulsion droplets after homogenization is generally greater than the theoretical minimum.

In order to attain the theoretical minimum droplet size, a homogenizer must be capable of generating a pressure gradient that is large enough to disrupt any droplets that are greater than  $r_{\min}$ . This pressure gradient is given by the LaPlace

pressure where  $\Delta p = 2\gamma/r$ . Some types of homogenizer are not capable of generating such high-pressure gradients and are therefore not suitable for producing emulsions with small droplet sizes, even though there may be sufficient emulsifier present (50). The emulsion must also spend sufficient time within the homogenization zone for all of the droplets to be completely disrupted. In general, the residence time of droplets within the homogenization zone is distributed and this distribution is impacted by operational conditions (flow speed) and the geometry of the homogenization zone. Design of the homogenization zone is therefore of crucial importance to the efficiency of the homogenization process.

A large variety of emulsifiers are used in the food industry, and each of these exhibits different characteristics during homogenization, e.g., the speed at which they adsorb, the maximum reduction in interfacial tension, and the effectiveness of the interfacial membrane to prevent droplet coalescence. A food manufacturer must select the most appropriate emulsifier for each type of food product, taking into account their performance during homogenization, solution conditions, cost, availability, legal status, ability to provide long-term stability, and the desired physicochemical properties of the product. It is generally recommended that food manufacturers closely consult with emulsifier manufacturers to select an appropriate emulsifier that is optimized for their particular application. In addition, classification schemes for emulsifiers are available that aide in the selection of a suitable emulsifier.

**4.5.2. Energy Input** The size of the droplets in an emulsion can be reduced by increasing the amount of energy supplied during homogenization (as long as there are a sufficient number of emulsifier molecules to cover the surfaces of the droplets formed). The energy input depends on the nature of the homogenizer. In a high-speed blender, the energy input can be enhanced by increasing the rotational speed or the length of time that the sample is blended. In a high-pressure homogenizer, it can be enhanced by increasing the homogenization pressure or recirculating the emulsion through the device, i.e., increasing the number of passes through the homogenizer. In a colloid mill, it can be enhanced by reducing the size of the gap between the stator and rotator system, increasing the rotational speed, using disks with roughened surfaces, or increasing again the number of passes through the homogenizer. In a high-intensity ultrasonicator, the energy input can be enhanced by increasing the intensity of the ultrasonic wave or by sonicating for a longer time. In a microfluidizer, the energy input can be increased by increasing the velocity at which the liquids are brought into contact with each other or by recirculating the emulsion. In a membrane homogenizer, the energy input can be enhanced by increasing the pressure at which the liquid is forced through the membrane. Under a given set of homogenization conditions (energy input, temperature, composition) there is a certain size below which the emulsion droplets cannot be reduced even with repeated homogenization, and therefore homogenizing the system any longer would be inefficient. It should be noted that increasing the energy input usually leads to increased manufacturing costs, which may offset the benefits gained through smaller droplet sizes. A food manufacturer should, therefore,

always establish processing conditions that provide an optimum compromise between droplet size, processing time, and cost (50).

Under most circumstances, the droplet size will decrease as the energy input is increased. Nevertheless, there may be occasions when increasing the energy actually leads to an increase in droplet size because the effectiveness of the emulsifier is reduced by excessive heating or exposure to high pressures. This can be particularly important for protein-stabilized emulsions, because the molecular structure and functional properties of proteins are particularly sensitive to changes in their environmental conditions. For example, globular proteins, such as  $\beta$ -lactoglobulin, are known to unfold and aggregate when they are heated above a certain temperature, which reduces their ability to stabilize emulsions.

**4.5.3. Properties of Component Phases** The composition and physicochemical properties of both the oil and aqueous phases influence the size of the droplets produced during homogenization (52). Variations in the type of oil or aqueous phase will alter the viscosity ratio,  $\eta_D/\eta_C$ , which determines the minimum size that can be produced under steady-state conditions. The interfacial tension of the oil-water interface depends on the chemical characteristics of the lipid phase, e.g., molecular structure or presence of surface-active impurities, such as free fatty acids, monoacylglycerols, or diacylglycerols. These surface-active lipid components tend to accumulate at the oil-water interface and lower the interfacial tension, thus lowering the amount of energy required to disrupt a droplet.

The aqueous phase of an emulsion may contain a wide variety of components, including minerals, acids, bases, biopolymers, sugars, alcohols, ice crystals, and gas bubbles. Many of these components will alter the size of the droplets produced during homogenization because of their influence on rheology, interfacial tension, coalescence stability, or adsorption kinetics. For example, the presence of low concentrations of short chain alcohols in the aqueous phase of an emulsion reduces the size of the droplets produced during homogenization because of the reduction in interfacial tension (57). The presence of biopolymers in an aqueous phase has been shown to increase the droplet size produced during homogenization due to their ability to suppress the formation of small eddies during turbulence (50). Protein-stabilized emulsions cannot be produced close to the isoelectric point of a protein or at high-electrolyte concentrations because the proteins are highly susceptible to aggregation.

Experiments have shown that the smallest droplet size that can be achieved using a high-pressure valve homogenizer increases as the disperse phase volume fraction increases (52). There are a number of possible reasons for this, (1) increasing the viscosity of an emulsion may suppress the formation of eddies responsible for breaking up droplets, (2) if the emulsifier concentration is kept constant, there may be insufficient emulsifier molecules present to completely cover the droplets, and (3) the rate of droplet coalescence is increased.

**4.5.4. Temperature** Temperature may influence the size of droplets produced during homogenization. The viscosity of both the oil and aqueous phases is

temperature dependent, and therefore, the minimum droplet size that can be produced may be altered because of a variation in the viscosity ratio,  $\eta_D/\eta_C$ . Heating an emulsion usually causes a slight reduction in the interfacial tension between the oil and water phases, which would be expected to facilitate the production of smaller droplets. However, certain types of emulsifiers lose their ability to stabilize emulsion droplets against aggregation when they are heated above a certain temperature. For example, when small molecule surfactants are heated close to their phase inversion temperature, they are no longer effective at preventing droplet coalescence, or when globular proteins are heated above a critical temperature, they unfold and aggregate. Alterations in temperature also influence the competitive adsorption of surface-active components, thereby altering interfacial composition (58).

The temperature is also important because it determines the physical state of the lipid phase. It is practically impossible to homogenize a fat that is either completely or substantially solid because it will not flow through a homogenizer or because of the huge amount of energy required to break up the fat crystals into small particles. There are also problems associated with the homogenization of oils that contain even small amounts of fat crystals because of partial coalescence. The crystals from one droplet may penetrate the surface of another droplet leading to the formation of an aggregate. Extensive aggregation leads to the generation of large particles and to a dramatic increase in the viscosity that, in the most extreme case, can cause the homogenizer to become blocked. For this reason, it is usually necessary to warm a sample prior to homogenization to ensure that the lipid phase is completely liquid. For example, milkfat is usually heated to about 40°C to melt all the crystals prior to homogenization (52).

#### 4.6. Other Processing Steps

Homogenization is only one of the processing operations involved in the production of a food emulsion. Postprocessing and preprocessing operations can have a direct impact on the properties of the final emulsions. One of the most common operations carried out prior to emulsion homogenization is to disperse the various ingredients into the phase in which they are most soluble. Oil-soluble ingredients, such as vitamins, colors, antioxidants, phospholipids, and lipophilic surfactants, are usually mixed with the oil, and water-soluble ingredients, such as proteins, polysaccharides, sugars, salts, vitamins, colors, antioxidants, and hydrophilic surfactants, are usually mixed with the aqueous phase. The intensity and duration of the mixing process depends on the time required to solvate and uniformly distribute the ingredients. Adequate solvation is important for the functionality of a number of food components, e.g., the emulsifying properties of proteins are often improved by allowing them to hydrate in water for a few hours prior to homogenization (59). If the lipid phase contains any crystalline material, it is necessary to warm it to a temperature where all the fat crystals melt prior to homogenization; otherwise it is extremely difficult to create a stable emulsion (52, 60). One of the most common operations after homogenization is thermal processing to improve the microbiological stability

of the product, e.g., pasteurization or sterilization. Thermal processing of an emulsion may have a significant impact on its quality and long-term physical stability because many ingredients used in food emulsions are heat sensitive, e.g. proteins, surfactants, and polysaccharides. Food emulsions may also be subjected to a variety of other processing operations during their manufacture that impact their quality and shelf life, including mixing, pumping, freezing, and drying.

## 5. PHYSICOCHEMICAL PROPERTIES OF FOOD EMULSIONS

### 5.1. Emulsion Stability

The term “emulsion stability” is broadly used to describe the ability of an emulsion to resist changes in its properties with time. The properties of an emulsion may evolve over time due to a variety of physical, chemical, or biochemical processes. From a technological standpoint, it is important to identify the dominant processes occurring in the system of interest because effective strategies can then be rationally designed to overcome the problem. A number of the most important physical mechanisms responsible for the instability of emulsions are shown schematically in Figure 5.

**5.1.1. Gravitational Separation** Gravitational separation is one of the most common forms of instability in food emulsions and may result in either creaming or sedimentation depending on the relative densities of the dispersed and continuous phases. Creaming is the upward movement of droplets due to the fact that their density is lower than that of the surrounding liquid, whereas sedimentation is the downwards movement of droplets due to the fact that they have a higher density than the surrounding liquid. Liquid edible oils normally have lower densities than water and, as a result, creaming is more prevalent in oil-in-water emulsions, whereas sedimentation is more prevalent in water-in-oil emulsions. If emulsions contain fully or partially crystalline fats, the density of the lipid phase may increase above the density of water causing sedimentation instead of creaming. The creaming velocity of an isolated rigid spherical particle suspended in a Newtonian liquid obeys Stokes' law:

$$v_{Stokes} = -\frac{2gr^2(\rho_2 - \rho_1)}{9\eta_1}, \quad (7)$$

where,  $r$  is the radius of the particle,  $g$  is the acceleration due to gravity,  $\rho$  is the density,  $\eta$  is the shear viscosity, and the subscripts 1 and 2 refer to the continuous and dispersed phases, respectively. The sign of  $v_{Stokes}$  determines whether the droplet moves upwards (+) or downwards (-). To a first approximation, the stability of a relatively dilute food emulsion to creaming can be estimated using Stokes' law. For example, an oil droplet ( $\rho_2 = 910 \text{ kg m}^{-3}$ ) with a radius of  $1 \text{ }\mu\text{m}$  suspended in water ( $\eta_1 = 1 \text{ mPa s}$ ,  $\rho_1 = 1000 \text{ kg m}^{-3}$ ) should theoretically cream at a velocity of about 17 mm per day. An emulsion containing droplets of this size would not

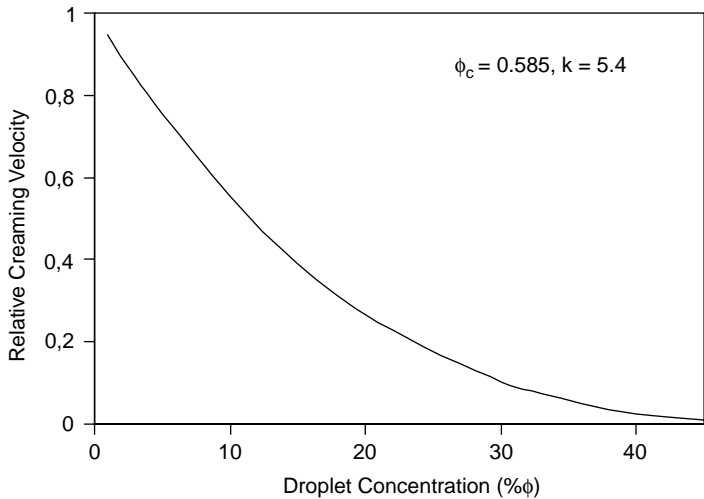
have a particularly long shelf life. Stokes' law highlights a number of strategies that food manufacturers can use to retard gravitational separation in emulsions, i.e., decreasing the density contrast between the two phases, decreasing the droplet radius, or increasing the viscosity of the continuous phase. Each of these strategies is used in the food industry, with the most appropriate one or combination depending on the nature of the emulsion.

It should be stressed that Stokes' law is inappropriate for accurately predicting the stability of many food emulsions due to gravitational separation because they do not exist as dilute suspensions of rigid spheres suspended in a Newtonian fluid. If the droplet concentration is high, single droplets will not move independent of each other and droplet movement may be retarded due to increased packing and hydrodynamic interactions. For this reason, the theory has been extended to take into account various other factors, such as droplet fluidity, droplet concentration, the interfacial membrane, and non-Newtonian continuous phases (4). A semiempirical equation that gives relatively good predictions of the creaming behavior of concentrated emulsions has been derived (Hunter, 1989):

$$v = v_{Stokes} \left( 1 - \frac{\phi}{\phi_c} \right)^{k\phi_c} \quad (8)$$

Here,  $\phi_c$  and  $k$  are parameters that depend on the nature of the spherical particles, i.e., their size, polydispersity, and colloidal interactions. Typically, the values of  $\phi_c$  and  $k$  for nonfloculated monodisperse colloidal suspensions are around 0.5–0.6 and 5.4, respectively. Normally,  $\phi_c$  is taken as the volume fraction at which the spherical particles become closely packed. This equation predicts that the creaming velocity decreases as the droplet concentration increases, until creaming is completely suppressed once a critical disperse phase volume fraction ( $\phi_c$ ) is exceeded (Figure 7). In general, the value of  $\phi_c$  depends on the packing of the droplets within an emulsion, which is governed by their polydispersity and colloidal interactions. Polydisperse droplets are able to fill the available space more effectively than monodisperse droplets because the small droplets can fit into the gaps between the larger ones (61), and so  $\phi_c$  is increased. When the droplets are strongly attracted to each other, they can form a particle gel at relatively low-droplet concentrations, which prevents any droplet movement (Figure 7). When the droplets are strongly repelled from each other, their effective size increases, which also causes complete restriction of their movement at lower values of  $\phi_c$  (62).

**5.1.2. Droplet Aggregation** The droplets in emulsions are in continual motion because of the effects of thermal energy, gravity, or applied mechanical forces, and as they move about, they frequently collide with their neighbors (63, 64). After a collision, emulsion droplets may either move apart or remain aggregated, depending on the relative magnitude of the attractive and repulsive interactions between them. Droplets aggregate when there is a minimum in the interdroplet pair potential that is sufficiently deep and accessible to the droplets (Figure 3). The three major



**Figure 7.** Reduction of relative creaming velocity in emulsions as a function of droplet concentration. In creamed layers with droplet concentrations above a critical value  $\phi_c$ , the relative creaming velocity may be virtually zero.

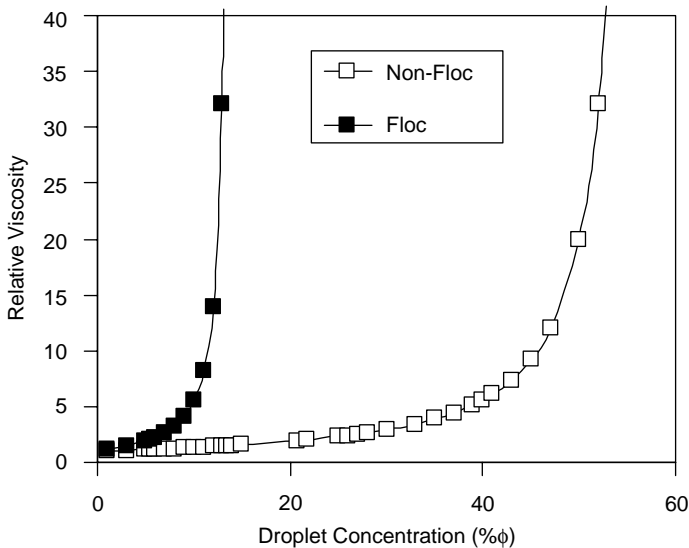
types of aggregation in food emulsions are flocculation, coalescence, and partial coalescence (1, 2, 17, 18).

**5.1.2.1. Flocculation** Droplet flocculation is the process whereby two or more droplets come together to form an aggregate in which the droplets retain their individual integrity. It may be either advantageous or detrimental to emulsion quality depending on the nature of the food product. Flocculation accelerates the rate of gravitational separation in dilute emulsions, which is undesirable because it reduces their shelf life (65). It also causes a pronounced increase in emulsion viscosity and may even lead to the formation of a gel (66, 67) (Figure 8). Some food products are expected to have a low viscosity and therefore flocculation is detrimental. In other products, a controlled amount of flocculation may be advantageous because it leads to the creation of a desirable texture. Improvements in the quality of emulsion-based food products, therefore, depends on a better understanding of the factors that determine the degree of floc formation, the structure of the flocs formed, the rate at which flocculation proceeds, and the effect that flocculation has on the bulk physicochemical properties of emulsions (4).

Flocculation may occur in emulsions through a variety of different processes, described below, that either increase the attractive forces or decrease the repulsive forces between the droplets.

**Reduced electrostatic repulsion.** Electrostatically stabilized emulsions may flocculate when the electrostatic repulsive interactions between the droplets are reduced. This can be achieved by altering the pH so that the electrical charge on the droplets is reduced, adding multivalent counter ions that bind to the surface





**Figure 8.** The viscosity of emulsion depends on droplet–droplet interactions. The viscosity of a flocculated emulsion at a given droplet concentration exceeds the viscosity of an unflocculated emulsions.

of the droplets and reduce the droplet charge, or increasing the ionic strength of the surrounding medium to screen the electrostatic interactions (21).

*Increased depletion attraction.* The presence of nonadsorbing colloidal particles, such as biopolymers or surfactant micelles, in the continuous phase of an emulsion causes an increase in the attractive force between the droplets due to an osmotic effect associated with the exclusion of colloidal particles from a narrow region surrounding each droplet. This attractive force increases as the concentration of colloidal particles increases, until eventually, it may become large enough to overcome the repulsive interactions between the droplets and cause them to flocculate (68–72). This type of droplet aggregation is usually referred to as *depletion flocculation* (17, 18).

*Increased hydrophobic attraction.* This type of interaction is important in emulsions that contain droplets that have some nonpolar regions exposed to the aqueous phase. A good example of this type of interaction is the effect of thermal processing on the flocculation stability of oil-in-water emulsions stabilized by globular proteins (66, 67, 73). At room temperature, whey-protein-stabilized emulsions (pH 7, 10 mM NaCl) are stable to flocculation because of the large electrostatic repulsion between the droplets, but when they are heated above 70°C they become unstable. The globular proteins adsorbed to the surface of the droplets unfold above this temperature and expose nonpolar amino acids that were originally located in the interior of the proteins (74, 75). Consequently, the droplet surface becomes more hydrophobic, which increases the hydrophobic attraction between droplets favoring flocculation (32, 67, 74).

*Formation of biopolymer bridges.* The addition of biopolymers to the aqueous phase of emulsions may promote flocculation by forming bridges between two or more droplets (64). Biopolymers may adsorb either directly to the bare surfaces of droplets or to the adsorbed emulsifier molecules that form the interfacial membrane (17). However, biopolymers will only bind to droplet surfaces if there is a sufficiently strong attractive interaction between segments of the biopolymer and the droplet surface. The principle molecular interactions that may be responsible for binding are hydrophobic and electrostatic (2, 76). For example, a positively charged biopolymer might adsorb to the surface of two negatively charged emulsion droplets, causing them to flocculate (77).

The development of a suitable strategy to prevent droplet flocculation in an emulsion, therefore, depends on identification of the physicochemical origin of flocculation in this particular system. In the most general terms, flocculation can be prevented by ensuring that the repulsive forces dominate the attractive forces.

**5.1.2.2. Coalescence** Coalescence is the process whereby two or more liquid droplets merge together to form a single larger droplet (Figure 5). Coalescence is the principal mechanism by which an emulsion eventually attains its thermodynamically most stable state because the contact area between the oil and water phases decreases over the course of the process. Coalescence also causes emulsion droplets to cream or sediment more rapidly because of the droplet size increase. In oil-in-water emulsions, coalescence eventually leads to the formation of a separate oil layer, a process that is referred to as *oiling off*. In water-in-oil emulsions, it leads to the merging, sedimentation, and finally phase separation of water droplets.

Coalescence requires that the molecules of liquid within two or more emulsion droplets come into direct contact (12, 17, 18, 78). Droplets, therefore, need to be in close proximity, which is the case in highly concentrated emulsions or in flocculated emulsions or creamed layers, for example. In a subsequent step, a disruption of the interfacial membrane must occur to allow the liquid molecules to come into direct contact. The rate at which coalescence proceeds and the physical mechanism by which it occurs is highly dependent on the nature of the emulsifier used to stabilize the system. Coalescence is an extremely complex process because it depends not only on the extent of gravitational, colloidal, hydrodynamic, and mechanical forces that act on the droplets but the intrinsic properties of the droplet membrane as well. Improving the stability of an emulsion to coalescence may be achieved by preventing droplet flocculation, preventing formation of a creamed layer, reducing the droplet concentration, and altering the rheological properties of the interfacial membrane to improve rupture resistance.

**5.1.2.3. Partial Coalescence** Partial coalescence occurs when two or more partially crystalline oil droplets come into contact and form an irregularly shaped aggregate. It is initiated when a solid fat crystal from one droplet penetrates the interface to the liquid phase of a second oil droplet (17, 28–31). Consequently,

the lipid crystal is surrounded by lipid molecules instead of water molecules, which is thermodynamically favored, i.e., the fat crystal is better wetted by liquid oil rather than water. Over time the droplets may continue to merge to further reduce the surface area of lipid that is exposed to water. Nevertheless, the aggregates partly retain the shape of droplets from which they were formed due to the low mobility of molecules in fat crystal networks (17, 19, 28, 60).

Partial coalescence only occurs in emulsions that contain partially crystalline regions. This is because one of the key requirements for partial coalescence is penetration into the liquid phase. (30). If all droplets were completely liquid, they would undergo normal coalescence. If all droplets were completely solid, they would undergo flocculation rather than partial coalescence because of the lack of liquid lipid regions that had sufficient molecular mobility required for merging. Thus, one can expect an "optimum" solid fat content at which partial coalescence would be highest. Indeed it has been found that increasing the solid fat content of the droplets causes an initial increase in the partial coalescence rate until a maximum value is reached, after which the partial coalescence rate decreases (30). The solid fat content at which this maximum rate occurs depends on the morphology and location of the crystals within the droplets, as well as the magnitude of the applied shear stresses (28, 31)

Partial coalescence is particularly important in dairy products, because milkfat globules are partly crystalline over a fairly wide temperature range (60, 79). The application of shear forces in combination with temperature cycling to cream-containing partly crystalline milkfat globules can cause partial coalescence, which leads to a marked increase in solution viscosity (28, 80, 81). Partial coalescence is an essential process in the production of ice cream, whipped toppings, butter, and margarine (1, 82–85). Oil-in-water emulsions are cooled to a temperature where the droplets are partly crystalline and a shear force is applied, which leads to droplet aggregation via partial coalescence (60). In butter and margarine, aggregation results in phase inversion (85), whereas in ice cream and whipped cream, the aggregated fat droplets form a network that surrounds incorporated air pockets thereby improving ice cream stability and texture (86, 87).

**5.1.3. Ostwald Ripening** Ostwald ripening is the process whereby large droplets grow at the expense of smaller ones because of diffusion driven mass transport of dispersed phase molecules from one droplet to another through the intervening continuous phase (88, 89). Ostwald ripening has traditionally not been recognized as a significant cause of emulsion instability. For example, it has been argued that the low solubility of triacylglycerols in water result in low mass transport rates (1). More recently however, it has been demonstrated that Ostwald ripening may in fact be the primary source of emulsion instability in oil-in-water emulsions that contain lipids with higher solubility (90) or in emulsions that contain alcohol, e.g., cream liquors (91). Due to the nature of the process, it should be noted that traditional methods to improve emulsion stability, such as increasing the emulsifier concentration or choosing an alternative emulsifier, may not be effective. Ostwald ripening occurs because the solubility of the molecules in a spherical droplet in

the surrounding aqueous phase in the vicinity of the droplet interface increases as the curvature of the interface decreases i.e., the size of the droplet decreases (88):

$$S(r) = S(\infty) \exp\left(\frac{2\gamma V_m}{RT r}\right). \quad (9)$$

Here,  $V_m$  is the molar volume of the solute,  $\gamma$  is the interfacial tension,  $S(\infty)$  is the solubility of the solute in the continuous phase for a droplet with infinite curvature (a planar interface), and  $S(r)$  is the solubility of the solute above a curved interface of radius  $r$ . Hence, there is a higher concentration of dissolved lipid molecules around a small droplet than around a larger one. Solubilized lipid molecules will move from the smaller droplets to the larger droplets because of this concentration gradient. Once steady state has been achieved, the rate of Ostwald ripening  $\omega$  is given by (88).

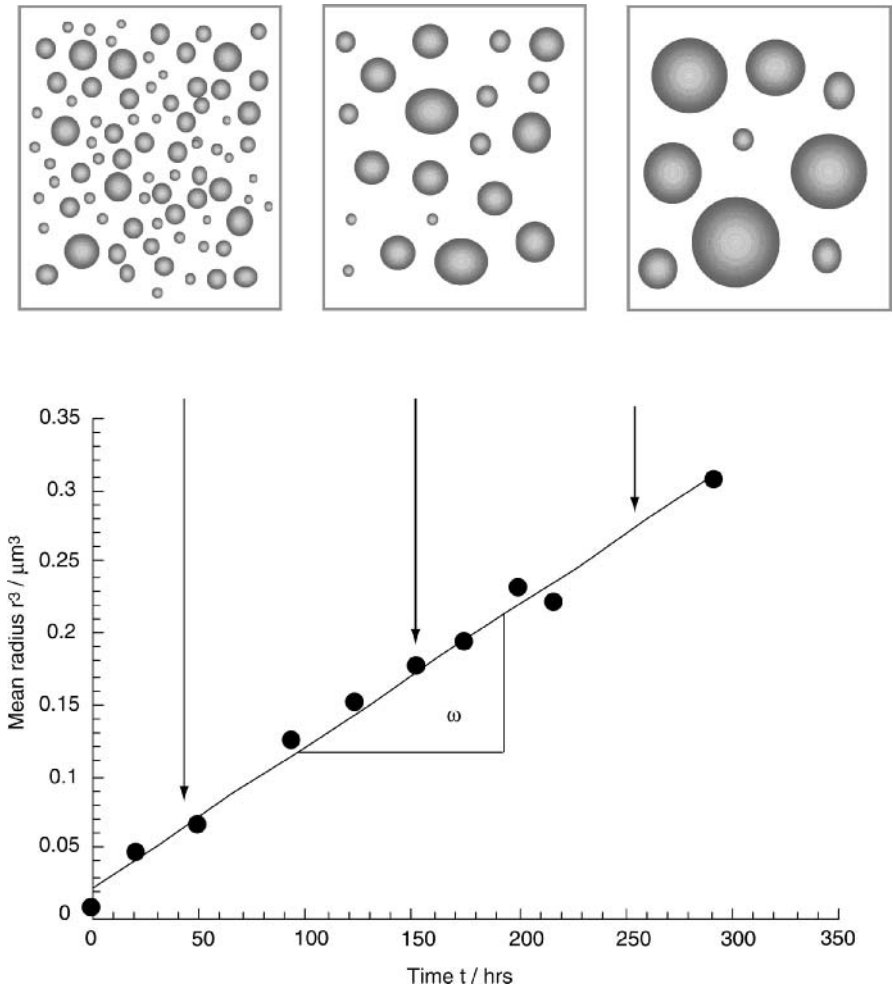
$$\omega = \frac{d\bar{r}^3}{dt} = \frac{8\gamma V_m S(\infty) D}{9RT}, \quad (10)$$

where  $D$  is the diffusion coefficient of the solute and  $\bar{r}$  is the mean size of the emulsion. This equation indicates that the change in droplet size with time becomes more rapid as the solubility of the molecules in the continuous phase increases (Figure 9). Therefore, Ostwald ripening may be reduced by reducing the solubility of the lipid in the aqueous phase or by reducing the surface tension (90).

**5.1.4. Phase Inversion** Phase inversion is the process whereby a system changes from an oil-in-water emulsion to a water-in-oil emulsion, or vice versa (Figure 5). Phase inversion is an essential step in the manufacture of a number of important food products, including butter and margarine (1, 60, 85). In most other foods, phase inversion is undesirable because it has an adverse effect on the products appearance, texture, stability, and taste and should therefore be avoided.

Phase inversion can be triggered by some alteration in the composition or environmental conditions of an emulsion, e.g., disperse phase volume fraction, emulsifier type, emulsifier concentration, solvent conditions, temperature, or mechanical agitation (2, 92). Only certain types of emulsion are capable of undergoing phase inversion, rather than being completely broken down into their component phases. These emulsions are capable of existing in a kinetically stable state after the phase inversion has taken place. It is usually necessary to agitate an emulsion during the phase inversion process, otherwise it will separate into its component phases. The physicochemical basis of phase inversion is believed to be extremely complex, involving aspects of flocculation, coalescence, partial coalescence, and emulsion formation.

**5.1.5. Chemical Instability** Molecular species present in an emulsion can be subject to chemical or biochemical reactions that alter their perceived quality. One of the most important types of chemical changes in food emulsions is the result



**Figure 9.** Large droplets grow at the expense of smaller ones in emulsions that undergo Ostwald ripening. The cube of the mean droplet size of such emulsions increases linearly with time.

of the oxidation of unsaturated lipids (93, 94). In some foods, a limited amount of lipid oxidation is desirable because it leads to the development of a characteristic taste or smell, e.g., cheeses (94). On the other hand, lipid oxidation is undesirable in many foods because it leads to the development of undesirable off-flavors (“rancidity”) and potentially toxic reaction products (95). The high susceptibility of polyunsaturated lipids to lipid oxidation has restricted their incorporation into many food products, which is unfortunate because greater consumption of polyunsaturated lipids is beneficial to health (96). Consequently, there is considerable interest in development of effective strategies for retarding the oxidation of unsaturated lipids in food emulsions.

A great deal of research has been carried out to elucidate lipid oxidation mechanisms in bulk fats and oils (97, 98). This research has provided important insights into the factors that influence lipid oxidation and strategies to control it. Nevertheless, the application of this knowledge to food emulsions is often limited because the lipids are dispersed as discrete phases dispersed in structurally and compositionally heterogeneous matrices (99). In these foods, the organization of the lipid molecules within the system, as well as their interactions with other types of molecules in their immediate vicinity, has a pronounced influence on their susceptibility to lipid oxidation (25). Recent experimental work has shown that the susceptibility of emulsified lipids to oxidation depends on a variety of factors. These include the chemical structure of the unsaturated lipids; the type, concentration, and location of antioxidants and prooxidants; the oxygen concentration; the temperature; the interfacial characteristics of the droplets, and the purity of the ingredients (27). Studies of the influence of structure and composition of emulsions on the rate of chemical reactions are likely to be an important area of research in the future.

## 5.2. Emulsion Rheology

The application of a stress to a material causes it to deform or to flow (100, 101). The extent of the deformation and flow depends on the physicochemical properties of the material. *Rheology* is the science that is concerned with the relationship between applied stresses and the deformation and flow of matter (101). Most rheological tests involve the application of a stress to a material and a measurement of the resulting flow or deformation (100). Sophisticated and sensitive analytical techniques are available for characterizing the rheological behavior of complex food emulsions, which are widely used in industrial, government, and university research laboratories. The knowledge gained from application of these techniques is important to food scientists for a number of reasons (1, 102–105). Many of the sensory attributes of food emulsions are directly related to their rheological properties, e.g., creaminess, thickness, smoothness, spreadability, pourability, flowability, brittleness, and hardness. A food manufacturer, therefore, must be able to design and produce a product that has the rheological properties expected by the consumer. The shelf life of many food emulsions depends on the rheological characteristics of the component phases, e.g., the creaming of oil droplets depends on the viscosity of the aqueous phase. Information about the rheology of food products is used by food engineers to design processing operations that depend on the way that a food behaves when it flows through a pipe, is stirred, or is packed into containers. Rheological measurements are also used by food scientists as an analytical tool to provide fundamental insights about the structural organization and interactions of the components within emulsions (11, 106).

**5.2.1. Mathematical Modeling of Emulsion Rheology** Food emulsions are compositionally and structurally complex materials that can exhibit a wide range of different rheological behavior, ranging from low-viscosity fluids (such as milk

and fruit juice beverages) to solids with elastic moduli (such as refrigerated margarine or butter). Our ability to control the rheological properties of food emulsions depends on a quantitative understanding of the relationship between rheology, composition, and microstructure. A variety of theories have been used to relate the rheological properties of emulsions to their composition and microstructure. In general, the apparent viscosity of an emulsion can be described by the following equation:

$$\eta = f(\eta_1, \eta_2, \phi, r, w(h), \tau), \quad (11)$$

where  $\eta_1$  is the viscosity of the continuous phase,  $\eta_2$  is the viscosity of the dispersed phase,  $\phi$  is the dispersed phase volume fraction,  $r$  is the droplet radius,  $w(h)$  is the interaction potential between the droplets, and  $\tau$  is the applied shear stress. The precise nature of the equation used to describe the rheological properties of an emulsion depends on the characteristics of the system, e.g. droplet concentration, droplet interactions, and continuous phase rheology. Exact expressions of the relationship between the rheology of colloidal suspensions and their composition/structure are only available in certain limiting cases, such as Einsteins' equation for a dilute suspension of rigid spherical particles given below.

$$\eta = \eta_1(1 + 2.5\phi). \quad (12)$$

This equation illustrates that the rheology of a dilute emulsion is proportional to the rheology of the continuous phase and increases with increasing droplet concentration. In concentrated emulsions, the rheology is influenced by hydrodynamic interactions associated with the relative motion of neighboring particles. At low-particle concentrations, hydrodynamic interactions mainly occur between pairs of particles, but as the particle concentration increases, three or more particles may be involved (107). As the particle concentration increases, the measured viscosity becomes larger than that predicted by the Einstein equation because these additional hydrodynamic interactions lead to a greater degree of energy dissipation. The Einstein equation can be extended to account for the effects of these interactions by including additional volume fraction terms (108):

$$\eta = \eta_0(1 + a\phi + b\phi^2 + c\phi^3 + \dots). \quad (13)$$

The value of the constants,  $a$ ,  $b$ ,  $c$ , etc., can either be determined experimentally or theoretically (107). For a colloidal dispersion of rigid spherical particles the value of  $a$  is 2.5. Therefore, Equation 13 equals the Einstein equation (Eq. 12) at low-volume fractions. A rigorous theoretical treatment of the interactions between pairs of droplets has established that  $b = 6.2$  for rigid spherical particles. Experiments have shown that Equation 13 can be used up to particle concentrations of about 10% with  $a = 2.5$  and  $b = 6.2$  for colloidal dispersions in the absence of long-range colloidal interactions (107). It is difficult to theoretically determine the value of higher order terms in Equation 13 because of the mathematical complexities involved in describing interactions between three or more particles.

Instead, a semiempirical approach is used to develop equations that describe the viscosity of concentrated colloidal dispersions. One of the most widely used equations was derived by Dougherty and Krieger and is applicable across the whole volume fraction range (Figure 9) (9, 109).

$$\eta = \eta_0 \left( 1 - \frac{\phi}{\phi_c} \right)^{-[\eta]\phi_c}, \quad (14)$$

where,  $[\eta]$  is the intrinsic viscosity and  $\phi_c$  is the maximum packing volume fraction, which is usually taken to be an adjustable parameter that is determined experimentally. Physically,  $\phi_c$  is related to the particle volume fraction at which the spheres become close packed. The intrinsic viscosity is 2.5 for spherical particles, but may be appreciably larger for nonspherical or aggregated particles (8). Typically, the value of  $\phi_c$  is between about 0.6 and 0.7 for spheres that do not interact via long-range colloidal interactions (9), but it may be considerably lower for suspensions in which there are strong long-range attractive or repulsive interactions between the droplets. This is because the effective volume fraction of the particles in the colloidal dispersion is greater than the actual volume fraction of the particles, so that the maximum packing volume fraction is reached at lower particle concentrations (62).

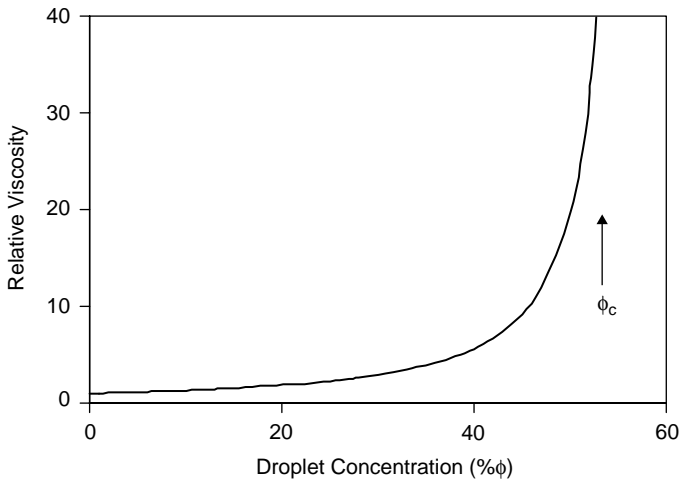
**5.2.2. Factors Influencing Emulsion Rheology** A variety of factors determine the rheological properties of food emulsions. Some of the most important of these factors are highlighted below.

*Continuous phase rheology.* The viscosity of most food emulsions is dominated by the rheology of the continuous phase (Equation 13). One of the most effective means of modifying the rheology of an emulsion is, therefore, to add a thickening or gelling agent to the continuous phase. The main exception to this rule is in systems that contain a network of aggregated particles. In these systems, the rheological properties are largely determined by the number and strength of the attractive forces between the aggregated particles.

*Disperse phase volume fraction.* The viscosity of food emulsions tends to increase with increased disperse phase volume fraction (Figure 10). The viscosity increases relatively slowly, with  $\phi$  at low droplet concentrations, but increases steeply when the droplets become closely packed together. At higher droplet concentrations, the particle network formed has predominantly elastic characteristics.

*Droplet-droplet interactions.* The nature of the colloidal interactions between the droplets in an emulsion is one of the most important factors determining its rheological behavior. When the interactions are long range and repulsive, the effective volume fraction of the dispersed phase may be significantly greater than its actual volume fraction ( $\phi_{\text{eff}} = \phi(1 + \delta/r)^3$ ), and so the emulsion viscosity increases. When interactions between the droplets are sufficiently attractive, the effective volume fraction of the dispersed phase is increased because of droplet flocculation, which results in an increase in emulsion viscosity. The rheological properties of an emulsion therefore depend on the relative magnitude of the attractive (mainly





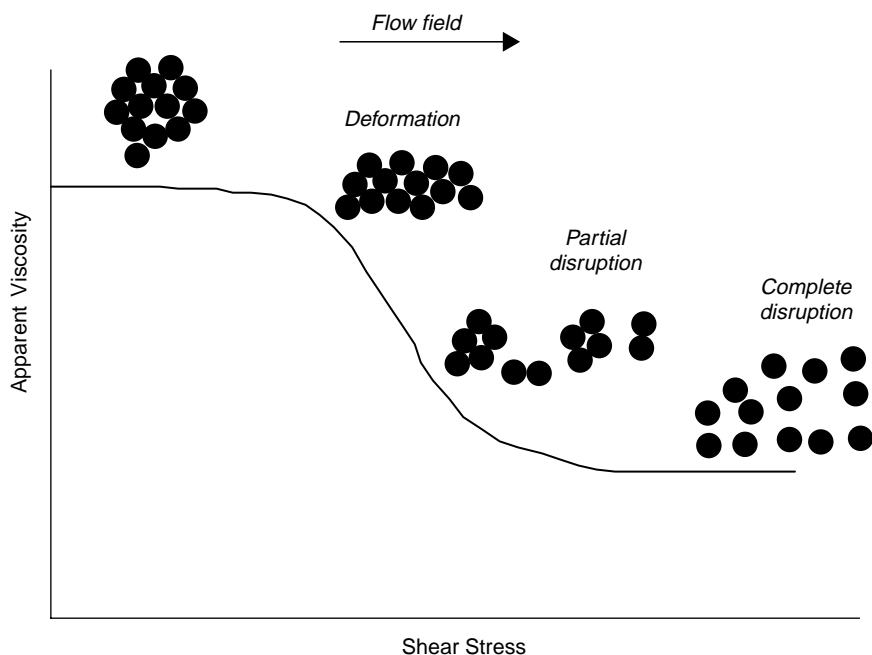
**Figure 10.** The relative viscosity of an emulsion depends on the droplet concentration. The emulsion viscosity increases linearly at small droplet concentrations and increases nonlinearly at droplet concentrations above 10%.

van der Waals, hydrophobic, and depletion interactions) and repulsive (mainly electrostatic, steric, and thermal fluctuation interactions) colloidal interactions.

**Droplet size.** Droplet size may influence the rheology of emulsions in a variety of ways. First, the viscosity of relatively concentrated suspensions (>30%) tends to decrease with increasing droplet size due to Brownian motion effects (108–110). This effect also causes emulsions to exhibit shear thinning behavior. At low-shear stresses, the particles have a three-dimensional isotropic and random distribution because of their Brownian motion (11). As the shear stress increases the particles become more ordered along the stream lines to form “strings” or “layers” of particles that offer less resistance to the fluid flow and therefore a decrease in viscosity (Figure 11). The viscosity decreases from a high constant value at low shear-stresses ( $\eta_0$ ) to a low constant value at high-shear stresses ( $\eta_\infty$ ). The shear thinning behavior of an emulsion is characterized by a critical shear stress, which corresponds to the stress where the viscosity has decreased by 50% between the low and high shear stress values. This critical shear stress increases with decreasing particle size, i.e., Brownian motion effects are more important for smaller droplets. Second, the droplet size influences the relative importance of the attractive and repulsive interactions between droplets, which may influence the rheology because it changes the effective volume fraction of the droplets (see above).

### 5.3. Emulsion Appearance

The appearance of an emulsion is one of the most important factors influencing its perceived quality, as it is usually the first sensory impression that a consumer makes of a product (4, 111). A better understanding of the factors that determine emulsion



**Figure 11.** Flocculated emulsions exhibit shear thinning. At increasing shear rates, the flocs are first deformed and then partially and finally completely disrupted.

appearance would therefore aid in the design of emulsion-based food products with improved quality. When a light beam is incident upon the surface of an emulsion, a portion of the incident light beam is transmitted through the emulsion while another portion is reflected. The relative proportions of light transmitted and reflected at different wavelengths depend on the scattering and adsorption of the light wave by the emulsion. Light scattering and absorption depend on size, concentration, refractive index, and spatial distribution of droplets, as well as the presence of any chromophoric materials (e.g., dyes). Hence, the overall appearance of an emulsion is influenced by its structure and composition. Scattering is largely responsible for the “turbidity,” “opacity,” or “lightness” of an emulsion, whereas absorption is largely responsible for “chromaticity” (blueness, greenness, redness, etc). It should be stressed that the overall appearance of an emulsion also depends on the nature of the light source and detector used (112–114). Finally, color of emulsion is also impacted by the nature of the lipids themselves that is the number and position of double bonds.

**5.3.1. Mathematical Modeling of Emulsion Color** Human beings have great difficulty objectively quantifying the color of objects, so color is normally quantified instrumentally in terms of “tristimulus coordinates,” such as the  $L^*a^*b^*$  system specified by the Commission Internationale de l’Eclairage (CIE) (114). The advantage of using the tristimulus coordinate system is that the color of an object can be

described in terms of just three mathematical variables. It is then possible to determine whether an object meets some predefined quality criteria in a quantitative manner. For example, in the  $L^*a^*b^*$  color space,  $L^*$  is lightness, and  $a^*$  and  $b^*$  are color coordinates: where  $L^* = 0$  is black,  $L^* = 100$  is white,  $+a^*$  is the red direction,  $-a^*$  is the green direction,  $+b^*$  is the yellow direction, and  $-b^*$  is the blue direction, (114). The overall color intensity of a product can be characterized in terms of its chroma,  $C = (a^2 + b^2)^{1/2}$ . One of the major advances in recent years has been the development of a theoretical approach to relate the tristimulus color coordinates of emulsions to their composition (dye and droplet concentration) and microstructure (particle size distribution) (115). This approach has led to the development of relationships given below.

$$L^* = f(\alpha(\lambda), c, \phi, r, n), \quad (15)$$

$$a^* = f(\alpha(\lambda), c, \phi, r, n), \quad (16)$$

$$b^* = f(\alpha(\lambda), c, \phi, r, n), \quad (17)$$

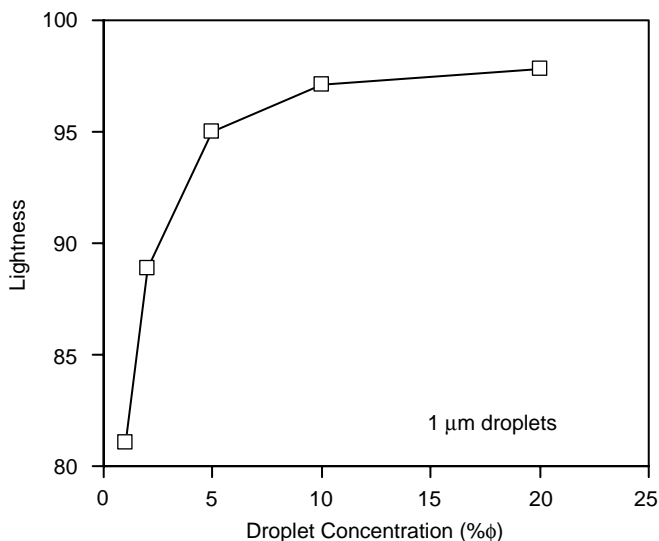
where  $\alpha(\lambda)$  is the absorption spectra of the dye solution,  $c$  is the concentration of dye present,  $\phi$  is the disperse phase volume fraction,  $r$  is the droplet radius, and  $n$  is the ratio of the refractive indices of the dispersed to the continuous phases. These equations can be used to predict the influence of emulsion composition and microstructure on product appearance, which reduces the number of time-consuming and laborious experiments required in a laboratory. A number of systematic experimental studies have recently been carried out to determine the influence of composition and microstructure on the color of oil-in-water emulsions (116–121). These measurements are in excellent qualitative agreement with predictions made using the light scattering theory mentioned above. However, the quantitative agreement is still fairly poor, mainly because of problems associated with accounting for the optical measurement system, although a number of methods of overcoming this problem have been proposed (115).

### 5.3.2. Factors Influencing Emulsion Color *Disperse phase volume fraction.*

As the droplet concentration is increased, more and more light is scattered. Emulsion lightness ( $L$ ) increases and emulsion chromaticity ( $C$ ) decreases with increasing disperse phase volume fraction (Figure 12).  $L$  and  $C$  change steeply when the droplet concentration is increased from 0 wt% to 5 wt%, but then remain relatively constant at higher droplet concentrations (117–119).

*Droplet size.* The magnitude and direction of light scattered by a particle depend on the ratio of its radius to the wavelength of light. Emulsion lightness increases with droplet radius from 0 nm to 100 nm, has a maximum value around 100 nm, and then decreases as the droplet radius is increased further (119).

*Relative refractive index.* The scattering efficiency of a particle increases as the contrast in refractive index between the particle and the surrounding liquid increases. Emulsion lightness, therefore, is high when the refractive index of the droplets is either much smaller or much greater than the refractive index of the



**Figure 12.** Appearance of emulsions are impacted by colloidal properties of the emulsion, e.g., droplet concentration. The lightness of an emulsion increases with increasing droplet concentration.

continuous phase, but decreases when the refractive index ratio tends towards unity. Indeed, it is possible to prepare optically transparent emulsions with high droplet concentrations by matching the refractive index of the continuous phase to that of the disperse phase by adding water-soluble solutes, such as glycerol or sucrose (62, 116). Refractive index matching is particularly useful in some emulsion studies because it means that the emulsions can be analyzed using spectroscopic techniques that require optically transparent solutions, such as UV-visible spectrophotometry, fluorescence, and CD.

## 5.4. Emulsion Flavor

Flavor plays a critical role in determining the quality of a food emulsion during consumption. The term “flavor” refers to those volatile components in foods that are sensed by receptors in the nose (aroma) and those nonvolatile components that are sensed by receptors in the tongue and the inside of the mouth (taste) (122, 123). In addition, certain components in foods may also contribute to flavor because of their influence on the perceived texture (mouthfeel) (124). The flavor of a food is therefore a combination of aroma, taste, and mouthfeel, with aroma usually being the most important (122). Flavor perception is an extremely complicated process that depends on a combination of physicochemical, biological, and psychological phenomena (125). Before a food is placed in the mouth, its flavor is perceived principally through those volatile components that are inhaled directly into the nasal cavity. After the food is placed in the mouth, the flavor is determined by nonvolatile

molecules, which leave the food and are sensed by receptors on the tongue and the inside of the mouth, as well as by those volatile molecules that are drawn into the nasal cavity through the pharynx at the back of the mouth (122). The interactions between flavor molecules and human receptors that lead to the perceived flavor of a food are extremely complicated and are still poorly understood (123). In addition, expectations and eating habits vary from individual to individual, so that the same food may be perceived as tasting different by two separate individuals or by a single individual at different times. This section focuses on the physicochemical aspects of flavor partitioning and release in foods, because these are the most relevant topics to emulsion science.

**5.4.1. Flavor Partitioning** The perception of a flavor depends on the precise location of the flavor molecules within an emulsion. The aroma is determined by the presence of volatile molecules in the vapor phase above an emulsion (122, 126). Most flavors are perceived more intensely when they are present in the aqueous phase, rather than in the oil phase (127, 128). Certain flavor molecules may associate with the interfacial region, which alters their concentration in the vapor and aqueous phases (129). It is therefore important to establish the factors that determine the partitioning of flavor molecules within an emulsion. An emulsion system can be conveniently divided into four phases between which the flavor molecules distribute themselves: the interior of the droplets, the continuous phase, the oil-water interfacial region, and the vapor phase above the emulsion. The relative concentration of the flavor molecules in each of these regions depends on their molecular structure and the properties of each of the phases (130, 131).

A number of the most important factors that influence the equilibrium distribution of flavors in food emulsions are listed below.

*Flavor partition coefficients.* The equilibrium distribution of a particular flavor molecule between two phases (e.g., oil-water, air-water, or air-oil) is characterized by an equilibrium partition function. These partition coefficients determine the distribution of the flavor molecules between the oil, water, and head space phases of an emulsion.

*Surface activity.* Many flavor molecules are amphiphilic in character, having both nonpolar and polar regions. These molecules will tend to accumulate at an oil-water interface.

*Droplet concentration.* The concentration of flavor molecules in the headspace of an emulsion depends on the disperse phase volume fraction, i.e., the ratio of oil to water. Previous studies have shown that there is a decrease in the fraction of a nonpolar flavor in the vapor phase as the oil content increases, whereas the amount of a polar flavor is relatively unaffected. Thus, nonpolar flavors in an emulsion become more odorous as the fat content is decreased, whereas the polar flavors remain relatively unchanged. This has important consequences when deciding the type and concentration of flavors to use in low-fat analogs of existing emulsion-based food products.

*Flavor Binding.* Many proteins and carbohydrates are capable of binding flavor molecules, and therefore altering their distribution within an emulsion (131–135).

Flavor binding can cause a significant alteration in the perceived flavor of a food. This alteration is often detrimental to food quality because it changes the characteristic flavor profile, but it can also be beneficial when the bound molecules are off-flavors. A flavor chemist must therefore take binding effects into account when formulating the flavor of a particular product.

*Solubilization.* Surfactants are normally used to physically stabilize emulsion droplets against aggregation by providing a protective membrane around the droplet. Nevertheless, there is often enough free surfactant present in an aqueous phase to form surfactant micelles. These surfactant micelles are capable of solubilizing the nonpolar molecules in their hydrophobic interior, which increases the affinity of nonpolar flavors for the aqueous phase. By a similar argument, reverse micelles in an oil phase are capable of solubilizing polar flavor molecules.

**5.4.2. Flavor Release** Flavor release is the process whereby flavor molecules move out of a food and into the surrounding saliva or vapor phase during mastication (126, 127). The release of flavors from a food material occurs under extremely complex and dynamic conditions (136). A food usually spends a relatively short period (typically 1 to 30 seconds) in the mouth before being swallowed. During this period, it is diluted with saliva, experiences temperature changes, and is subjected to a variety of mechanical forces. Mastication may therefore cause dramatic changes in the structural characteristics of a food emulsion.

During mastication, nonvolatile flavor molecules must move from within the food, through the saliva to the taste receptors on the tongue, and the inside of the mouth, whereas volatile flavor molecules must move from the food, through the saliva and into the gas phase, where they are carried to the aroma receptors in the nasal cavity. The two major factors that determine the rate at which these processes occur are the equilibrium partition coefficient (because this determines the initial flavor concentration gradients at the various boundaries) and the mass transfer coefficient (because this determines the speed at which the molecules move from one location to another). A variety of mathematical models have been developed to describe the release of flavor molecules from oil-in-water emulsions.

## 6. CONCLUSIONS

Over the past few years, there has been a growing emphasis on the understanding of the colloidal basis of properties of food emulsions, rather than just treating them as a “black box” whose properties could be characterized in terms of certain empirical parameters. Researchers are attempting to quantitatively relate the properties of food emulsions to the characteristics, interactions, and spatial distribution of the droplets they contain. A wide variety of analytical, mathematical, and computational techniques are being developed and utilized to achieve this objective. Powerful commercial instruments are widely available to quantify the colloidal characteristics of both dilute and concentrated emulsions, e.g., droplet size, concentration, and electrical charge (137–139). Theoretical, computational, and

experimental work is providing a much better understanding of the various types of colloidal interactions that operate between emulsion droplets (140, 141). The cost, sensitivity, and range of commercial rheometers are continually improving (109). New analytical instruments are being developed that will enable researchers to measure changes in structure and bulk physicochemical properties. Traditional microscopic techniques are being refined so that they can be used to characterize the microstructure of delicate materials, such as emulsions (142). In addition, new microscopic technologies are being developed to characterize the organization of molecules at an interface (143–145). Advances in our understanding of the relationship between emulsion properties and colloidal characteristics are also being made through development of more comprehensive physical theories (107, 146, 147) and the utilization of powerful computational techniques (36, 37, 148). The application of these new concepts and tools will eventually lead to a much better understanding of the colloidal basis of emulsion properties. This knowledge will enable food manufacturers to design foods in a more rational fashion, which should eventually lead to improvements in product quality and reductions in manufacturing costs.

## REFERENCES

1. E. Dickinson and G. Stainsby, *Colloids in Foods*, Applied Science, London, U.K., 1982.
2. E. Dickinson, *Introduction to Food Colloids*, Oxford University Press, Oxford, U.K., 1992.
3. S. E. Friberg and K. Larsson, *Food Emulsions*, Marcel Dekker, New York, 1997.
4. D. J. McClements, *Food Emulsions: Principles, Practice and Techniques*, CRC Press, Boca Raton, Florida, 1999.
5. P. Sherman, *Emulsion Science*, Academic Press, New York, 1968.
6. P. Becher, *Emulsions: Theory and Practice*, Chapman & Hall, London, U.K., 1957.
7. P. Becher, *Encyclopedia of Emulsion Technology—Basic Theory*, Marcel Dekker, New York, 1983.
8. P. C. Hiemenz and R. Rajagopalan, *Principles of Colloid and Surface Chemistry*, Marcel Dekker, New York, 1997.
9. R. J. Hunter, *Foundations of Colloid Science*, Oxford University Press, Oxford, U.K., 1986.
10. R. J. Hunter, *Foundations of Colloid Science*, Oxford University Press, Oxford, U.K., 1989.
11. R. J. Hunter, *Introduction to Modern Colloid Science*, Oxford University Press, Oxford, U.K., 1993.
12. D. F. Evans and H. Wennerstrom, *The Colloidal Domain: Where Physics, Chemistry, Biology and Technology Meet*, VCH Publishers, New York, 1994.
13. A. E. Sloan, *Food Technol.*, **48**(7), 89 (1994).
14. A. E. Sloan, *Food Technol.*, **50**(7), 55 (1996).
15. F. Katz, *Food Technol.*, **51**(6), 55–59 (1997).
16. P. Hollingsworth, *Food Technol.*, **49**(2), 67 (1995).

17. P. Walstra, *Encyclopedia of Emulsion Technology*, 1996, ch. 1.
18. P. Walstra, in O. Fennema, ed., *Food Chemistry*, 1996, ch. 3.
19. E. Dickinson and D. J. McClements, *Advances in Food Colloids*, Chapman & Hall, London, U.K., 1995.
20. J. Evison, E. Dickinson, R. K. Owusu-Apenten, and A. Williams, in D. E. Lorient and D. Lorient, eds., *Food Macromolecules and Colloids*, Royal Society of Chemistry, Cambridge, U.K., 1995, 235–243.
21. J. N. Israelachvili, *Intermolecular and Surface Forces*, Academic Press, London, U.K., 1992.
22. C. E. Stauffer, *Emulsifiers*, Eagen Press Handbook, St. Paul, Minnesota, 1999.
23. J. M. Carr, K. Sufferling, and J. Poppe, *Food Technol.*, **49**(7), 41 (1995).
24. C. Orr, *Encyclopedia of Emulsion Technology*, 1988, ch. 3.
25. J. N. Coupland and D. J. McClements, *Trends Food Sci. Technol.*, **7**, 83 (1996).
26. P. Landy, J. L. Courthaudon, C. Dubois, and A. Voilley, *J. Agriculture Food Chem.*, **44**, 526 (1996).
27. L. Mei, D. J. McClements, J. Wu, and E. A. Decker, *Food Chem.*, **61**(3), 307–312 (1998).
28. K. Boode, *Partial Coalescence in Oil-in-Water Emulsions*, Wageningen Agricultural University, 1992.
29. K. Boode, P. Walstra, and A. E. A. Degrootmostert, *Colloids Surfaces A.*, **81**, 139 (1993).
30. K. Boode and P. Walstra, *Colloids Surfaces A.*, **81**, 121 (1993).
31. K. Boode and P. Walstra, in E. Dickinson and P. Walstra, eds., *Food Colloids and Polymers: Stability and Mechanical Properties*, Royal Society of Chemistry, Cambridge, U.K., 1993, p. 23.
32. D. G. Dalgleish, in J. Sjoblom, ed., *Emulsions and Emulsion Stability*, Marcel Dekker, New York, 1996, ch. 5.
33. D. G. Dalgleish, *Food Res. Int.*, **29**, 541 (1996).
34. P. J. Wilde, *Curr. Opin. Colloid Interface Sci.*, **5**, 176–181 (2000).
35. E. Dickinson, *Colloid Surface, B.*, **20**, 197–210 (2001).
36. J. F. M. Lodge and D. M. Heyes, *J. Rheol.*, **43**, 219–244 (1999).
37. E. Dickinson, *J. Colloid Interface Sci.*, **225**, 2–15 (2000).
38. P. Manoj, A. J. Fillery-Travis, A. D. Watson, D. J. Hibberd, and M. M. Robins, *J. Colloid Interface Sci.*, **207**, 283–293 (1998).
39. E. Dickinson, in B.P. Binks, ed. *Modern Aspects of Emulsion Science*, Royal Society of Chemistry, Cambridge, U.K., 1998, pp. 145–174.
40. M. Loncin and R. L. Merson, (1979).
41. K. S. Birdi, *Handbook of Colloid and Surface Chemistry*, CRC Press, New York, 1997.
42. F. Asinger, *Chemistry, Physics and Application of Surface Active Substances*, Gordon & Breach Science Publishers, New York, 1967.
43. K. Shinoda, B. Yamamushi, T. Nakagawa, and T. Isemura, *Colloidal Surfactants—some Physicochemical Properties*, Academic Press, New York, 1963.
44. C. Paquot, *Chemistry, Physics and Application of Surface Active Substances*, Gordon and Breach Science Publishers, New York, 1976.



45. J. F. G. Overbeek, *Chemistry, Physics and Application of Surface Active Substances*, Gordon and Breach Science Publishers, New York, 1967.
46. R. Zana, *Surfactant Solutions*, New York, New York, 1987.
47. D. Myers, *Surfactant Science and Technology*, VCH Publishers, New York, 1988.
48. S. Ross and I. D. Morrison, *Colloidal Systems and Interfaces*, John Wiley & Sons, New York, 1988.
49. P. Walstra, *Principles of Emulsion Formation*, 1993.
50. P. Walstra, *Encyclopedia of Emulsion Technology*, 1983, ch. 2.
51. S. S. Dukhin, G. Kriechamer, and M. R., *Dynamics of Adsorption at Liquid Interfaces: Theory, Experiment and Application*, Elsevier, Amsterdam, The Netherlands, 1995.
52. L. W. Phipps, *Nat. Inst. Res. Dairying*, **4**, 12 (1985).
53. H. A. Stone, *Annu. Rev. Fluid Mechanics*, **26**, 65 (1994).
54. H. Karbstein and H. Schubert, *Chemical Eng. Processing*, **34**, 205 (1995).
55. H. Schubert and H. Armbruster, *Int. J. Chemical Eng.*, **32**, 14 (1992).
56. M. Stang, H. Karbstein, and H. Schubert, *Chemical Eng. Processing*, **33**, 307 (1994).
57. W. Banks and D. D. Muir, in E. Dickinson and G. Stainsby, eds., *Advances in Food Emulsions and Foams*, Elsevier Applied Science, London, U.K., 1988.
58. E. Dickinson and S. K. Hong, *J. Agric. Food Chem.*, **13**, 876 (1994).
59. J. E. Kinsella and D. M. Whitehead, *Adv. Food Nutrition Res.*, **33**, 343 (1989).
60. H. Mulder and P. Walstra, *The Milk Fat Globule: Emulsion Science as Applied to Milk Products and Comparable Foods*, 1974.
61. A. K. Das and P. K. Ghosh, *Langmuir*, **6**, 1668 (1990).
62. J. Weiss and D. J. McClements, *Langmuir*, **16**, 2145–2150 (2000).
63. S. Dukhin and J. Sjoblom, in J. Sjoblom, ed. *Emulsions and Emulsion Stability*, Marcel Dekker, New York, 1996.
64. A. Lips, T. Westbury, P. M. Hart, I. D. Evans, and I. J. Campbell, *Food Colloids and Polymers*, **31** (1993).
65. H. Luyten, M. Jonkman, W. Kloek, and T. van Vliet, *Food Colloids and Polymers: Stability and Mechanical Properties*, **224** (1993).
66. K. Demetriades, J. Coupland, and D. J. McClements, *J. Food Sci.*, **62**, 342–347 (1997).
67. K. Demetriades, J. N. Coupland, and D. J. McClements, *J. Food Sci.*, **62**, 462 (1997).
68. E. Dickinson, M. Golding, and M. J. W. Povey, *J. Colloid Interface Sci.*, **185**, 515 (1997).
69. E. Dickinson, M. I. Goller, and D. J. Wedlock, *Colloids Surfaces A*, **75**, 195 (1993).
70. E. Dickinson and M. Golding, *Food Hydrocolloids*, **11**, 13 (1997).
71. M. P. Aronson, *Emulsions—A Fundamental and Practical Approach*, Kluwer Academic Publishers, Netherlands, 1992, 75.
72. P. Jenkins and M. Snowden, *Adv. Colloid Interface Sci.*, **68**, 57 (1996).
73. J. A. Hunt and D. G. Dalgleish, *J. Food Sci.*, **60**, 1120 (1995).
74. F. J. Monahan, D. J. McClements, and J. B. German, *J. Food Sci.*, **61**, 504 (1996).
75. S. Damodaran, *Food Proteins and Their Applications*, Marcel Deckker, New York, 1997.
76. E. Dickinson, *J. Colloid Interface Sci.*, **132**, 274 (1989).
77. H. Ogawa, K. Sigita, K. Saito, M. Kim, M. Tamada, A. Katakai, and T. Sugo, *J. Chromatogr. A*, **954**(1–2), 89–97 (2002).

78. A. Kabalanov and W. H., *Langmuir*, **12**, 276 (1996).
79. P. Walstra and E. C. H. van Beresteyn, *Netherlands Milk Dairy J.*, **29**, 35 (1975).
80. M. A. J. S. van Boekel and P. Walstra, *Colloids Surfaces*, **3**, 109 (1981).
81. K. Boode, C. Bispermick, and P. Walstra, *Colloids Surfaces*, **61**, 55 (1991).
82. N. M. Barfod and M. Krog, *J. Amer. Oil Chem. Soc.*, **64**, 112 (1987).
83. H. D. Goff, M. Liboff, W. K. Jordon, and J. E. Kinsella, *Food Microstruct.*, **6**, 193 (1987).
84. N. M. Barfod, N. Krog, G. Larsen, and W. Bucheim, *Fat Sci. Technol.*, **93**, 24 (1991).
85. D. P. J. Moran and K. K. Rajah, *Fats in Food Products*, 1994.
86. H. D. Goff, in E. Dickinson and P. Walstra, eds., *Food Colloids and Polymers: Stability and Mechanical Properties*, Royal Society of Chemistry, Cambridge, U.K., 1993, p. 71.
87. N. M. Barfod, N. Krog, and W. Bucheim, in D. E., ed. *Food Emulsions and Foams*, Royal Society of Chemistry, Cambridge, U.K., 1987.
88. A. S. Kabalnov and E. D. Shchukin, *Adv. Colloid Interface Sci.*, **38**, 69 (1992).
89. P. Taylor, *Colloids Surfaces A*, **99**, 175 (1995).
90. J. Weiss, *Mass Transport Phenomena in Oil-in-Water Emulsions*, The University of Massachusetts, Amherst, Massachusetts, 1999.
91. D. J. McClements, S. R., Dungan, J. B. German, and J. E., Kinsella, *Colloids Surfaces A*, **81**, 203 (1993).
92. K. Shinoda and S. Friberg, *Emulsions Solubilization*, 1986.
93. E. N. Frankel, S. W. Huang, J. Kanner, and J. B. German, *J. Agric. Food Chem.*, **42**, 1054 (1994).
94. W. W. Nawar, in O. Fennema, ed. *Food Chemistry*, Marcel Dekker, New York, 1996.
95. B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Clarendon Press, London, U.K., 1991.
96. D. Kritchevsku, S. A. Tepper, S. Wright, A. Kuksis, and T. A. Hughes, *Nutrition Res.*, **18**(2), 259–264 (1998).
97. B. Halliwell, R. Aeschback, J. Loeliger, and O. I. Aruoma, *Food Chem. Toxicol.*, **33**(7), 601–617 (1995).
98. E. N. Frankel, *Lipid Oxidation*, Matreya, Incorporated, Pleasant Gap, Pennsylvania, 1998.
99. E. A. Decker, *Trends Food Sci. Technol.*, **9**(6), 241–248 (1998).
100. R. W. Whorlow, *Rheological Techniques*, Ellis Horwood, New York, 1992.
101. C. W. Macosko, *Rheology: Principles, Measurements and Applications*, 1994.
102. P. Sherman, *Industrial Rheology With Particular Reference To Foods, Pharmaceuticals and Cosmetics*, Academic Press, London, U.K., 1970.
103. M. A. Rao, *Engineering Properties of Foods*, 1995, ch. 1.
104. V. N. M. Rao, R. A. M. Delaney, and G. E. Skinner, *Engineering Properties of Foods*, 1995, ch. 2.
105. C. F. Shoemaker, J. Nantz, S. Bonnans, and A. C. Noble, *Food Technol.*, **46**, 98 (1992).
106. T. F. Tadros, *Colloids Surfaces*, **91**, 30 (1994).
107. R. G. Larson, *The Structure and Rheology of Complex Fluids*, Oxford University Press, Oxford, U.K., 1999.

108. R. Pal, Y. Yan, and J. H. Masliyah, *Emulsions: Fundamentals and Applications in the Petroleum Industry*, 1992, ch. 4.
109. J. Mewis and C. W. Macosko, *Rheology: Principles, Measurements and Applications*, 1994, ch. 10.
110. S. Liu and J. H. Masliyah, in L.L. Schramm, ed. *Suspensions: Fundamentals and Applications in the Petroleum Industry*, American Chemical Society, Washington, D.C., 1996, ch. 3.
111. J. B. Hutchings, *Food Colour and Appearance*, Blackie Academic and Professional, London, U.K., 1994.
112. D. B. Judd and G. Wyszecki, *Color in Business, Science, and Industry*, Wiley, New York, 1963.
113. F. W. Billmeyer and M. Saltzman, *Principles of Color Technology*, Wiley, New York, 1981.
114. G. Wyszecki and W. S. Stiles, *Color Science: Concepts and Methods, Quantitative Data and Formulae*, Wiley, New York, 1982.
115. D. J. McClements, *Curr. Opin. Colloid Interface Sci.*, **7**(5–6), 451–455 (2002).
116. W. Chantrapornchai and D. J. McClements, *J. Food Sci.*, **12** (2001).
117. W. Chantrapornchai, F. Clydesdale, and D. J. McClements, *Colloids Surface A*, **155**(373–382) (1999).
118. W. Chantrapornchai, F. Clydesdale, and D. J. McClements, *J. Colloid Interface Sci.*, **218**, 324–330 (1999).
119. Chantrapornchai, F. W. Clydesdale, and D. J. McClements, *J. Agric. Food Chem.*, **46**, 2914–2920 (1998).
120. D. J. McClements, W. Chantrapornchai, and F. Clydesdale, *J. Food Sci.*, **63**, 935–939 (1998).
121. R. Chanamai and D. J. McClements, *Food Hydrocolloids*, **1** (2000).
122. A. J. Taylor and R. S. T. Linforth, *Trends Food Sci. Technol.*, **7** (1996).
123. D. M. H. Thomson, *Develop. Food Flavours*, **1** (1986).
124. J. L. Kokini, *J. Food Eng.*, **6**, 51 (1987).
125. G. A. Bell, *Trends Food Sci. Technol.*, **7**, 425 (1996).
126. P. Overbosch, W. G. M. Afterof, and P. G. M. Haring, *Food Rev. Int.*, **7**, 137 (1991).
127. P. B. McNulty, *Food Struct. Behaviour*, (1987).
128. J. E. Kinsella, in D.B. Min and T.H. Smouse, eds., American Oil Chemists Society, Champaign, Illinois, 1989, p. 376.
129. B. L. Wedzicha, *Advances in Food Emulsions*, 1988, ch. 10.
130. R. Baker, *Controlled Release of Biologically Active Agents*, Wiley, New York, 1987.
131. J. Bakker, in A.G. Gaonkar, ed. *Ingredient Interactions: Effects on Food Quality*, Marcel Dekker, New York, 1995.
132. K. L. Franzen and J. E. Kinsella, *Chem. Ind.*, **21**, 505 (1975).
133. T. E. O'Neill, *Flavor-Food Interactions*, 1996, ch. 6.
134. A. P. Hansen and D. C. Booker, in R. J. McGorrin and J. V. Leland, eds. *Flavor-Food Interactions*, American Chemical Society, Washington, D.C., 1986.
135. M. Y. M. Hau, D. A. Gray, and A. J. Taylor, in R. J. McGorrin and J. V. Leland, eds. *Flavor-Food Interactions*, American Chemical Society, Washington, D.C., 1996.

136. D. G. Land, in R. J. McGorin and J. V. Leland, eds. *Flavor-Food Interactions*, American Chemical Society, Washington, D.C., 1996.
137. R. J. Hunter, V. A. Hackley, and J. Texter, *Handbook on Ultrasonic and Dielectric Characterization Techniques for Suspended Particles*, American Chemical Society, Westerville, Ohio, 1998.
138. B. P. Hills, H. R. Tang, P. Manoj, and C. Destruel, *Magnetic Resonance Imaging*, **19**, 449–451 (2001).
139. R. A. Meyers, *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation*, Wiley, New York, 2000.
140. P. M. Claesson, E. Blomberg, and E. Poptoshev, in J. Sjoblom, ed. *Encyclopedic Handbook of Emulsion Technology*, Marcel Decker, New York, 2001, pp. 305–326.
141. B. A. Bergenstahl and P. M. Claesson, in S. Friberg and K. Larsson, eds. *Food Emulsions*, Marcel Decker, New York, 1997.
142. E. Dickinson, *New Physico-chemical Techniques for the Characterization of Complex Food Systems*, Chapman & Hall, London, U.K., 1995.
143. J. M. R. Patino and M. R. R. Nino, *Colloid Surface B*, **15**, 235–252 (1999).
144. K. P. Plucknett, S. J. Pomfret, V. Normand, D. Ferdinando, C. Veerman, W. J. Frith, and I. T. Norton, *J. Microscopy-Oxford*, **201**, 279–290 (2001).
145. V. J. Morris, A. R. Mackie, P. J. Wilde, A. R. Kirby, E. C. N. Mills, and A. P. Gunning, *Lebensmittel Wissenschaft und Technologie*, **34**, 3–10 (2001).
146. A. G. Marangoni, *Phys. Rev.*, **62**, 13951–13955 (2000).
147. L. G. B. Bremer, B. H. Bijsterbosch, P. Walstra, and T. Van Vliet, *Adv. Colloid Interface Sci.*, **46**, 117 (1993).
148. M. Whittle and E. Dickinson, *J. Colloid Interface Sci.*, **242**, 106–109 (2001).

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## *Dietary Fat Substitutes*

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### 1. INTRODUCTION

Lipids are a heterogeneous group of substances made up primarily or exclusively of nonpolar groups. As a result of their nonpolar character, lipids have a low solubility in water and a high solubility in nonpolar solvents. The lipids of our diet are known as fats or oils, depending on their physical state at room temperature. Phospholipids, sphingolipids, and steroids are physiologically important, but, chemically and functionally, quite different from the triacylglycerols (TAGs), which constitute our dietary fats and oils. Lipids are made up of fatty acids that belong to three major categories of saturated, monounsaturated, and polyunsaturated. Saturated fats, but not all, have a hypercholesterolemic effect. These are found predominantly in animal products (butter, cheese, and meat), but are also present in coconut and palm oils. Saturated fat may raise blood cholesterol levels more than anything else in the diet, even more than dietary cholesterol. Intake of monounsaturated fats in oils, such as olive oil, is thought to be preferable to consumption of polyunsaturated fats and oils, such as corn oil, because the monounsaturated fats apparently do not lower high-density lipoprotein (HDL) cholesterol levels (1). Unsaturated fats originate primarily from vegetable oils (safflower, corn, soybean, cottonseed,

sesame, and sunflower oils), nuts, and seeds, although fish is a good source of unsaturated fatty acids. Most of the essential fatty acids are found in unsaturated fat, so foods high in saturated fat and cholesterol (animal fat, dairy products, and eggs) should be eaten sparingly.

Fat is found at some level in most foods. The principle sources of fat in the U.S. diet are fats and oils (butter, margarine, shortening, salad, and cooking oils), red meats, poultry, fish, and dairy products. The most recent data from the Economic Research Service of the U.S. Department of Agriculture (2) indicate that use of fats and oils has increased from 64.6 lb per capita to 65.4 lb per capita during 1991 through 1995 to 64.2 lb per capita to 74.5 lb per capita during 1996 through 2000. Little variation in red meats consumption occurred between 1991 and 2000; levels varied from 111.6 lb per capita to 113.5 lb per capita. Poultry use has increased from 58.2 lb per capita to 66.5 lb per capita during 1991 through 2000. Consumption of fish has remained relatively stable, ranging from 14.8 lb per capita in 1991 to 15.2 lb per capita in 2000. The use of dairy products has risen sharply from a level of 564 lb per capita to 593 lb per capita during the year 1991 through 2000. Although there was a marked consistent reduction in consumption of whole milk and a partially offsetting increase in the use of fat-free milk, there has also been an increase in the intake of cheese, yogurt, and fluid cream products, whereas consumption of ice cream has remained unchanged (2). In the U.S. food supply, 30% of the total fat comes from red meat, poultry, and fish. Grain products and dairy products account for 25% and 18% of the total fat, respectively. On the other hand, fats and oils (mainly tablespreads and salad dressings) account for 11% of the total fat. Collectively, these food ingredients account for 84% of the fat in the food supply. Hence, the effort to reduce total fat and caloric intake has focused largely on reducing the fat content of foods in these categories (3).

Dietary fats have been classified as “visible” and “invisible” sources of fat. Visible fats are those that have been isolated from animal tissues, oilseeds, or vegetable sources and are used in such products as margarine, shortening, and salad oil. These fats and oils comprise about 43% of the total fat available for consumption in the U.S. diet. Invisible fats are those that are consumed as part of the animal tissues or the vegetables in the diet. These comprise the remaining 57% of the fat available for consumption in the U.S. diet (4).

### 1.1. Roles of Dietary Fat

Natural fats have many useful functions in the diet. They are among nutrients essential for proper growth and development and maintenance of good health. They furnish fat-soluble Vitamins A, D, E, and K and assist in their absorption in the intestine. They are the only source of essential fatty acids (EFAs), such as linoleic and  $\alpha$ -linolenic acids, which play a functional role in all tissues of the body. Adequate intakes of EFAs are thought to range between 5% and 10% of total energy intake. Deficiency symptoms can be prevented when 1–2% of dietary energy is derived from n-6 fatty acids and 1% from n-3 fatty acids (5). Fats also play important roles in food preparation and consumption. As a food ingredient, oils

and fats contribute favorably to the taste, odor, consistency, stability, and palatability of foods and help us feel full after eating. Fat content also affects the structure and color of a food. In addition, as a result of this unique functional properties, lipids are important in determining the flavor or aroma characteristics of some foods, because many flavor or aroma components are fat soluble. Fats serve as a heating medium and are frequently used to cook and preserve foods. Their reversible solid-to-liquid phase transition properties allow them to function as frying shortenings, pastry fats, and confectionery applications. Also, fats contribute gloss and surface appearance to products such as snacks, crackers, confections, and fried foods. Fat may also have an important effect on the texture of food as perceived during eating. The physical state of the fat is important. Pure oil is unpleasant to swallow for most individuals, but an emulsion may be perceived as nicely creamy. An oil-in-water emulsion gives quite a different sense from a water-in-oil emulsion of the same chemical composition. Thus, full-fat milk, cream, and butter each have their individual sensory characteristics. Fats can crystallize into different crystal forms. The consistency, plasticity, graininess, and other physical characteristics of many food products, such as butter, margarine, shortening, lard, and cocoa butter, all depend on a particular polymorphic form of the TAGs present. Fat is calorie dense: It contains 9 kcal/g, compared with 4 kcal/g for proteins and carbohydrates. It is an important source of calories for many adults as well as infants and toddlers, who have the highest energy need per kg of body weight of any age group. Fats should make up only 10% to 25% of the caloric intake.

## 1.2. Rationale for Use of Fat Substitutes

There is an overconsumption of fats and oils in the average Western diet. This has led to an increase in health risk among population groups consuming a diet high in animal fats (i.e., ice cream, chocolates, fast foods, and desserts). Eating excessive fat may lead to undesirable weight gain. In 1999, an estimated 61% of U.S. adults were overweight, along with 13% of children and adolescents, and this has continued to increase in recent years. Obesity among adults has doubled since 1980, while overweight statistics among adolescents has tripled (6). Statistics from the Center for Disease Control and Prevention (CDC) indicate that almost 87% of obese and 80% of overweight individuals are trying to lose or maintain their weight (7). There are many diet-related human disorders that are found almost exclusively in the Western World; these include coronary heart disease (CHD), stroke, diabetes, and cancer, among others. A 2001 U.S. Surgeon General's report attributed 300,000 U.S. annual deaths to fat-related health issues (8).

Although it is well known that a high-fat diet can cause many chronic health problems, what is perhaps less well known is that low- or no-fat diets bring their own set of problems. Nutrition professionals encourage people to select diets low in total fat, saturated fat, and cholesterol. National Academy of Sciences, U.S. Surgeon General, American Heart Association, National Cholesterol Education Program, American Cancer Society, American Dietetic Association, National

Institutes of Health, USDA, and the Department of Health and Human Services are among the many health and government authorities that advocate reduction of dietary fat for most consumers. These scientific groups recommend that total fat be limited to amounts that will provide no more than 30% of calories (9). Dietary Guidelines for Americans also recommend limiting saturated fat to no more than 10% of the intake. Cholesterol intake should be limited to no more than 300 mg/day (10). Saturated fat and cholesterol are the substances in fat that contribute to the formation of plaque, which clogs arteries, leading to heart disease. To reach recommended levels of 30% of calories from total fat and 10% of calories from saturated fats, many Americans will have to cut out one-fifth of their current fat intake. Advice to reduce fat and energy consumption has led to the production of foods with a lower content of fat and to the development of fat substitutes.

As a result of their high caloric content, fat is the number one consideration in caloric reduction. Methods to reduce total fat intake include low-fat cookery, such as boiling, broiling, steaming, stewing, baking, and roasting. Consumers may also trim visible fat from meat and poultry products and limit fat spreads, dressings, gravies, and other sauces. Another way to achieve lower total fat intake is to take stock of fat-rich desserts and snack foods and substitute fruits and vegetables for rich desserts and oily snacks. Recent research suggests that individuals who consume a diet that is reduced in fat and calories and includes use of fat-modified products have a better overall nutrient profile than do individuals who do not use any fat-modified products (3). A survey conducted in 2000 by Calorie Control Council, an international association of manufacturers of low-calorie, low-fat, and diet foods and beverages, indicated that low-fat, reduced-fat, and fat-free products remained popular among the general public (7). Seventy-nine percent of the adult U.S. population uses such products, with more women (82%) using fat-modified products than men (75%). Sixty-five percent of those surveyed reported consuming the same amount of the reduced-fat version as the full-fat variety. Consumer's favorite reduced-fat products include milk, cheese, salad dressings, potato chips, mayonnaise, margarine, ice cream, and frozen desserts (Table 1) (7). Another Council survey shows that two-thirds of adults believe there is a need for food ingredients that can replace the fat in food products.

Fat substitutes are becoming an important part of the American diet. Many Americans are looking for ways to enjoy their favorite foods while maintaining a

**TABLE 1. Most Popular Reduced-Fat Products.**

Type of Food Product	Percent of Adult Consumers
Low-fat or skim milk	56
Reduced-fat cheese/dairy products	49
Reduced-fat salad dressings/sauces/mayonnaise	46
Reduced-fat margarine	34
Reduced-fat chips/snack foods	33

Adapted from (7).



**TABLE 2. Potential Benefits and Drawbacks of Fat Substitutes.**

Possible Advantages	Possible Disadvantages
Reduce total fat intake in the diet	Reduce intake of fat-soluble vitamins
Reduce energy intake in the diet	Reduce essential fatty acids intake
Replace saturated fat in the diet	Compensatory energy intake
Reduce intake of cholesterol	Increase cost
Reduce absorption of cholesterol	Alters the function of the digestive tract (for high intake of nonabsorbable substances)
Reduce serum and plasma triacylglycerol levels	May cause gastrointestinal tract disturbances
Reduce coronary heart disease risk factors	
Increase in intake of complex carbohydrates	
No flavor compromise	
No <i>trans</i> -fatty acids	
Increase oxidative stability	
Maintain mouthfeel, texture, and moisture	

low-calorie diet. Food scientists are developing new food additives that will mimic the function of fats in foods while keeping the calorie contents of foods low. Dietary fat substitutes are food constituents able to replace, completely or partially, dietary fat in such a manner that certain physical and organoleptic properties of the food product involved are left unaltered as far as possible. There are two principal approaches to the replacement of dietary fat. The first involves hydratable carbohydrates and proteins with the mouthfeel of fats. The second includes nonabsorbable synthetic substances with the physical properties and technical function of fat within foods. Today, a broad range of fat substitutes can be found. Each has its own strengths, weaknesses, and restrictions. Potential advantages and disadvantages of fat substitutes are summarized in Table 2. Fat-replacement ingredients can be categorized as follows: (1) fat substitutes based on esters and ethers, (2) fat replacers based on carbohydrates and proteins (also referred to as "fat mimetics"), and (3) calorie-reduced structured lipids.

### 1.3. Labeling of Foods Containing Fat Substitutes

Labeling foods for their fat components has received considerable attention because of the increased consumer demand and modified intake of dietary fats. It is expected that the increased availability of nutrition labels on food products would improve public health and assist consumers in following dietary recommendations. A 1995 survey by the Calorie Control Council found that 72% of respondents who said they look for "light" foods said they were most attracted to food products claiming to be "reduced in fat" (10). According to a 1998 survey conducted for the Calorie Control Council, 54% of consumers found "reduced in both fat and calories" an appealing descriptor when selecting light products. Food labels indicating a reduction in fat are, therefore, important to both the consumer and the food industry.

The labels on fat-modified products must adhere to the Nutrition Labeling and Education Act (NLEA) criteria for the use of fat- and calorie-related items (11). The NLEA of 1990 requires most foods to bear nutrition labeling and requires food labels that bear certain health and nutrient-content claims to comply with specific requirements. The NLEA permits the use of energy conversion factors to determine the caloric availability of food ingredients. For instance, in the case of salatrim (BENEFAT), a value of 5 kcal/g has been established. Hence, salatrim should be listed at the appropriate point in the ingredient declaration and accounted for at 5 kcal/g. In 1996, the Food and Drug Administration (FDA) proposed a rule for the labeling of products containing fat-based fat substitutes (61 CFR 67243). The FDA requires that the labels on foods containing fat substitutes list the analytical fat amount on the "Nutrition Facts" label with a footnote indicating the amount that is bioavailable. For example, the available fat content of salatrim has been determined to be 5/9 grams of fat per gram of salatrim. Under the label's "Nutrition Facts" panel, manufacturers are required to provide information on certain nutrients. The mandatory components and the order in which they must appear are: total calories; calories from fat; total fat; saturated fat; cholesterol; sodium; total carbohydrates; dietary fiber; sugars; proteins; Vitamin A; Vitamin C; calcium; and iron (12). The NLEA also requires that all foods have ingredient statements and that claims for nutrient content and claims for diet-disease relationships be defined by regulation.

In response to the U.S. food labeling regulations in 1994, the FDA has proposed to define nutrient-content claims such as fat free, low fat, and reduced fat. Products labeled "fat free" and "low fat" must contain less than 0.5 g of fat per serving and less than 3 g of fat per serving, respectively. "Reduced" or "less fat" may be used on the labels of products that contain 25% less fat than regular (full-fat) products. Products labeled "percent fat free" should be based on 100 g, when product meets the definition of low fat or a 100% fat free, claim can be made when products meet the definition of fat free (contains no added fat) (12). Proposals have also been published to permit health claims for a relationship between the level of dietary fats and cardiovascular disease, as well as for a relationship between the level of dietary fats and cancer.

The fat-labeling claims do not provide any indication of the caloric content of the food product. However, products containing one-third to one-half fewer calories of the reference food may be labeled as "light." If more than 50% of calories in a food are derived from fat, the fat content of the "reduced-fat" version must be reduced by 50% or more. The term "calorie free" and "low calorie" only can be used on products with less than 5 calories per serving and less than 40 calories per serving, respectively, and "reduced" or "fewer calories" only can be used on products that have less than 25% of the calories in the regular product (12).

Although labeling regulations do not require a specific listing of fat substitutes on the "Nutrition Facts" panel, the amount of fat substitutes added to foods will affect labeling information. Fat substitutes, like all other ingredients, must be listed in order of predominance in the list of ingredients. The number of calories listed in the nutrition label must equal the digestible calories contained in a serving of

food. In the case of a nondigestible ingredient, the manufacturer must have data to demonstrate the level of digestible calories (12).

#### 1.4. Safety Considerations of Fat Substitutes

The safety of fat substitutes is based on the consideration of each compound's toxicological significance, nutritional profile, effect on overall diet, and expected level of use by various age groups of the population. The FDA makes decisions about safety of fat substitutes via two major routes (10). Each route has its own set of regulatory requirements. The first approach involves a food manufacturer either claiming that a substance qualifies as Generally Recognized as Safe (GRAS) or petitioning the FDA to grant the ingredient such a status. Ingredients that the FDA determines are derived from common food components and are generally recognized by scientific experts to be safe for specific applications, based on a long history of safe use in foods or extensive scientific evidence, can be approved for inclusion in foods as GRAS status. Examples of GRAS substances used as fat-replacement ingredients include various carbohydrate-based fat mimetics, microparticulate proteins, whey proteins, and fat emulsifiers, among others. In general, GRAS substances do not have to undergo rigorous testing before they are used in foods. The second approach entails a manufacturer's request for a new ingredient's approval as a food additive. The 1958 Food Additive Amendment to the Federal Food, Drug and Cosmetic (FD&C) Act established a premarket approval process for food additives for their intended use in food. Guidelines for the safety testing of new food ingredients are outlined in the FDA monograph "Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives used in Food," referred to as the Red Book (13). A food additive is defined as an ingredient not previously found in food whose intended use results, or may reasonably expect to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (14). A food manufacturer's request requires submission of extensive results on the ingredient safety and intended use, and awaits FDA approval before using them in food. Once approved, the FDA establishes recommended limits on consumption and may require monitoring of use and safety over a period of time. This latter route was followed for olestra.

An important step in assuring the safety of new food ingredients, such as fat substitutes, is the calculation of the level of exposure to an individual if the product is approved for use. This step is referred to as exposure assessment (15). It is necessary to use an exposure assessment model that is based on food consumption data from national probability surveys. These surveys indicate both the frequency of consumption and the serving size of individual foods. When estimated exposure levels proved to be higher than safety data, they can be lowered by limiting product content or the categories of food in which the product may be used (15).

Unlike food additives that are typically consumed in minor quantities per day, a fat substitute could replace a substantial proportion of the fat in the diet. As a result, special consideration must be given for the toxicological and nutritional

evaluations of these substitutes. If the substance is not digested or poorly digested, the absorption of essential nutrients by an individual may be reduced. Another nutrition concern with nonabsorbable fat substitutes is the effect on the total intake of nutrients. The potential effect of fat substitutes on the microflora in the intestine should also be considered. Some of these intestinal bacteria that are associated with the synthesis of certain nutrients, such as Vitamin K, biotin, and volatile fatty acids, may be altered and, hence, may exert long-term effects on health. For non-digestible fat substitutes, effects on intestinal epithelium, bile acid physiology, and pancreatic function should also be considered (16). The laxative effects of ingredients that are not digested may also be of concern. For fat substitutes that are absorbed, absorption, distribution, metabolism, and elimination of the substance should be considered.

## 2. REDUCED-CALORIE STRUCTURED LIPIDS AS FAT SUBSTITUTES

For several years, structured lipids with less than 9 kcal/g have been on the market. These engineered lipid molecules contain fatty acids that are less digestible to create a reduced-calorie content. These low-calorie TAGs are characterized by the presence of short-chain fatty acids (SCFAs) or medium-chain fatty acids (MCFAs) and long-chain fatty acids (LCFAs) in a single TAG structure. The caloric content of constituent SCFAs is lower compared with that of their LCFA counterparts. The products have all the functional properties of full-calorie fats, including their ability to act as carriers for fat-soluble ingredients. Reduced-calorie structured lipids are intended for use in baking chips, coatings, dips, bakery and dairy products, or as cocoa butter substitutes. Currently, such structured lipids are produced by random chemical interesterification between a short-chain triacylglycerols (SCTs) or medium-chain triacylglycerols (MCTs) and long-chain triacylglycerols (LCTs). The most prominent examples of low-calorie fats are Caprenin, Salatrim, Bohenin, and MCT preparations (Table 3). The following sections describe each of these products.

### 2.1. Caprenin

Caprenin is a reduced-calorie structured lipid contributing about 5 kcal/g compared with 9 kcal/g of conventional fats and oils (17). It has a defined structure of caprocapylobehenin ( $C_{8:0}-C_{10:0}-C_{22:0}$ ) (Figure 1a). It is a TAG formed by esterification of glycerol with the medium-chain saturated fatty acids caprylic acid ( $C_{8:0}$ ) and capric acid ( $C_{10:0}$ ) and the very long-chain saturated fatty acid behenic acid ( $C_{22:0}$ ). All of these fatty acids are derived from natural food sources. Caprylic and capric acids are obtained by fractionation of palm kernel and coconut oils. Behenic acid is produced from rapeseed oil (18) and is also found in peanuts and marine oils. The behenic acid passes through the digestive system without being absorbed and the MCFAs provide fewer calories than absorbable LCFAs. This

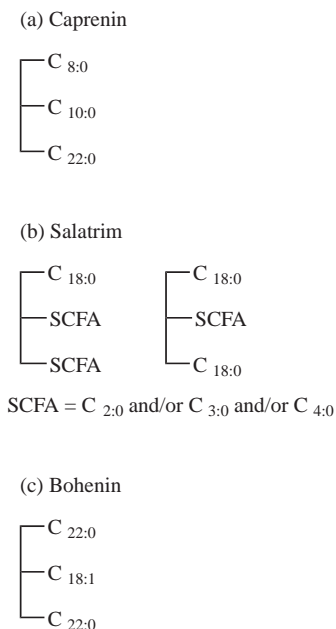
**TABLE 3. Examples of Lipid-Based Fat Replacers and Their Applications.**

Type of Fat Replacer	Commercial Names	Applications/Potential Applications
Salatrim	Benefat	Baked goods, dairy products, confections, snacks, margarines, and spreads
Structured lipid	Caprenin	Soft candies, confections
Structured lipid	Bohenin	Confections
Medium-chain triacylglycerols	Neobee, Grindsted MCT, Captrin, Captex, Miglyol	High-energy protein bars, ready-to-drink nutritional beverages, high-performance wellness foods, snack products
Sucrose polyester/Olestra	Olean	Baked goods, fried foods, mayonnaise, salad dressing
Sorbitol polyester	Sorbestrin	Baked goods, fried foods, formulated products
Polyglycerol esters	Grindsted PGE	Margarine, shortening, bakery products, frozen desserts, confections
Esterified Propoxylated Glycerol	EPG	Formulated products, baked goods, fried foods
Emulsifiers	Dur-Lo-, EC-25, Dur-Em	Cookies, cake mixes, icings, vegetable dairy products
Diacylglycerol oil	Enova	Salad dressings, mayonnaise, beverages, nutrition bars, soups and sauces, pizza, bakery products, frozen dinner entrees

product was developed by Procter & Gamble Company (Cincinnati, Ohio). During the synthesis of caprenin, glycerol is thermally esterified with behenic acid derived from hydrogenated rapeseed oil. The glycerol monobehenate is subsequently esterified with capric and caprylic acids or their anhydrides from coconut and palm kernel oils. The final product is purified by molecular distillation and a combination of one or more of thermal winterization, bleaching, and steam deodorization (19).

Caprenin has functional properties similar to cocoa butter and is intended to replace some of the cocoa butter in soft candies and confectionery coatings. It is digested, absorbed, and metabolized by the same pathway as other TAGs (20). The Procter & Gamble Company filed a GRAS affirmation petition for use of caprenin as a confectionery fat in soft candy bars and in confectionery coatings. However, caprenin is not suitable for frying foods.

Absorption, digestion, and metabolism studies have been conducted on caprenin (21, 22). Several clinical studies suggested that, despite its lower energy content, caprenin slightly increased lipoprotein levels. In one study, with 30 adult males fed caprenin at 34 g/day for eight weeks, total serum cholesterol, LDL, and HDL were increased by 8%, 20%, and 13%, respectively (23).



**Figure 1.** Chemical structures of (a) caprenin, (b) salatrim, and (c) bohenin.

## 2.2. Salatrim (BENEFAT)

Salatrim, similar to caprenin, is also a reduced-calorie structured lipid, and the caloric value is claimed to be 5 kcal/g, or 55% of the caloric value of conventional fats. It is composed of a mixture of SCFAs (acetic, propionic, or butyric acids) and LCFAs (principally stearic acid) (24). The chemical structure of Salatrim is given in Figure 1b. The SCFAs are chemically *trans*-esterified with vegetable oils such as highly hydrogenated canola or soybean oil (25). The calorie reduction is achieved by the combination of low-calorie SCFAs and the long-chain saturated stearic acid, which is only partially absorbed by the body. The physical and functional properties of Salatrim are determined by the composition of short- and long-chain fatty acids, their positional distribution on the glycerol backbone, and their ratio to one another. Salatrim was developed by Nabisco Foods Group (East Hanover, New Jersey) (18) and is now marketed by Danisco Cultor USA, Inc. (New Century, Kansas) under the registered trademark BENEFAT. It has the taste, texture, and functional properties of conventional fats and oils. It can be produced to have different melting profiles by adjusting the amounts of SCFAs and LCFAs used in their chemical synthesis. Salatrim, like caprenin, cannot be used for frying. As a result of the SCFAs, these fats may be prone to hydrolysis during the high heat encountered during frying conditions, which may cause the development of off-flavors. It has the ability to function effectively in low-moisture foods and to overcome most of the problems

caused by other fat-based replacers (26), such as polyol esters. One product in the market that contains BENEFAT is the reduced-fat baking chips introduced in 1995 by Hershey Food Corporation (Hershey, Pennsylvania). A GRAS affirmation petition for Salatrim was accepted for filing by the FDA in June 1994. Following this, Salatrim can now be used in the United States as a cocoa butter substitute. Salatrim is intended as a low-calorie fat for use in chocolate-flavored coatings, chips, caramel, fillings for confectionery and baked goods, peanut spreads, savory dressings, dips and sauces, and in dairy products (27). A shortening form of Salatrim has been developed and is marketed by Danisco Cultor USA, Inc. (New Century, Kansas) under the brand name BENEFAT B. The shortening is a 1:1 replacement for fat in bakery applications such as cookies, cakes, pie crust, cream fillings, and muffins.

Salatrim has recently been approved for use by the food-processing industry in Europe by the Commission of the European Communities (EC). The committee declared that Salatrim is safe for human consumption, but all products containing Salatrim should carry a statement that excessive consumption ( $>30$  g/day) may cause gastrointestinal disturbances. Furthermore, mandated wording on labels should identify products as containing "reduced energy fat" and state that the products are not suitable for children to eat.

### 2.3. Bohenin

Bohenin is a reduced-calorie TAG containing behenic acid at the *sn*-1 and *sn*-3 positions and oleic acid at the *sn*-2 position (Figure 1c). Bohenin is produced by the interesterification of TAGs containing oleic acid, and ethyl behenate in the presence of a suitable lipase (*sn*-1,3-specific) preparation. The manufacturing process is identical to that used to produce a similar TAG, cocoa butter substitute, which the FDA has affirmed as GRAS (21 CFR 184.1259). Bohenin's high melting point ( $\sim 52^{\circ}\text{C}$ ) and its ability to form  $\beta_2$ -crystalline polymorphic structures are important properties that bear on its functionality as an antibloom agent and facilitate the chocolate-tempering process. The specifications for bohenin are consistent with the Food Chemical Codex specifications for edible fats and oils (28). Bohenin is being manufactured by Fuji Oil Co., Ltd. (Osaka, Japan), and filed an FDA GRAS affirmation petition in 1994. Bohenin is hydrolyzed to behenic acid and 2-oleoyl glycerol prior to absorption. Behenic acid is a very long-chain saturated fatty acid that occurs naturally in peanuts, most seed oils, animal milkfat, and marine oils. Oleoyl-monoacylglycerol is a common product of human digestion of edible fats and oils typically used in Western diets. Since 1994, Fuji Oil Co., Ltd. has submitted to the FDA a number of scientific articles relating to bohenin. These include (28):

- a. Physical characteristics and functionality of bohenin
- b. Sampling, detection, and analysis of commercial fats and oils
- c. Detection and analysis of bohenin in food

- d. Consumption of behenic acid from currently consumed fats and oils
- e. Metabolism of TAGs containing bohenic acid
- f. Hydrolysis of bohenin to behenic acid and 2-oleoyl glycerol prior to absorption
- g. Safety of other TAGs containing behenic acid
- h. Safety of behenic acid
- i. Unpublished toxicity and mutagenicity studies conducted with bohenin
- j. A recent history of use of bohenin in Japan

Bohenin is intended for use as a tempering aid and as an antibloom agent in the manufacture of chocolate and chocolate coatings. In 2000, the FDA stated that it has no objections to the Fuji Oil claim that bohenin is GRAS under the intended conditions of use (28). After extensive review of the Fuji Oil's GRAS submission, the agency has listed the notice as No. GRN 000050. The FDA has not, however, made its own determination regarding the GRAS status of bohenin. There have been no published studies to determine the caloric availability of bohenin. Nonetheless, given its fatty acid composition, and using the 0.29 absorption coefficient determined for behenic acid in caprenin, the caloric value of behenic should be approximately 5 kcal/g (19).

## 2.4. Medium-Chain Triacylglycerols (MCTs)

Medium-chain triacylglycerols (MCTs) contain predominantly 8-carbon (caprylic) and 10-carbon (capric) saturated fatty acids esterified to the glycerol backbone of TAGs. The primary sources for MCTs are fractions of coconut and palm kernel oils. Hence, MCTs are manufactured from these oils via hydrolysis followed by fractionation of the resulting fatty acids to concentrate caprylic and capric acids, and re-esterification with glycerol to form new TAGs (29, 30). Caprylic and capric acids comprise more than 96% of the fatty acids in MCT preparations. MCTs are readily hydrolyzed by digestive enzymes, and the fatty acid end products are rapidly absorbed into the bloodstream (31). MCFAs do not require carnitine to cross the double mitochondrial membrane of the hepatocyte, thus they quickly enter the mitochondria and undergo rapid  $\beta$ -oxidation. Hence, MCFAs are used as immediate sources of energy by the liver, yielding fewer calories per gram than LCFAs. The gross energy content of MCTs is 8.3 kcal/g vs 9 kcal/g for fat or LCTs. However, the net caloric energy value of MCT preparation is 6.8 kcal/g (32). MCTs are relatively stable at high temperatures and are less susceptible to oxidation (33). MCTs are much more soluble in water than LCTs. MCTs are a translucent and odorless liquid at room temperature. Although completely saturated, it is not solid in consistency like other saturated fats, because of the shorter chain lengths of the fatty acids within the oil.

MCTs were originally developed for therapeutic purposes to provide a source of energy for individuals with compromised gastrointestinal systems; thus, they are beneficial for AIDS and cancer patients, premature infants, burn victims, and individuals with shortened bowel syndrome. MCTs have been used extensively in the



manufacture of parenteral and enteral nutrition formulations. MCTs are beneficial for such applications because they are more readily hydrolyzed and metabolized much more rapidly than LCTs. MCTs have specific nutritional applications such as infant formulas, energy bars and drinks, geriatric preparations, and sports nutrition products. In addition, they are used as carriers for colors, flavors, and vitamins and provide gloss and prevent sticking on confectionery products. It is reported that feeding diets, wherein a fat source of LCTs was replaced with MCTs, to laboratory animals and humans resulted in decreased body weight gain and reduced fat deposition (34, 35). Such results have led to the suggestion that replacing conventional source of dietary fat, which composed primarily of LCTs, with MCTs may yield food with lower caloric content. In the sports world, MCTs have been positioned as an easily absorbed and oxidized fuel source and have been marketed to body builders and athletes as a fat source that is less likely to deposit as body fat.

**2.4.1. Neobee** Neobee is composed of capric and caprylic acids and produced by Stepan Company (Northfield, Illinois). This class of specialty lipids includes different products. For example, Neobee 1053 and Neobee M-5 contain both capric and caprylic acids, whereas Neobee 1095 is made up of only capric acid (36). Neobee 1095 is a solid product. Therefore, in certain applications that require solid fats, this product may be suitable. Neobee 1814 is a MCT derivative made by interesterifying MCT with butter oil (37). This product contains half of the long-chain saturated fatty acids found in conventional butter oil and is suitable to replace butter oil in a variety of applications. Neobee 1814 may serve as a flavor carrier and functions as a textural component for low-fat food products (36).

**2.4.2. Captrin** Captrin was the proposed common name for the randomized TAGs of primarily capric or caprylic fatty acids. It is a class of structured TAGs produced by the interesterification of glycerol with MCFAs derived from coconut and palm kernel oils. The predominant fatty acids available from coconut and palm kernel oils include caproic, caprylic, capric, and lauric acids. Fractional distillation of these fatty acids affords fractions that can be enriched in specific components. Captrin contains mainly capric and caprylic acids. Bomb calorimetry measurements showed that captrin provided 8.3 kcal/g (19). Captrin may be labeled as MCTs, capric/caprylic TAGs or glyceryl tri(caprylate/caprate). Its primary use at this time is as flavor carrier for fat-free food products. A first line of products was based on interesterification of butter oil and MCTs. In June 1994, the FDA accepted for filing a GRAS petition from Stepan Co. (Northfield, Illinois) for captrin.

**2.4.3. Captex** Captex products are manufactured by ABITEC Corporation (Columbus, Ohio). Captex 300 EP and 355 EP, commonly known as MCTs, are produced via esterification of C<sub>8</sub> and C<sub>10</sub> fatty acids with glycerol. These C<sub>8</sub> and C<sub>10</sub> fatty acids are derived from fractionation. Captex 300 EP and 355 EP function in nutritional foods as a readily available energy source for athletes, bodybuilders, and exercise enthusiasts. They have the potential to replace some or all long-chain fatty acid components in food, creating a lower calorie food without losing the

organoleptic properties. Potential applications for Captex products are snacks, main meals, frozen, and refrigerated foods. The smoke, flash, and fire points of these products are lower than those of conventional fats and oils, making them more suitable for low-heat applications.

**2.4.4. Miglyol** Miglyol 810 and Miglyol 812, marketed by Huls America, Inc. (Piscataway, New Jersey), are TAGs of the fractionated coconut oil fatty acids  $C_8$  (50–65%) and  $C_{10}$  (30–45%). These products are labeled as MCTs, fractionated coconut oil, or caprylic/capric triacylglycerols. Miglyol 810 and 812 differ only in ratio of  $C_8$  to  $C_{10}$  fatty acids. As a result of its low  $C_{10}$  content, the viscosity and cloud point of Miglyol 810 is lower. The fatty acids used for the production of Miglyol 810 and 812 comply with the FDA's proposed regulation, 21 CFR 172.860, and are classified as GRAS. These products are not heat sensitive. Even in hot climates, cooling is not necessary. At low temperatures, parts of TAGs may crystallize. However, this phenomenon is completely reversible. These products have a very low moisture content, and are therefore resistant to hydrolytic and microbial splitting. Miglyol 810 and 812 are used as part of fatty emulsions for parenteral nutrition.

### 3. FAT SUBSTITUTES BASED ON ESTERS AND ETHERS

Synthetic fats may offer the functionality of fat without the calories. This includes the elusive ability to be used for frying. Typically, these ingredients use food-grade materials to create a molecule that resists lipase hydrolysis of the ester bond during the digestion process. The methods include substituting the fatty acid and glycerol with a fatty alcohol and organic acid, replacing a sugar molecule for glycerol, or adding to the structure of the glycerol so the fatty acid is no longer adjoining that portion of the molecule. Table 4 shows various lipid-based fat substitutes that can be used in foods and their chemical composition.

#### 3.1. Carbohydrate Fatty Acid Polyesters (CPE)

Carbohydrate fatty acid polyesters (CPEs) have been the focus of a broad spectrum of scientific research ranging from studies of naturally occurring glycolipids to synthetic carbohydrate polyesters. Recently, CPEs have received considerable attention as possible fat substitutes because of their unique properties. Some of the research interest in these molecules has been based to the structural variations that can be introduced into these systems, such as the nature of the esterified fatty acyl chains, the degree of esterification, and the type and number of the carbohydrate moieties. Among the CPEs, particular attention has been paid to sucrose fatty acid polyesters (SPEs). Extensive research studies on SPEs have led to the discovery of a wide range of applications based on their physicochemical properties (38). The ability of SPE to behave as a lipid-like molecule is likely a result of its high degree of esterification with long-chain fatty acids. Furthermore, these

**TABLE 4. Selected Lipid-Based Fats and Oils Substitutes.**

Product	Digestibility	Composition	Manufacturer
Salatrim	Partially digestible	SCFA (C <sub>2:0</sub> -C <sub>4:0</sub> ) and LCFA (Predominantly C <sub>18:0</sub> )	Nabisco Foods Group (East Hanover, New Jersey)
Caprenin	Partially digestible	Caprylic (C <sub>8:0</sub> ), capric (C <sub>10:0</sub> ), and behenic (C <sub>22:0</sub> ) acids	Procter & Gamble Co. (Cincinnati, Ohio)
Bohenin	Partially digestible	Oleic (C <sub>18:1</sub> ) and behenic (C <sub>22:0</sub> ) acids	Fuji Oil Co. Ltd. (Osaka, Japan)
Captex	Partially digestible	Medium-chain triacylglycerol	ABITEC Co. (Columbus, Ohio)
Neobee	Partially digestible	Caprylic (C <sub>8:0</sub> ), capric (C <sub>10:0</sub> ), and LCFA	Stepan Co. (Northfield, Illinois)
Captrin	Partially digestible	Caprylic (C <sub>8:0</sub> ) and capric (C <sub>10:0</sub> ) acids	Stepan Co. (Northfield, Illinois)
Olestra	Nondigestible	Sucrose fatty acid polyester	Procter & Gamble Co. (Cincinnati, Ohio)
Sorbestrin	Nondigestible	Sorbitol fatty acid polyester	Cultor Food Science, Inc. (Ardsley, New York)
EPG	Partially digestible	Esterified propoxylated glycerol	ARCO Chemical Technology (Greenville, Delaware)
TATCA	Partially digestible	Trialkoxytricarballlylate	CPC International, Inc. (Englewood Cliffs, New Jersey)
DDM	Partially digestible	Dialkyl dihexadecylmalonate	Frito-Lay Inc. (Plano, Texas)
Jojoba oil	Partially digestible	Predominantly C <sub>20:1</sub>	Nestec Ltd. (Switzerland)
PGE	Partially digestible	Polyglycerol ester	Danisco Cultor USA, Inc. (New Century, Kansas)
PEP	Nondigestible	Partially esterified polysaccharides	ARCO Chemical Technology (Greenville, Delaware)
DAG oil	Partially digestible	LCFA (Predominantly C <sub>18:1</sub> , C <sub>18:2</sub> , and C <sub>18:3</sub> )	ADM Kao LLC (Decatur, Illinois)

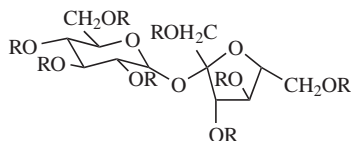
molecules are resistant to the hydrolytic action of pancreatic lipase and, hence, are noncaloric because they are not absorbed by the gastrointestinal tract and pass through the human body unmetabolized. SPE is effective as a nonabsorbable lipophilic binding agent, which can reduce intestinal absorption of certain lipophilic molecules. For instance, the presence of SPE as a low-calorie alternative for dietary TAGs has been shown to decrease levels of low-density LDL cholesterol (39).

The most investigated lipid-based fatty acid polyesters with a carbohydrate backbone for use as fat substitutes are SPEs, sorbitol polyesters, raffinose polyesters, and alkyl glycoside-based fatty acid polyesters. Sucrose serves as the backbone of SPEs, but other sugars, such as methyl glucose, sorbitol, and raffinose, can also be used. By using a variety of fatty acids and carbohydrates, one can manufacture products with a wide range of physical and biological properties. The fatty acids can be saturated or unsaturated, and the particular combination used will affect the functional characteristics, melting point, consistency, and stability of the

product. Fat substitutes of this type are not hydrolyzed by digestive enzymes because they are unable to penetrate the bulky nonpolar region around the sucrose center. Mattson and Volpenhein (40) reported that both sorbitol hexaoleate and sucrose octaoleate were not hydrolyzed by pancreatic lipase.

The physical properties and thermal stability of SPEs are comparable with those of conventional fats and oils with the same fatty acid profiles. The physical characteristics of SPEs, similar to those of TAGs, are determined by the properties of the fatty acid side chains (38). For instance, SPEs produced from predominantly highly unsaturated fatty acids are clear liquids resembling conventional vegetable oils. On the other hand, SPEs made with more saturated fatty acids are opaque solids, resembling solid fats with a higher melting point (41).

**3.1.1. Olestra** Olestra is a member of the family of compounds known as SPEs and is derived from sucrose and vegetable oils. Olestra is unique among the SPEs in that it is manufactured according to strict criteria specified by the FDA (42). It is a mixture of hexa-, hepta-, and octaesters synthesized by esterifying sucrose with long-chain (predominantly  $C_{18}$ ) fatty acids. Each molecule contains six to eight fatty acids—at least 70% octa-fatty acid esters with 1% or less hexaesters. The structure of olestra is analogous to that of the TAGs; however, olestra differs from TAGs in that it is comprised of a core of sucrose, rather than glycerol, esterified with 6–8, rather than three fatty acid moieties (Figure 2). Olestra can be manufactured from saturated and unsaturated fatty acids of chain length  $C_{12}$  to  $C_{20}$  and higher from conventional edible vegetable oils such as palm, corn, soybean, coconut, and cottonseed oils (43). Its contribution to caloric intake is zero because its molecular constitution prevents its hydrolysis by gastrointestinal enzymes. The large molecule of olestra has the ability to pass unaltered through the gastrointestinal tract, because the fatty acids that are crowded around the sucrose core prevent the digestive enzymes to find a breaking point in order to metabolize the olestra molecule into smaller pieces and be absorbed by the body. This ingredient has a 140°F melting point, a 480°F smoke point, and a 550°F flash point. It has an AOM peroxide value at two hours of 100 ppm. Olestra is stable under ambient and high-temperature storage conditions and has an acceptable flavor. The main advantage of olestra is that it has all of the characteristics of fat without adding calories. Its appearance, texture, oral feel, thermostability, and half-life are similar to those of conventional fats and oils. The manufacturer of olestra, Procter &



R = Acyl group of fatty acids ( $R-\overset{\text{O}}{\parallel}{\text{C}}-$ )

**Figure 2.** Chemical structure of olestra.

Gamble Co. (Cincinnati, Ohio), petitioned the FDA in 1987 for use of olestra as a calorie-free replacement for fats and oils. The petition included results of more than 150 safety studies, several chronic feeding studies, and clinical studies. According to the FDA, these studies included (44):

- a. Animal and human studies that indicated that olestra does not break down in the digestive tract.
- b. Animal studies that showed that olestra is not absorbed into the body.
- c. Animal studies that showed that olestra does not cause birth defects.
- d. Animal studies that showed that a diet containing olestra is not associated with a higher incidence of cancer.
- e. Animal and human studies that showed that adding fat-soluble vitamins (A, D, E, and K) to diet containing olestra can offset by providing olestra-containing foods with these vitamins.
- f. Animal and human studies that showed that olestra does not decrease the absorption of five key water-soluble nutrients (folate, Vitamin B12, calcium, zinc, and iron) that are hard to absorb or are limited in the U.S. diet.
- g. Human studies that showed that olestra does not affect normal intestinal microflora functions.
- h. Human studies that showed that at usual snack-food consumption levels, olestra's potential to cause cramping, bloating, loose stools, diarrhea, and other gastrointestinal symptoms in healthy adults and healthy children and in adults with inflammatory bowel disease is not different from that of the full-fat snack foods.
- i. Animal and human studies that showed that olestra does not affect the absorption of some commonly used drugs, especially drugs that attach to fat in the body, such as oral contraceptives.

In 1990, the petition was amended to seek only the approval for fat replacement of up to 100% in savory snacks, including fried snacks and snack crackers. Procter & Gamble has decided to use the brand name Olean for the ingredient. Olestra has the FDA GRAS status as of January, 1996. Hence, olestra can replace 100% of the vegetable oil used in the preparation of savory snacks (CFR 172.867c). Savory snacks include flavored and unflavored chips such as potato chips, corn chips, and tortilla chips as well as snacks such as cheese puffs, cheese curls, and crackers. Olestra also has a potential for use in margarines, salad dressings, and frozen desserts. However, Olestra has its drawbacks. As it has the physical properties of fat, certain fat-soluble vitamins, such as A, D, E, and K, and carotenoids can be partially partitioned into the Olestra and eliminated from the body (45). Factors that influence the level of partitioning include (1) the degree of fat solubility (the more fat soluble the nutrient, the more it will be partitioned into olestra), (2) the relative amount of olestra to nutrient (partitioning increases as the amount of olestra per measure of nutrient increases), and (3) the timing of olestra and nutrient consumption (partitioning can occur when olestra and the fat-soluble nutrients

are in the gastrointestinal tract at the same time) (3). Based on these factors, it is predicted that any effect of olestra on the nutritional status be offset by supplying an extra amount of the affected nutrient to the diet. As a result, the FDA insisted that foods made with olestra be fortified with fat-soluble vitamins. Animal and clinical studies showed that these vitamins could be offset by incorporating greater amounts of fat-soluble vitamins into olestra-containing foods (46, 47). When fed to animals (46) and healthy adult humans (47–51), olestra had no effect on the absorption of macronutrients, such as carbohydrates, TAGs, and proteins, water-soluble micronutrients, and Vitamins D and K. Olestra may cause gastrointestinal cramping, potential anal leakage, diarrhea, and loose stools in some individuals (52, 53). It was suggested that soft stools be prevented by using olestra with semisolid consistency or by increasing the viscosity of the olestra or product. To warn consumers of potential gastrointestinal and nutrient side effects, products made with olestra must contain the following label statement, per the FDA: “This product contains olestra. Olestra may cause abdominal cramping and loose stools. Olestra inhibits the absorption of some vitamins and other nutrients. Vitamins A, D, E, and K have been added.” The safety studies, based on animal and human trials, showed that olestra is not toxic, mutagenic, carcinogenic, or teratogenic, and it is considered safe. This is because olestra, for the most part, is not digested or absorbed.

Olestra appears to offer some other health benefits. This product can help people lose weight, benefit individuals at high risk of heart disease, obesity, and colon cancer, and possibly lower cholesterol levels. It can also inhibit cholesterol absorption and reduce blood cholesterol levels (54). In a double-blind crossover study, twenty men with normal cholesterol levels were fed 750 mg of cholesterol per day along with either butter or a butter-olestra blend. Olestra was fed at a level of approximately 14 g/day. The group receiving the olestra absorbed about 18% less cholesterol than the group receiving all butter (55), a response similar to that was obtained when triacylglycerol was replaced with olestra in animal studies (56).

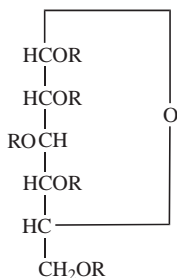
In another study, 24 healthy, normal-weight men with normal cholesterol levels were fed diets that contained 300 mg or 800 mg of cholesterol per day in ten-day periods, and in a 21-day period, less than 50 mg per day of cholesterol. Subsequently, 8 g, 16 g, or 25 g of olestra were added to each of the diets in ten-day periods. The diets were of the typical American variety and contained approximately 20% of their calories as protein, 40% as carbohydrates, and 40% as fat. Care was taken so that there were no significant reductions in weight. Adding olestra to the diet lowered both total and LDL cholesterol at all levels of cholesterol feeding (57).

Crouse and Grundy (58) also studied the effects of sucrose polyester on cholesterol metabolism in obese men. In this study, 11 overweight men were fed low-calorie (1000 kcal/day), low cholesterol (19 mg/day) diets containing 21% of calories from fat with and without 62 g of olestra per day for six-week periods. Beyond the 20% decrease in plasma cholesterol caused by weight loss, olestra feeding resulted in another 12.5% reduction in plasma cholesterol in six subjects, but had no significant effect on plasma cholesterol in the other five subjects. Grundy et al. (59) reported that in nondiabetic patients, calorie-restricted diet plus sucrose

polyester exhibited a reduction in total and LDL cholesterol by 20% and 26%, respectively. In diabetic patients with hypertriacylglycerolemia, caloric restriction showed a marked reduction in plasma triacylglycerols with or without sucrose polyesters. Calorie-restricted diet apparently reduced cholesterol by decreasing cholesterol synthesis (59). Sucrose polyester had little effect on the concentration of HDL cholesterol (60).

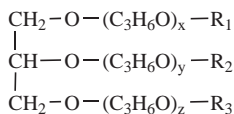
Sensory evaluation studies have shown that olestra can reduce the fat content of snack foods without affecting their taste. The reduction in fat intake accomplished by substitution of olestra snacks for full-fat snacks can be a positive step for an individual toward improved health and weight control. For instance, a 30 g bag of potato chips made with vegetable oil contains about 10 g fat and 150 calories. This same bag of potato chips made with olestra contains no fat and only about 70 calories. A reduction in fat intake of 10 g per day, which could be accomplished by substituting one bag of potato chips made with olestra for regular potato chips per day, would save the same number of calories over a year as those in 3.6 kg of fat (61). Numerous studies have confirmed that olestra can help people reduce the percent of calories from fat in their diets. However, there have also been reports on negative aspects of olestra consumption (62).

**3.1.2. Sorbestrin** Sorbestrin, a reduced-calorie fat substitute, is a mixture of tri-, tetra-, and pentaesters of sorbitol. The metabolically available energy of sorbestrin is about 1–2 kcal/g. It is a clear liquid and has a cloud point of 15°C. It has a bland oil-like taste and can serve as a reduced-calorie fat substitute. Sorbestrin was discovered in the late 1980s by Pfizer Food Science Group and is currently under development by Danisco Cultor America Inc. (Ardsley, New York). The chemical structure of sorbestrin is shown in Figure 3. It is thermally stable and is intended for use in frying and baking of foods. Sorbestrin is produced by *trans*-esterification of sorbitol with fatty acid methyl or ethyl esters. The fatty acids are derived from conventional vegetable oils, such as those from sunflower, soybean, safflower, or cottonseed. The fatty acid profile of sorbestrin generally consists of 80% oleic (18:1), 10% linoleic (18:2), 4% stearic (18:0), 4% palmitic (16:0), and less than



R = Acyl group of fatty acids ( $\text{R}-\overset{\text{O}}{\parallel}{\text{C}}-$ )

Figure 3. Chemical structure of sorbestrin.



**Figure 4.** Chemical structure of esterified propoxylated glycerol (EPG) (where:  $R_1$ ,  $R_2$ , and  $R_3$  are aliphatic acyl groups of 4–23 carbons and  $x$ ,  $y$ , and  $z$  are 1–7).

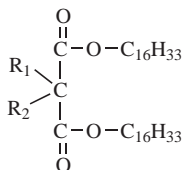
1% of linolenic (18:3), arachidic (20:0), eicosenoic (20:1), and behenic (22:0) acids. As no regulatory approval has yet been obtained for sorbestrin, it is still not in commercial use.

### 3.2. Esterified Propoxylated Glycerols (EPGs)

Esterified propoxylated glycerols (EPGs) are the result of reacting glycerol with propylene oxide to form a polyether polyol, which is subsequently esterified with one or more fatty acids (Figure 4). The number of propylene oxide (sometimes referred to as oxypropylene) groups and selection of fatty acids (i.e., carbon chain length, the level of unsaturation) give the products a range of functionality. The average number of oxypropylene groups, which are incorporated into glycerol, is called the propoxylation number (63). Their contribution to caloric intake is zero because they resist hydrolytic enzymes, and they are stable enough to be used for baking and frying. The physical properties of the finished product, similar to natural TAGs, depend on the type of fatty acids esterified. Short-term animal studies indicate that EPG is safe and resistant to hydrolysis (64, 65). Patented by ARCO Chemical Technology (Greenville, Delaware) and CPC International, Inc. (Englewood Cliffs, New Jersey), EPGs can replace fats and oils in margarines, fried foods, frozen desserts, salad dressings, and bakery products. However, there has been no evidence of regulatory submissions of this product.

### 3.3. Dialkyl Dihexadecylmalonate (DDM)

Dialkyl dihexadecylmalonate (DDM) is an ester of a fatty alcohol with malonic and alkylmalonic acids (Figure 5). DDM was developed in the early 1980s by researchers at Frito-Lay, Inc. (Plano, Texas). It is a low-calorie fat-based substitute suitable for high-temperature frying and baking. DDM, consisting of a monomer and a dimer, has a melting point below body temperature but higher than that of



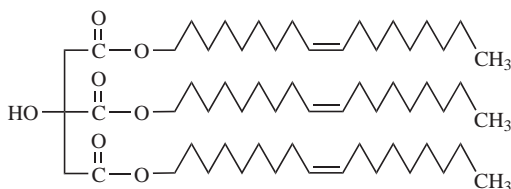
**Figure 5.** Chemical structure of dialkyl dihexadecyl malonate (DDM) (where:  $R_1$  and  $R_2$  are fatty alkyl groups).



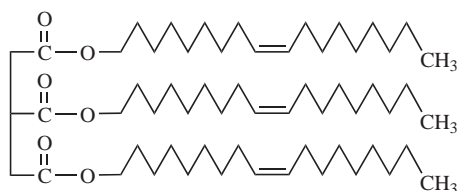
conventional frying oils. As it is stable when heated and not absorbed, DDM has the potential for use in a blend with conventional frying oils to make reduced-calorie frying oils. Mixtures of DDM with other oils, such as soybean oil, constitute frying oils with caloric values 33–60% lower than the natural oil. Rat studies with DDM showed that less than 0.1% of it is absorbed (66). Rat studies have also found no toxic effects. To date, there is no evidence of regulatory submissions or commercial development of this product.

### 3.4. Polycarboxylic Acid Esters and Ethers

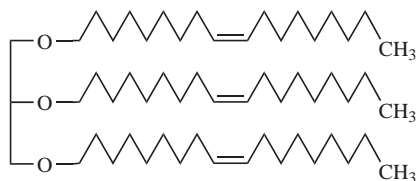
Polycarboxylic acids have two to four carboxylic acid groups in a polycarboxylic acid backbone esterified with saturated or unsaturated straight or branched long-chain alcohols (from C<sub>8</sub> to C<sub>30</sub>). These substances have been proposed as thermally stable low-calorie fat substitutes (67, 68). They have physical and functional properties similar to those of the typical TAGs, but cannot be digested and contribute no energy to the diet. Typical examples of polycarboxylic acid esters and ethers include trialkoxytricarballylate (TATCA), trialkoxycitrate (TAC), and trialkoxyglyceryl ether (TGE), among others (Figure 6). TATCA is a tricarboxylic acid



Trialkoxycitrate (TAC)



Trialkoxytricarballylate (TATCA)



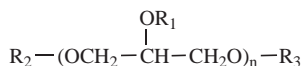
Trialkoxyglyceryl ether (TGE)

**Figure 6.** Chemical structures of polycarboxylic acid esters and ethers.

esterified with saturated or unsaturated alcohols (Figure 6). It is a nonhydrolyzable, edible, oil-like compound and is currently being evaluated by researchers at Best Foods, a division of CPC International, Inc. (Englewood Cliffs, New Jersey), as a fat replacer for use in margarines and mayonnaises. It can also be used to replace vegetable oil in cooking applications. TATCA resembles a natural TAG with tricarballylic acid replacing the glycerol and saturated or unsaturated alcohols replacing the fatty acids (Figure 6). Hence, it appears to be a flexible candidate in terms of its direct substitution as a low-calorie replacement for edible fats and oils in food applications. Animal studies indicated that TATCA was poorly absorbed (67). Weight gain results in rats showed that TATCA has a low-caloric value compared with corn oil (66, 67). Evidence indicates that moderate to high dose levels (1.0–3.0 g) of TATCA cause anal leakage, depression, weakness, and death among animals (67, 68). Trialkoxycitrate (TAC) has a structure similar to TATCA, with a hydroxyl group on the central carbon. Studies showed that TAC did not appear to have the thermal stability to be used for frying (67). Although limited animal research has been reported for these compounds, the approval of these products for human use has not occurred thus far.

### 3.5. Polyglycerol Esters (PGEs)

Polyglycerol fatty acid esters (PGEs) have been used in the United States and Europe since the 1940s. In the 1960s, the PGEs were approved for food applications in the United States. PGEs are mixtures of the esters of fatty acids with the polyglycerol mixture that function as emulsifiers in various food applications. The structural formula of PGEs is shown in Figure 7. During the synthesis, the first step involves the preparation of polyglycerols via polymerization of glycerol under alkaline conditions at 230°C (69). These glycerol molecules are linked by an ether group between  $\alpha$ -hydroxyl groups. The polyglycerol is then esterified with vegetable oil fatty acids. They are derived from edible oil sources such as cottonseed, corn, soybean, palm, peanut, safflower, and sesame oils and are approved for use as food additives under the U.S. Code of Federal Regulations 21 CFR 172.854 (70). PGEs can be purified through fractionation, molecular distillation, or solvent crystallization. The fractionated PGEs are more functional and can be used at lower concentrations (71). There are many different types of PGEs, which are widely used in food, cosmetics, and as surfactants, owing to differences in the extent of polymerization of the polyglycerol, hydrophilic group, and in the degree of esterification. Examples of PGEs include decaglycerol decaoleate, triglycerol monostearate, octaglycerol monostearate, and octaglycerol monopalmitate, among others. The



Where  $R_1$ ,  $R_2$ , and  $R_3$  are aliphatic fatty acyl moieties and  $n$  is no more than 3

**Figure 7.** Structural formula of polyglycerol esters (PGEs).

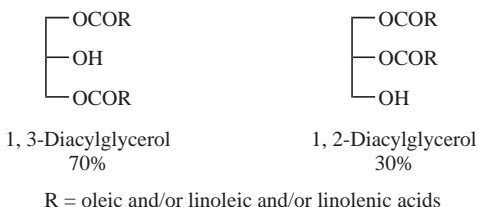
high molecular weight of PGEs is responsible for its reduced hydrolysis and bio-availability. PGEs have an estimated caloric value of 6–7 kcal/g, however, the net caloric value may be as low as 2 kcal/g as a result of partial absorption (72). Currently, PGEs are used as emulsifiers and dietetic aids and can also be used in margarines, shortenings, bakery products, frozen desserts, and confectionery products.

Animal studies suggested that PGEs are regarded as safe. In one study, groups of 100 rats were fed on diets containing 0%, 2.5%, 5.0%, or 10.0% of PGEs for three months. There were no adverse effects on survival, growth, organ weights, and body weight ratios (73). In another study, mice were fed on a diet containing 1% of PGEs for a period of 15.5 months. There were no significant differences between growth rate and longevity of test and control animals (74).

### 3.6. Other Reduced-Calorie Lipids

**3.6.1. Diacylglycerol (DAG) Oil** Diacylglycerol (DAG) oil is composed of greater than 80% DAGs as a mixture of 1,3-DAGs and 1,2-DAGs at a ratio of 7:3 (Figure 8). This product is made from edible oils using a patented process. The method of manufacture of DAG oil involves enzyme-catalyzed esterification of fatty acids derived from natural edible plant oils and either MAG or glycerol with *sn*-1,3 specific Lipozyme RMIM as the biocatalyst (75). Component fatty acids may be derived from soybean oil, canola oil, corn oil, and olive oil. The main fatty acid constituents of DAG oil are oleic (20–65 wt%), linoleic (15–65 wt%), and  $\alpha$ -linolenic (<15 wt%) fatty acids (76). The FDA has affirmed that MAGs and DAGs are GRAS (21 CFR 184.1505) when used in food at levels not exceeding the current good manufacturing practice for a variety of technical effects, such as an emulsifier, stabilizer, thickener, and texturizer.

DAG oil may help reduce body weight and fat mass when used as part of a diet. Although the oil contains the same amount of calories and fat as conventional fats, it is metabolized differently, which gives the oil its unique properties. This product is being marketed under the name Enova by ADM Kao LLC, a joint venture between Archer Daniels Midland Company (Decatur, Illinois) and Kao Corporation of Japan. Enova oil has been sold in Japan since 1999 and is the leading premium oil brand in Japan. The oil, which has a bland odor and moderate taste, may be substituted for conventional cooking oils in home frying, baking, salad dressings, and



**Figure 8.** Chemical structure of diacylglycerol oil.

cooking or in virtually any recipe that vegetable oil is used. However, this oil is not intended for use in repetitive, institutional frying applications. DAG oil can also be incorporated into a wide range of prepared foods, including dairy-based products, bakery products, meal replacements, frozen dinner entrees, nutrition bars, pizza, spreads, soups, gravies, and sauces. In 2000, DAG oil received FDA GRAS status for use in home cooking oil and vegetable oil spreads. GRAS use has now been established for other food products including salad dressings, mayonnaise, beverages, nutrition bars, soups and sauces, pizza, bakery products, and frozen dinner entrees.

FDA GRAS status of DAG oil was based on the following criteria, demonstrating the safety of DAG oil and the manufacturing process:

- a. MAGs and DAGs have been approved as food additives for more than 50 years.
- b. DAG components occur naturally in the diet and in the human body.
- c. The enzyme, Lipozyme RMIM, used in the manufacture of DAG oil has been approved since 1989.
- d. There has been more than 2 years of expanding consumer use in Japan.
- e. Studies indicated that there was no caloric difference between TAGs and DAGs.
- f. DAG oil was readily absorbed and metabolized by the body and did not affect the absorption of fat-soluble vitamins in the diet.
- g. Clinical studies indicated that there were no DAG-related toxic or other adverse effects.
- h. Research studies were reviewed by experts and government officials in Japan as well as in the United States by expert scientific panels.
- i. The DAG oil was granted “Food for Specific Health Use” (FOSHU) status by the Ministry of Health and Welfare in Japan in 1998 for use as a cooking oil.
- j. Both animal and human studies have demonstrated the safety of DAG oil.

As indicated above, this product consists of 80% DAGs. Seventy percent of these DAGs are the *sn*-1,3 form of DAGs. The remainder of DAG oil is comprised of TAGs, MAGs, FFAs, and Vitamin E added as an antioxidant. The predominant DAGs present in the oil have their fatty acids only in the *sn*-1 and *sn*-3 positions on the glycerol backbone, and hence more of the oil is metabolized directly by the body as energy, rather than stored as fat, as is the case with TAGs.

DAG oil is digested and absorbed in the small intestine, with fewer fat-rich particles being reassembled to enter the bloodstream as TAGs. That means a portion of the fat molecules are available for use immediately as energy rather than stored as fat. Studies have shown that DAG oil helps curb the level of TAGs or blood fats that usually increase after eating. Lower TAGs in the blood may help prevent the accumulation of body fat, particularly fat deposits in and around internal organs. The body digests the product like a conventional oil, so there is not the same risk of digestive disturbances and side effects that has been observed with some fat

substitutes. Research has shown that DAG oil does not influence the absorption of important vitamins in the diet, including fat-soluble Vitamins A, D, E, and K. In human subjects, it has been observed that DAG oil does not influence the absorption of fat-soluble vitamins in the diet. Subjects were fed 20 g of DAGs or TAGs either in mayonnaise or an emulsion drink once a day for 12 weeks. At 4, 8, and 12 weeks, fasting blood samples were drawn and serum levels of Vitamin A, D, and E were measured. The study concluded that DAGs and TAGs had no effect on fat-soluble vitamin levels (77).

Studies have shown that, over a 24-week period, overweight people who incorporated this oil into a calorie-controlled diet decreased their body weight by an average of 3.6% and their body fat mass by 8.3%. This was a significant reduction compared with the conventional fats. In addition, this oil can help reduce postmeal serum TAGs by 30–50% (78). More recently, the long-term effects of dietary DAGs and TAGs on the development of obesity in mice were investigated (79). Compared with mice ingested the high-TAG diet, mice fed the high-DAG diet accumulated significantly less body fat during the 8-month study period. Within the first ten days, dietary DAGs stimulated  $\beta$ -oxidation and lipid metabolism-related gene expression in the small intestine, but not in the liver, skeletal muscle, or brown adipose tissues. This suggests the predominant contribution of DAGs to intestinal lipid metabolism. Thus, in mice, dietary DAGs decreased body weight gain that accompanied the stimulation of intestinal lipid metabolism, and these effects may be related to the characteristic metabolism of DAGs in the small intestine (79). More research is needed in human subjects to clarify the role of intestinal fat metabolism in body weight management.

**3.6.2. Emulsifiers** Emulsifiers, like conventional fats and oils, provide 9 kcal/g of food; however, less is used and thus the product contains less fat and calories. The food industry refers to mono- and diacylglycerols as functional ingredients because they are added to products to give or aid in providing specific characteristics. Mono- and diacylglycerols have been developed as emulsifiers that help disperse fat in a watery medium. These ingredients help stretch fats or spread them more widely throughout food, thereby allowing less fat to be used in the product. By reducing the size of fat globules, emulsifiers cause their specific surface area to increase, which allows less fat to be used without any loss of viscosity. The ability of emulsifiers to do this is because of their amphiphilic nature; their lipophilic moiety, generally a long alkyl chain, dissolves well in nonaqueous phase, whereas their polar moiety dissolves well in water. In mixtures such as oil and water, emulsifiers can be used with water to replace all or part of the shortening content in cookies, cake mixes, icings, and selected dairy products.

**3.6.3. Jojoba Oil** Oils from novel plant sources have also gained interest as potential fat substitutes. One such example is jojoba oil. Typically, jojoba plant can reach up to 4.5 m in height and live for more than 150 years. The plant is suitable for hot climates and can be grown on salty soils and even in deserts. The oil is obtained from an evergreen shrub of the Buxaceae family, *Simmondsia chinensis*,

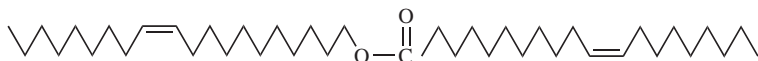


Figure 9. Chemical structure of jojoba wax ester.

which yields a nut that contains 50% oil. Jojoba oil is composed of a mixture of linear esters of monounsaturated fatty acids and fatty alcohols containing 20–22 carbons each. The chemical structure of jojoba wax ester is shown in Figure 9. The main fatty acid components of jojoba oil are 20:1 (70.7%), 22:1 (16.3%), 18:1 (9.0%), 24:1 (3.0%), and 16:0 (1.0%) (66). It is a liquid at 20°C. In 1979, Nestec Ltd. (Switzerland) started research on the possible use of jojoba oil as a low-calorie food ingredient. The physical properties of jojoba oil were generally similar to those of the typical edible oils (67). Early indications are that its functional qualities in foods are marginally useful (80).

Results showed that jojoba oil is not fully hydrolyzed by the pancreatic lipase because of its structure. Weight gain data in rats showed that jojoba oil has a low-caloric value compared with corn oil (67). There were problems associated with the consumption of jojoba oil. For instance, in rat feeding trials, anal leakage, depression, weakness, and death were observed when jojoba oil was fed at moderate-to-high doses (67). Animal studies with jojoba oil also showed that approximately 40% of the oil was absorbed (67), and therefore might serve as a low-calorie replacement for edible fats and oils in food use provided this compound was proven safe for food applications. Hence, possible applications of this oil are in control of obesity and calories.

#### 4. FAT MIMETICS BASED ON CARBOHYDRATES

Carbohydrate-based fat mimetics are used to replace fat in foods because of their textural and organoleptic properties. Carbohydrates generally mimic the fat by binding water, thus providing lubrication, slipperiness, body, and mouthfeel. There are many carbohydrate-based fat replacers available for use in foods. Most are modified starch products produced by hydrolysis or substitution. Maltodextrin and starch-based ingredients form a smooth, viscous solution or soft gel on hydration. These materials can provide the finished product with textural and functional characteristics of their full-fat counterparts. Microparticulated intact starch granules can also mimic the mouthfeel and structural contributions of fat crystals. They are totally digested and provide 4 kcal/g. Carbohydrate-based fat replacers can be successfully used for reducing the fat in a low moisture food system, but not for completely replacing it. The FDA has affirmed many of these carbohydrate-based fat replacers as GRAS for functional uses. Examples of various carbohydrate-based fat replacers used in foods and their applications are summarized in Tables 5 and 6.

Cellulose can replace some or all of the fat in dairy products, sauces, frozen desserts, and salad dressings. Finely ground microparticles of cellulose disperse throughout the food to provide a noncaloric network with the smoothness and

**TABLE 5. Examples of Carbohydrate-Based Fat Replacers in Food Products.**

Type of Fat Replacer	Commercial Names	Applications
Cellulose	Avicel, Methocel, Solka-Floc, Cellulose gel, Just Fiber	Mayonnaise, salad dressings, frozen desserts, processed cheese, fried foods, sauces
Dextrins	Amylum, N-Oil, Stadex	Salad dressings, puddings, spreads, dairy-type products, frozen desserts, meat products
Gums	Kelcogel, Keltrol, Rhodigel, Fibrex, Jaguar, Uniguar, Dycol, Rhodigum, Viscarin, Gelcarin, Novagel	Frozen desserts, processed meats, dairy products, fat-free salad dressings, reduced-fat margarines, soups, and sauces
Fiber	Z-Trim, Opta, Oat fiber, Betatrim, Oatrim, TrimChoice, Fibrim, Opta, Snowwhite, Ultracel	Reduced-fat baked goods, meats, extruded products, spreads, fat-free cheeses, cookies, fruit bars, muffins, frozen desserts
Maltodextrins	Paselli SA2, Paselli Excel, Paselli D-Lite, Maltrin, CrystaLean, Lycadex, Lorelite, N-oil, Star-Dri, Stellar, Rice-Trim	Dairy products, baked goods, frozen desserts, frostings, fillings, processed meat, salad dressings, spreads, sauces, beverages
Polydextrose	Litesse, Litesse II, Litesse Ultra, Sta-Lite	Frozen desserts, baked goods, fruit spreads and fillings, nutrition bars, beverages, confections

flow properties similar to fat. Powdered cellulose, microcrystalline cellulose, and cellulose gels present a wide variety of possibilities in terms of water-binding, film-forming, viscosity, pulpiness, and gelling.

Insoluble fiber is an important tool for fat and caloric reduction. Fiber-based products, such as gum arabic, guar gum, xanthan gum, locust bean, carrageenan,

**TABLE 6. Examples of Carbohydrate-Based Fat Replacers in Food Products (continued).**

Type of Fat Replacer	Commercial Names	Applications
Pectin	Splendid, Slendid, Grindsted	Baked goods, cookies, frostings, dressings, spreads, frozen desserts, gravies, soups, sauces
Starch/Modified Food Starch	Instant Stellar, Amalean I & II, N-Lite, OptaGrade, Sta-Slim, Pure-gel	Baked goods, dairy products, processed meats, salad dressings, fillings, frostings, sauces
Inulin	Fibruline, Raftiline, Fruitafit, Oliggo-Fiber	Baked goods, processed meats, frozen desserts, dairy products, whipped cream, icings, fillings
Chitosan	ChitoClear	Noodles, snacks, bread sticks, soups
$\beta$ -Glucan	Nu-TrimX	Baked goods

alginate, and pectin have virtually no calories. These have a wide variance in functional characteristics, including heat, shear and pH stability, type of texture, and thermoreversibility. Low concentrations of gums form gels, which can increase viscosity, provide texture and fat-like mouthfeel, and promote a creamy texture within the food. Gums can be used in reduced-calorie and fat-free salad dressings. They can also be used to reduce the fat content in formulated foods such as processed meats and desserts.

Dextrins can replace all or some of the fat in such products as in salad dressings, puddings, spreads, frozen desserts, and dairy foods. They provide 4 kcal/g of food. Most dextrins are derived from tapioca. A typical example is N-oil, marketed by National Starch and Chemical Corp. (Bridgewater, New Jersey), which can partially or totally replace fat or oil in foods, giving the illusion of a high-fat content.

Maltodextrin is an acid or enzyme-catalyzed hydrolysis product of starch, which has a dextrose equivalent of  $< 20$ . Maltodextrins are GRAS substances (21 CFR 184.1444) and can be used as a fat replacer, texture modifier, or bulking agent in dairy products, salad dressings, spreads, sauces, baked goods, frozen meat, and frozen desserts. Most maltodextrins are derived from corn, potato, tapioca, and wheat starches. Some examples of commercial products are Paselli SA2, Maltrin, and Stellar.

Modified food starch is a reduced-calorie fat replacer, supplying 1–4 kcal/g of food. This material mimics the mouthfeel that fat provides in foods. The ingredient is manufactured as a fine powder. When liquid is added, a slurry is made and sheared to form a smooth, cream-like substance that has similar properties to shortening. Modified food starch (21 CFR 172.892) can also be used as a bulking agent and texture modifier. It is used in combination with emulsifiers, gums, proteins, and other food starches to make dairy products, salad dressings, sauces, baked goods, and fillings.

Polydextrose is a reduced-calorie fat replacer, supplying 1 kcal/g of food. It is often used in conjunction with fat replacers to provide additional bulk and viscosity with reduced calories. Polydextrose contains minor amounts of sorbitol and citric acid. This product was approved by the FDA in 1981 for use in products in several food categories (21 CFR 172.841). Use of polydextrose in some of the products in these categories results in substantial reductions in fat. Polydextrose is currently used in baked foods, chewing gums, gelatins, puddings, and frozen dairy desserts.

## 5. FAT MIMETICS BASED ON PROTEINS

A protein's contribution to fat replacement is determined by the extent of denaturation, which influences flavor, as well as the protein solubility, gelling properties, and temperature stability. Proteins are important whipping agents, emulsion stabilizers, and dough strengtheners. Several fat mimetics are derived from a variety of protein sources, including egg, milk, whey, gelatin, soy, and wheat gluten. Some of these products are microparticulated to form microscopic, coagulated, round, deformable particles that mimic the texture and mouthfeel of natural fats and



**TABLE 7. Examples of Protein-Based Fat Replacers and Their Applications.**

Type of Fat Replacer	Commercial Names	Applications
Microparticulated Protein	Simplese	Baked goods, milk/dairy products, salad dressings, frozen desserts, mayonnaise-type products, margarine-type products, coffee creamer, soups, sauces
Modified Whey Protein Concentrate	Dairy-Lo	Dairy products, mayonnaise-type products, baked goods, frostings, salad dressing
Other	Lita, Ultra-Bake, K-Blazer, Ultra-Freeze, UltraBake, Powerpro, Proplus, Supro, Trailblazer	Frozen desserts, baked goods, spreads, butter, salad dressings

oils. Protein-based fat mimetics are commonly used in margarines, butter, cheese, dairy products, sour cream, salad dressings, mayonnaise-containing products, soups, sauces, baked goods, and frozen desserts. These substances generally give a better mouthfeel than do carbohydrate-based counterparts. However, similar to carbohydrate-based substances, protein-based fat mimetics cannot be used for frying. Typical examples of various protein-based fat mimetics used in foods and their applications are reported in Table 7.

Simplese, a low-calorie and cholesterol-free fat substitute, is manufactured from whey protein concentrate by a patented microparticulation process. Through this special process of heating and blending, egg protein and milk protein are combined and formed into minute particles that are 1–1.5  $\mu\text{m}$  in size. These particles are spherical and smooth, which allows the mouth to perceive them as fat. This product was introduced in 1988 by the NutraSweet Corporation, a subsidiary of Monsanto Corporation (St. Louis, Missouri), and is currently marketed by CP Kelco US, Inc. (Wilmington, Delaware). In petitioning the FDA for approval of simplese, NutraSweet compared the fat, cholesterol, and caloric content of a super-premium vanilla ice cream containing 16% butter fat with a frozen dessert using simplese. A 4-ounce serving of the ice cream provided 19 g of fat, 97 mg of cholesterol, and 274 calories, whereas the same size serving of frozen dessert containing simplese had less than 1 g fat, 14 mg of cholesterol, and 120 calories (81). Simplese received FDA GRAS status (21 CFR 184.1498) in 1990 and is approved for use as a thickener or texturizer in ice creams and other frozen dessert products. It can replace up to one-third of fat in frozen foods. This product is also suitable for use in yogurt, cheese spreads, cream cheese, and sour cream as well as oil-based products such as salad dressings, mayonnaise, and margarine. The caloric value of simplese is 1–2 kcal/g. It provides fat-like creaminess. However, similar to other proteins, it tends to mask flavor. As it is made from proteins, it cannot be used in foods that require high-temperature applications such as frying or baking. When it is heated, protein gel and the texture effects are lost. Products containing

simplese may not be suitable for people on protein restricted diets. People who are allergic to milk proteins or egg proteins may have an allergic reaction to this product.

Modified whey protein concentrate (Dairy-Lo), a GRAS substance, is manufactured from high-quality whey protein concentrate. This product contributes only 4 kcal/g. Modified whey protein helps improve texture, flavor, and stability of low-fat foods. It is typically used in sour cream, frozen dairy desserts, cheese, baked goods, yogurts, dips, and sauces. Its ability to prevent shrinkage and iciness in frozen foods makes it especially desirable as a fat replacement ingredient in those products.

## REFERENCES

1. D. M. Dreon, K. M. Vranizan, R. M. Krauss, M. A. Austin, and P. D. Wood, *J. Amer. Med. Assoc.*, **263**, 2462 (1990).
2. Economic Research Service, U.S. Department of Agriculture, *Agricultural Outlook: Statistical Indicators*, Washington, D.C., January 2003.
3. J. Wylie-Rosett, *AHA Scientific Statement, Circulation*, **105**, 2800 (2002).
4. Institute of Shortening and Edible Oils, in *Food Fats and Oils*, 8th ed., Washington, D.C., 1999, p. 25.
5. Anonymous, *J. Amer. Diet. Assoc.*, **98**, 463 (1998).
6. Anonymous, *FDA Consumer*, **36**(2), 8 (2002).
7. Calorie Control Commentary, vol. 24, no. 1, Calorie Control Council, Fall 2002.
8. P. Hollingsworth, *Food Technol.*, **57**(4), 17 (2003).
9. S. E. Gebhardt and R. G. Thomas, U.S. Department of Agriculture, Agricultural Research Service, Home and Garden Bulletin 72, 2002.
10. P. Kurtzweil, *FDA Consumer*, **30**(6), 7 (1996).
11. U.S. Food and Drug Administration, *A Food Labeling Guide-Appendix A*, Washington, D.C., June 1999.
12. FDA Backgrounder BG 99-5, *The Food Label*, U.S. Food and Drug Administration, Washington, D.C., May 1999.
13. U.S. Food and Drug Administration, *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*, Bureau of Foods, Washington, D.C., 1982.
14. U.S. Food and Drug Administration, Department of Health and Human Services, *Code of Federal Regulations, Title 21- Food and Drugs, Part 170- Food Additives*. U.S. Government Printing Office, vol. 3, Revised as of April 1, 2001, p. 5.
15. J. E. Vanderveen and W. H. Glinsmann, *Annu. Rev. Nutr.*, **12**, 473 (1992).
16. S. N. Gershoff, *Nutr. Rev.*, **53**(11), 305 (1995).
17. D. R. Webb and R. A. Sanders, *J. Amer. Coll. Toxicol.*, **10**, 3 (1991).
18. J. W. Finley, A. McDonald, and L. P. Klemann, in F. D. Gunstone and F. B. Padley, eds., *Lipid Technologies and Applications*, Marcel Dekker, Inc., New York, 1997, p. 501.
19. M. H. Auerbach, L. P. Klemann and J. A. Heydinger, in *Structural and Modified Lipids*, Marcel Dekker, Inc., New York, 2001, p. 485.

20. W. E. Artz and S. L. Hansen, in R. E. McDonald and D. B. Min, eds., *Food Lipids and Health*, Marcel Dekker, Inc., New York, 1996, p. 385.
21. D. R. Webb and R. A. Sanders, *J. Amer. Coll. Toxicol.*, **10**, 325 (1991).
22. D. R. Webb, J. C. Peters, and R. J. Jandacek, *J. Amer. Coll. Toxicol.*, **10**, 341 (1991).
23. G. M. Wardlaw, J. T. Snook, S. M. Park, P. K. Patel, F. C. Pendley, M. S. Lee, and R. J. Jandacek, *Amer. J. Clin. Nutr.*, **61**, 535 (1995).
24. R. E. Smith, J. W. Finley, and G. A. Leveille, *J. Agric. Food. Chem.*, **42**, 432 (1994).
25. L. P. Klemann, K. Aji, M. M. Chrysam, R. P. D'Amelia, J. M. Henderson, A. S. Huang, M. S. Otterburn, and R. G. Yarger, *J. Agric. Food. Chem.*, **42**, 442 (1994).
26. J. Giese, *Food Technol.*, **4**, 78 (1996).
27. R. Kosmark, *Food Technol.*, **50**, 98–101 (1996).
28. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, *Agency Response Letter: GRAS Notice No. GRN 000050*, Office of Premarket Approval, Washington, D.C., 2000.
29. V. K. Babayan, *Lipids*, **22**, 417 (1987).
30. A. C. Bach, Y. Ingenbleek, and A. Frey, *J. Lipid Res.*, **37**, 708 (1996).
31. V. K. Babayan, *J. Amer. Oil Chem. Soc.*, **51**, 260 (1974).
32. D. L. Ingle, A. Driedger, K. A. Traul, and D. K. Nakhasi, *J. Food Sci.*, **64**, 960 (1999).
33. V. K. Babayan and J. R. Rosenau, *Food Technol.*, **45**, 111 (1991).
34. N. Baba, E. F. Brace, and S. A. Hashim, *Amer. J. Clin. Nutr.*, **35**, 678 (1982).
35. A. C. Bach and V. K. Babayan, *Amer. J. Clin. Nutr.*, **36**, 950 (1982).
36. J. A. Heydinger and D. K. Nakhasi, *J. Food Lipids*, **3**, 251–257 (1996).
37. V. K. Babayan, G. L. Blackburn, and B. R. Bistran, *U.S. Patent* 952,606, 1990.
38. R. J. Jandacek and M. R. Webb, *Chem. Phys. Lipids*, **22**, 163 (1978).
39. R. B. Toma, D. J. Curtis, and C. Sobotor, *Food Technol.*, **42**, 93 (1988).
40. F. H. Mattson and R. A. Volpenhein, *J. Lipid Res.*, **13**, 325 (1972).
41. A. B. R. Thomson, R. H. Hunt, and N. L. Zorich, *Aliment Pharmacol. Ther.*, **12**, 1185 (1998).
42. Food and Drug Administration (FDA), *Fed. Regist.*, **61**, 3118 (1996).
43. J. Giese, *Food Technol.*, **3**, 130 (1996).
44. FDA Backgrounder BG 95-18, *Olestra and Other Fat Substitutes*, U.S. Food and Drug Administration, Washington, D.C., November 28, 1995.
45. D. A. Cooper, D. R. Webb, and J. C. Peters, *J. Nutr.*, **127**, 1699S (1997).
46. D. A. Cooper, D. A. Berry, M. B. Jones, A. L. Kiorpes, and J. C. Peters, *J. Nutr.*, **127**, S1589 (1997).
47. T. Schlagheck, J. Kesler, M. Jones, N. Zorich, L. Dugan, and J. Peters, *J. Nutr.*, **127**, S1666 (1997).
48. D. Y. Jones, K. W. Miller, B. P. Koonsvitsky, M. L. Ebert, P. Y. Lin, M. B. Jones, and H. F. DeLuca, *Amer. J. Clin. Nutr.*, **53**, 1281 (1991).
49. D. Y. Jones, B. P. Koonsvitsky, M. L. Ebert, M. B. Jones, P. Y. Lin, B. H. Will, and J. W. Suttie, *Amer. J. Clin. Nutr.*, **53**, 943 (1991).
50. G. Dasher, D. Cooper, K. Riccardi et al., *FASEB J.*, **8**, A443 (1994).
51. N. Zorich, G. Dasher, D. Cooper et al., *FASEB J.*, **8**, A735 (1994).
52. R. G. La Barge, *Food Technol.*, **1**, 84 (1988).

53. M. J. Mellies, C. Vitale, R. J. Jandacek, G. E. Lamkin, and C. J. Glueck, *Amer. J. Clin. Nutr.*, **41**, 1 (1985).
54. J. W. Finley and G. A. Leveille, in E. E. Ziegler and L. J. Filer, Jr., eds., *Present Knowledge in Nutrition*, 7th ed., ILSI Press, Washington, D.C., 1996, p. 581.
55. R. J. Jandacek, M. M. Ramirez, and J. R. Crouse, *Metabolism*, **39**, 848 (1990).
56. F. H. Mattson, R. J. Jandacek, and M. R. Webb, *J. Nutr.*, **106**, 747 (1976).
57. C. J. Glueck, F. H. Mattson, and R. J. Jandacek, *Amer. J. Clin. Nutr.*, **32**, 1636 (1979).
58. J. R. Crouse and S. M. Grundy, *Metabolism*, **28**, 994 (1979).
59. S. M. Grundy, J. V. Anastasia, Y. A. Kesaniemi, and J. Abrams, *Amer. J. Clin. Nutr.*, **44**, 620 (1986).
60. M. J. Mellies, R. J. Jandacek, J. D. Taulbee, M. B. Tewksbury, G. Lamkin, L. Baehler, P. King, D. Boggs, S. Goldman, A. Gouge, R. Tsang, and C. J. Glueck, *Amer. J. Clin. Nutr.*, **37**, 339 (1983).
61. R. Hunt, N. L. Zorich, and A. B. R. Thomson, *Can. J. Gastroenterol.*, **12**(3), 193 (1998).
62. F. M. Fouad, O. A. Mamer, F. Souriel, F. Shahidi, and G. Ruhenstroth-Bauer, in A. Spanier, F. Shahidi, T. H. Parliment, C. Mussinan, C-T. Ho, and G. Tratras Coutis, eds., *Food Flavors and Chemistry: Advances of the New Millennium*, Royal Society of Chemical, Cambridge, United Kingdom, 2001, p. 684.
63. B. C. Sekula and J. W. Golebiowski, *U.S. Patent* 6,361,817, 2002.
64. J. F. White and M. R. Pollard, *U.S. Patent* 4,861,613, 1989.
65. L. W. Masten, *Eur. Patent* 00571219/EP B1, 1997.
66. Anonymous, *J. Amer. Oil Chem. Soc.*, **66**, 868 (1989).
67. D. J. Hamm, *J. Food Sci.*, **49**, 419 (1984).
68. D. J. Hamm, *U.S. Patent* 4,508,746, 1985.
69. R. C. McIntyr, *J. Amer. Oil Chem. Soc.*, **56**, 835A (1979).
70. U.S. Food and Drug Administration, Department of Health and Human Services, *Code of Federal Regulations, Title 21- Food and Drugs, Part 172- Food Additives Permitted for Direct Addition to Food for Human Consumption*, vol. 3, U.S. Government Printing Office, Revised as of April 1, 2002, p. 89.
71. M. Martinez-Serna Villagran, S. P. Zimmerman, J. D. Reed, P. Seiden, and S. M. Brower, *U.S. Patent* 6,436,459, 2002.
72. W. R. Michael and R. H. Coots, *Toxicol. Appl. Pharmacol.*, **20**, 334 (1971).
73. W. R. King, W. R. Michael, and R. H. Coots, *Toxicol. Appl. Pharm.*, **20**, 327 (1971).
74. J. Bickel, A. J. Therkelsen, and A. Stenderup, *Arzneimittel Forsch.*, **14**, 238 (1964).
75. N. Goto, T. Nishide, Y. Tanaka, T. Yasukawa, and K. Masui, *U.S. Patent* 6,326,050, 2001.
76. N. Goto, T. Nishide, Y. Tanaka, T. Yasukawa, and K. Masui, *U.S. Patent* 6,139,897, 2000.
77. H. Watanabe, K. Onizawa, S. Naito, H. Taguchi, N. Goto, T. Nagao, N. Matsuo, I. Tokimitsu, T. Yasukawa, R. Tsushima, H. Shimasaki, and H. Itakura, *Ann. Nutr. Metabol.*, **45**, 259 (2001).
78. D. E. Pszczola, *Food Technol.*, **57**(3), 42 (2003).
79. T. Murase, M. Aoki, T. Wakisaka, T. Hase, and I. Tokimitsu, *J. Lipid Res.*, **43**, 1312 (2002).
80. R. G. La Barge, in L. O. Nabors and R. C. Gielari, eds., *Alternative Sweeteners*, 2nd ed., Marcel Dekker Inc., New York, 1991, p. 423.
81. M. Segal, *U.S. Food and Drug Administration: FDA Consumer*, December 1990.

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## *Structural Effects on Absorption, Metabolism, and Health Effects of Lipids*

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### **1. INTRODUCTION**

#### **1.1. Food Lipids and Dietary Fats**

Standard analytical procedures to determine the amount of fat in a food indirectly define food lipids. A food lipid can be considered as the material that can be extracted from a food with one or more organic solvents under well-specified conditions (which often are dependent on the type of food analyzed). Food lipids consist of an extremely large number of different molecules. Each of them, in mutual combination or in combination with other dietary components, may have effects

that have an impact on health. The major components of most food lipids are triacylglycerols (TAGs, also known as triglycerides). TAG molecules can differ from each other in the fatty acids they contain and in the combination and/or stereochemical implantation of different fatty acids. Other food lipid components with distinct nutritional effects are phospholipids, sterols, fat-soluble vitamins, antioxidants, and other fat-soluble components, some of which can affect gene expression.

Dietary fats are defined here as fats that can be incorporated into foods, e.g., bread and margarine, among others, or used for the preparation of foods. By chemical and enzymatic processes, the structure of TAGs can be modified and minor natural fat components such as monoacylglycerols (MAGs, also known as monoglycerides), diacylglycerols (DAGs, also known as diglycerides), and phytosterol esters can be produced in large quantities. These have been shown to have special nutritional properties. This is also the case for TAGs with changed stereospecific position or changed combination of fatty acids such as interesterified fats, structured lipids, salatrims, and sucrose polyesters, among others.

Structural effects of food lipids on several of their physiological, nutritional, and pathophysiological properties can be understood on basis of the specificity of enzymes that are involved in lipid metabolism.

## 1.2. Definition of Lipid Structure

From a nutritional point of view, we propose that the term *lipid structure* refers to classes of lipids containing ester bound fatty acids such as triacylglycerols, partial acylglycerols, phospholipids, sterol esters, sucrose polyesters, and so on. Each lipid class thus has common structural characteristics, but it can also have molecules that differ in grouping or positioning of fatty acids on the alcohol backbone. For pure acylglycerols, the structure can be unequivocally described by the stereospecific numbering convention (prefix *sn*-) of Hirschmann (1). A number of other prefixes are commonly used to designate the position of substituents in acylglycerols. Alpha,  $\alpha$ , refers to the two primary hydroxyl groups and  $\beta$  to the secondary hydroxyl groups, *rac* precedes the names of acylglycerols that are equal mixtures of enantiomers, and X is used when the positioning of the substituents is either unknown or unspecified. Food lipids contain complex mixtures of acylglycerols. For nutritional studies, they are often described according to their occurrence in nature (e.g., butterfat, soybean oil, etc) or by the way they are modified (e.g., enzymatically interesterified butterfat). For semisynthetic lipids, we propose the prefixes *eq* - for thermodynamic equilibrium mixtures and *random* for mixtures where the fatty acids occupy the acylglycerols positions according to chance. Thus *eq*-monoacylglycerols (MG) would contain about 45% *sn*1-MG, 10 % *sn*2-MG and 45% *sn*3-MG, whereas *random*-MG would contain, respectively, 33 mole% of each. Near *random*-triacylglycerols are formed by interesterification of a physical mixture of triacylglycerols. From a nutritional point of view, these notations make sense because most nutritional studies concerning the effect of fat structure are done with these types of acylglycerols.

### 1.3. Nutritional Effects of Food Lipids

There is a vast amount of data on the effects different fatty acids have when fed as usual food lipids. These will not be dealt with here. It is sufficient to stress that it is important to avoid generalizations. All saturated fatty acids do not raise cholesterol. Not all polyunsaturated fatty acids reduce cholesterol, and the physiological effects of different *trans*-fatty acids are different. In general, each fatty acid has distinct effects. We concentrate here on how fat structure can modulate the known effects of fatty acids. Furthermore, it is important to keep in mind that the nutritional effects of edible fats and oils do not only depend on the fatty acids they contain but also on the presence of other components.

### 1.4. Lipids Used for Evaluating Stereospecific Effects

The ideal situation to evaluate stereospecific effects of lipids is that at least two lipid mixtures are available that differ only in the stereospecific structure one wants to study. This is seldom the case. Studies with TAG molecules that have the same fatty acids but at different position are rare because such components are very expensive. Often physical and interesterified mixtures are compared. These fats do not only differ in the position of fatty acids but also in combination of fatty acids in the TAGs. Moreover, the interesterification process may affect minor components that may have an effect on the parameter measured. As an example, reduced atherogenicity of interesterified peanut oil could well be caused by reduced lectin content and not by changed TAG composition (2). A mixture of two previously interesterified fats before and after interesterification has the same fatty acid composition at the different stereospecific positions but differs in the grouping of fatty acids in the TAG molecules. To the best of our knowledge, such fats have not been compared. An interesterified mixture of trimyristin with peanut oil has been used to determine the effect of combinations of fatty acids in the molecules on cholesterolemia (3). As peanut oil has no 1,2,3 random structure, not only the combination of fatty acids but also their composition at the different stereospecific conditions is changed. Another approach that is sometimes used is comparing the effect of two different natural fats with comparable fatty acid composition but with a different position of specific fatty acids in the TAGs (e.g., seal and squid oil) (4).

## 2. STEREOSPECIFIC EFFECTS OF FAT DIGESTION AND RELATED PHENOMENA

Digestion of usual food fats and subsequent absorption of their digestion products have recently been reviewed (5). In addition, the *in vitro* digestion profiles of long- and medium-chain acylglycerols and the phase behavior of their lipolytic products has also been reported (6). In this chapter, the stereospecific aspects will be highlighted.

## 2.1. Gastric Phase

**2.1.1. Physicochemical Reactions/day in the Stomach** An average Western diet contains about 100–150-g food lipid, about 97% of which are TAGs. The remainder is made up of phospholipids (4–8 g/day, mainly phosphatidylcholine, PC), cholesterol, and other sterols and minor lipid components. When they are the only lipids present in an aqueous environment, TAGs form unstable emulsion droplets, whereas PC form relatively stable lamellar structures. When TAGs and PC are present together in proportions as found in the diet, phospholipids acts as an emulsifier by coating the TAG droplets, thus forming stable emulsions. These are substrates for gastric lipase.

**2.1.2. Gastric Digestion** Gastric lipase starts the hydrolysis of dietary lipids. It has positional, substrate but probably no fatty acid specificity (7). Gastric lipase only splits primary ester bonds. It catalyses the hydrolysis of the fatty acid at the *sn*-3 position preferentially to that at *sn*-1 position and prefers TAGs with short- and medium-chain lengths (8). Thus, TAGs with these fatty acids in the *sn*-3 position, which are present in bovine milk and its products, are preferential substrates. The main digestion products of gastric lipase are free fatty acids (FFA) and *sn*1,2-DAGs (9). It has been demonstrated that medium-chain fatty acids liberated in the stomach can be absorbed directly into the portal blood and thus can affect hepatic metabolism. The specific activity of gastric lipase in vivo is comparable with that of pancreatic lipase for liquid fats and higher for solid fats. Of course, pancreatic lipase output is much higher than gastric lipase output. High-fat diets increase gastric lipase output in humans (10). About 10% of the dietary triacylglycerol fatty acids are liberated by gastric lipase from a meal containing long-chain fatty acids but considerably higher amounts from a test meal containing short- or medium-chain fatty acids (11). Even for long-chain fatty acid containing TAGs, this corresponds to the breakdown of about one-third of the TAGs into DAGs and FFA in the stomach. Furthermore, there is evidence that gastric lipase is further active in the upper small intestine (12). The importance of gastric digestion is that its hydrolysis products affect the excretion of some hormones involved in fat digestion, help to stabilize the surface of the TAG emulsion, promote binding of pancreatic colipase later in the small intestine, and thus make the emulsion particles a better substrate for pancreatic lipase (13). There is a compensatory increase in gastric lipase when pancreatic lipase is low. Nonetheless, when pancreatic lipase is low, the capacity for lipid absorption remains limited when usual food fats are fed. It can be expected that in these conditions, semisynthetic triacylglycerols with a short-chain fatty acid at the *sn*-3 position and long-chain fatty acids, among which essential fatty acids, at the other positions may be the lipids of choice to enable feeding more fat and provide for energy and essential fatty acids.

**2.1.3. Gastric Emptying** The composition of the fat that is emptied in the upper small intestine depends on the rate of gastric digestion and on the time the dietary fat was retained in the stomach. The physicochemical and chemical forms in which



dietary fats are consumed affect the rate of gastric emptying. Fats that are ingested as emulsion are partitioned into an aqueous phase and an oil phase. In contrast, fats consumed as nonemulsions form an oil phase. Fats in the aqueous phase are readily emptied from the stomach, whereas fats in an oil phase are emptied much slower (14). The chemical composition of the oil phase also influences the rate of emptying (15). The delaying effect depends on the saturation (16) and the chain length (17) of the fatty acids. No data are available as to the effect of the chemical structure of the fat if any. In healthy individuals, the rate of gastric emptying can be rate limiting for fat absorption (17).

## 2.2. Small Intestinal Phase

### 2.2.1. Reactions in the Lumen of the Small Intestine

**2.2.1.1. Endogenous Dilution and Interactions** The lipids that are emptied from the stomach are diluted by lipids from endogenous origin (~10–25 g/day when fasting). The two major sources of endogenous lipids originate from slaughtered intestinal cells and from biliary secretions. The amount of phospholipids (PL) from biliary origin (7–22 g/day; mainly PC) is considerably larger than from dietary origin (3–8 g/day). The same is also often true for cholesterol (~1 g/day of endogenous origin). In bile, bile salt–phospholipid mixed micelles exist in cylindrical arrangement. In the intestinal lumen, bile salts and PC molecules are likely to partly adsorb to the lipid emulsion droplets (18).

Lipolysis starts at the water/emulsion interphase. The major enzymes involved are pancreatic lipase, pancreatic phospholipase A<sub>2</sub>, and pancreatic cholesterol esterase, the latter also named bile-salt-dependent lipase.

**2.2.1.2. Intestinal Digestion and Accompanying Reactions** The extent of intestinal digestion and the nature and the relative amounts of the digestion products taken up by the intestinal cells depend on the activity and specificity of the lipases, the rate of isomerization, and the rate of absorption of the products formed by lipase action.

Human pancreatic lipase catalyses the equilibrium reaction between ester formation and their hydrolysis. It is specific for primary alcohols and esters of such alcohols. Thus, under certain conditions, pancreatic and other lipases can be used for synthesis of TAGs with certain stereospecific structure (19). In the beginning of and during fat digestion, the lipid/water interface is rich in water and the digestion products are continuously removed from the interface favoring lipolysis. Pancreatic lipase thus splits off fatty acids from the *sn*1- and *sn*3-position of TAGs. It is more active against *sn*1,2- and *sn*2,3 DAGs thus formed than against TAGs (20). Thus, it can break down the TAGs into *sn*1,2- and *sn*2,3-DAGs, which can be further hydrolyzed into *sn*2-MAGs. These are no longer substrates for pancreatic lipase. Pancreatic lipase is less active against polyunsaturated fatty acids with a double bond close to the ester bond, in particular when these are located in the *sn*-3 position and clearly prefers the *sn*-1 position of both TAGs and DAGs (21). Thus, if there would

be neither isomerization nor absorption of TAGs and DAGs, the ultimate digestion products of TAGs would be a 2/1 mixture of FFA and *sn2*-MAGs. However, both reactions do occur.

“Linear” partial acylglycerols are thermodynamically favored over “branched” partial acylglycerols. MAGs with equilibrium isomeric composition consist of about 45% *sn1*-, 45% *sn3*-, and 10% *sn2*-MAGs (22). For DAGs, the isomer ratio is about 70/30 (23) for *sn1,3*-DAGs and *sn1,2*- plus 2,3-DAGs, respectively. Isomerization of partial acylglycerols yields products with primary ester bonds, which are substrates for pancreatic lipase and thus results in more complete digestion with the liberation of glycerol. As MAGs are readily absorbed, the rate of isomerization relative to that of uptake is important to be further digested. Isomerization rate increases with unsaturation and decreases with chain length (24). Anyhow, spontaneous isomerization of long-chain fatty acids containing *sn2*-MAGs in model systems is low compared with their intestinal uptake (25). About 15–20% of *sn2*-MAGs would isomerize in the intestinal lumen (26).

There is some suggestive evidence that small amounts of TAGs and DAGs can be taken up without prior digestion. After feeding medium-chain triacylglycerols (MCT), small amounts of these triacylglycerols were demonstrated in ascites fluid from a patient with obstruction of the thoracic duct (27). In concurrence di-medium-chain mono-long-chain TAGs increased in lymph of rats when MCT were fed together with long-chain TAGs (28). These findings in combination with the concept that medium-chain fatty acids cannot be converted into acyl-coenzyme A (CoA) derivatives (29) and consequently cannot be incorporated in TAGs in the intestinal cell can be seen as an indication that intact TAGs (at least MCT) and intact DAGs (at least di-medium-chain DAGs) can be taken up, the latter serving as acceptors of long-chain fatty acids. The concept that medium-chain fatty acids cannot be converted into CoA derivatives has been challenged, however (30). The finding that caprylic acid was found in the TAG fraction of lymph in rats after feeding 1,3 dioctanoyl-2-linoleyl-*sn*-glycerol was seen as an indication that caprilic acid must have been converted into the CoA derivative for incorporation into TAG (28). An alternative explanation would be absorption of intact caprilic acid containing DAGs. The possibility should be considered that DAGs with a fatty acid at the outer position that is not easily split off by pancreatic lipase may be absorbed as such to some degree. Absorption of intact DAGs could explain the finding that the combination of fatty acids in the dietary TAG molecules affects some of its physiological effects (3, 31).

The presence of intact PL in the intestinal lumen retards TAG digestion (32), affects the partitioning of lipid digestion products between the oil and micellar phase (33), and slows down the uptake of lipid digestion products from the micellar phase (34). Phosphatidylcholine is digested, however, under the action of phospholipase A<sub>2</sub>, which hydrolyses the fatty acid esterified at the *sn2* position to yield lyso-PC and free fatty acids (34). In contrast to PC, the resulting lyso-PC favors micellar solubilization and uptake of fat digestion products (35) and other dietary lipophilic substance (36). Lyso-PC is taken up by the intestinal cell, affects intestinal

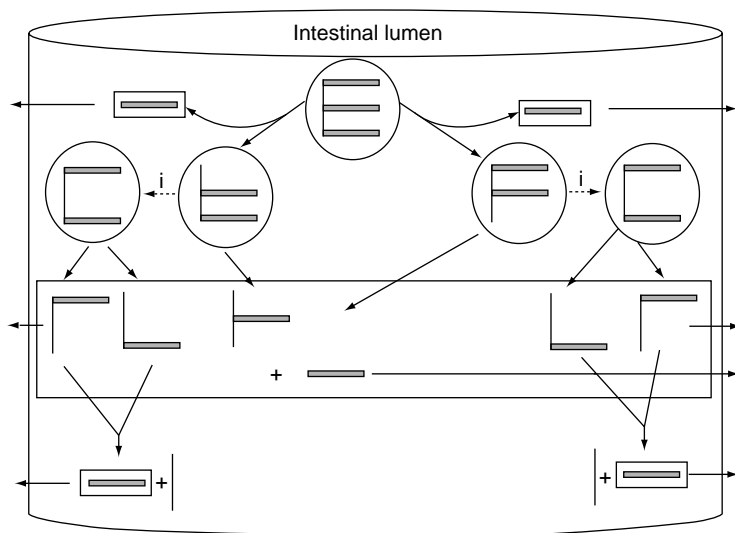
metabolism (37), and can be converted into PC again. There is evidence that PC can be taken up intact and excreted as part of intestinal lipoproteins in the lymph (38).

Cholesterol esterase hydrolyses sterol esters. Cholesterol is absorbed as free sterol (39), and the effects of phytostanol esters and phytosterol esters are mediated by the free phytostanol/sterol.

**2.2.1.3. Physicochemical Aspects** For a lipid to be a candidate for absorption, it must be able to reach the absorptive surface of the small intestine. This is the surface of the microvilli. Geometrically, they are so close to each other that emulsion droplets are too large to gain access between them. Moreover, an “unstirred” water layer lines the microvilli. Passage through this layer is rate limiting for lipid absorption. MAGs and FFA leave the emulsion/water interface where they were formed by the action of pancreatic lipase and form mixed micelles with conjugated bile acids. Only in this form can they easily access the absorptive surface of the small intestine and be absorbed. To the best of our knowledge, no data are available on the effect of isomer structure or fatty acid composition of the MAGs on the rate that they are transferred from the emulsion phase to the micellar phase if any. Partitioning of fatty acids between the emulsion interface and the micellar phase depends on pH. When pH is low, such as in the absence of pancreatic juice, protonated fatty acids remain at the interface of the emulsion and inhibit further digestion. The interior of the MAG/FFA/conjugated bile salt mixed micelle is a lipophilic environment in which lipophilic substances such as cholesterol, carotenes, and fat-soluble vitamins also can dissolve. It is in this way that they can reach the absorptive surface and are candidates for uptake. Phytosterols/-stanols can compete with cholesterol for incorporation in the micelles and thus interfere with cholesterol absorption. Reaching the absorptive surface of the small intestinal cells is not enough for uptake. Bile salts, for instance, are not absorbed at this site, and absorption of phytosterols is very limited. It cannot be excluded that the stereospecific structure of the MAGs in the mixed micelles affects the extent of micellar solubilization and uptake of lipophilic substances, but experimental data are lacking. The composition of mixed micelles that exist in the intestinal lumen and several of their physicochemical properties have been described (40).

**2.2.1.4. End Products of TAG, DAG, and MAG Digestion** Intestinal digestion and absorption are interrelated phenomena that go on simultaneously. The chemical form in which fatty acids present in food fats are taken up depends on rates of digestion and isomerization relative to rates of uptake. The chemical form in which a fatty acid is present in the intestinal lumen or is taken up in the intestinal cells is of utmost importance because it influences its further metabolism.

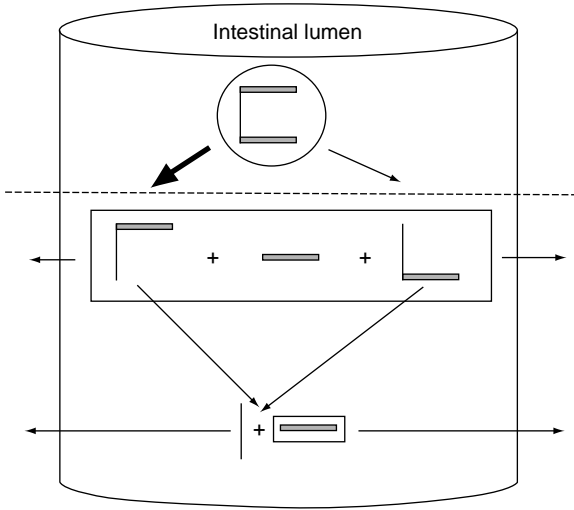
For long-chain triacylglycerols, it can be stated that fatty acids fed in the *sn*1,3-position are taken up mainly as free fatty acids, whereas those at the *sn*2-position are mainly taken up as *sn*2-MAGs. This is schematically shown in Figure 1.



**Figure 1.** Schematic representation of TAG digestion and absorption. In circle: in emulsion phase; in rectangles: in micellar phase; horizontal arrows: absorption or isomerisation (i); descending arrows: digestion.

In contrast, medium-chain fatty acids are mainly but not exclusively taken up as FFA whatever their position in medium-chain or mixed-chain TAGs.

*eq*-DAGs and *eq*-MAGs are easily prepared industrially in large quantities, and *eq*-DAGs are on the market as edible oils in Japan and their approval for the U.S. market is solicited (2002). DAGs and MAGs are also used in smaller quantities as emulsifiers in some foods. In *eq*-DAGs, the 1,3 isomers are the major components. In a diet, TAGs are always present. When *eq*-DAGs are also present in the diet, it is expected on theoretical grounds that they will form emulsion droplets together with the TAGs. The *rac*-1,2 isomers are normal intermediates of TAG digestion. The *sn*1,3-isomers would only be formed from TAGs in very small quantities by isomerization of their intermediary *sn*1,2- and *sn*2,3-DAG digestion products. If pancreatic lipase preferentially attacks the *sn*-1-position of 1,3 DAGs as it does in TAGs, the major first digestion product would be *sn*3-MAGs, but *sn*1-MAGs would be formed as well. This would be of no consequence if both MAG stereoisomers, which are substrates for pancreatic lipase, would be broken down completely to glycerol and fatty acids before absorption. However, if there will be substantial absorption of the intact MAGs, their isomeric composition could be of importance because it has been shown that they are handled differently in the intestinal cells (*vide infra*). There is indirect evidence that *sn*1(3)-MAGs are taken up intact from the intestinal lumen (*vide infra*). Theoretical possibilities for intraluminal handling of DAGs and MAGs fed as such are given in Figure 2.



**Figure 2.** Schematic representation of DAG (total panel) and MAG (lower panel) digestion and absorption. In circle: in emulsion phase; in rectangles: in micellar phase; horizontal arrows: absorption; descending arrows: digestion.

### 3. DIFFERENCES IN METABOLISM BETWEEN ESTERIFIED AND FREE FATTY ACIDS

#### 3.1. In the Intestinal Lumen

Saturated fatty acids are better absorbed as MAGs than as FFA, as the latter can form less-absorbable hydrated acid soaps and complexes with calcium and other divalent ions (41). The effect is more pronounced when the amount of calcium in the diet is high (42). In the presence of insoluble soaps, cholesterol absorption may be reduced (43). Thus, the stereospecific position of fatty acids in the TAGs can have an effect on their absorption and on the absorption of minerals and other lipid constituents.

#### 3.2. In the Intestinal Cell

**3.2.1. Intestinal Handling of FFA** In the cells of the small intestine, several enzymes can act on free but not on esterified fatty acids. In contrast to MAGs, FFA are diluted with fatty acids originating from the plasma free fatty acid pool (44). After activation they can be oxidized, elongated, chain desaturated, and converted into complex lipids (45, 46). The relative rates depend on the nature of the fatty acids and on the presence of other components in the intestinal cells (47). Conversion of saturated fatty acids in monounsaturated ones when they are absorbed as FFA, i.e., when they were present in the outer position of the dietary TAGs, could

contribute to their lower hypercholesterolemic effect than when originally present at the inner position. FFA also affect intestinal gene expression (48) and the production of apolipoproteins, and these effects are fatty acid dependent (49).

### 3.2.2. Intestinal Handling of MAGs

**3.2.2.1.  $\beta$ -monoacylglycerols** After feeding usual food fats, the major digestion products that are absorbed are *sn2*-MAGs and FFA. In the small intestinal cells, most of the *sn2*-MAGs react with activated free fatty acids to be converted by a multienzyme complex into TAGs. The excess of FFA reacts with *sn3*-glycerophosphate to form lysophosphatidic acids, which are further converted into phosphatidic acids. Dephosphorylation results in *sn1,2*-DAGs, which are precursors of both TAGs and PL.

**3.2.2.2.  $\alpha$ -monoacylglycerols** What will happen after influx of *sn1,3*-DAGs or 1(3) *sn*-MAGs in the intestinal cell is less clear. A series of competing reactions must be considered such as complete digestion, acylation, transacylation, phosphorylation, and intact excretion out of the intestinal cells.

Lipases that hydrolyze MAGs are present in the intestinal cells, but their action seems to be reduced by other intestinal cell constituents (50). Some of them show considerable fatty acid specificity (51). Both *sn1*- and *sn3*-MAGs can be acylated to form *sn1,3*-DAGs. This transformation is fatty acid and species dependent (51). Anyhow, *sn1,3*-DAGs are poor substrates for intestinal TAG synthesis (51, 52). *Sn1*- but not *sn3*-MAGs can be phosphorylated into lysophosphatidic acid (53) and possibly be metabolized further as described above. If MAGs would escape intestinal metabolism and could leave the cell intact, they could be transported by portal blood bound to albumin (54). The different metabolic pathways of 1(3)-MAGs that have been demonstrated in vitro in intestinal tissue are given in Figure 3.

There is some indirect evidence that after MAG feeding, intact *sn1*-MAGs or their phosphorylated derivatives reach the liver with retention of the *sn1* ester bond. In the liver, TAGs are mainly formed from *sn3*-glycerophosphate. *Sn3*-glycerophosphate acyltransferase shows fatty acid chain length selectively, incorporating about 60% fatty acids with 16 carbon atoms and 40% fatty acids with 18 carbon atoms at the *sn1*- position. The other enzymes involved in hepatic synthesis of TAGs that will be secreted in plasma mainly incorporate unsaturated fatty acids with 18 carbon atoms (about 80%). As a result, the main chain length combination of fatty acids in TAGs in fasting plasma will be 16/18/18. *Sn1*-MAGs can be phosphorylated in the intestine and in the liver. Thus, by feeding *sn1*-MAGs, the fatty acid selectivity of *sn3*-glycerophosphate could be bypassed if *sn1*-MAGs were precursors of lysophosphatidic acid and TAGs with an unusual fatty acid at the *sn1*-position could be formed. If this actually happens was checked by analysis of the chain length combination of fatty acids in TAGs by carbon number analysis. Carbon number composition of TAGs found in fasting serum after long-term TAG and *eq*-MAG feeding in humans are given in Table 1.

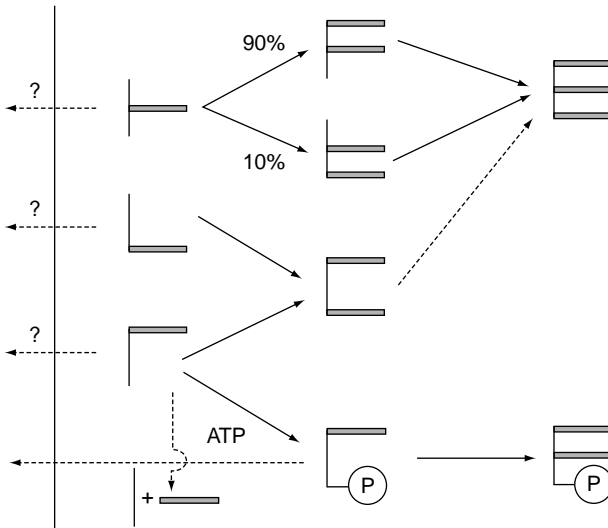


Figure 3. Intestinal metabolism of monoacylglycerols.

TABLE 1. Carbon Number Composition\* of TAGs in Fasting Plasma After Long-Term TAG or MAG Feeding.

Carbon Number	Chain Length Combinations	After TAG Feeding** (n = 10)	After MAG Feeding** (n = 4)
50	16/16/18	20.5 ± 3.8	12.5 ± 4.5
52	16/18/18	55.8 ± 3.6	57.0 ± 4.7
54	18/18/18	15.2 ± 3.1	25.0 ± 5.2

\*mole% ± SD.

\*\*Fatty acid composition of sunflower seed oil.

The reduced fraction of TAGs with carbon number 50 and the increased ratio of TAGs with fatty acid combination 18/18/18 to these with 16/18/18 in fasting serum (55) after long-term *eq*-monolinoleate feeding suggests that the MAGs were transported into the liver with structure retention and used for TAG synthesis. If the MAGs contain at the *sn*1-position a fatty acid such as linoleic acid that normally is not incorporated into lysophosphatidic acid by *sn*3-glycerophosphate acyltransferase, TAGs may be formed with unusual structure. Such plasma TAGs, which may also occur after DAG feeding in case these are taken up as MAGs, may be turned over at different rates and consequently affect their fasting concentration.

#### 4. POSTPRANDIAL EFFECTS

After TAG feeding, long-chain fatty acids leave the intestinal cell mainly as TAGs incorporated in intestinal lipoproteins with the size of chylomicrons. These are

transported via the lymphatic route. When they enter the blood stream, there is an increase in chylomicronemia, triglyceridemia, and a change of the fatty acid composition of plasma TAGs toward that of the fat fed. Chylomicron-TAGs are hydrolyzed by lipoprotein lipase, resulting in the formation of remnants and liberation of free fatty acids. As a consequence, the composition of the nonesterified fatty acids in plasma also changes toward that of the fat fed. Chylomicron remnants are taken up by the liver. Medium-chain fatty acids provided in the diet as MCT or as structured lipid are transported mainly as free fatty acids by the portal vein.

After isomeric *eq*-MAG loading, the well-known postprandial chylomicronemia, triglyceridemia, and shift of the fatty acid composition of serum triacylglycerols and nonesterified fatty acids toward that of the fat fed is much less pronounced than after natural fat feeding (56). However, when MAGs are fed together with FFA, effects similar to these of TAG feeding are obtained. This finding is in concurrence with the finding that the availability of long-chain free fatty acids in the intestine is critical for chylomicron synthesis. The reduced hypertriglyceridemia and chylomicronemia after MG loading in combination with the absence of increased stool fats or appearance of MG or DG in blood in the postprandial state suggest that at least part of the fatty acids fed as monoglycerides is not transported by the lymphatic system but by the portal vein. The influx of these components in the liver could initiate a cascade of reactions, which may explain the appearance of "unusual" triacylglycerols in the circulation (see above) (55), lower adiposity, and lower insulinemia and leptinemia (23).

The concept of portal transport of MAGs based on findings in humans contrasts with findings in rats. Docasahexaenoic acid administered intragastrically as an emulsion in lymph cannulated rats resulted in higher lymphatic output, mainly as TAG, when administered as MAG than as TAG (57). It is not known whether this is caused by the differences in experimental setup or by species differences.

Feeding MAGs together with FFA resulted postprandially in TAG effects. This cannot be caused by the presence of FFA as these have effects different from those of TAGs or MAGs when fed as sole source of fats.

*eq*-DAG feeding has postprandial effects distinct from feeding TAGs with the same fatty acid composition. For instance, in rat liver, decreased activities of

**TABLE 2. Postprandial Lipemic and Lipoproteinemic Effects of Feeding TAGs\*, *eq*-DAGs\*\*, *eq*-MAGs\*, and a 2/1 Mixture of FFA plus *eq*-MAGs\*.**

Fat Fed	Chylomicronemia	Effects Increase in VLDL	Increase in Triglyceridemia	On Fatty Acids <sup>a</sup>
TAGs	high	low	high	high
DAGs	lower		lower	
MAGs	low	low	low	low
FFA	low	high	high	high
MAGs + FFA	high	low	high	high

\* With the fatty acid composition of sunflower seed oil; from Christophe and Verdonk (56).

\*\* With the fatty acid composition of olive oil; from Tagushi et al. (58).

<sup>a</sup> Change of the fatty acid composition of fasting plasma triglycerides toward that of the fat fed.



enzymes involved in fatty acid synthesis and increased activity in those involved in fatty acid oxidation were found after DAG compared with TAG feeding. It is conceivable that when *sn*1,3-DAGs are fed, they will be partially digested and taken up as *sn*1(3)-MAG and FFA. Indeed some of their postprandial effects mimic those of isomeric equilibrium MG feeding (e.g., lower increase of chylomicron TAG [58]).

Postprandial lipemic/lipoproteinemic effects effects of feeding TAGs, DAGs, and TAGs are summarized in Table 2.

## **5. EFFECTS OF STEREOSPECIFIC STRUCTURE OF DIETARY ACYLGLYCEROLS ON CHYLOMICRON CLEARING AND TISSUE TARGETING**

The composition of the fatty acids in the *sn*2-position of chylomicron triacylglycerols is similar to that of the *sn*-2 position of the long-chain fatty acids of the fat fed. Chylomicron clearing is slowed down by saturated fatty acids in the *sn*2-position compared with unsaturated fatty acids (59). More general, plasma clearing of chylomicrons depends on the specific arrangement of acyl chains of the constituting TAGs and not necessarily on their overall saturation (60). The rate of chylomicron clearing relative to uptake of chylomicron remnants is of importance as it affects remnant concentration. Chylomicron remnant removal is strongly influenced by the type of dietary fat with slower clearance with saturated fats with high melting points (61). High concentrations of chylomicron remnants have been correlated with accelerated atherosclerosis (62).

Relative to fatty acids esterified in the 1(3)-position of chylomicron TAGs, fatty acids in the *sn*2-position are delivered to a greater extent to the liver, whereas the fatty acids originally present at the outer positions are taken up to a greater extent by peripheral tissues (63). As biological effects of fatty acids are dependent on their nature, their tissue targeting could be of importance.

## **6. EFFECTS OF STEREOSPECIFIC STRUCTURE OF DIETARY TRIACYLGLYCEROLS ON THEIR HEALTH-RELATED NUTRITIONAL EFFECTS**

The effect different fatty acids have on cholesterolemia is well known. Whatever the kind of the effect (hyper- or hypocholesterolemic), it seems to be more pronounced when the fatty acids are esterified at the inner than at the outer positions of TAGs. The lower hypercholesterolemic effect of saturated fatty acids at the outer positions (64) can be the result of a combination of different factors such as reduced absorption by unabsorbable soap formation, which in turn interferes with cholesterol absorption in the intestinal lumen, partial desaturation and oxidation in the small intestinal cells, and reduced targeting into the liver (63). The stronger hypocholesterolemic effect of polyunsaturated fatty acids at the *sn*-2-position (65) could be the result of increased influx in the liver (66).

Symmetrical stearic acid containing TAGs with oleic acid in the *sn*-2-position (cocoa butter) is absorbed faster than asymmetrical TAGs with saturated fatty acids in the *sn*-2-position, leading to higher postprandial lipemia and activated factor VIIa levels (67).

The atherogenicity of fats containing palmitic acid is higher when this fatty acid is esterified at the *sn*-2-position than at the outer positions, even when cholesterol-emia is not affected (68).

Higher conversion of "parent" essential fatty acids into their longer and more unsaturated metabolites has been described when the parent essential fatty acids were esterified into the *sn*-2-position (66). This may be explained by increased influx in the liver (66).

When infants were fed the same amount of palmitic acid, significantly lower values of high-density lipoprotein-cholesterol and of apolipoprotein A and significantly higher levels of apoB were found in infants when the same amount of palmitic acid was fed in TAGs in the *sn*-2-position than in the *sn*1(3)-position (69). This illustrates that lipoprotein metabolism can be affected by fatty acid distribution in TAGs.

Lipids with palmitic acid mainly in the 1(3)-position caused a larger increase in triglyceridemia and lower increase in insulinemia in the beginning of the postprandial period in postmenopausal women (70).

Clear differences between feeding seal oil, with eicosapentaenoic acid and docosahexaenoic acid mainly at the outer positions of TAG, or fish or squid oil with these fatty acids mainly at the inner position, were obtained on eicosanoid production (71), reduction of triglyceridemia (4), and serum phospholipid arachidonic acid content (72). In general, the positive effects of these fatty acids seem to be more pronounced when they are esterified at the outer position dietary of triacylglycerols. Moreover, fatty acid effects are not only dependent on the nature of the fatty acid and its positional incorporation in acylglycerols but also on coingestion with other fats (73) and other dietary macronutrients (74).

## 7. EFFECTS RELATED TO TRIACYLGLYCEROL HYDROPHOBICITY

The nature of the fatty acids in TAGs determines their hydrophobicity/hydrophilicity and diffusional mobility. In an aqueous/lipid environment, such as adipose tissue or lipoproteins in plasma, the relative hydrophilicity of the TAGs determines their partitioning between the interfacial phase and the apolar phase. This may have far stretching consequences. For instance, the rate and selectivity of fatty acid mobilization from fat cells may affect levels and composition of the nonesterified fatty acids in plasma. These in turn affect lipid homeostasis. Rate and selectivity of fatty acid mobilization from adipose stores are not related to the positional distribution of fatty acids on the glycerol backbone (75). They are related, however, to triacylglycerol hydrophilicity and thus to TAG structure (76).

## 8. EFFECT OF ACYLGLYCEROL STRUCTURE ON NUTRITIONAL EFFECTS

### 8.1. Diacylglycerols

Although the energy value and digestibility of *eq*-DAG are similar to those of TAGs (77), replacing the latter by the former in a mildly reduced-energy diet enhances loss of body weight and fat (78). Long-term *eq*-DAG feeding results in lower triglyceridemia than TAG feeding (79) and enhances the cholesterol-lowering effect of phytosterols (80). DAG feeding does not affect fat-soluble vitamin status (81).

### 8.2. Monoacylglycerols

It is very hard to incorporate relatively large amounts of MAGs in a diet that is acceptable to humans. Although MAGs derived from a polyunsaturated oil are liquid as such, they form sticky phases when they come into contact with water (81), giving an unpleasant mouthfeel when eaten. Even tube feeding can cause problems because the tube can become blocked when the MAGs come in contact with water. Nonetheless, long-term administration of *eq*-MAGs has been achieved in some patients who benefited from them. Often, the MAGs had to be frozen, cut in small pieces, and swallowed as such. Compared with TAGs with the fatty acid composition of sunflower seed oil, long-term MAG administration resulted in higher triglyceridemia and lower cholesterolemia (22). If the properties of MAGs would be indicated for clinical nutrition, structured lipids with the long-chain fatty acid in the *sn*1(3)-position might substitute for them. Structured lipids with the long-chain fatty acid either at the *sn*1- or *sn*3-position could possibly be used to determine differential effects of both isomers. As far as we know, this strategy has not been explored yet.

### 8.3. Phospholipids

Differences in nutritional effects between PLs and TAGs can be caused by several factors not related to their fatty acid composition, such as the presence of a phosphate group and a nitrogen base (mainly choline) that may interact in several metabolic pathways (82). Moreover, several glycerophospholipid preparations studied can contain other components such as cholesterol, cerebrosides, sphingomyelins also depending on their source, method of isolation, and purification. These components may also affect the nutritional properties. In this chapter, the metabolic fate of constituent fatty acids of PLs and TAGs will be compared.

Enrichment of the dietary fat with PLs or TAGs may or may not affect fecal excretion of fat and minerals and may increase or decrease saturated fat absorption depending on the PL and TAG source (83). Long-chain polyunsaturated fatty acids were better absorbed in preterm infants when fed as PLs than as TAGs (84). Feeding long-chain polyunsaturates as PLs or TAGs influences the distribution of these fatty acids in plasma lipoprotein fractions, affects their content in different plasma

lipid fractions, and affects the composition of HDL and LDL phospholipids (83). Arachidonic acid was shown to be more effective for brain arachidonic acid accretion when fed as PL compared with TAG (85).

## **9. SEMI-SYNTHETIC FOOD FATS WITHOUT ACYLGLYCEROL STRUCTURE**

### **9.1. Sucrose Polyesters**

Sucrose polyesters have no primary ester bonds and are not digested. As a result, they remain in the oil phase and are not taken up and are excreted with the stools. In the small intestine, they have some effect on the partitioning of fat-soluble components between the emulsion and micellar phase and as a consequence on their absorption. Reduced absorption of fat-soluble vitamins can be avoided by enriching the sucrose polyesters with these vitamins. Their main use is related to the fact that they can replace usual food fats in many prepared foods but that they do not provide for calories.

### **9.2. Phytosterol and Stanol Esters**

Plant sterols and stanols derived from wood pulp and vegetable oils lower total and low-density lipoprotein cholesterol by inhibiting cholesterol absorption from the intestine in humans. The effect is dose dependent, and significant reductions of apo B are already obtained with doses as low as 1 g/day (86). Because of their solubility properties, sterols/stanols cannot be incorporated in foods to any large extent. Esterification has allowed their incorporation into various foods such as margarine without changing the taste and texture of those foods. During intestinal transit, sterol esters are hydrolyzed to a large extent and reduce hydrolysis of cholesterol esters. The free phytosterols are incorporated into micelles, thus reducing micellar solubilization of cholesterol (87) and consequently its intestinal uptake (88). Unless in some rare diseases, phytosterols/stanols are poorly absorbed and are resecreted into the intestinal lumen through bile (89). They may also have some negative effect on carotenoid absorption, but a healthy diet rich in carotenoids is effective in maintaining normal blood carotenoid levels (90). There are no changes in serum fat-soluble vitamins when a normal diet is consumed (91). Sterol and stanol esters seem to have comparable cholesterol-lowering activity (92). The serum cholesterol-lowering effect of phytosterols is higher when fed together with DAGs than with TAGs (80). Except possessing cholesterol-lowering potency, phytosterols have been suggested to have anticancer properties (93).

## **10. CONCLUSION**

Modern methodology allows the synthesis of special food fats that are not digested or that can yield in high-yield digestion products with stereochemical structures that

are formed only in very small quantities after feeding the common food fats. Triacylglycerols with a different combination of fatty acids and/or with a different stereochemical structure can also be produced. These fats can either have improved long-term health effects or may be useful for clinical nutrition.

## REFERENCES

1. H. Hirschmann, *J. Biol. Chem.*, **235**, 762–2767 (1960).
2. D. Kritchevsky, S. A. Tepper, and D. M. Klurfeld, *Lipids*, **33**, 821–823 (1998).
3. S. Mukherjee, R. Duta, and C. Bandhyopadhyay, *J. Atheroscl. Res.*, **10**, 51–54 (1960).
4. H. Yoshida et al., *J. Nutr. Sci. Vitaminol.*, **45**, 411–421 (1999).
5. C. T. Phan and P. Tso, *Front. Biosci.*, **6**, D299–D319 (2001).
6. L. Sek, C. J. Porter, A. M. Kaukonen, and W. N. Chapman, *J. Pharm. Pharmacol.*, **54**, 29–41 (2002).
7. R. G. Jensen, F. A. De Jong, L. G. Lambert-Davis, and M. Hamosh, *Lipids*, **29**, 433–435 (1994).
8. E. Levy et al., *J. Pediatr. Gastroenterol. Nutr.*, **5**, 784–789 (1984).
9. M. Hamosh, H. L. Klaeveman, R. O. Wolf, and R. O. Scow, *J. Clin. Invest.*, **55**, 908–913 (1975).
10. M. Armand et al., *Am. J. Clin. Nutr.*, **62**, 74–80 (1995).
11. F. Carriere, J. A. Barrowman, R. Verger, and R. Laugier, *Gastroenterology*, **105**, 876–888 (1993).
12. T. H. Liao, M. Hamosh, and P. Hamosh, *P. Pediatr. Res.*, **18**, 402–4090 (1984).
13. R. Jian, N. Vigneron, Y. Najean, Y. Bernie, and J. J. Bernie, *Dig. Dis. Sci.*, **27**, 705–711 (1982).
14. B. D. Maes et al., *Eur. J. Clin. Invest.*, **28**, 197–204 (1998).
15. A. Cortot, S. F. Philips, and J. R. Malagelada, *Gastroenterology*, **82**, 877–881 (1982).
16. J. N. Hunt and M. T. Knox, *J. Physiol.*, **19**, 327–336 (1968).
17. B. D. Maes et al., *Gut*, **38**, 23–27 (1996).
18. M. Wickham, P. Wilde, and A. Fillereley-Travis, *Biochim. Biophys. Acta*, **1580**, 110–122 (2002).
19. X. Xu, L. B. Fomuso, and C. C. Akoh, *J. Agric. Food Chem.*, **48**, 3–10 (2000).
20. F. Carriere et al., *Bioorg. Med. Chem.*, **5**, 429–435 (1997).
21. A. Lykidis, V. Mougios, and P. Arzoglou, *Eur. J. Biochem.* **230**, 892–898 (1995).
22. A. Christophe and G. Verdonk, *Biochem. Soc. Trans.*, **5**, 1041–1042 (1977).
23. T. Murase et al., *J. Lipid Res.*, **42**, 372–378 (2001).
24. G. Boswinkel, J. P. T. Derksen, K. van't Riet, and F. P. Cuperus, *J. Amer. Oil Chem. Soc.*, **73**, 707–711 (1996).
25. G. Lyubachesskaya and E. Boyle-Rode, *Lipids*, **35**, 1353–1358 (2000).
26. V. P. Skipski, M. G. Morehous, and H. J. Duel, *Arch. Biochem. Biophys.*, **81**, 93–104 (1959).
27. A. Christophe, G. Verdonk, M. Mashali, and P. Sandra, *Lipids*, **17**, 759–761 (1982).

28. H. Mu and C. E. Hoy, *Lipids*, **37**, 329–331 (2002).
29. Y. Ohkubo et al., *Digestion*, **51**, 42–50 (1992).
30. R. Lehner and A. Kuksis, *J. Biol. Chem.*, **268**, 24726–24733 (1993).
31. P. R. Redden, X. Lin, J. Fahey, and D. F. Horrobin, *J. Chromatog A*, **704**, 99–111 (1995).
32. D. R. Saunders and J. Sillery, *J. Lipids*, **11**, 830–832 (1976).
33. G. Nalbhone et al., *Biochim. Biophys. Acta*, **620**, 612–625 (1980).
34. B. Borgstrom, *Gastroenterology*, **78**, 954–962 (1984).
35. A. J. Rampone and L. R. Long, *Biochim. Biophys. Acta*, **486**, 500–510 (1977).
36. T. Sugawara et al., *J. Nutr.*, **131**, 2921–2927 (2001).
37. P. J. A. O'Doherty, I. M. Yousef, and A. Kuksis, *Can. J. Biochem.*, **52**, 726–733 (1974).
38. O. Zierenberg, J. Odenthal, and H. Betzing, *Atherosclerosis*, **34**, 259–276 (1979).
39. R. Shamir et al., *Biochem.*, **16**, 6351–6358 (1995).
40. C. M. Mansbach, R. S. Cohen, and P. B. Leff, *J. Clin. Invest.*, **56**, 781–791 (1975).
41. D. M. Small, *Ann Rev. Nutr.*, **11**, 413–434 (1991).
42. Y. Shahkhalili et al., *Am. J. Clin. Nutr.*, **73**, 246–252 (2001).
43. T. Aoyama et al., *J. Nutr.*, **126**, 225–231 (1996).
44. B. Boucroy and J. Clement, *Biochim. Biophys. Acta*, **187**, 59–72 (1969).
45. A. Nilsson and B. Becker, *Am. J. Physiol.*, **268**, G732–G738 (1995).
46. Y. S. Huang, J. W. Lin, K. Koba, and S. N. Anderson, *Mol. Cell. Biochem.*, **18**, 121–130 (1995).
47. Q. Chen and A. Nilsson, *J. Lipid Res.*, **35**, 601–609 (1994).
48. T. Murase, H. Kondo, T. Hase, I. Tokimitsu, and M. Saito, *Biochim. Biophys. Acta*, **1530**, 15–22 (2001).
49. I. R. Sanderson and S. Naik, *S. Ann Rev. Nutr.*, **20**, 311–338 (2000).
50. B. J. De Jong and B. C. Hulsmann, *Biochim. Biophys. Acta*, **27**, 36–46 (1978).
51. A. M. Hanel and M. H. Gelb, *Biochemistry*, **20**, 7807–7818 (1995).
52. R. Lehner, A. Kuksis, and Y. Itabashi, *Lipids*, **28**, 29–34 (1993).
53. R. Paris and G. Clement, *Proc. Soc. Exptl. Biol. Med.*, **131**, 363–365 (1969).
54. S. M. Duff, S. Kalambur, and E. Boyle-Roden, *J. Nutr.*, **131**, 774–778 (2001).
55. A. Christophe and G. Verdonk, *Arch. Intern. Physiol. Biochim.*, **88**, B221–B222 (1980).
56. A. Christophe and G. Verdonk, *Arch. Intern. Physiol. Biochim.*, **80**, 954–955 (1972).
57. F. Banno, S. Doisaki, N. Shimizu, and K. Fujimoto, *J. Nutr. Sci. Vitaminol.* **48**, 30–35 (2002).
58. H. Tagushi et al., *J. Am. Coll. Nutr.*, **19**, 789–796 (2000).
59. B. C. Mortimer, M. A. Kenrick, D. J. Holthouse, R. V. Stick, and T. G. Redgrave, *Biochim. Biophys. Acta*, **1127**, 67–73 (1992).
60. B. C. Mortimer, D. J. Holthouse, I. J., Martins, R. V. Stick, and T. G. Redgrave, *Biochim. Biophys. Acta*, **1211**, 171–180 (1994).
61. C. T. Phan, B. C. Mortimer, I. J. Martins, and T. G. Redgrave, *Am. J. Clin. Nutr.*, **69**, 1151–1161 (1999).
62. K. C. Yu and A. D. Cooper, *Front Biosci.*, **6**, D332–D354 (2001).
63. D. A. Pufal, P. T. Quinlan, and A. M. Salter, *Biochim. Biophys. Acta*, **1258**, 41–48 (1995).

64. I. Yamamoto, M. Sugano, and M. Wada, *Atheroscl.*, **13**, 171–184 (1971).
65. C. E. Elson, L. R. Dugan, L. J. Bratzler, and A. M. Parson, *Lipids*, **1**, 322–324 (1967).
66. S. C. Renaud, J. C. Ruf, and D. Pethitory, *J. Nutr.*, **125**, 229–237 (1995).
67. T. A. Sanders, S. E. Berry, and G. J. Miller, *Am. J. Clin. Nutr.*, **74**, 777–782 (2003).
68. D. Kritchevski, in A. B. Christophe, ed., *Structural Modified Food Fats: Synthesis, Biochemistry and Use*, AOCS Press, Champaign, Illinois, 1998, pp. 183–188.
69. C. M. Nelsson and S. M. Innis, *Am. J. Clin. Nutr.*, **70**, 62–69 (1999).
70. K. Yli-Jokipii et al., *J. Lipid Res.*, **42**, 1618–1628 (2001).
71. I. Ikeda et al., *Lipids*, **33**, 897–904 (1998).
72. H. Yoshida, I. Ikeda, and M. Tomooka, *J. Nutr. Sci. Vitaminol.*, **47**, 242–247 (2001).
73. L. D. Lawson and B. G. Hughes, *Biochim. Biophys. Res. Comm.*, **156**, 960–963 (1988).
74. S. Kaku et al., *Biosci. Biotechnol. Biochem.*, **65**, 315–351 (2001).
75. T. Raclot, C. Leray, A. C. Bach, and R. Groscolas, *Biochem. J.*, **311**, 911–916 (1995).
76. T. Raclot, *Progr. Lipid Res.*, **42**, 257–288 (2003).
77. H. Taguchi et al., *Lipids*, **36**, 379–382 (2001).
78. K. C. Maki et al., *Am. J. Clin. Nutr.*, **76**, 1230–1236 (2002).
79. K. Yamamoto et al., *J. Nutr.*, **131**, 3204–3207 (2001).
80. S. Meguro et al., *Eur. J. Clin. Nutr.*, **55**, 513–517 (2001).
81. H. Wanatabe et al., *Ann. Nutr. Metab.*, **45**, 259–264 (2001).
82. S. H. Zeisel and J. K. Blusztajn, *Annu. Rev. Nutr.*, **14**, 269–296 (1994).
83. L. Amate, A. Gil, and M. Ramirez, *J. Nutr.*, **131**, 3216–3221 (2001).
84. L. Amate and M. Ramirez, *J. Nutr.*, **131**, 1250–1255 (2001).
85. V. Wijendran et al., *Pediatr. Res.*, **51**, 265–272 (2002).
86. M. A. Hallikainen, E. S. Sarkinen, and M. I. Uusitupa, *J. Nutr.*, **130**, 767–776 (2000).
87. M. Nissinen, H. Gylling, M. Vuoristo, and T. A. Miettinen, *Am. J. Physiol.*, **282**, G1009–G1015 (2002).
88. P. J. Jones et al., *J. Lipid Res.*, **41**, 679–705 (2000).
89. T. A. Miettinen et al., *Am. J. Clin. Nutr.*, **71**, 1095–1102 (2000).
90. F. Y. Ntanios and G. S. Duchateau, *Int. J. Vitam. Nutr. Res.*, **72**, 32–39 (2002).
91. M. Raeini-Sarjaz, F. Y. Ntanios, C. A. Vanstone, and P. J. Jones, *Metabolism*, **51**, 652–656 (2002).
92. M. A. Hallikainen et al., *Eur. J. Clin. Nutr.*, **54**, 715–725 (2000).
93. A. B. Awad and C. S. Fink, *J. Nutr.*, **130**, 2127–2130 (2000).

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## *Modification of Fats and Oils via Chemical and Enzymatic Methods*

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### 1. INTRODUCTION

Edible fats and oils (lipids) are derived from plant, animal, and marine sources. Fats and oils differ in that fats are solids at normal room temperature whereas oils are liquids under similar conditions. Lipids are recognized as essential nutrients in both human and animal diets. They provide the most concentrated source of energy of any foods. The caloric value of lipids (9 kcal/g) exceeds twice that of proteins and carbohydrates (4 kcal/g). Lipids not only contribute to flavor, color, odor, and texture of foods, but also confer a feeling of satiety after eating. Lipids also act as carriers of fat-soluble vitamins, supply essential fatty acids, and increase the palatability of foods. Dietary fats are often categorized as “visible” or “invisible”



fats: Visible fats are those consumed in the diet as foods such as butter, margarine, spreads, salad oil, lard, and shortening; invisible fats are those naturally occurring in basic foods, such as milk, cheese, and meat.

Triacylglycerols (TAGs) are the predominant component of most edible fats and oils. Lipids, however, usually contain small amounts of various minor components and include monoacylglycerols (MAGs), diacylglycerols (DAGs), phospholipids, pigments, sterols, free fatty acids (FFAs), fatty alcohols, fat-soluble vitamins, and other substances. The functional properties and quality characteristics of fats and oils are directly related to the type of TAGs in the fats and oils. The types of TAGs are determined by the fatty acid composition and stereospecific distribution of fatty acids on the individual TAG molecules. The physical characteristics of edible fats and oils depend on the nature of their constituent fatty acids (i.e., degree of unsaturation, carbon chain length of the fatty acids, the presence of geometric isomers of fatty acids, and positional distribution of the fatty acids), molecular configuration of TAGs, and polymorphic forms. In effect, the unique fatty acid distribution patterns of some natural fats limit their industrial application.

Hydrogenation, interesterification, fractionation, and blending are distinct processes that can be applied to modify the physical or chemical properties of fats and oils in order to improve their usefulness. These processes can be used alone or in combination with each other. By combining hydrogenation, fractionation, and interesterification with the simple blending of native and modified oils, it is possible to engineer a wide variety of fats and oils with characteristics suited to specific applications. Hydrogenation is used to convert liquid oils into products having different consistencies, melting points, and textures. On the other hand, interesterification produces changes in physical properties by rearrangement or redistribution of fatty acids within and among the TAGs of oils. Fractionation provides a means of producing fats and oils with sharply defined melting characteristics.

## 2. HYDROGENATION

Hydrogenation is the process most widely employed to change the physical characteristics of natural fats and oils to make them better suited for specific applications. Hydrogenation of edible fats and oils involves the addition of hydrogen, in the presence of a catalyst, to the carbon-carbon double bonds present in the fatty acid chains. It is a process that creates consistency in fats and oils by converting unsaturated low melting TAGs into higher melting solid TAGs. The purpose of the hydrogenation process is two fold: (1) to convert liquid oils into semisolid or plastic fats for specific food applications, and (2) to improve the oxidative stability of the oil to maintain its organoleptic acceptability as a human food for an extended period of time. During the hydrogenation of fats and oils, three important reactions occur simultaneously: (1) saturation of double bonds with hydrogen, (2) *cis-trans*-isomerization of double bonds, and (3) migration of double bonds to new positions in the fatty acid carbon chain. Changes to reaction conditions will have an effect on the relative rates of these three reactions.

Hydrogenation is a three-phase reaction in which solid catalyst, gaseous hydrogen, and liquid oil interact. Both batch and continuous processes are used. In the process of hydrogenation, the oil is mixed with an appropriate catalyst and heated to a suitable temperature and hydrogen is then added at high pressure (up to 60 psig). At elevated temperatures and pressures, the solubility of hydrogen in the oil is increased, thus increasing the rate of the reaction. Only hydrogen, which is dissolved in the oil, is available for the reaction. After the hydrogenation process is completed, the catalyst is removed from the fat by filtration. In commercial practice, two types of hydrogenation are performed: nonselective hydrogenation, under conditions such as 50 psig, 0.05% (wt% of oil) catalyst at 121°C; and selective hydrogenation, under conditions such as 5–14 psig, 0.05% catalyst at 177°C (1). These processes are discussed in another chapter in this series.

### 3. FRACTIONATION

Fractionation of edible fats and oils has become an important and a versatile oil-modification process. Fractionation separates fats and oils into fractions with different melting points. Fractionated fats and oils have been used to prepare a variety of foods such as margarines, shortenings, salad oils, frying oils, and confectionery products. Fractionation also is employed to split diacylglycerols (DAGs) and monoacylglycerols (MAGs) to produce various fractions with desirable properties for manufacturing of soap, or for oleochemical and pharmaceutical applications.

The principle of oil fractionation is based on the difference in solubility of the component TAGs. The difference in solubility is directly related to the types of TAGs in the fats and oils. Three major processes are employed commercially to produce value-added fractionated fats and oils: (1) dry fractionation, (2) solvent fractionation, and (3) aqueous detergent fractionation.

Dry fractionation of fats and oils is based on the gradual cooling of the oil under controlled conditions without solvent. In this process, no additives or chemicals are used. When the oil reaches the desired temperature, the cooling is stopped and the solid TAGs are allowed to separate from the liquid TAGs. The separation of the liquid (olein) and solid (stearin) fractions may be performed by centrifugation. Dry fractionation is generally used for the separation of the stearin and olein fractions of palm oil and lauric fats.

Solvent fractionation is the term used to describe the crystallization process of a desired fat fraction from oil that is solubilized in a suitable solvent. Fat fractions may be selectively crystallized at different temperatures, after which the fractions are separated and the solvent removed. This procedure is suitable for the preparation of value-added products. The production of cocoa butter equivalents, cocoa butter substitutes, cocoa butter replacers, and medium-chain triacylglycerols (MCTs) are well-known applications for this type of fractionation.

In detergent fractionation, an aqueous detergent solution (5% sodium lauryl sulfate) is added to the crystallized material to assist in the separation of the liquid olein and the solid stearin fractions. The separation of the two phases can be

performed by centrifugation. Traces of detergent in the olein fraction can be removed by water washing. This process is widely employed for palm oil and tallow.

#### 4. BLENDING

After the refining process, edible fats and oils may be blended together to produce a product with a specified fatty acid composition, or consistency and stability requirements. Blending of two or more fats is widely used in the production of margarines, shortenings, frying oils, salad oils, and some specialty oils. This process is economical, requires little capital investment, and does not change the chemical characteristics of the blend components. The mixtures of oils are blended to meet both the desired fatty acid composition and consistency of the final product. The consistency of the product can be established using various analytical criteria, such as fatty acid composition, solid fat index, iodine value, and melting point. For example, shortening and margarine oil blends are manufactured to a uniform specification by blending to a given solid-fat content.

The blending process requires scale tanks and meters to proportion the starting materials accurately for each type of product. The blend tanks should be equipped with agitators and heating devices to ensure uniform blend of products. Nitrogen blanketing also should be provided to protect the oil from oxidation.

Simple physical blending of medium-chain triacylglycerol (MCTs) and long-chain triacylglycerols (LCTs) has been used in certain enteral and parenteral TAGs applications. Blending is a simple and economical way for providing medium-chain fatty acids (MCFAs) to individuals who require them. Incorporation of MCFAs into structured lipids, via chemical or enzymatic reactions, provides a more nutritionally available source of MCFAs than simple physical blending.

#### 5. INTERESTERIFICATION

Chemical interesterification is one of the major reactions used by the industry for modification of natural fats and oils. It is a process that is used to modify the physical and functional properties of TAG mixtures in fats and oils (2). In its simplest form, interesterification corresponds to an exchange of acyl groups between two TAGs, resulting in the formation of new TAGs that have chemical and physical properties deemed superior to those of the starting TAGs (3). In general, chemical interesterification involves the random removal of fatty acids from the original TAGs, shuffling these fatty acids, and random replacement of them on the new TAGs.

##### 5.1. Types of Interesterification

The term “interesterification” is often used to describe reactions that involve the exchange of acyl residues between an ester and an acid (acidolysis), an ester and an alcohol (alcoholysis), or an ester with another ester (*trans*-esterification).

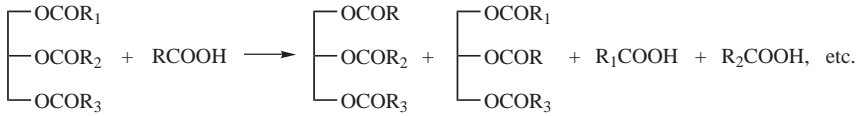
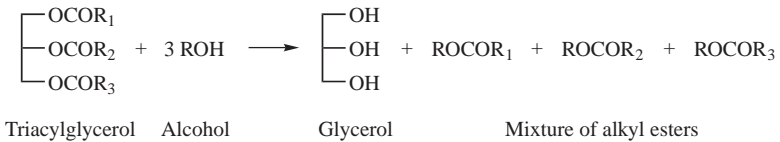


Figure 1. Acidolysis reaction.

**5.1.1. Acidolysis** Acidolysis involves the transfer of an acyl group between a free fatty acid (FFA) and a TAG (Figure 1). This reaction takes place under acidic conditions. The added acid catalyst may catalyze the partial hydrolysis of fatty acids from the TAGs producing the required DAGs and MAGs. Fatty acids can now react with the free hydroxyl groups of the DAGs and MAGs, which results in the synthesis of new TAGs (4). These reactions can produce an equilibrium mixture of reactants and products or can be driven to completion by physically removing one of the reaction products. Theoretically, the reaction could also be pushed to higher yields by using excess amounts of free fatty acids, however, this is not practical because of the high cost of fatty acids and the need to separate large amounts of FFAs from the final product once the acidolysis reaction is completed (4). Acidolysis is an effective means of incorporating novel fatty acids into TAGs and may be used to incorporate eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) into vegetable and fish oils to improve their nutritional properties. For example, borage oil and DHA can be reacted to partially replace the linoleic acid of borage oil with DHA (5).

**5.1.2. Alcoholysis and Glycerolysis** Alcoholysis is the esterification reaction between an alcohol and a TAG (Figure 2a). In the alcoholysis of vegetable oils, a TAG reacts with an alcohol in the presence of a strong acid or base, producing a mixture of alkyl esters and glycerol. The alcoholysis process is a sequence of

(a) Alcoholysis



(b) Glycerolysis

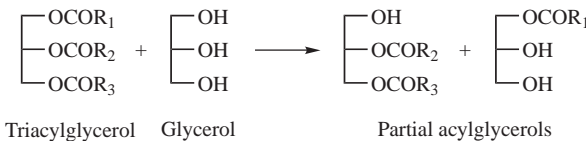


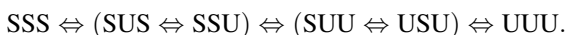
Figure 2. Alcoholysis and glycerolysis.

consecutive and reversible reactions, in which MAGs and DAGs are produced as intermediates (6). The stoichiometric reaction requires one mole of TAG and three moles of alcohol. An excess of the alcohol, however, may be used to increase the yield of the alkyl esters and to allow phase separation from the glycerol formed.

Glycerolysis is the exchange of acyl groups between glycerol and a TAG (Figure 2b). Most of the MAGs and DAGs are produced via chemical-catalyzed glycerolysis of TAGs. In this process, TAGs are mixed with an excess of glycerol at elevated temperatures in the presence of an alkaline catalyst, usually sodium hydroxide. The reaction mixture is kept at an elevated temperature until the fatty acid groups of the TAGs are redistributed at random among the available hydroxyl groups of the glycerol. The reaction mixture after reaching equilibrium is cooled and the catalyst inactivated by addition of a food-grade acid, usually phosphoric acid. The deactivated catalyst must then be removed by filtration. The excess glycerol dissolved in the reaction mixture must be subsequently removed by vacuum distillation. This process yields substantial amounts of MAGs and DAGs.

Enzymatic glycerolysis of fats and oils under atmospheric pressure and ambient temperatures has been investigated as an alternative method to the conventional chemical procedures used for industrial production of MAGs and DAGs (7). There are several ways to produce MAGs, which are of great importance in the food industry as surface-active agents and emulsifiers. MAGs may be produced by ester exchange between TAGs and glycerol, or by reaction of FFAs and glycerol, with the former reaction termed glycerolysis and the latter acidolysis.

**5.1.3. *Trans-esterification (Ester Interchange)*** *Trans-esterification* is the most widely employed type of interesterification reaction used by the food industry. *Trans-esterification* results in the “shuffling” of fatty acids within a single TAG molecule (intraesterification) and among TAG molecules until an equilibrium is achieved in which all possible combinations are formed (2). Figure 3 shows the complete randomization of fatty acids in a typical TAG containing stearic, oleic, and linoleic acids (1-stearoyl-2-oleoyl-3-linoleoyl glycerol; SOL). During the reaction, the first step involves the cleavage of ester bonds linking fatty acyl residues to the glycerol backbone. The newly liberated fatty acids form a fatty acid pool that are re-esterified onto a new position, either on the same glycerol (intraesterification) or onto another glycerol (interesterification) molecule (8). Once the reaction reaches equilibrium, a mixture of disaturated, monosaturated, triunsaturated, and trisaturated TAG components is obtained (Figure 3). The equilibrium may be simplified and represented as:



## 5.2. Chemical Interesterification

Chemical interesterification results in a complete randomization of acyl groups in the TAGs. Interesterification, alone or in combination with other processes, extends

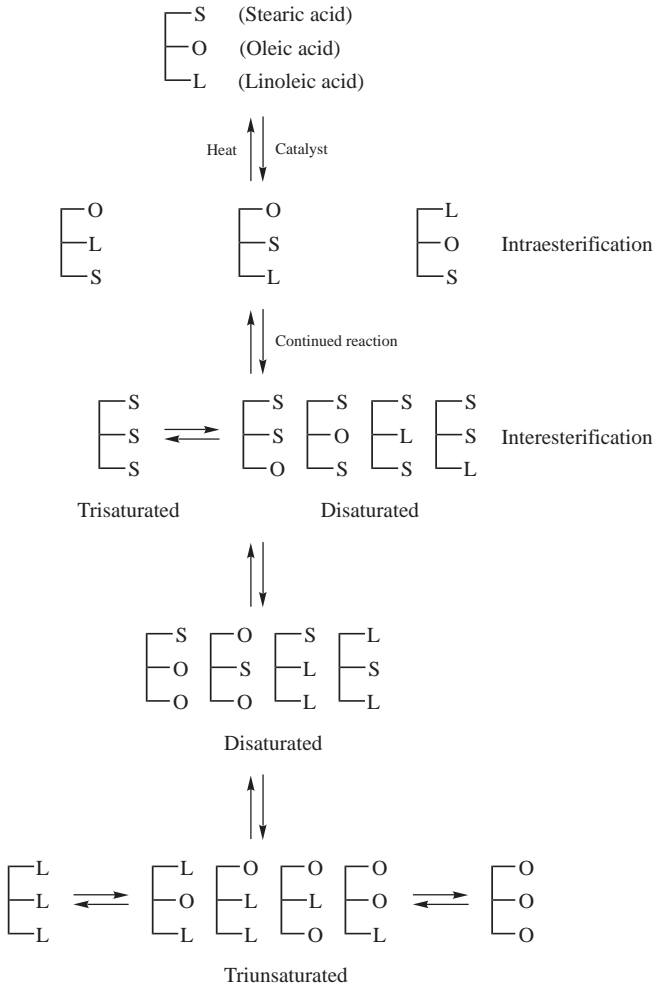


Figure 3. Formation of triacylglycerols during interesterification [Adapted from (2)].

the utility of edible oils, and such modified oils are extensively employed in a wide variety of food applications. Commercially, chemical interesterification is used for processing of edible fats and oils to produce margarines, spreads, cooking oils, shortenings, confectionery fats, and reduced-calorie lipids.

Two mechanisms have been proposed for alkaline-catalyzed chemical interesterification; enolate anion formation and carbonyl addition.

**5.2.1. Enolate Ion Formation** This mechanism suggests that the reaction begins when the sodium methoxide (catalyst) attacks the acidic hydrogen from the carbon  $\alpha$  to the carbonyl carbon to produce an enolate structure (Figure 4). This reaction produces a carbanion, which is a strong nucleophile. The enolate anion then reacts

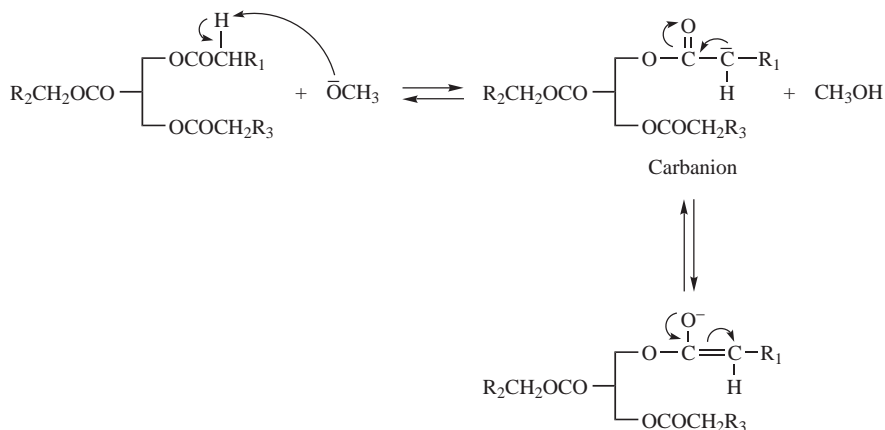


Figure 4. Enolate anion formation.

with another ester group in the TAG molecule to produce a  $\beta$ -keto ester and a glycerylate, which, in turn, reacts further with other carbonyl carbons to afford other  $\beta$ -keto esters. In this way, all ester groups in the TAG may react and move around from their initial positions. The same mode of action applies to ester interchange between two or more TAG molecules. The intra-ester interchange is believed to predominate in the initial stages of the reaction. This mechanism is referred to as the Claisen condensation pathway.

**5.2.2. Carbonyl Addition** In this reaction, the alkylate ion (nucleophile) adds on to the slightly positively-charged carbonyl carbon at one of the three fatty acylglycerol ester bonds and forms a tetrahedral intermediate (Figure 5). The fatty acid methyl ester is then released, regenerating a glycerylate anion for further reaction.

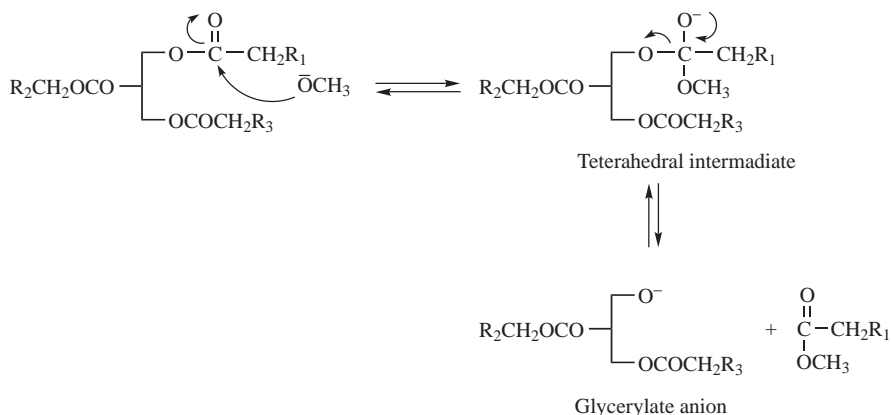
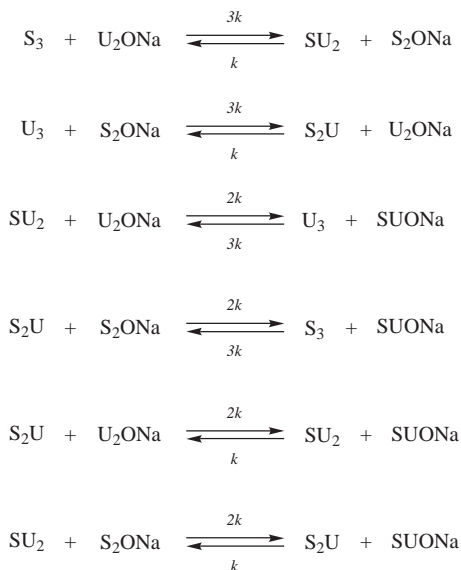


Figure 5. Carbonyl addition.

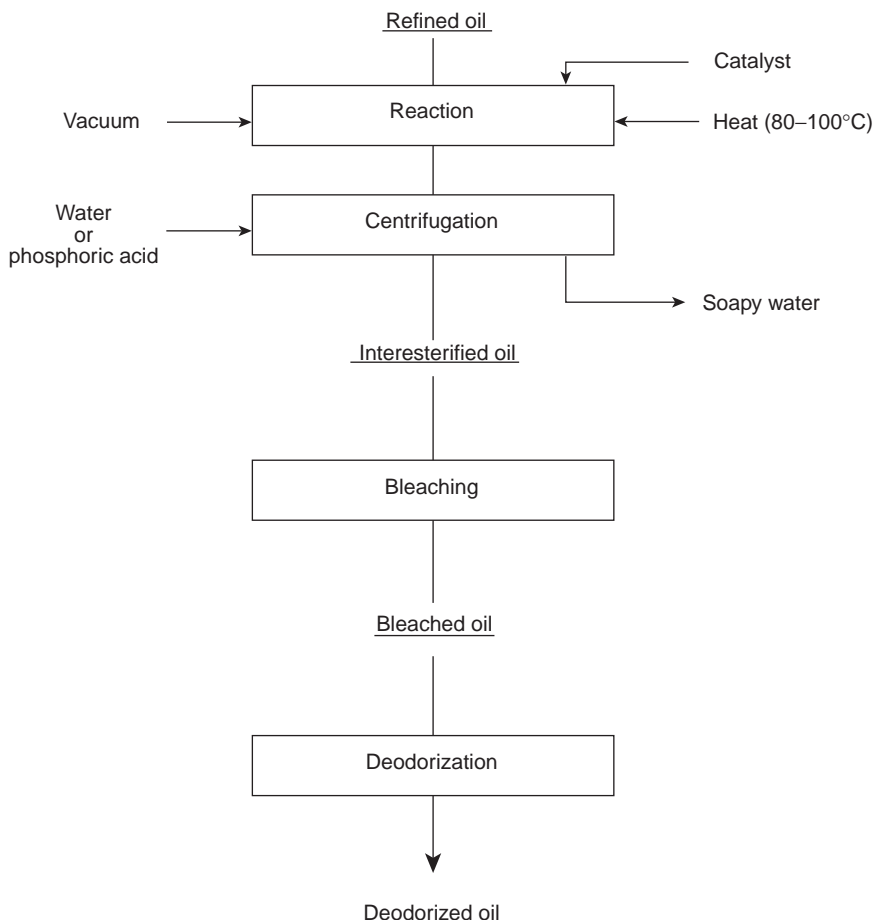


**Figure 6.** Kinetics of interesterification via carbonyl addition ( $S$ ,  $S_2$ ,  $S_3$  and  $U$ ,  $U_2$ ,  $U_3$ : mono-, di-, and trisaturated and unsaturated, respectively) [Adapted from (9)].

The glycerylate anion functions as the real catalyst and transfers acyl groups around the glycerol backbone. It should be noted that with the carbonyl addition mechanism, both intra- and interesterification reactions are possible (4). The similarity in the two mechanisms is that glycerylate anions are formed via both mechanisms. The basic difference is that, in the first instance, both  $\beta$ -keto esters and glycerylate ions are the acyl donors for interesterification, and in the second, only glycerylate ions are the acyl donors. Ester interchange between fully saturated  $S_3$  and fully unsaturated  $U_3$  molecules is shown in Figure 6, as a model for the randomization, which occurs via the carbonyl reaction mechanism (9). The kinetics were demonstrated with six possible reactions between various glycerylate anions with equilibrium constants of  $3k$ ,  $2k$ , and  $2/3k$  (Figure 6).

**5.2.3. Chemical Interesterification Process** Interesterification results when oil is heated at relatively high temperatures ( $\sim 200^\circ\text{C}$ ) for a considerably long period of time. However, when carried out in the presence of catalysts, chemical interesterification can be performed at lower temperatures (as low as  $50^\circ\text{C}$ ) than when it is carried out without catalyst. The catalyst used can be acids, bases, or their corresponding salts and metals. Approximately 0.1% catalyst is required (wt% of oil). The most commonly used catalysts are sodium methylate or ethylate, sodium-potassium alloy (NaK), and sodium or potassium hydroxide. The advantages of using metal alkylate catalysts are high activity, ready availability, low cost, and ease of handling. These compounds may not be the real catalysts, but serve as initiators in the process of forming the "true catalyst." When the catalyst is





**Figure 7.** Typical chemical interesterification process.

dispersed in a dried oil maintained at 60–80°C, a white slurry is formed. A few minutes after the catalyst is added, the oil acquires a reddish brown color, which deepens as the reaction continues. The reddish brown color is a result of the formation of a complex between the sodium and the TAGs and is believed to be the active catalyst. At the end of the interesterification reaction, the catalyst must be inactivated and removed. Most chemical catalysts can be removed by washing the reaction mixture with water or phosphoric acid. A typical example of a chemical interesterification is shown in Figure 7. Methods employed for defining the end point of the interesterification reaction include melting point, cloud point, and solid-fat content, among others.

The oil and catalyst must be extremely dry because moisture can deactivate the catalyst and cause soap formation. As even trace amounts of water decrease the potency of the catalyst tremendously, the oil should contain < 0.01% (w/w) water

(4). In addition to moisture, FFAs and peroxides also will poison the catalyst. For successful interesterification, most of the crude oil impurities must be removed, and refining operations should be performed beforehand.

Two basic types of chemical interesterification processes involving the use of metal catalysts are employed: random and directed.

**5.2.4. Random Chemical Interesterification** Random interesterification is the most commonly used approach to interesterification. In random chemical interesterification, fatty acyl groups freely move from one position to another in a single TAG molecule or from one TAG to another. This continues until the arrangement of fatty acids in the TAGs is completely random. As the fatty acids rearrange, they reach an equilibrium. A change from ordered to random arrangement results in an alteration of physical characteristics of the starting fats and oils, such as melting profile and melting point. Three distinct steps are involved in the random chemical interesterification process: (1) pretreatment of the oil, (2) reaction with the catalyst, and (3) deactivation of the catalyst. Random chemical interesterification can be done using either batch or continuous processes. Interesterification normally does not result in the production of either *trans*-fatty acids or positional isomers of fatty acids.

**5.2.5. Directed Chemical Interesterification** This process modifies the fatty acid randomization by altering the reaction equilibrium in the mixture. In directed chemical interesterification, one or more of the TAG products of the interesterification reaction is selectively removed from the ongoing reaction. As this reaction is directed to produce a particular type of TAG, it is referred to as directed interesterification, which is usually done at low temperature. Interesterification is directed away from randomness because the oil is maintained at a temperature below the melting point of the highest melting component that might result. This results in selective crystallization of the trisaturated TAG species, with the effect of removing them from the reaction mixture as they crystallize on formation. Interesterification thus proceeds with the formation of more trisaturated TAGs than would have otherwise occurred. During directed interesterification, two distinct reactions take place simultaneously. As trisaturated TAGs are formed, they crystallize from solution and the fatty acid composition of the remaining liquid phase keeps changing. The reaction equilibrium in the liquid phase is pushed toward increased production of the crystallization of trisaturated TAGs (10). The process continues until most of the trisaturates in the oil have precipitated from the reaction medium. Directed interesterification reactions normally take considerably longer because the temperature used is much lower than that for a random interesterification and the additional time is required for TAG crystallization. In this process, only catalysts that are active at low temperatures are used. Sodium-potassium alloy is more suitable for this type of reactions as it is highly active at low temperatures. Continuous processes are normally used for directed chemical interesterification because batch processes are difficult to control.

### 5.3. Enzymatic Interesterification

Microbial lipases may be used as biocatalysts for interesterification. Lipase-assisted interesterification offers possibilities for transformation of lipids beyond those possible using chemical interesterification (11). Enzymatic interesterification has several advantages over the chemical-assisted reactions, such as mild reaction conditions leading to reduced energy consumption and less thermal damage to reactants and products, the possibility of lipase specificity toward their natural substrates, as well as high catalytic efficiency. Lipase-catalyzed interesterification reactions, in contrast to those carried out with chemical catalysts, yield different types of products depending on the specificity of the lipase used.

Lipase-catalyzed interesterification of fats and oils can be accomplished using either a batch or continuous process. Among these, continuous interesterification is the preferred reaction and can be performed using a fixed-bed reactor. The advantages of using continuous interesterification process are catalyst recovery and reduced reaction time. Processing conditions (temperature, pH, and moisture content) must be properly controlled and impurities in the starting material kept at a minimum to prevent inactivation of the enzyme catalyst. The continuous interesterification begins by dissolving the starting oil in a solvent followed by treatment to remove enzyme inhibitors and particulate matter. This solution is partially saturated with water before pumping through a bed of hydrated biocatalyst particles. The reaction products are a mixture of TAGs and FFAs. After the reaction, the FFAs are removed by distillation. The TAGs free from FFA are then solvent fractionated to yield the desired TAG composition.

To date, lipase-catalyzed interesterification has been applied almost exclusively to the production of value-added products. One of the best examples is the manufacture of cocoa-butter-type TAGs. The potential of *sn*-1,3-specific lipases for production of cocoa butter substitutes relies on enzyme-catalyzed *trans*-esterification or acidolysis of palm oil with tristearin or stearic acid, respectively.

### 5.4. Applications

Intesterification has a number of applications in the food industry. Chemical interesterification is used commercially to produce modified edible fats and oils for margarines and spreads, shortenings, confectionery fats, reduced-calorie fats and oils, and infant formula.

**5.4.1. Margarines and Spreads** Margarines and spreads are prepared by blending fats and oils with other ingredients, such as water, milk products, edible proteins, salt, flavorings, colorings, and Vitamins A and D. In the United States and Canada, margarine is marketed as a butter alternative and must contain 80% fat. There are many formulations for making margarines; practically all of which contain partially hydrogenated oils and, therefore, appreciable levels of *trans*-fatty acids. Attempts are being made to reduce the level of *trans*-fatty acids in these products as a result of recent nutritional concerns on such fatty acid isomers (12).

Interesterification has been applied to the production of high-stability margarine blends and hard butters that have desirable melting qualities. Margarines, spreads and shortenings made from partially hydrogenated canola, soybean, coconut, olive, peanut, and sunflower oils tend to develop  $\beta$ -type polymorph crystals. This can be prevented by the incorporation of some cottonseed oil, palm olein, hydrogenated palm oil, tallow, or hydrogenated fish oils, all of which stabilize crystals in the  $\beta'$  form. The  $\beta'$ -type crystals are desirable for butter, margarine, and spreads and in the latter two cases, a serious “graininess” defect may result from the formation of large coarse  $\beta$  crystals. Potential problems of “graininess” may be overcome by using  $\beta$ -type fats as the “soft” component of the fat blend, although other conditions may be manipulated to favor the formation of  $\beta'$ -type crystals. For this reason, interesterification may be used to enable margarine and spreads to be made from vegetable oils without problems associated with  $\beta$ -type crystal formation. Interesterification affects the crystallization behavior of fats and oils. Oils that normally have  $\beta$  crystallization tendencies shift significantly toward  $\beta'$  after the interesterification process. Soybean oil generally tends to crystallize in the  $\beta$  form. It has been shown that an interesterified blend of liquid soybean oil and fully hydrogenated soybean oil crystallizes in the more desirable  $\beta'$  form (13). The primary purposes of using interesterification are to minimize *trans*-fatty acid content in margarines and spreads and, to a lesser extent, promote crystallization in the  $\beta'$  form.

**5.4.2. Shortenings** The term “shortenings” was originally used to describe the function performed by solid fats such as lard and butter in baked foods. Shortenings are 100% fat products that vary in consistency according to their temperature and the proportion of solid fat to liquid oil. Lard and other animal fats as well as many types of vegetable oils, including palm, soybean, corn, sunflower, canola, and cottonseed, can be used in shortening products.

The most important crystal forms in the production of shortenings are  $\alpha$ ,  $\beta$ , and  $\beta'$ , of which the last two are the more stable forms. The desired form for most shortenings is the  $\beta'$  structure. Interesterification is employed mostly in the manufacture of shortenings and has been used to improve the physical properties of lard. Shortenings made from natural lard possess a grainy consistency, poor creaming capacity, limited plastic range, and poor performance in baking. Natural lard, as a result of its high proportion of disaturated TAGs with palmitic acid predominantly (64%) in the *sn*-2 position, forms relatively large and coarse crystals, even when scraped surface heat exchange equipment is used to minimize this  $\beta$  crystal form tendency. During interesterification, the proportion of palmitic acid in the *sn*-2 position is reduced from approximately 64% to 24%. Interesterification also helps to reduce the solid-fat content of lard at 20°C, prevent the development of graininess, and improve its plastic range, which makes it a better shortening. Hence, interesterification stabilizes the lard in the  $\beta'$  form by rearrangement of its palmitic acyl residues, which improves the functionality of lard for most shortening applications. Directed interesterification, on the other hand, may result in a product with a higher total solids content at high temperatures and, thus, an extended plastic range.

**5.4.3. Cocoa Butter Alternative Fats** Cocoa butter alternative fats are substances with physical and functional properties similar to cocoa butter. Cocoa butter is a solid fat obtained from cocoa beans along with cocoa powder. The use of interesterification makes it possible to produce cocoa butter alternatives from other vegetable oils that better mimic the composition and properties of cocoa butter. Cocoa butter alternatives can be categorized into three main groups: (1) cocoa butter equivalents (CBE), (2) cocoa butter substitutes (CBS), and (3) cocoa butter replacers (CBR). CBE have the same general chemical composition and, hence, similar melting and crystallization properties as cocoa butter. They include tropical oils, such as those from palm, illipe, shea, sal, and kokum. These oils can be blended to give mixtures of POP, POST, and StOSt very similar to cocoa butter. CBS are predominantly produced from lauric fats such as coconut and palm kernel and may involve processing techniques such as hydrogenation, interesterification, and fractionation. CBS share some of the physical properties of cocoa butter, but have a different chemical composition. The best material for CBS is fully hydrogenated palm kernel stearin with an iodine value of  $< 1$  (14). This product has a very steep melting curve, similar to cocoa butter, thus ensuring that the organoleptic properties of the end product are comparable. CBR are derived from vegetable oils (palm, soybean, or cottonseed oil) that have been subjected to partial hydrogenation or a combination of hydrogenation and fractionation processes; these are suitable for bakery products and confectionery applications.

**5.4.4. Milkfat and Milkfat Substitutes** Milkfat is a natural fat with unique physical, chemical, and biological properties. It is an important source of dietary fat that imparts a characteristic flavor, texture, appearance, and mouthfeel when present in formulated food products. Milkfat is characterized by its numerous different chain length fatty acids compared with other fats of animal or vegetable origin. This difference from other fats imparts to a low average molecular weight, which is indicated by a high saponification value and a low refractive index. Milk lipids are mainly TAGs (97–98%), 0.2% to 1% phospholipids, 0.2% to 0.4% free sterols, traces of FFAs, and varying amounts of the fat-soluble Vitamins A, D, E, and K. The fatty acid profile of milkfat is complex and includes 7% short-chain fatty acids ( $C_4$  to  $C_8$ ), 15–20% medium-chain fatty acids ( $C_{10}$  to  $C_{14}$ ), 73–78% long-chain fatty acids ( $C_{16}$  and higher), and minor quantities fatty acids, including uneven carbon number, branched chain, and keto and hydroxy fatty acids. The saturated fatty acids present in the largest amount in milkfat are myristic (14:0), palmitic (16:0), and stearic (18:0) acids. The major unsaturated fatty acids are oleic (18:1), linoleic (18:2), and linolenic (18:3) acids. Oleic acid is the main mono-unsaturated fatty acid in milkfat.

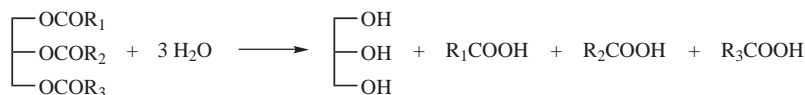
Modification of milkfat aimed at improving its nutritional quality may take the form of changes in the fatty acyl residues on the glycerol backbone of TAGs. Currently, available techniques for commercial modification of milkfat include blending, hydrogenation, and fractionation. Interesterification has been proposed as a means of modifying milkfat, milkfat fractions, or mixtures of milk and vegetable fats, either as a means of improving the nutritional status or spreading properties.

Betapol<sup>®</sup>, a human milkfat substitute produced by Loders Croklaan (Wormerveer, the Netherlands), is produced by interesterification of vegetable oils in which the component TAGs have been modified to more closely resemble those found in breast milk. This product closely mimics the specific structure and fatty acid composition of human milkfat and resembles breast milk in terms of its nutritional value and high content of palmitic acid at the *sn*-2 position than other milkfat substitutes. In addition, the use of Betapol<sup>®</sup> in infant formula can lead to improved mineral and fat absorption and less calcium soap formation within the intestinal lumen resulting in softer stools.

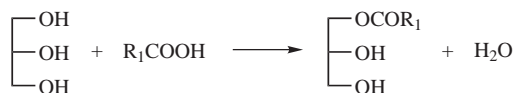
## 6. HYDROLYSIS AND ESTERIFICATION

Hydrolysis of fats and oils refers to the splitting of their TAGs into their constituent fatty acids and glycerol in the presence of water (Figure 8a). There are three distinct routes used for the hydrolysis of TAGs to FFAs and glycerol: high-pressure steam splitting, alkaline hydrolysis (saponification), and enzymatic hydrolysis. In current industrial practices, fats and oils are hydrolyzed to produce FFAs or soaps and glycerol. For production of free FFAs, fats and oils are hydrolyzed using steam in a countercurrent continuous process operated at high temperature (250°C) and

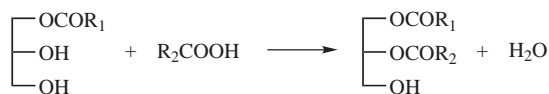
(a) Hydrolysis



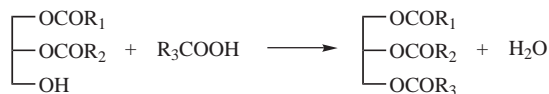
(b) Esterification



Glycerol      Fatty acid                      Monoacylglycerol



Monoacylglycerol      Fatty acid                      Diacylglycerol



Diacylglycerol      Fatty acid                      Triacylglycerol

**Figure 8.** Hydrolysis and esterification.

pressure (50–60 bars). This is an efficient process giving an aqueous phase containing 10% glycerol and oil phase containing 97% FFAs. As a result of the high temperature used in this process, oils containing highly unsaturated fatty acids undergo decomposition yielding undesirable oxidation products. The products obtained by high-pressure steam splitting are usually dark-colored and, hence, require distillation. High-pressure steam splitting requires high capital investment and energy costs. Alkaline hydrolysis also consumes a large amount of energy and the resulting soap requires acidification to yield free fatty acids. Enzyme-catalyzed hydrolysis of fats and oils offers an alternative process for production of fatty acids and may be performed at ambient or moderate temperatures and pressures. Therefore, this process can be used to obtain fatty acids from oxidatively unstable oils that contain highly unsaturated fatty acids or conjugated fatty acyl residues. Lipase-catalyzed hydrolysis of fats for the production of fatty acids and glycerol appears to be less economical than conventional chemical processes mainly because of the relatively high cost of the lipases. Such processes, however, would be economically attractive for the production of specific value-added products. Hydrolysis of marine and fish oils by lipases for the production of n-3 polyunsaturated fatty acids is a possible application of enzymatic hydrolysis of oils for the preparation of products of reasonable commercial value.

Esterification is the reverse of the hydrolysis process. It is carried out by reacting fatty acids with glycerol. In addition to esters, water is also a product of esterification. The reaction is reversible and proceeds to completion only if water is removed from the medium. The equilibrium between the forward reaction (hydrolysis) and the reverse reaction (esterification) is controlled by water content of the reaction mixture. In the presence of excess water, hydrolysis predominates, whereas under water-eliminating conditions, esterification is favored (3, 4).

When the esterification reactants are properly adjusted, MAGs, DAGs, and TAGs may be produced by esterification of fatty acids with glycerol. The esterification of glycerol and fatty acids consists of a sequence of three equilibrium reactions, as shown in Figure 8. The products of these reactions are always a mixture of MAGs and DAGs and contain variable quantities of unreacted glycerol. These products have many applications, among which food, cosmetic, and pharmaceutical emulsifiers and stabilizers are prominent examples (15, 16).

Lipase-mediated esterification of glycerol and fatty acids is also an equilibrium reaction. The extent of esterification depends on the water content of the medium. The accumulation of water during esterification is a concern because it enhances the hydrolysis of the resultant esters. The water, which is produced during this reaction, may be continuously removed and this is usually accomplished by carrying out the reaction in the presence of molecular sieves (17). A small amount of water, however, is needed in the reaction medium to maintain the activity of the enzyme. The ester concentration at equilibrium is dependent on various medium properties, i.e., the water activity of the reaction system. Usually, a low water activity is necessary to obtain a high ester concentration. Water activity, however, is not the only parameter that determines the equilibrium position. The thermodynamic activities of other reaction variables also are important.

## 7. LIPASES IN LIPID MODIFICATION

Lipases, or TAG acylhydrolases (E.C. 3.1.1.3), are widely diversified in their enzymatic properties and substrate specificity. The need for novel lipases is obvious, and the industry continues to look for lipases with high catalytic activity, high selectivity, and lower costs. Lipases occur widely in nature and are active at the oil/water interface in heterogeneous reaction systems and are used for modification of lipids. Lipases are enzymes that preferentially catalyze the hydrolysis and synthesis of esters and TAGs. The physiological function of lipases is to hydrolyze lipids for conversion to accessible energy for the cells or organisms. Lipases are ubiquitous in nature, being present in the animal, microbial, and plant kingdoms. The most well-known and well-studied lipases are human pancreatic lipase, porcine pancreatic lipase, and several microbial lipases. The common feature that distinguishes lipases from esterases, which also catalyze the hydrolysis of ester bonds, is that lipases are activated by the oil-water interface (18). In contrast to esterases, lipases exhibit low activity toward monomeric substrates; but as soon as the substrate can form micelles or a separate phase, lipase activity increases dramatically. This phenomenon is referred to as interfacial activation (19).

The major advantage of lipase-catalyzed reactions over those carried out with chemical catalysts lies in the fact that a wide variety of products having different composition and properties can be prepared, depending on substrate specificity or stereospecificity of the lipase used. Further advantages of lipases-catalyzed reactions include mild reaction conditions leading to reduced energy consumption and less thermal damage of reactants and products.

Although enzymes have been used for many years to modify the structure and composition of foods, only recently have they become available for large-scale use in the industry. According to enzyme manufacturers, progress in genetic engineering and modern processing technology may now enable the enzyme industry to offer enzymes with improved properties at a reduced cost (20). For the industrial exploitation of enzyme-catalyzed reactions, reuse of the enzyme is certainly necessary. To this end, enzymes have been immobilized in a variety of ways. Immobilization of enzymes can simply be accomplished by mixing an aqueous solution of the enzyme with a suitable support material and removing the water under reduced pressure; for activation of the enzyme, a small amount of water is retained by the support material or added to the reaction mixture.

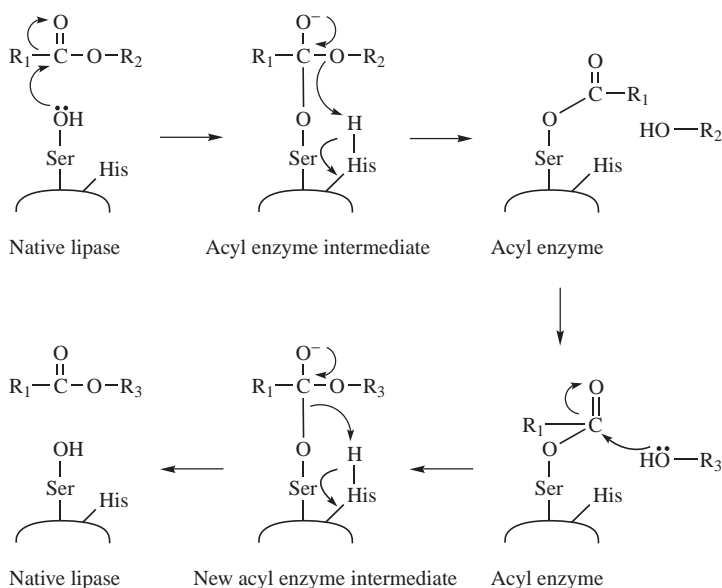
Lipases also may be used to catalyze interesterification reactions by restricting the amount of water in the medium, whereupon the interesterification reaction predominates over hydrolysis. This is generally achieved by performing the reaction in an organic solvent, which also helps dissolve the reactants. It is insufficient, however, to only lower the water concentration; reversal of the hydrolysis reaction requires operation at a water activity ( $A_w$ ) below 1 (21). Thus, attempts for interesterification in a predominantly aqueous environment have resulted in poor product yields (22).



## 7.1. Structure-Function Relationship

As stated earlier, lipases act at the interface between hydrophobic and hydrophilic regions, a characteristic that distinguishes lipases from esterases. Similar to serine proteases, lipases share the nucleophile-histidine-acidic residue catalytic triad that manifests itself as either a Ser-His-Asp triad or a Ser-His-Glu triad. The enzyme's catalytic site often is buried within the protein structure, surrounded by relatively hydrophobic residues. An  $\alpha$ -helical polypeptide structure acts as a cover, making the site inaccessible to solvents and substrates. For the lipase to be active, the  $\alpha$ -helical lid structure has to open so that the active site is accessible to the substrate. The phenomenon of interfacial activation is often associated with reorientation of the lid, increasing the hydrophobicity of the surface in the vicinity of the active site and exposing it. The opening of the lid structure may be initiated on interaction with an oil/water interface.

The mechanism of acylation and deacylation of the substrate, which may be an ester such as a TAG, fatty ester of a monohydric alcohol, or fatty acid, in the active site is illustrated in Figure 9. During acylation, the substrate binds to the active site and the carboxyl carbon is positioned in close proximity to the hydroxyl oxygen in the serine side chain (Figure 9). This oxygen makes a nucleophilic attack on the carbonyl carbon of the substrate and a tetrahedral transition state, an acyl enzyme intermediate, is formed. The serine is made a stronger nucleophile by the close proximity of the neighboring histidine and aspartic acid residues. The hydrogen from the hydroxyl group of serine is transferred temporarily to the histidine residue close to the serine residue. The intermediate rearranges and the hydrogen



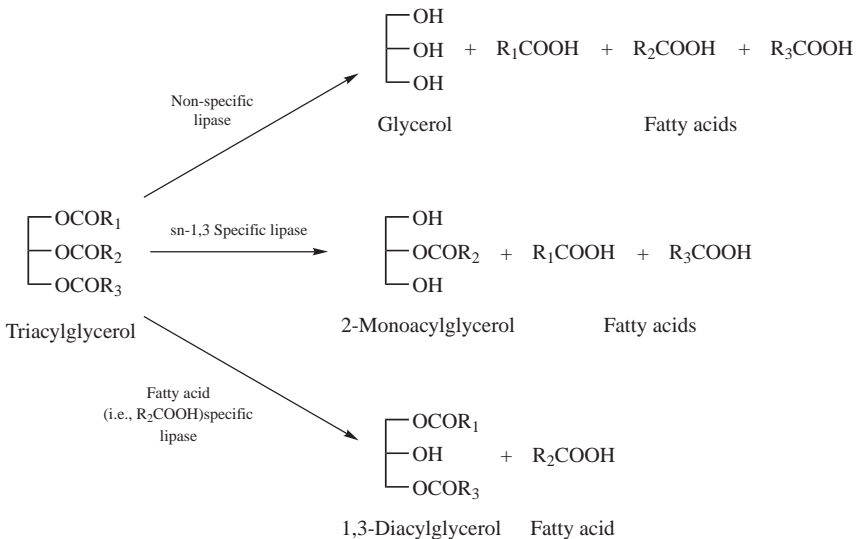
**Figure 9.** Mechanism for lipase catalysis.

is transferred from the histidine to the alcohol moiety of the substrate ester and an alcohol is formed, which then leaves the active site. The fatty acyl moiety is now covalently linked to the enzyme by an ester bond, which is called the acyl enzyme. In the next step, the reversal of the acylation of the lipase occurs and the acyl enzyme is attacked by an alcohol or a water molecule. Again, an acyl enzyme intermediate is formed, which rearranges and a new ester is released. As it is a multisubstrate reaction, this type of mechanism is referred to as a Ping-Pong Bi-Bi reaction mechanism (23).

## 7.2. Lipase Specificity

Specificity is one of the most striking properties of enzyme molecules. Enzyme specificity can be defined as a comparative difference in rates of catalysis of certain reactions. After an enzyme is identified as a lipase, several specificities within the class are identified or can be expected to occur. The main advantage of lipases, which differentiate enzymatic reactions from chemically-catalyzed reactions, is lipase specificity. Lipases have turned out to be very useful enzymes for catalyzing various types of reactions with a rather wide substrate specificity. The fatty acid specificity of lipases has been exploited to produce structured lipids and to enrich lipids with specific fatty acids to improve the nutritional characteristics of lipids (24). Certain lipases display positional specificity (regiospecificity) toward fatty acyl groups in a TAG molecule as well as fatty acid selectivity.

Lipases can be categorized into three groups based on their specificity; random (nonregiospecific), *sn*-1,3-specific (regiospecific) toward TAGs, or specific for a particular fatty acid or, more generally, a class of fatty acids (Figure 10). Examples



**Figure 10.** Regiospecificity of lipases.

of nonspecific lipases include those from *Candida cylindraceae*, *Corynebacterium acnes*, *Staphylococcus aureus*, *Penicillium expansum*, and *Pseudomonas cepacia*; *Geotrichum candidum* shows fatty acid specificity (11, 25). The hydrolysis of TAGs using nonspecific lipases leads to complete breakdown of TAGs to glycerol and FFAs. DAGs and MAGs are intermediates in the reaction, but do not normally accumulate to a high level during the reaction, presumably because they are hydrolyzed more rapidly than TAGs.

Lipases that are *sn*-1,3 specific include those from *Mucor miehei*, *Mucor javanicus*, *Aspergillus niger*, *Pseudomonas fluorescens*, *Rhizopus delemar*, *Rhizopus arrhizus*, and pancreatic lipase (11, 25–27). These enzymes cleave the fatty acids only from the *sn*-1 and *sn*-3 positions of the glycerol backbone of TAGs. Thus, with these lipases, TAGs are hydrolyzed to afford FFA, 1,2 (2,3)-DAGs and 2-MAGs.

The lipase from *Geotrichum candidum* is an example of a lipase possessing fatty acid specificity toward C<sub>18</sub> fatty acids containing a *cis*-9 double bond (25, 28). This lipase will hydrolyze acylglycerol esters of these fatty acids regardless of their position on the glycerol backbone. These specificities will never be achieved in chemically catalyzed hydrolysis, esterification, or interesterification reactions. Moreover, most lipases exhibit enantioselectivity toward racemic ester, alcohol, and acid substrates. This property has been used for carrying out resolution of enantiomers from racemic mixtures of the substrates and is highly important in organic synthesis (29). Thus, the unique specificity of *Geotrichum candidum* lipase may be useful for production of special fatty acid fractions. For example,  $\gamma$ -linolenic acid (GLA) in borage oil has been concentrated in esterification reactions catalyzed by this lipase (30).

Short-chain fatty acid preference also is a lipase specificity that can be exploited on an industrial scale. Such lipases may be used in the production of low-calorie structured lipids or in the dairy industry to obtain specific flavor components by the release of short- or medium-chain fatty acids from milkfat (25).

### 7.3. Factors that Affect Lipase-Catalyzed Reactions

In enzyme-catalyzed reactions, parameters such as concentration of water, reaction temperature, pH, enzyme immobilizing agents, and the nature of any solvent may be of great importance. A brief description of each of these factors is given below.

**7.3.1. Water Content** It is well known that a certain level of water is necessary for a lipase-catalyzed reaction to occur in organic media, but the content of water has varying effects on various lipases. Therefore, the water content has to be adjusted for individual reaction systems to achieve an acceptable product yield. It is possible for TAGs to be easily hydrolyzed even in the presence of small amounts of water.

Water plays different functions in enzyme-catalyzed reactions. A certain level of water in the reaction medium is required to maintain a layer of water around the enzyme molecules to maintain their activity. This water is important for lipases in that it maintains their active three-dimensional conformational state. Water

also contributes to structural integrity, active site polarity, and protein stability. It is recognized that the amount of water present is important for both the thermal stability and catalytic function of enzymes in nonaqueous media. To favor synthesis, the thermodynamic activity of water should be maintained as low as possible. When the amount of water reaches a critical level it promotes hydrolysis, but complete elimination of water from the system results in lipase deactivation and, hence, no biochemical reaction.

Enzyme activity is markedly influenced by water activity ( $a_w$ ). Lipases from different sources have been shown to have completely different behavior: some exhibit high activity at low  $a_w$ , whereas others show a high activity at intermediate or very high  $a_w$  (31). The  $a_w$  also affects the apparent  $K_m$  and  $V_{max}$  values measured for lipase-catalyzed hydrolysis and esterification reactions (32). Thermodynamic  $a_w$  can be used to define the relationship between water and other components of the reaction system (33). Thermodynamic  $a_w$  of a solution is defined as the water vapor pressure over the solution divided by the water vapor pressure over pure water at a given temperature. The water content of the biocatalyst is more important in dictating enzyme activity than the total water content in the system. Novozym-435<sup>®</sup> (Novo Nordisk, Franklinton, North Carolina) from *Candida antarctica* has a water content of 1–2% (w/w) (34). This enzyme preparation has been shown to give high yields of TAG products under several nonaqueous reaction conditions (5, 35, 36).

**7.3.2. Temperature and pH** It is generally stated that the rate of a chemical reaction doubles with every 10°C increase in the reaction temperature. As lipases are proteins, they undergo thermal denaturation at high temperatures. Thus, enhancement of enzyme activity with increasing temperature is compromised by the competing effect of general protein denaturation at elevated temperatures. Hence, the catalytic efficiency will increase with temperatures over a finite range, then decrease significantly above some critical temperature that is characteristic of protein denaturation.

The pH of a solution can affect lipase activity in a number of ways. Like all proteins, enzymes have a tertiary structure that is sensitive to pH. In general, denaturation of enzymes occurs at extreme low and high pH values. At extremes of pH, the tertiary structure of the protein may be disrupted and the protein denatured. Many proteins aggregate on pH-induced denaturation and this behavior can be observed by visual inspection. If the activity of an enzyme is plotted against the pH, a bell-shaped curve usually results, with either a sharp or broad pH optimum.

**7.3.3. Solvent Type** The affect of different organic solvents on various enzyme catalytic activities has been demonstrated (37). From a mechanistic standpoint, the effect of organic solvents on enzyme catalysis is still debated (38). Conformational changes in enzymes, when suspended in organic solvents, have been reported to result in alteration of substrate specificity and affinity of substrates for enzymes (39). The polarity of organic solvents affects lipase-catalyzed reactions as do other variables that are critical to enzyme activity in organic media. The nature of the

solvent is crucial for maintaining a layer of essential water around the enzyme, which greatly influences the catalytic properties of the lipase. Enzyme activity can be correlated with solvent hydrophobicity following its dependency on water; thus, solvent suitability may be predicted by a measure of solvent hydrophobicity. The most frequently used parameter for quantitatively describing the effect of solvents on enzyme reactions is  $\log P$ , where  $P$  is the partition coefficient of the solvent between water and 1-octanol. In general, enzyme catalysis in organic solvents is governed by the following rules: (1) solvents with  $\log P$  values of less than two are not suitable for enzymatic reactions, as they more strongly distort the essential water about the enzyme, thereby inactivating them; (2) solvents with  $\log P$  values between two and four are weak water distorters and affect enzyme activity in an unpredictable manner; and (3) solvents with  $\log P$  values of greater than four do not distort the essential water layer, and therefore are more suited for enzyme-catalyzed reactions (38). The sensitivity of a lipase to  $\log P$  of the solvent also is dependent on the source of the lipase.

Organic solvents may help to keep a low  $a_w$ , which then decreases the thermodynamic barrier for reactions, such as esterification vs hydrolysis. Moreover, higher solubility of organic substrates in organic solvents results in higher reaction rates. Generally, polar organic solvents compete with the available water required to maintain the three-dimensional structure of the enzyme, which may disrupt the enzyme activity. Thus, water-immiscible hydrophobic solvents are ideal for non-aqueous enzymology. Organic solvents, such as *n*-hexane, play several functions, including increasing the solubility of nonpolar substrates and shifting the reaction toward synthesis rather than hydrolysis (37). Lipases seem to be more active in *n*-hexane than other solvents, such as isooctane, acetone, petroleum ether, benzene, toluene, and ethyl acetate (5).

In solvent-free reaction systems, the absence of solvent facilitates downstream processing as fewer components are present in the reaction mixture; moreover, the elimination of solvent from the production step offers significant cost savings. In some ways, solvent-free reaction systems are similar to solvent systems in that reactants serve as a reaction medium. With the absence of solvent, high purity products (solvent-free) often can be obtained. One drawback to solvent-free systems is the possibility of high media viscosity, which may result in poor mixing and, hence, slow reaction rates.

**7.3.4. Immobilizing Agents** As a result of the high cost of enzymes, the regeneration and reuse of the lipase by immobilization is an attractive feature in enzyme biocatalysis. An immobilized enzyme is one that has been attached to or enclosed by an insoluble support medium or one where the enzyme molecules have been cross-linked to each other, without loss of catalytic activity. Immobilization of lipases provides some benefits for their commercial applications. Immobilization, to some extent, may protect the lipase from solvent denaturation and permits repeated use of the lipase and, at the end, give a product free of catalyst. Immobilization also could increase lipase stability against pH and heat. In general, immobilized enzymes, are easily separated from the reaction mixtures, which is of great

industrial importance. Hence, the reaction can be stopped by physical removal of the immobilized enzyme and the enzyme can be reused. For these reasons, immobilized lipases are ideal for use in continuously operated processes. At present, continuous industrial processes involving immobilized lipases are usually carried out in simple stirred-tank reactors or in packed-bed reactors.

Enzymes can be immobilized by several techniques: (1) cross-linking of two enzyme molecules; (2) covalent attachment to an inert molecule; (3) adsorption onto an insoluble matrix; (4) precipitation of insoluble enzyme particles in an organic liquid phase; and (5) entrapment in a three-dimensional polymeric matrix. An immobilizing agent thus comprises the following: (1) covalent forces as in covalent attachment, intermolecular cysteine bridges, and cross-linking; (2) hydrogen bonds as in intermolecular interaction between hydrogen atoms and electronegative atoms; (3) van der Waal's forces as in hydrophobic adsorption, microencapsulation, and reversed micelles; and (4) ionic forces as in ion exchange, intermolecular cysteine, or salt bridges.

Immobilizing agents are relatively larger in size than the enzyme molecule to which they are directly bound or in which the enzyme is confined and have the function of helping to create an enzyme-rich phase. This support material is absent in the case of intermolecular cross-linking and precipitation of the enzyme in an organic solvent. Immobilizing agent may be a liquid, as in reversed micelles, or a solid, as in most of the commonly employed immobilization techniques. Examples of solid supports include celite, amberlite resins, cellulose, silica gel, clay, carbon, and alumina, among others.

Support materials for immobilized lipases should allow the effective use of the enzyme by having the enzyme molecules remaining accessible to the substrates. On immobilization, stability improvement has been observed for some lipases (40). This might possibly be attributed to the ability of the support material to retain just the right amount of moisture for the enzyme to remain active. Support materials affect the amount of water in the vicinity of the enzyme molecules and partitioning of reactants or products in the reaction mixture. Immobilization on hydrophilic support materials may cause loss of lipase activity as the enzyme undergoes some conformational changes. Hydrophilic (i.e., polar) supports also may reduce the solubility of hydrophobic substrates in hydrophilic regions, thereby reducing the accessibility of substrates to the enzyme's active site.

The water-absorbing capacity of an enzyme support material may be characterized by the term "aquaphilicity" ( $Aq$ ), which is the ratio of the amount of water on the support to the amount of water in the solvent under standard conditions (41). Support materials with high  $Aq$  are hydrophilic, and hence might deprive the enzyme of its essential water of hydration. Lipase activity is generally higher with hydrophobic (i.e., essentially nonpolar) supports such as celite and Bonopore (42).

As lipases are proteins, their conformation can be affected by the temperature and pH and, hence, their catalytic activity is expected to have an optimum for these two parameters as happens with most enzymes. The optimum pH for lipase-catalyzed reactions is slightly shifted toward a more alkaline range after immobilization

(43). After immobilization, one can also notice a shift toward higher optimum temperatures. As immobilization provides a rigid external backbone for the lipase molecules, the effect of higher temperatures in breaking the interactions that are responsible for the catalytically active structure becomes less prominent, and so temperature optimum is expected to increase. Immobilization also affixes the enzyme in one conformation, which reduces the susceptibility of the enzyme to denaturation by heat. Immobilized lipases, such as those from *Mucor miehei* and *Candida antarctica* (34), show good thermal stability at temperatures required to process most fats and oils and are therefore most appropriate for the biomodification of fats and oils.

#### 7.4. Applications of Lipases

Enzyme-catalyzed reactions have been employed for production of TAGs used for confectionery fat formulations and nutritional applications. In the area of confectionery fats, interesterification of high oleic sunflower oil and stearic acid using immobilized *Rhizomucor miehei* lipase produces mainly 1,3-di-2-stearoyl-oleoyl-glycerol (StOSt). Other reactants also may be used for production of TAGs useful as confectionery fats. In particular, there are many reports on enzymatic interesterification of palm oil fractions with stearic acid or stearate esters to produce fats containing high concentrations of StOSt and 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POSt) (11), which are the main TAGs components of cocoa butter. Hence, enzymatic interesterification can produce fats with compositions and physical properties very similar to cocoa butter. In this example, the selectivity of most lipases for the *sn*-1 and *sn*-3 positions of the glycerol backbone has been found to be advantageous, because most important TAGs present in cocoa butter have palmitoyl and stearoyl residues primarily at the *sn*-1,3 positions and oleoyl residues predominantly at the *sn*-2 position. Chemically catalyzed interesterification is not selective. Cocoa butter substitutes have also been produced using *sn*-1,3 specific lipases via acidolysis of palm oil fraction with myristic acid (44) or by acidolysis of olive oil with palmitic acid using lipozyme from *Mucor miehei* as the biocatalyst (45).

Enzyme-catalyzed reactions are used to produce human milkfat substitutes for use in infant formulas (46–48). Acidolysis reaction of a mixture of tripalmitin and unsaturated fatty acids using a *sn*-1,3-specific lipase as a biocatalyst afforded TAGs derived entirely from vegetable oils rich in 2-position palmitate with unsaturated fatty acyl groups in the *sn*-1 and *sn*-3 positions (44). These TAGs closely mimic the fatty acid distribution found in human milkfat, and, when used in infant formula instead of conventional fats, the presence of palmitate in the *sn*-2 position of the TAGs improved digestibility of the fat and absorption of other important nutrients such as calcium (46, 49).

The possible application of enzyme-assisted reactions for production of lower value nonspecialty lipids such as margarine hardstocks and cooking oils has been reported (50). When nonspecific lipases, such as those from *Candida cylindraceae* and *C. antarctica*, are used as biocatalysts for interesterification of oil blends, the TAG

products are very similar to those obtained by chemical interesterification (11). Therefore, replacement of chemical interesterification by an enzyme process giving similar products is technically feasible, although it has not yet been adopted on a commercial scale, largely because of the comparatively high process and catalyst costs.

Another application is in the manufacture of fats and oils containing nutritionally important PUFAs, such as EPA and DHA. For example, various vegetable and marine oils have been enriched with EPA and DHA using enzyme-catalyzed reactions (35, 51, 52). Use of this technique to produce structured lipids with MCFAs and PUFAs located specifically in either the *sn*-2 or *sn*-1,3 positions of the TAGs has been described. Enzymatic processes are particularly suitable for the production and modification of lipids containing PUFAs, because these unstable fatty acids are susceptible to damage under the more severe conditions used for chemical processing.

If regio- or stereospecific lipases are used to interesterify oil blends, the products formed are different from those obtained by chemical interesterification, and may exhibit better functional properties. For example, interesterification of blends of canola and palm oils, using the *sn*-1,3-specific *Rhizopus delemar* lipase as a biocatalyst, gave oils with improved fluidity compared with the original blends or chemically interesterified products.

Intesterification of blends of palm and hydrogenated canola oils and cottonseed and hydrogenated soybean oils using *sn*-1,3-specific lipases as biocatalysts gave fats with a low *trans*-fatty acid content that were effective as margarine hardstocks (53). Reaction of mixtures of palm stearin and lauric fats using immobilized *Rhizomucor miehei* as a biocatalyst also produced fats that were functional as margarine hardstocks (54). With these enzymatically interesterified fats, margarine could be formulated without using hydrogenated fats.

## 8. MODIFICATION OF FATS AND OILS TO PRODUCE STRUCTURED LIPIDS

Structured lipids may be defined as TAGs restructured or modified to change the fatty acid composition or their positional distribution in glycerol molecules by a chemical or enzymatic process. These specialty lipids have been developed to fully optimize the benefits of various fatty acid moieties. Structured lipids have been reported to have beneficial effects on a range of metabolic parameters including immune function, nitrogen balance, and improved lipid clearance from the bloodstream (46). Structured lipids are also synthesized to improve or change the physical or chemical properties of TAGs, such as melting point, solid-fat content, iodine, and saponification values, as discussed earlier.

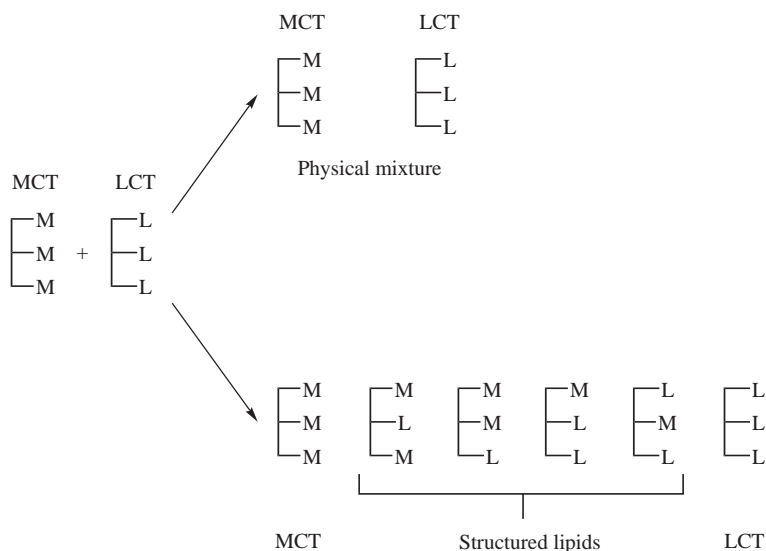
Structured lipids are often referred to as a new generation of lipid that can be considered as “nutraceutical” (55). Nutraceutical is a term used to describe foods that provide health benefits beyond those ascribed to their nutritional effects (56). These products may be referred to as functional foods or functional lipids if they are



incorporated into products that have the usual appearance of food, but to which they may be added and provide specific health benefits (56). Regardless of the definition, structured lipids can be designed for use as medical or functional foods as well as nutraceuticals, depending on the type of application.

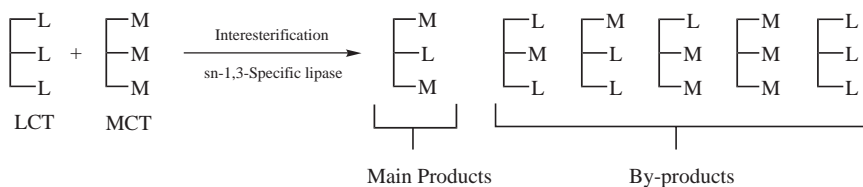
One way of preparing structured lipids may be by hydrolysis of fatty acyl groups from a mixture of TAGs followed by random re-esterification onto the glycerol backbone (57). Typically, a variety of fatty acids are used in this process, including different classes of saturated, monounsaturated, and polyunsaturated fatty acids, depending on the desired metabolic effect. Thus, a mixture of fatty acids is incorporated onto the same glycerol molecule. These manufactured lipids are structurally and metabolically quite different from the more simple, random physical mixtures of medium-chain triacylglycerols (MCTs) and long-chain triacylglycerols (LCTs). Starting with an MCT and an LCT, six possible fatty acid combinations could result on the structured lipids: two MCFAs and one LCFA; one MCFA and two LCFAs; the two positional isomers; and small amounts of the starting MCT and LCT (Figure 11).

Based on their high regioselectivity, lipases are effective biocatalysts for the manufacture of structured lipids that have a predetermined composition and distribution of fatty acids on the glycerol backbone. Structured lipids resembling TAGs of human milk have been produced by *trans*-esterification of tripalmitin, derived from plant oil, with oleic acid or PUFAs, obtained from plant oils, using *sn*-1,3-specific lipases as biocatalysts (46). Such TAGs were found to closely mimic the fatty acid distribution of human milk and may be used in infant food formulations. Apart from imitating the human milk more closely, the occurrence of palmitic acid

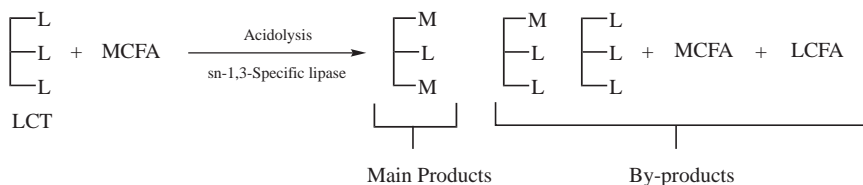


**Figure 11.** Triacylglycerol physical mixture vs structured triacylglycerols.

## (a) Interesterification of LCT and MCT



## (b) Acidolysis of LCT with MCFA



**Figure 12.** Enzyme-catalyzed synthesis of specific structured lipids.

in the *sn-2* position of the TAGs has been shown to improve digestibility and increase the absorption of other important nutrients such as calcium.

Intesterification of a LCTs with MCTs or MCFAs by means of *sn-1,3*-specific lipases may yield TAGs containing medium-chain acyl moieties at the *sn-1,3* positions and LCFAs at the *sn-2* position. Such products do not occur in nature and are difficult to synthesize by chemically catalyzed reactions. Two possible methods for the synthesis of these structured lipid products are shown in Figure 12. A key point for both strategies is the use of *sn-1,3* specific lipases. The goal is to substitute the fatty acid residues specifically at the *sn-1* and *sn-3* positions of the TAGs with desired ones by a *sn-1,3*-specific lipase, leaving the fatty acid residues at the *sn-2* position unchanged.

Structured lipids possessing *n-3* PUFAs, such as EPA and DHA, located at mid position, with MCFAs at the end positions, have gained considerable attention as nutritional and health supplements. These TAGs provide rapid delivery of energy via oxidation of the MCFAs and, at the same time, supply metabolically functional fatty acids in the same molecule. Senanayake and Shahidi (51) used an immobilized *sn-1,3*-specific lipase from *Mucor miehei* to incorporate capric acid into seal blubber oil containing EPA and DHA. On enzyme-catalyzed acidolysis, a structured lipid containing 27.1% capric acid, 2.3% EPA, and 7.6% DHA was achieved. Lipase from *Mucor miehei* incorporated capric acid predominantly at the *sn-1,3* positions of the structured lipid.

Structured lipids containing both GLA and *n-3* PUFAs may be of interest because of their desired health benefits. Performing an acidolysis between  $\gamma$ -linolenic acid (18:3 $n-6$ ; GLA) rich oils, such as those from borage and evening primrose, and free EPA and DHA, Senanayake and Shahidi (36) used the nonspecific lipase PS-30

from *Pseudomonas sp.* to incorporate n-3 PUFAs into these oils. Under optimum reaction conditions, the incorporation of n-3 PUFAs (EPA+DHA) into borage and evening primrose oils was 35.5% and 33.6%, respectively. The amounts of GLA retained in the structured lipids were 17.1% and 7.6%, respectively. GLA has shown therapeutic benefits in a number of disease conditions, notably atopic eczema, dermatitis, inflammation, hypertension, and premenstrual syndrome. On the other hand, n-3 PUFAs have potential for prevention of cardiovascular disease, arthritis, hypertension, immune and renal disorders, diabetes, and cancer. Another structured lipid containing GLA and DHA was successfully produced via Novozym 435 (*Candida antarctica*)-catalyzed acidolysis using borage oil as the main substrate (5). The structured lipid so prepared contained 27.4% DHA and 17.0% GLA. These tailor-made lipids may be useful in the treatment of certain clinical disorders.

The fatty acid compositions of borage and evening primrose oils were also modified by incorporation of EPA using lipase PS-30 from *Pseudomonas sp.* as the biocatalyst (58). After acidolysis reaction, borage oil-based structured lipid contained 26.8% EPA and 15.2% GLA. Meanwhile, evening primrose oil-based structured lipid had EPA and GLA contents of 25.2% and 7.6%, respectively (58).

Another area of interest in the field of structured lipids is the synthesis of reduced-calorie TAGs. The most familiar classes of low-calorie lipids are Caprenin (Procter & Gamble, Cincinnati, Ohio), Salatrim (Danisco Cultor USA, Inc., New Century, Kansas), and MCTs preparations. These will be discussed in more detail in another chapter.

## REFERENCES

1. T. L. Mounts, *NATO ASI Series: Series A, Life Sciences*, **131**, 201 (1987).
2. V. Sreenivasan, *J. Amer. Oil Chem. Soc.*, **55**, 796 (1978).
3. W. M. Willis and A. G. Marangoni, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry, Nutrition and Biotechnology*, Marcel Dekker, Inc., New York, 1998, p. 665.
4. A. G. Marangoni and D. Rousseau, *Trends Food Sci. Tech.*, **6**, 329 (1995).
5. S. P. J. N. Senanayake and F. Shahidi, *J. Amer. Oil Chem. Soc.*, **76**, 1009 (1999).
6. A. Millqvist, P. Adlercreutz, and B. Mattiasson, *Enzyme Micorb. Technol.*, **16**, 1042 (1994).
7. H. Noureddini and S. E. Harmeyer, *J. Amer. Oil Chem. Soc.*, **75**, 1359 (1998).
8. D. Rousseau and A. G. Marangoni, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry, Nutrition and Biotechnology*, Marcel Dekker, Inc., New York, 1998, p. 301.
9. J. W. E. Coenen, *Rev. Fr. Corps Gras*, **21**, 403 (1974).
10. H. K. Hawley and G. W. Holman, *J. Amer. Oil Chem. Soc.*, **33**, 29 (1956).
11. A. R. Macrae, *J. Amer. Oil Chem. Soc.*, **60**, 291 (1983).
12. G. List, *Food Technol.*, **58**(1), 23 (2004).
13. G. List, E. Emken, W. Kwolek, and T. Simpson, *J. Amer. Oil Chem. Soc.*, **54**, 408 (1977).
14. M. C. Willson, *Inform*, **13**, 904 (2002).
15. J. A. Arcos and C. Otero, *J. Amer. Oil Chem. Soc.*, **73**, 673 (1996).

16. R. Rosu, M. Yasui, Y. Iwasaki, and T. Yamane, *J. Amer. Oil Chem. Soc.*, **76**, 839 (1999).
17. F. Ergan, M. Trani, and G. Andre, *Biotechnol. Lett.*, **10**, 629 (1988).
18. R. Verger and G. H. de Haas, *Annu. Rev. Biophys. Bioeng.*, **5**, 77 (1976).
19. R. Verger, in B. Borgstrom and H. L. Brockman, eds., *Lipases*, Elsevier Applied Science, New York, 1984, p. 121.
20. E. N. Vulfson, *Trends Food Sci. Technol.*, **4**, 209 (1993).
21. P. J. Halling, *Enzyme Microbial Technol.*, **6**, 513 (1984).
22. R. W. Stevenson, F. E. Luddy, and H. L. Rothbart, *J. Amer. Oil Chem. Soc.*, **56**, 676 (1979).
23. H. R. Reyes and C. G. Hill, Jr., *Biotechnol. Bioeng.*, **43**, 171 (1994).
24. R. Reena, S. Divakar, and B. R. Lokesh, *J. Amer. Oil Chem. Soc.*, **79**, 555 (2002).
25. P. Villeneuve and T. A. Foglia, *INFORM*, **8**, 640 (1997).
26. K. D. Mukherjee, *Biocatalysis*, **3**, 277 (1990).
27. N. N. Gandhi, *J. Amer. Oil Chem. Soc.*, **74**, 621 (1997).
28. R. G. Jensen, F. A. Dejong, and R. M. Clark, *Lipids*, **18**, 239 (1983).
29. P. E. Sonnet, *J. Amer. Oil Chem. Soc.*, **65**, 900 (1988).
30. T. A. Foglia and P. E. Sonnet, *J. Amer. Oil Chem. Soc.*, **72**, 417 (1995).
31. R. Valivety, P. J. Halling, A. D. Peilow, and A. R. Macrae, *Biochim. Biophys. Acta*, **1122**, 143 (1992).
32. R. Bovara, G. Carrea, G. Ottolina, and S. Riva, *Biotechnol. Lett.*, **15**, 937 (1993).
33. P. J. Halling, *TIBtech*, **7**, 50 (1989).
34. Novo Nordisk, Product Sheet, B 606c-GB, Franklinton, North Carolina, 1999.
35. S. P. J. N. Senanayake and F. Shahidi, *Food Chem.*, **77**, 115 (2002).
36. S. P. J. N. Senanayake and F. Shahidi, *J. Agric. Food Chem.*, **47**, 3105 (1999).
37. A. M. Klibanov, *Chemtech.*, **16**, 354 (1986).
38. C. Laane, S. Boeren, and K. Vos, *Trends Biotechnol.*, **3**, 251 (1985).
39. J. S. Dordick, *Enzyme Microb. Technol.*, **11**, 194 (1989).
40. H. F. De Castro, W. A. Anderson, M. Moo-Young, and R. L. Legge, in J. Tramper, M. H. Vermue, H. H. Beefink, and U. von Stockar, eds., *Biocatalysis in Non-Conventional Media*, Elsevier Science Publishers, Amsterdam, 1992, p. 475.
41. M. H. Vermue and J. Tramper, *Pure Appl. Chem.*, **67**, 346 (1995).
42. A. R. M. Yahya, W. A. Anderson, and M. Moo-Young, *Enzyme Microb. Technol.*, **23**, 438 (1998).
43. V. M. Balcao, A. L. Paiva, and F. X. Malcata, *Enzyme Microb. Technol.*, **18**, 392 (1996).
44. R. A. Wisdom, P. Dunnill, M. D. Lilly, and A. Macrae, *Enzyme Microb. Technol.*, **6**, 443 (1984).
45. T. Nielsen, *Fette Seifen Anstrichmittel*, **87**, 15 (1985).
46. P. Quinlan and S. Moore, *INFORM*, **4**, 580 (1993).
47. T. C. Christensen and G. Holmer, *Milchwissenschaft*, **48**, 543 (1993).
48. K. D. Mukherjee, in C. C. Akoh and D. B. Min, eds., *Food Lipids: Chemistry, Nutrition and Biotechnology*, Marcel Dekker, Inc., New York, 1998, p. 589.
49. A. Lucas, P. Quinlan, S. Abrams, S. Ryan, S. Meah, and P. J. Lucas, *Arch. Dis. Child.*, **77**, F178 (1997).

50. Z. Zainal and M. S. A. Yusoff, *J. Amer. Oil Chem. Soc.*, **76**, 1003 (1999).
51. S. P. J. N. Senanayake and F. Shahidi, *Food Res. Intl.*, **35**, 745 (2002).
52. S. P. J. N. Senanayake and F. Shahidi, in F. Shahidi, ed., *Seafood in Health and Nutrition. Transformation in Fisheries and Aquaculture: Global Perspectives*, ScienceTech Publishing Co., St. John's, Newfoundland, 2000, p. 29.
53. H. M. A. Mohamed and K. Larsson, *Fat Sci. Technol.*, **96**, 56 (1994).
54. L. H. Posorske, G. K. LeFebvre, C. A. Miller, T. T. Hansen, and B. L. Glenvig, *J. Amer. Oil Chem. Soc.*, **65**, 922 (1988).
55. J. P. Kennedy, *Food Technol.*, **11**, 76 (1991).
56. F. W. Scott and N. S. Lee, *Report of the Bureau of Nutritional Science Committee on Functional Foods*, Food Directorate, Health Protection Branch, Ottawa, Canada (1996).
57. V. K. Babayan, *Lipids*, **22**, 417 (1987).
58. S. P. J. N. Senanayake and F. Shahidi, *J. Agric. Food Chem.*, **50**, 477 (2002).

## *Novel Separation Techniques for Isolation and Purification of Fatty Acids and Oil By-Products*

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### 1. INTRODUCTION

Lipids are composed of a group of compounds that are classified according to their solubility in organic solvents, thus, include several related groups of compounds with diverse chemical and physical characteristics. The predominant component of most fats and oils are triacylglycerols (TAGs), which are actually esters of glycerol with three fatty acids. During extraction of oil from seeds, pericarp, or

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kernels, other components that are associated with TAGs, such as phospholipids, pigments, tocopherols, and sterols, are also removed. Thus, when edible oils are involved, these coextracted lipid-soluble compounds may contribute to the development of off-flavors, undesirable colors, and sometimes a shorter shelf life. With the development of technology related to edible oil industry, crude lipids are refined in order to obtain mainly TAGs. During processing steps, several components with high commercial value are also removed. With the growing scientific evidence about the potential health benefits and functional applications of associated components, more effort has been made to separate and concentrate such minor components. Although TAGs are the predominant molecular form of edible fats and oils, it might be necessary to subject them to separation according to their chemical composition or to modify them in different ways.

## 2. METHODS OF OBTAINING FATTY ACIDS

Among the products obtained from fats and oils, fatty acids are most extensively produced and find uses ranging from mineral oil purification to food and pharmaceutical applications. In nature, fatty acids exist either free or mostly in the esterified form. These fatty acids are characterized by carbon chain length and the number of double bonds. The characteristic composition of fatty acids is specific for each kind of fat. Numerous methods have been established to isolate (or concentrate) and recover specific fatty acids and their derivatives (i.e., esters, free fatty acids, triacylglycerols, etc.) from various naturally occurring sources, but only few are suitable for large-scale production. The available methods include chromatography (adsorption and partitioning), fractional or molecular distillation, enzymatic splitting, low-temperature crystallization, supercritical fluid extraction, and urea complexation. Each technique has its own advantages and drawbacks.

Majority of fatty acids exist in the form of glycerol ester and these constitute a major esterified to glycerol fraction of fats of oils. As an example, in palm oil, the natural mixture of fatty acids is separated into two fractions, namely "olein," which contains the lowest possible amount of saturated fatty acids, and "stearin," which contains the lowest possible amount of unsaturated fatty acids. Independent of the process of separation employed, the starting mixture of fatty acids or "feed stock" should meet certain specifications. A high-quality product with undamaged fatty acids (e.g., less oxidation, minimum isomerization) and fatty acids in the form of mono-, di-, or triacylglycerols, or salts of fatty acids with minimum amount of impurities is necessary for the feed stock (1).

The first documented fatty acid separation was reported by Guesserow (2). Separation of linear saturated fatty acids from linear unsaturated fatty acids by first preparing the lead salt derivative, dissolving the mixed salts in diethyl ether or ethyl alcohol, and then separating the less soluble saturated acids from the more soluble unsaturated acids has been reported. This was a very efficient method and involved large amounts of solvent and pre-preparation of fatty acid salts that made this process difficult to adopt in commercial scale (3). The classic commercial process of

fatty acid separation includes panning and pressing (4, 5), Solexol process (6–11), Emersol process (12–15), Armour–Texaco process (16, 17), and the Henkel process (18–22).

The classic panning and pressing method uses the melting point differences to separate olein and stearin fractions. The *Lanza*, *Henkel*, or *Lipofrac* processes use the melting point difference in developing the automated separation process. The *Solexol* and *Emersol* processes use liquid-liquid countercurrent extraction in combination with melting point differences to separate fatty acids to a higher degree of purity. The *Armour–Texaco* process is similar to the *Emersol* process, but uses acetone instead of methanol as the solvent. Most of these methods are focused on recovering of stearic and oleic acids. These processes are not very suitable for high-value fatty acid separation. Present day demand for a variety of fatty acids, including long-chain polyunsaturated fatty acids (PUFA), may require a combination of different processes and further steps of separation.

## 2.1. Classical Methods and their Developments

Principles of separating fatty acids are based on specific properties of each acid or acid group. Two major properties (vapor pressure and melting point difference) are used in developing separation techniques. The vapor pressure of a mixture of fatty acids varies significantly with the chain length of fatty acids involved, which is used in fractional distillation as a means of separating short- and long-chain fatty acids. However, vapor pressure does not change much with the degree of unsaturation. In the other method, the melting point of fatty acids changes considerably with the degree of unsaturation, which could be used to separate a mixture of fatty acids into saturated and unsaturated components. By changing the temperature of the mixture, fatty acids can be separated according to the degree of unsaturation at their respective crystallization temperature.

**2.1.1. Common Chromatographic Methods** Smith (23) and Elsdon (24) have reported classic chromatographic separation of short-chain fatty acids. Use of adsorption chromatography to purify methyl arachidonate on alumina (25) and unsaturated fatty acids on silica (26) has also been reported. Using latex as the stationary phase fatty acids had been separated by partition chromatography (27, 28). Aqueous acetone or methanol was used as the mobile phase to separate long-chain PUFA and acylglycerol oils; silicone oil or synthetic polymers were used to impregnate filter paper. Boldingh (28) has reported separation of fatty acids using reversed phase column chromatography in which latex powder saturated with peanut oil was used as the stationary phase and methanol/acetone or methanol/water as the mobile phase. The gas-liquid chromatography of fatty acids was first published in 1952 (29), where separation of fatty acids from 1 to 12 carbon atoms was described. The same research group later discovered that prior methylation of fatty acids improved the volatility and separation at high temperatures. The stationary phase used was silicone greases incorporated into Celite 545 as the solid powdered support packed into a glass column. Use of a liquid polyester phase as the stationary phase



to separate saturated and unsaturated fatty acids has also been described by Orr and Callen (30). Later, capillary columns with very thin films of various polar phases were introduced to improve the separation of fatty acids according to their carbon number, unsaturation, and isomerization, among others.

Chromatographic separation of fatty acids is widely used in order to obtain individual fatty acids with high purity. Several patents have been granted for different types of adsorbents and techniques to separate fatty acids. A stationary bed chromatographic system may employ a nonpolar stationary phase (i.e., a reverse phase chromatographic system) or a polar stationary phase (i.e., a normal phase chromatographic system). Fatty acid methyl esters can be used as alternatives to fatty acids in the production of many oleochemicals (fatty alcohols, alkanolamides,  $\alpha$ -sulfonated methyl esters, sucrose esters, and other fatty esters). Methyl esters are preferable to fatty acids as they yield higher purity finished products and require milder conditions during synthesis. Methyl esters are obtained by methanolysis of fats and oils in the presence of an alkaline catalyst, usually sodium methoxide, or splitting the fat by energy- and capital-intensive Colgate–Emery process followed by esterification of the resulting fatty acids with methanol (31).

Molecular sieve adsorbents have been widely studied and employed to separate saturated, mono- and di-saturated fatty acids or to concentrate them in a mixture. Logan and Underwood (32) patented a method to use zeolite as the adsorbent to separate esters of fatty acids according to their degree of unsaturation. Use of non-ionic, hydrophobic cross-linked polymers has also been successful in selective separation of fatty acids (33). The fatty acids are first allowed to adsorb onto the solid bed of a particular adsorbent and then the adsorbed acids may be desorbed by employing a suitable desorbent.

Although several gas chromatographic separation methods are available for analytical purposes, liquid chromatographic methods have been more applicable for preparative and industrial scale separation of fatty acids or their esters. Liquid chromatographic separation of fatty acids has also been used to obtain concentrates from particular fatty acids or their esters from a mixture for food and pharmaceutical products. In order to obtain individual long-chain PUFA in pure form, chromatographic methods have been studied in detail. Nakahara et al. (34) have reported the isolation of triacylglycerols (TAG) containing docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) residues from marine microalgae (i.e., *Schizochytrium*) by reverse-phase high-performance liquid chromatography using acetone/acetonitrile as the mobile phase and octadecylsilane (ODS) as the stationary phase. This stationary phase separates the TAG based on the strength of van der Waals forces between the stationary phase and the fatty acid residues of the TAG. Separation of fatty acids using silver nitrate-silica gel has been a big step in isolating individual fatty acid methyl esters according to their number of double bonds and by the configuration (*cis*-/*trans*-) of the double bonds. The principle of this separation technique is that the metal ion (e.g., a silver ion) coordinates reversibly with electrons of a  $\pi$  orbital of a double bond between carbon atoms of an unsaturated fatty acid residue to form a metal complex. The strength of the complex tends to be dependent on the chain length, number of carbon-carbon double bonds, position of the double

bonds, and their configuration (*cis*- or *trans*-) in the fatty acids involved. Argentiation (silver ion) chromatography has also been used to separate fatty acids with high-performance liquid chromatography (HPLC). The dual nature of separation mechanism of reversed-phase and silver ion high-performance chromatography allows the separation of triacylglycerols according to the degree of unsaturation and PUFAs involved (35). By employing capillary supercritical fluid chromatography (25% cyanopropyl-75% methylpolysiloxane stationary phase), it has been possible to separate TAGs with an identical acyl carbon number and degree of unsaturation (36).

Teshima et al. (37) used silver nitrate-impregnated silica gel column to separate eicosapentaenoic acid (EPA) and DHA from squid liver oil fatty acid methyl esters with a purity of 85–96% EPA and 95–98% DHA and a yield of 39% and 48%, respectively. Application of HPLC column comprising of silica gel and silver or magnesium ion has been used by Corley et al. (38) to isolate TAG rich in DHA from marine algal oils. Hayashi and Kishimura (39) have isolated 63–74% pure DHA from skipjack tuna eye orbital oil by stepwise elution with *n*-hexane, diethyl ether/*n*-hexane, and diethyl ether on a silicic acid column. Adlof and Emiken (40) were able to enrich the  $\omega$ 3 content of commercial  $\omega$ 3-PUFA concentrates from 76.5% to 99.8% using isocratic elution from a silver resin column. In another study, the same authors fractionated 100 mg of concentrated fish oil methyl esters containing 29.1% EPA and 20.5% DHA into fractions of 87.7% EPA and 95.4% DHA with increasing amounts of acetonitrile (0–30%, v/v) in methanol. They also fractionated nonenriched menhaden oil methyl esters and fatty acids, which contained approximately 12.5% EPA and 11.1% DHA. The separation was done isocratically using 40% (v/v) acetonitrile in acetone to yield one eluted fraction containing approximately 69% EPA and DHA in total.

The choice of solvent for chromatographic separation of fatty acid esters depends on the desired purity of the eluted fractions and their end use as well as production requirements. Tetrahydrofuran/methanol/water (25 : 55 : 20, v/v/v) (41) and methanol/water (90 : 10, v/v) (42) have been described to yield EPA and DHA with high purity (75–96%) as fatty acid ethyl esters from fish oil. Krzynowek et al. (43) obtained higher purity fractions of EPA and DHA using the tetrahydrofuran (THF) system of Tokiwa et al. (41). However, THF oxidizes readily yielding peroxides that initiate oxidative decomposition of PUFA and is potentially explosive. Ethanol and water would be the solvents of choice if the end product is to be consumed by humans.

**2.1.2. Countercurrent Chromatography** Countercurrent chromatography (CCC) or centrifugal partition chromatography (CPC) has gained much attention in recent years for isolation of polyunsaturated fatty acids. This new liquid chromatographic technique uses liquid-liquid partition, countercurrent distribution of solute mixture between two liquid phases, in the absence of a solid support, to perform separation of complex mixture of chemical substances (44, 45).

Centrifugal partition chromatography (CPC), pioneered by Ito in 1964, is a liquid-liquid chromatography without a sorbent, requiring two immiscible solvent

phases, which is basically an outgrowth of countercurrent distribution, as developed by Craig (46). In the most distinct variants of CPC, one liquid phase remains stationary while the second solvent phase passes through the stationary phase solvent. The principle of separation involves the partition of a solute between two immiscible solvents. The relative proportion of solute passing into each of the two solvent phases is determined by the respective partition coefficients. CPC is a process-scale separation technique, which offers distinct advantages for the separation, isolation, and purification of natural products compared with traditional liquid-solid separation methods, such as normal column chromatography and HPLC.

Centrifugal partition chromatography does not use a solid support as the stationary phase. Therefore, the possibility of irreversible retention of highly retentive sample components is eliminated. For this reason, the chromatographer is virtually assured of almost 100% recovery of the compounds from a chromatography. Any two-phase solvent system may be used; many partition systems can be prepared with nontoxic, commonly available solvents. The volume ratio of the stationary phase to the total column (rotor) volume is greater in CPC than in conventional liquid chromatography. Therefore, large quantities of sample materials can be retained in the stationary phase. Decomposition and denaturation of valuable sample components, often encountered with conventional packed chromatographic columns, are virtually nonexistent under the mild operating conditions used in CPC. The CPC separation method may be performed at almost any pH. The pH constraints imposed by solid supports, such as silica, alumina, etc., are not a consideration in CPC. The other advantage of CPC is the low solvent consumption and that both normal and reversed-phase elution may be conducted with the same solvent pair. CPC can be readily adapted for large-scale continuous separations. The entire process is performed in the liquid phases, in a closed system. Environmental problems are minimal, and solvent may be completely recovered and recycled (45).

CPC instrument is basically of two types: the centrifugal partition chromatography instrument manufactured by Sanki Engineering, Ltd. (Kyoto, Japan) and the multicoil countercurrent system designed by Ito and manufactured by Pharma-Tech Research (Baltimore, MD). Similar to most chromatographic systems, the CPC has basic components such as pumps for solvent delivery, valves to control solvent delivery and sample injection, detector, and recorder. The main component of the instrument is its rotor. The rotor is made of stacking engraved poly-phenylene sulfide (PPS) or poly-chlorotrifluoroethylene (DAIFLON) disks separated by Teflon seal sheets and stainless-steel plates. The disks consist of channels and ducts on each side. The liquid stationary phase is held in these channels and the centrifugal field generated by the spinning rotor holds the stationary phase sufficiently enabling a mobile phase to be pumped through. Details of the instrument and its function are described in the review by Wansunsudara and Fedec (45).

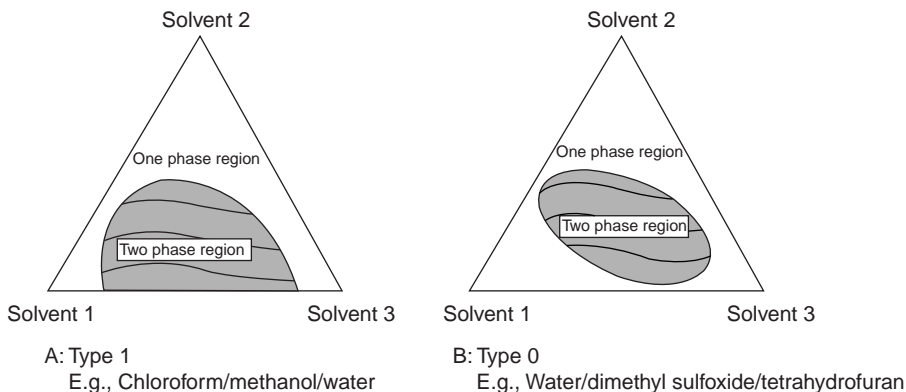
Recently, another type of CPC instrument has been manufactured by Kromaton (Angers, France), and this model is now available for commercial use. Another commercially available rotating coil instrument is the multicoil countercurrent chromatograph, introduced by Pharma-Tech Research (Baltimore, MD). The multicoil

chromatograph consists of two or three identical multilayer coils arranged symmetrically around the rotor frame of the centrifuge, thus eliminating the need for balancing with a counterweight. Each coil column undergoes synchronous planetary motion in such a way that it revolves around the central axis of the centrifuge and simultaneously rotates around its own axis at the same angular velocity (47, 48). The columns are equipped with flow tubes arranged in such a fashion that they do not twist, allowing seal-free operation of the instrument. The three-coil instrument is available in different modules with different column capacities (Models TCC-1000, CCC-3000, and CCC-1000 column capacities of 4 ml, 40ml, and 800ml, respectively). As no solid support, exists retention of injected samples depends solely on the respective partition coefficients of the components present in the mixture between the immiscible two-phase solvents. The number of theoretical plates of the column can be easily modified by increasing the number or length of the coils, by reducing the inside diameter of the coiled column, or by reducing the helical diameter (49).

The correct choice of a solvent system is of paramount importance for a successful CPC separation. Selection of a two-phase (biphasic) solvent system for CPC is similar to choosing the solvents for other chromatographic methods such as for a normal column or HPLC (50). Important criteria are the polarity of the sample components and their solubility, charge state, and their ability to form complexes, etc. The most critical points in selecting solvent systems for CPC are two-fold: one is solubility of the sample and the second is the difference in partition coefficients of the molecular species that are to be separated.

The chromatography literature contains numerous examples of solvent systems used in different countercurrent chromatographic separations (51, 52), and these references may give some leads to possible systems useful for the separation in question. Alternatively, a classic chloroform/methanol/water (polar) or n-hexane/ethyl acetate/methanol (less polar) system can be selected as the starting point, and the proportions of the individual solvents may be changed until the required distribution of the sample between the two phases is obtained (53). Chloroform-based solvent systems provide large density differences and relatively high interfacial tension between the two solvent phases. Consequently, they are frequently employed for the separation of natural products by CPC. As a result of their short setting times, the chloroform/methanol/water systems normally produces satisfactory phase retention. However, one drawback of chloroform system is that it readily leads to overpressure problems with the Sanki apparatus; another drawback is the serious health hazard of chloroform.

The other approach for selecting proper solvent systems for CPC is using the solvent phase diagrams. Ternary diagrams for many solvent systems have been compiled by Sorensen and Arlt (54), and two of the diagrams are as given in Figure 1. They often consist of two immiscible solvents plus a third solvent that is soluble in the two primary solvents. Most systems conform to type 1, comprising one solvent miscible with two other immiscible solvents (Figure 1A). A typical example for the type 1 ternary system is chloroform/methanol/water. Very few systems are like type 0, made with three solvents fully miscible by pairs, but for which a zone exists



**Figure 1**

in the ternary diagram where a biphasic system occurs when mixing them in a suitable ratio (Figure 1B). A good example of type 0 system is water/dimethyl sulfoxide/tetrahydrofuran. Foucault (50) has suggested following three criteria to be considered when ternary diagrams are used for the selection of solvents.

1. Select the solvent(s) in which the sample can be completely dissolved. As CPC has a preparative goal, the final solvent mixture must be able to dissolve large amounts of sample, and thus it should contain at least one of the “best” solvents that make the sample freely soluble.
2. Aided by the polarities of the solvents (numerous polarity scales may be found in the literature), choose a solvent on each side of the selected best solvent, that is, one less polar and one more polar, in order to obtain a biphasic system where the best solvent will partition into the two other solvents.
3. Adjust the ratio of the best solvent to disperse the sample into two phases; that is, the less polar fraction of the sample will preferentially go into the less polar phase and the more polar fraction will preferentially go into the more polar phase, so that the average partition coefficient will stay around 1.
4. Common biphasic solvent systems that are used in separation of natural products are listed in Table 1.

Bousquet et al. (55, 56) have tested CPC separation of EPA and DHA from microalgal oil and were able to isolate pure EPA and DHA from this oil with excellent yields. The first separation used heptane as the stationary phase and acetonitrile/water (3% v/v) as the mobile phase was able to remove fatty acids of minor importance, leaving a mixture of four major polyunsaturated fatty acids. A crude acid mixture (2.4 g) yielded a mixture (1 g) of the fatty acids, i.e., C18:3 (43%), C18:4 (7.5%), EPA (45%), and DHA (4.5%). This mixture was subjected to another separation, using heptane as the stationary phase and methanol-water as the mobile

**TABLE 1. Common Biphasic Solvent Systems Employed in the Separation of Natural Products by CPC (45).**

	Compounds	Solvent System
Water soluble	Proteins, nucleic acids sugars	Ethanol/Aqueous salt Acetonitrile/Aqueous salt
	Peptides, sugars	Butanol/Acetic acid/Water Butanol/Methanol/Water
	Nonionic (polyphenols, saponins)	Butanol/Acetic acid/Water Butanol/Methanol/Water Butanol/Ethyl acetate/Water
Water insoluble	High polar (Polyphenols, tannins)	Butanol/Acetic acid/Water Butanol/Ethanol/Water Butanol/Ethyl acetate/Water
	Medium polar (Polyphenols, Terpenoids)	Chloroform/Methanol/Water Methy-t-butyl ether/Acetonitrile/Water Heptane/Ethyl acetate/Water
	Low polar (Fatty acids, Tocopherols, Phytosterols)	Hexane/Acetonitrile Hexane/Methanol/Water Hexane/Ethanol/Water

phase. Under these conditions, a good separation was achieved, and isolation of pure EPA and DHA resulted with an excellent yield. Murayama et al. (57) have successfully separated a mixture of ethyl esters of C18:0, C18:1, C18:2, and C18:3 fatty acids because their partition coefficients are distributed over a wide range in the two-phase solvent, n-hexane/acetonitrile (1:1 v/v). The fatty acids C18:2 and C18:3 were separated during the first normal ascending elution, whereas C18:1 was recovered by switching the elution mode. The CPC may also be applied to separate EPA and DHA ethyl esters with a solvent system comprising hexane/dichloromethane/acetonitrile (5:1:4 v/v). CPC separation has been used to purify DHA and DPA from algal oil containing 39.7% DHA and 15.2% DPA (45). The free fatty acid (FFA) mixture obtained from algal oil was eluted with hexane/methanol/water (100:95:5 v/v) two-phase solvent system in normal phase ascending mode of CPC. Under these conditions, DHA was purified up to 84.6% and DPA up to 84.9%; however, coelution of C14:0 fatty acid with DHA occurs under these conditions. In order to isolate ultra-pure DHA (99%, fine chemical grade), prepurification of algal oil-FFA was needed by complexing with urea to remove the coeluting C14:0 fatty acid (45). Purification of gamma linolenic acid (GLA) is difficult by classic chromatographic methods because of its instability; however, GLC with 98+% purity could be achieved by CPC separation of borage oil-FFA (21.8% GLA in starting oil) under the conditions and solvent systems described above.

Highly purified phospholipids have been isolated and purified from egg yolk using CPC. An ethanol extract of egg yolk has yielded phosphatidyl choline (PC, lecithin) of 98.3% purity in a single run through the CPC (58). Byproducts of this

purification include phosphatidyl ethanolamine (PE), lyso-phosphatidyl choline (LPC), sphingomyelin (SPM), and triacylglycerol (TAG) with reasonably good purity. Boudimant et al. (58) have applied the CPC technique for purification of PC to homogeneity from a crude phospholipid extract of squid. The starting extracts contained PE (24.1%), phosphatidyl inositol (PI, 17.5%), phosphatidyl serine (PS, 4.9%), PC (48.3%), and SPM (5.1%). The crude extract was submitted to a first run using n-heptane/ethyl acetate/acetonitrile (1:0.65:1, v/v) as the solvent system. The first elution was performed in a normal ascending mode allowing the removal of all other components except PC, and then most polar PC was eluted from the rotor in a reversed descending mode. Isolation and purification of natural tocopherol isomers is also possible by employing the CPC separation (45). A starting mixture containing 70% of total tocopherols was separated into  $\gamma$ - and  $\delta$ -tocopherols (97% pure) and  $\alpha$ -tocopherol (99% pure) with high recovery using Sanki instrument in the ascending mode elution with hexane/acetonitrile/ethanol (40:25:10, v/v/v) solvent system.

CPC is a powerful process-scale separation technology, which offers distinct advantages over other methods for the separation, isolation, and purification of fatty acids. It is also complementary to HPLC. Although capital investment for a CPC often runs higher than that for HPLC, operating costs are generally an order of magnitude lower than when the same separation is performed with conventional liquid chromatography or preparative HPLC. Scaling up problems associated with HPLC because of variability of column packing chemistry, surface morphology, and geometry are not a consideration in CPC, because no solid stationary phase exists in this system. Another advantage is that the option exists of running the separation under a blanket of nitrogen or other inert gas, when working with substances prone to oxidation processes, especially for highly unsaturated fatty acids. So far it has demonstrated its potential for the separation of lipid molecules at the level of a few grams and expected to develop into production at kilogram levels (45).

**2.1.3. Crystallization** It has long been known that the higher saturated acids are much less soluble than their corresponding unsaturated counterparts and may be partially separated from a mixture. Therefore, crystallization at ordinary, zero, and subzero temperatures has been a useful method of purifying fatty acids and their derivatives. In the 1930s, Brown and Stoner (59) and Brown and Shinowara (60) showed that low-temperature crystallization can be used for partial separation of fatty acids from cottonseed oil and for the preparation of oleic acid from olive oil. Low-temperature crystallization was originally developed to separate certain TAG, fatty acids, esters, and other lipids that are highly soluble in organic solvents at temperatures above 0°C, but become sparingly soluble at lower temperatures down to -80°C (61). In 1950, Bailey and co-workers emphasized that the solubility of any given acid is closely related to its melting point and to a certain degree dependent on the nature of the solvent.

Fractionation by crystallization using the differences of melting point of fatty acids or triacylglycerols is done in two ways. Dry fractionation, also known as

the low-temperature crystallization, is the enrichment of oil with the more unsaturated triacylglycerols by removing saturated, high melting components at low temperature. The other method is solvent fractionation (crystallization), which involves use of organic solvents such as acetone or hexane in order to improve the yield of each fraction.

**2.1.3.1. Low-Temperature Crystallization** This method of fatty acid separation does not involve organic solvents; therefore, it is fractionated without solvents or referred to as low-temperature crystallization. The solubility of fats in organic solvents decreases with increasing mean molecular weight and increases with increasing unsaturation (62). In general, even number carbon saturated and unsaturated acids become less soluble with increase in molecular weight; for unsaturated fatty acids, solubility increases with the number of double bonds present. Singleton (63) and Stout et al. (64) have determined the solubility of a large number of fatty acids and esters in a variety of solvents and discerned the following rules: when acids are saturated, long-chain acids are less soluble than short chain acids; saturated acids are less soluble than monoenoic and dienoic acids of equal chain length; *trans*-isomers; are less soluble than *cis*-isomers; and normal acids are less soluble than branched acids. The melting point of fatty acids changes considerably with the type and degree of unsaturation, thus separation of mixtures of saturated and unsaturated fatty acids is possible. The *trans*-acid (higher melting) of a *cis-trans* pair is less soluble than the *cis*-acid (lower melting). Saturated acids are usually solid at ambient temperature and can crystallize at temperatures down to 0°C. Crystals are separated from the mother liquor by any conventional filtration procedure. At low temperatures, long-chain saturated fatty acids that have higher melting points crystallize out and PUFAs remain in the liquid form. However, unsaturated fatty acids with lower melting points and higher solubilities must be crystallized at lower temperatures (0–100°C) and filtration must be carried out at an appropriate low temperature. The solution temperature should be reduced to the crystallization temperature slowly (1–6 h) and maintained there for 4–24 h. This produces larger crystals, which are easier to filter. Low-temperature crystallization is a mild procedure that is especially suitable for polyenoic acids that are susceptible to oxidation at elevated temperatures. Crystallization of saturated fatty acids at low temperatures has been in practice since the 1940s and has developed into a commercial technique for separation of saturated fatty acids from triacylglycerols of natural sources. The fatty acid crystals formed at lower temperatures are separated by a filtration process and special equipment is developed to accomplish this at lower temperatures.

The solubility of a given fatty acid in a mixture could be approximated to its solubility in the pure state; however, intersolubilization could occur and may also form mixed crystals at temperatures that do not permit precipitation of a particular acid (62). The removal of high melting fully saturated fatty compounds is comparatively easy when more unsaturated compounds are present; however, this is not always possible for natural mixtures of fatty acids. Retention of liquid phase in



the solid material separated (entrainment) is also a problem in the fractionation by crystallization, which happens because of occlusion of uncrystallized liquid within the crystallized particles or aggregates as well as to liquid retention between the particles.

The use of membrane filter press enables dry fractionation to compete very effectively with solvent fractionation, especially in palm mid-fraction separation. Specification for palm mid-fraction is the proportion of symmetrical monounsaturated triacylglycerols and the ratio of symmetrical to asymmetrical monounsaturated triacylglycerols in the fraction, which also characterize cocoa butter equivalents. According to Deffense (65), two-stage solvent (acetone) fractionation produces similar enrichment of symmetrical triacylglycerols of the mid-fraction of palm oil, which is usually produced by three-stage dry fractionation. Willner et al. (66) showed that two-stage solvent (acetone) fractionation produces similar composition mid-fraction as two-stage dry fractionation combined with a membrane filter press for first stage and high-pressure "hydrofilter" press for the second stage. Introduction of membrane filter press has improved the applicability of dry fractionation in commercial separation of fats and oils components.

Slow cooling that is achieved by long crystallization time helps to produce stable polymorphic form of triacylglycerol crystals. In order to achieve this situation, one way is to use the oil temperature as the control parameter for the coolant (e.g., use in Fractionment Tirtiaux, Fleurus, Belgium). Control of agitation during crystallization is also important in optimizing the yield of olein and quality of the product. The other is the horizontal vacuum belt filter that was introduced in the early 1970s (Fractionment Tirtiaux, Fleurus, Belgium), which enables one to improve the yield of olein (from 67% to 72%). However, these two processes still could not reduce the entrainment problem. The membrane filter press that was introduced in the early 1980s permitted applying pressure to the cake in the press chamber while giving a lower level of liquid entrainment at the filter cake. The filter presses were further developed to operate at pressures up to 50 bars, but at a high operating cost (Hydrofilter, Krupp Maschinentechnik, GmbH, Hamburg, Germany). Use of countercurrent crystallization (feed material is in contact with a progressively solid depleted liquid fraction in successive stages of a multistage crystallization process) may enhance the fractional crystallization efficiency of edible oils (67).

Use of crystallization-modifying substances to accelerate, retard, or inhibit crystallization of triacylglycerols may be used for dry fractionation (68). These crystallization modifiers (or crystal habit modifiers) are fatty acid polyesters of sucrose, especially those of palmitic or stearic acid esters (69–71) or glucose and derivatives, e.g., dextrin (72). The crystal habit modifiers can change the habit of triacylglycerol crystals in a way that the crystallized stearin base can be separated more effectively from the liquid olein phase. Palmitic or stearic acid as the main substituting agent in sucrose (4 to 8 average degree of substitution) is an emulsifier and added to fat at high temperatures (about 50°C). Once they are mixed with the fat, temperature is lowered and fine, rigid crystals of specific components of fat are formed. Filtration under vacuum may be used to separate the resultant crystals. Separation efficiency also depends on the mode of crystallization, stagnant or stirred.

**2.1.3.2. Solvent Crystallization** The low-temperature crystallization process may be carried out on the neat liquid in the absence of a solvent or in a selected solvent/solvent mixture. Liquid-liquid countercurrent extraction has been developed and used for fractionation and refining of oils and fatty acids. Crystallization of saturated fatty acids from a solution of fatty acid mixture in organic solvents, especially apolar type, is a promising way of separation. Table 2 provides solubility of different fatty acids in different organic solvents as a function of temperature. For unsaturated fatty acid mixtures, Hildith and Riley (73) have shown that when fatty acid mixtures of sunflower, sesame, and peanut oil were cooled to  $-30^{\circ}\text{C}$  in acetone (5ml/g) the portion left in the solution after crystals were separated contained most of the linoleic acid. The crystallized solids had low iodine value and mostly contained saturated fatty acids. Later, this procedure was employed for separation of a variety of natural sources of unsaturated fatty acids (61). Separation of unsaturated fatty acid methyl esters by crystallization at low temperatures ( $-20^{\circ}$  to  $-80^{\circ}\text{C}$ ) is feasible because of the easy crystallization of saturated and monoenoic acid esters. The commonly used solvents are methanol and acetone, and these have been employed to separate "stearic" and "oleic" fractions. Attempts have also been made to use other solvents such as hexane and methyl formate (74). The *Emersol* process and *Armour-Texaco* process use the principles of fractional crystallization of fatty acids from solvents at low temperatures. Fatty acids are dissolved in methanol and then passed through a series of precoolers and coolers/crystallizers that are maintained at temperatures below freezing ( $<-15^{\circ}\text{C}$ ).

Use of low-temperature crystallization in fractionation of PUFA is very promising because of the mild conditions involved. It has been reported that use of different organic solvents and temperatures affects the concentration of PUFA (61, 75). With proper choice of solvent and temperature, PUFA can be concentrated into the noncrystallized fraction. The fungal oil extracted from *Mortierella genus* was used for concentration of  $\gamma$ -linolenic acid (GLA) by low-temperature crystallization (75, 76). The concentration of GLA in different solvents was in the order of acetone ( $-20^{\circ}\text{C}$ ) > n-hexane ( $-20^{\circ}\text{C}$ ) > acetone ( $4^{\circ}\text{C}$ ) > petroleum ether ( $-20^{\circ}\text{C}$ ) at a solvent to oil ratio of 5 : 1 (v/v). Solvent crystallization of fatty acids is an indispensable method for preparing pure fatty acids. This method requires the least number of unit operations and the simplest equipment (77, 78). Briefly, the process consists of the following: cooling of the oil or fatty acids in a solvent, holding for a specified period of time, and removing the crystallized fraction by filtration. Studies carried out on solvent crystallization of PUFA from seal blubber oil (SBO) showed that fatty acids in the free or TAG form can be concentrated into the non-crystalline fraction (79). Table 3 shows the enrichment of total  $\omega$ 3-PUFA following low-temperature crystallization of SBO in the TAG form using hexane and acetone as solvents. The content of  $\omega$ 3-PUFA in the noncrystalline fraction (the concentrate) was increased with lowering of the crystallization temperature. Under all temperature conditions, acetone afforded the highest concentration of total  $\omega$ 3-PUFA (79).

Low-temperature crystallization of SBO, in the free fatty acid form, at  $-60^{\circ}\text{C}$  and  $-70^{\circ}\text{C}$  in hexane, resulted in total  $\omega$ 3-PUFA contents of up to 58.3% and 66.7% in the preparation with concentrate recoveries of 39.0% and 24.8%,

**TABLE 2. Solubility (g acid/100-g solution) of Fatty Acids in Various Organic Solvents at Different Temperatures (61).**

Temperature °C	Solvent					
	Acetone	Diethyl Ether	Ethyl Acetate	<i>n</i> -Heptane	Methanol	Toluene
<i>Stearic acid, 18:0</i>						
10	0.54	2.40	0.58	0.080	0.26	0.00
0	0.11	0.95	0.13	0.018	0.090	0.080
-10	0.023	0.38	0.027	0.004	0.031	0.015
-20	0.005	0.15	0.006	—	0.011	0.003
-30	—	0.051	—	—	—	—
<i>Oleic acid, 18:1 cis</i>						
-20	5.20	—	5.95	2.25	4.02	—
-30	1.68	—	1.00	0.66	0.86	3.12
-40	0.53	5.15	0.62	0.19	0.29	0.96
-50	0.17	1.80	0.20	0.050	0.10	0.28
-60	0.055	0.61	0.057	0.011	0.03	0.075
-70	—	0.21	—	—	—	—
<i>Elaidic acid, 18:1 trans</i>						
0	—	—	—	0.59	—	—
-10	—	—	—	0.19	0.48	0.86
-20	0.26	1.40	0.29	0.060	0.18	0.20
-30	0.092	0.60	0.10	0.019	0.064	0.056
-40	0.029	0.23	0.027	0.007	0.020	0.013
-50	0.009	0.10	0.008	—	0.010	—
<i>Linoleic acid, 18:2 cis</i>						
-50	4.10	—	4.4	0.98	3.10	—
-60	1.20	—	1.38	0.20	0.90	—
-70	0.35	—	0.39	0.042	0.25	—
<i>Arachidic acid, 20:0</i>						
10	0.13	0.90	0.14	0.028	0.080	0.12
0	0.035	0.38	0.036	0.005	0.028	0.026
<i>Eicosenoic acid, 20:1, cis</i>						
-20	1.10	—	1.30	—	0.80	—
-30	0.54	3.90	0.60	0.45	0.35	1.10
-40	0.27	1.70	0.26	0.15	0.15	0.30
-50	0.12	0.68	0.11	0.048	0.06	0.07
-60	0.05	0.22	0.04	0.01	0.02	—
<i>Palmitic acid, 16:0</i>						
10	1.60	—	—	0.30	1.30	1.41
0	0.66	2.95	0.52	0.08	0.46	0.36
-10	0.27	1.35	0.18	0.02	0.16	0.086
-20	0.10	0.56	0.060	0.005	0.050	0.018
-30	0.038	0.21	0.018	—	—	—
<i>Behenic acid, 22:0</i>						
10	0.050	0.48	0.055	0.012	0.019	0.012
0	0.014	0.18	0.016	0.002	0.007	0.002
-10	0.004	0.068	0.004	—	0.002	—
<i>Erucic acid, 22:1, cis</i>						
-10	—	—	—	0.35	0.40	—
-20	0.28	—	0.31	0.11	0.19	0.68
-30	0.10	1.20	0.11	0.030	0.068	0.16
-40	0.037	0.49	0.040	0.008	0.024	0.044
-50	—	0.18	—	—	0.007	—

**TABLE 3. Fractionation of PUFA (%) of Seal Blubber Oil (SBO) by Low-Temperature Crystallization in Different Solvents.**

Crystallization Temperature (°C)	TAG		Free Fatty Acids	
	Hexane	Acetone	Hexane	Acetone
-10	23.2	23.5	23.8	24.3
-20	23.9	26.9	24.5	25.4
-40	26.2	36.4	31.0	40.6
-60	30.5	43.8	58.3	56.8
-70	35.1	47.9	66.7	46.8

Total  $\omega$ 3-fatty acids content of original SBO is 20.1%.

Adapted from (79).

respectively. However, the content of total  $\omega$ 3-PUFA in acetone increased up to 56.7% and 46.8%, but the recovery of the concentrates was 15.9% and 12.9%, respectively.

**2.1.4. Distillation Method** Hydrolysis of triacylglycerols and saponification has been described by Chevruel (80). The process of alkaline hydrolysis of acylglycerols under refluxing conditions in ethanolic media to produce soap has been in practice. In 1895 and 1896, Henriques reported that the saponifying process can be carried out at room temperature. The oldest record of distillation process dates back to about 3600 B.C. An apparatus consisting of essential parts for distillation has been found at Tape Gowra in Mesopotamia, which must have been used for preparing perfumes (81). The physical laws of Dalton (1766–1844) and Roullet (1830–1901) were the fundamentals of developing the science of distillation. Laboratory vacuum distillation was introduced in 1869 by Dittmar, and also by Kekule in 1872. Separation of pure palmitic and stearic acids to use in the synthesis of triacylglycerols was reported by Kraft (82) and Kreiser (83). Fractional distillation of methyl esters obtained from coconut oil has been reported by Haller et al. (84). In the same year, Bull (85) reported isolation of 9-eicosenoic acid from methyl esters of cod liver oil. Quantitative analysis of the composition of fractionated coconut oil and palm kernel oil was reported by Elsdon (86, 87). Analytical fractionation of fatty acid methyl esters in order to determine the composition of peanut, sunflower, and olive (88–91) as well as soybean oil (92), unsaturated fatty acids of menhaden oil and beef brain lipids has been in practice (93, 94).

Invention of an actual distillation apparatus was reported in 1932–1935, which the inventor is not clearly identified. Fractionation of fatty acid esters using open tubes and spiral-type columns has been illustrated in Reily's book (95). The objective of this distillation process was to obtain fractions containing "no more than two adjacent homologous saturated and unsaturated members and having chain length differing by 2-carbon units" (96). Columns consisting of rotatable fractionating sections were described by Podbielniak in 1935 (97) and later modified by

Baker (98) to improve the separation efficiency by increasing the number of theoretical plates. Privett et al. (99) have used this device and were able to fractionate methyl esters of pork liver lipids into 38 distilled fractions with chain lengths ranging from C14 to C22. Technical improvements of this distillation method were developed into molecular distillation and isolation of Vitamin D (100), esters of fish oil (101, 102), cholesterol, tocopherol (103), and fish oil to obtain high-purity DHA (104).

Distillation has been used for partial separation of mixtures of fatty acid esters. This method takes advantage of existing differences in the boiling point and molecular weight of fatty acids under reduced pressure (61) and requires high temperatures of approximately 250°C (105). Short-path distillation or molecular distillation uses lower temperatures and short heating intervals. However, fractionation of PUFA-rich oils (e.g., marine oil esters) is difficult because separation of these components becomes less effective with increasing molecular weight (106, 107).

At present, the most widely used distillation procedure is fractional distillation of methyl esters under reduced pressure (0.1 to 1.0 mmHg). Even under these conditions, moderately high temperatures are required; the more highly unsaturated acids, especially  $\omega$ 3-PUFA, are more prone to oxidation, polymerization, and isomerization of double bonds. Heated columns packed with glass helices or some form of metal packing are in common use despite the disadvantage of a significant hold up and pressure drop through the column. Spinning band columns are designed to avoid these disadvantages. Distillation at lower pressures has been used in the isolation of some highly unsaturated acids, and is particularly valuable in polymerization studies to separate monomeric, dimeric, and polymeric materials and in the separation of monoacylglycerols from di- and triacylglycerol mixtures. Exposure of long-chain  $\omega$ 3-PUFA to high temperatures during distillation may induce hydrolysis, thermal oxidation, polymerization, and isomerization. Possible degradation products of long-chain PUFA are cyclic fatty acids and high-molecular-weight polymers (108, 109). Privett et al. (110) and Privett and Nickell (111) found a marked decomposition of arachidonic acid (C20:4  $\omega$ 6) when it was distilled slowly in a spinning band column. When the catalytic effect of metal parts was eliminated by using an all-glass apparatus, still the high temperature and exposure to oxygen caused a major loss of  $\omega$ 3-PUFA (112). Therefore, design of a method for preparation of  $\omega$ 3-PUFA concentrates involving low process temperature and time to minimize thermal damage to labile molecules is desirable.

**2.1.5 Solubility differences of fatty acid salts** In 1828, Gusserow introduced a method that lead salts or soaps of fatty acids in ether can be separated depending on the solubility differences. Saturated and unsaturated fatty acids form salts with metallic ions (e.g., Li) whose solubilities in water and organic solvents vary with the nature of the metallic ion and the chain length, degree of unsaturation, and other characteristics of the acid radicals. Substitution of ethanol for diethyl ether (113) allows better separation.

The alkali salts of saturated fatty acids crystallize more readily than those of PUFA containing four or more double bonds when the saponified solution is cooled.

Therefore, concentration of  $\omega$ 3 fatty acids from PUFA-rich oils in the form of fatty acid salts may be achieved by employing a lower alcohol using solubility differences of the salts. In order to obtain high content of total  $\omega$ -3 fatty acid with a good recovery, the water content of the medium should be maintained at a 3% level. Han et al. (114) were able to increase the total  $\omega$ 3 fatty acids of sardine oil from 33.2% to 75.9%.

## 2.2. Novel Methods

**2.2.1. Enzymatic Methods** The use of enzymes to produce fatty acids and fatty acid-derived products has been a focus in both academic and industrial circles. Lipases may catalyze esterification, hydrolysis, or exchange of fatty acids in esters (115). These processes can be selected by choosing appropriate substrates and reaction conditions. Lipase-catalyzed processes have attracted attention because of the mild reaction conditions under which they occur and the selectivity displayed by these catalysts. In both respects, they differ from typical chemical reactions. As enzymatic reactions occur under mild temperature and pH conditions and at ambient pressure, they generally require less energy and are conducted in equipment of lower capital cost than many other chemical processes. Another advantage of enzymatic process is related to the selectivity of many lipases, which allows obtaining products that are difficult to produce by more conventional chemical reactions.

**2.2.1.1. Lipase-catalyzed Hydrolysis** Bottino et al. (116) have illustrated the mechanism of resistance of lipases toward long-chain  $\omega$ 3-PUFA in marine oils. The presence of *cis*-carbon-carbon double bonds in the fatty acids results in bending of the chains. Therefore, the terminal methyl group of the fatty acids lies close to the ester bond, which may cause a steric hinderance effect on lipases. The high bending effect of EPA and DHA caused by the presence of 5 and 6 double bonds, respectively, enhances the steric hinderance effect; therefore, lipases cannot reach the ester-linkage between these fatty acids and the glycerol moiety. However, saturated and monounsaturated fatty acids do not present any barriers to lipases and, thus, may be easily hydrolyzed. Therefore, fatty acid selectivity of a lipase for EPA and DHA allows their separation and concentration from other components present in the remaining portion of marine oils. In addition, lipases have frequently been used to discriminate between EPA and DHA in concentrates containing both of these fatty acids, thus providing the possibility of concentrating  $\omega$ 3-PUFA (116).

Microbial lipases from *Aspergillus niger* (AN), *Candida cylindracea* (CC), *Pseudomonas* spp. (PS), *Chromobacterium viscosum* (CV), *Rhizopus delemer* (RD), and *Rhizopus javanicus* (RJ) have been widely used in modifying PUFA-rich oils (117). The fatty acid specificity of lipases (discrimination of PUFA over short-chain fatty acids) is a crucial factor when considering the application of enzymes to modify marine oils rich in PUFA (118–121). Lipases from *Rhizopus* spp are known to be 1,3-position specific (122). Wanasundara (79) and Wanasundara and Shahidi (123, 124) (seal blubber oil; SBO and menhaden oil; MHO) and Tanaka et al. (117)

**TABLE 4. Enrichment of Total  $\omega$ 3 PUFA in the Nonhydrolyzed Fraction of Seal Blubber (SBO) and Menhaden (MHO) Oils on Hydrolysis by Different Microbial Lipases.**

Source and Enzyme	Hydrolysis Time, h	Total $\omega$ 3-PUFA, %	
		MHO	SBO
No enzyme	0	20.2	30.1
<i>Aspergillus niger</i> (AN)	12	20.8	30.3
	75	23.3	35.0
<i>Pseudomonas</i> spp. (PS)	12	22.4	35.0
	75	26.1	39.5
<i>Candida cylindracea</i> (CC)	12	40.2	40.9
	75	45.0	45.8
<i>Rhizopus oryzae</i> (RO)	12	24.2	39.2
	75	33.1	45.7
<i>Geotrichum candidum</i> (GC)	12	30.0	40.1
	75	30.0	42.2

Adapted from (79).

(tuna oil) have shown that lipase-assisted hydrolysis of marine oil may enrich  $\omega$ 3-PUFA in the acylglycerols of the nonhydrolyzed fraction. Table 4 shows that in MHO, the total content of  $\omega$ 3-PUFA was increased from 30% in the original oil to 45.7%, 45.8%, and 42.2% after a 75 h hydrolysis by RO-, CC-, and GC-lipases, respectively. Corresponding increases in the content of DHA in this oil were from 10.1% in the original oil to 25.6%, 18.2%, and 15.5%, respectively. In SBO, maximum increase in the content of total  $\omega$ 3-PUFA, from 20.2% to 45.0%, was attained using CC-lipase under similar experimental conditions.

**2.2.1.2. Lipase-Catalyzed Esterification** When the TAG form and alkyl (methyl or ethyl) esters of PUFA were compared in laboratory animals, alkyl esters showed impaired intestinal absorption (125, 126). Therefore, TAG form is nutritionally more favorable than methyl or ethyl esters of fatty acids. Yang et al. (127) have shown that methyl and ethyl esters are hydrolyzed slower than their corresponding TAG. From a marketing point of view, triacylglycerols of PUFA are often promoted as being more “natural” than other fatty acid derivatives. In order to include preferred fatty acids into the TAGs lipase-catalyzed esterification of fatty acids is widely employed and structured lipids with high biological value are generated.

When inclusion of PUFA are concerned, direct esterification of glycerol with individual free fatty acids, including EPA and DHA, had been carried out by employing CV- and CC-lipases (128). Several researchers have reported that this method affords a high degree of incorporation of targeted fatty acids into the TAG molecule, which has been observed with microbial lipases for marine oils (128–130) and plant oils (131–133). All these studies have pointed out that the water content in the reaction medium is a crucial factor determining the extent of the esterification

reaction. A high water content in the reaction medium shifts the chemical equilibrium toward hydrolysis, whereas a reduced water content shifts the equilibrium toward esterification. The optimum content of water in the esterification reaction should be kept to a minimum in order to discourage occurrence of partial hydrolysis of products and formation of glycerol as well as mono- and diacylglycerols. However, the content of water in the reaction medium should be sufficiently high in order to prevent enzyme deactivation. The water requirement for different enzymes varies considerably, typically from 1% to 4% for interesterification reactions of TAG (133). Starting with previously enriched material (high content of targeted fatty acids by other means, such as urea fractionation), it is possible to obtain very high levels of incorporation (134, 135).

**2.2.2. Supercritical fluid extraction** Supercritical fluid extraction (SFE) uses compressed gas as the extraction medium and circumvents some of the problems associated with the use of classical separation techniques involving organic solvents. This technique combines features of distillation (i.e., separation because of differences in component volatiles) and liquid extraction (i.e., separation of components that exhibit little difference in their relative volatilities or that are thermally labile). A number of gases, when compressed isothermally at a temperature greater than their critical temperature and to pressures greater than their critical pressure, exhibit an enhanced solvating power (136), which has been known since the nineteenth century (137, 138), but its actual applications did not come to practice until the late twentieth century.

The region in which a substance exists as a supercritical fluid is defined by its critical pressure ( $P_c$ ) and temperature ( $T_c$ ). Supercritical fluids can have liquid-like densities and, at the same time, values of transport properties such as viscosity and diffusion coefficients, intermediate between those typical for gases and liquids. The solubility of a solute in a supercritical fluid is mainly a density-driven phenomenon and can be significantly increased with increasing pressure. Near the critical point, the fluid is highly compressible and a small increase in pressure can induce a large increase in fluid density. At pressures near the critical point, a moderate temperature increase may cause a large decrease in fluid density resulting in a decrease in solute solubility, which is known as retrograde behavior (139). The fluid becomes less compressible at much higher pressures and an increase in temperature causes density decrease to a lesser extent. Therefore, at higher pressures, an increase in temperature may bring about an increase in solubility, i.e., a nonretrograde behavior (140). These pressures are very high and in the order of 1000 psig to 2000 psig. For edible applications, CO<sub>2</sub> is chosen because it has moderate critical temperature and pressure and is inert, inexpensive, nonflammable, environmentally acceptable, readily available, and safe (141). Carbon dioxide has critical temperature of 31.1°C (at 1070 psig), which allows the extractions to be carried out at temperatures below 100°C. The single-component pressure-temperature phase diagram defines the critical region for the specific case of CO<sub>2</sub>. Readers are referred to the reviews provided by Paul and Wise (142) and McHugh and Krukoni (143) for the details of basic concepts of this technique.



The use of supercritical fluid carbon dioxide (SFCO<sub>2</sub>) for the extraction of oil from oilseeds (144), and decaffination of coffee (145) is currently in practice in an industrial scale. The good solvating power with low viscosity and high diffusion coefficients of supercritical fluids make them suitable for use as a mobile phase in chromatography with high velocities. The solvating power of supercritical fluids depends on their specific gravity, which is a function of pressure and temperature. Görner and Perrut (146) were able to separate fatty acid methyl esters (C<sub>18</sub>–C<sub>22</sub>, both saturated and unsaturated) on a silica column using SFCO<sub>2</sub> as the mobile phase.

The solvent strength of supercritical carbon dioxide, even at high density, is not sufficient for the elution of polar solutes. The solubility of the solute in the supercritical phase can be greatly influenced by adding modifiers to the mobile phase. Addition of modifiers, usually organic solvents, increases the polarity of the extracting SFCO<sub>2</sub> (147). Using SFCO<sub>2</sub> with modifiers (acetonitrile or isopropanol) as the mobile phase and silica-based cation exchanger impregnated with silver nitrate as the stationary phase, separation of geometric isomers of diene- and triene-fatty acid methyl esters of fish oils has been achieved (148).

Merkle and Larick (149) have separated triacylglycerols from beef tallow using SFCO<sub>2</sub> as the extracting medium at 40°C and at pressures of 10.3–27.6 MPa. Concentrated TAGs were obtained under ambient temperature and pressure. Saturated and monounsaturated TAGs could be extracted based on the solvent density and molecular weight. When a model mixture containing fatty acid methyl esters and cholesterol was extracted with SFCO<sub>2</sub> (1500 psig, 328.2°K), a selective extraction of cholesterol was observed, demonstrating that the removal of cholesterol is possible from the mixture (150). Supercritical fluid extraction (SFE) has been effectively used to refine fish oils and to remove cholesterol, polychlorinated biphenyls (PCB), Vitamin E, and other components (151). The separation of PUFA by SFE is dependent on the molecular size of the components involved rather than their degree of unsaturation; therefore, a prior concentration step is needed to achieve a high concentration of PUFA in the final product (141). Oils to be used for enriching PUFA by SFE require preparation steps of extraction, hydrolysis, and esterification by conventional methods (151, 152). Several research groups have reported the fractionation of mixtures of mono-, di-, and triacylglycerols using various fluids (153, 154). Some enhancement of the content of ω3-PUFA present in fish oil TAG has been reported (155) Stout and Spinelli (156) have demonstrated that fish oil esters could be fractionated by SFE to produce an oil with a DHA content of 60–65%. The fractionation of free fatty acids using SFE has also been reported (145). Both of these studies have shown a low recovery of ω3-PUFA during the process, the reasons for which remain speculative. However, use of extremely high pressures and high capital costs might limit the widespread use of this method for industrial-scale concentrate production. Recently, propane has gained more attention in the extraction technology, especially in the nutraceutical industry (157). However, more research and development will be required to determine the extent of its use for separation of PUFA from different oils.

**2.2.3. Urea Complexation** Urea alone crystallizes in a tightly packed tetragonal structure with channels of 5.67 Å diameter. However, in the presence of long straight-chain molecules, it crystallizes forming a hexagonal structure with inner channels of 8–12 Å diameter (158). The channels formed, in the presence of long-chain unbranched molecules, are sufficiently large to accommodate aliphatic chains. The applications of urea inclusion compounds (UIC) in separation of fatty acids involve isolation of PUFA and cyclic free fatty acids in the noninclusion fraction. UIC-based fractionation is very efficient in removing saturated fatty acids as UIC fraction. The main selectivity for separation of fatty acids via forming UIC includes (i) increased discrimination against inclusion as the number of double bonds per molecule increases, (ii) preference of molecules of longer chain length, (iii) preference for *trans*-versus *cis*- double bonds with *trans*- monoenes are often preferred over the corresponding saturate and (iv) sensitivity for double bond position (159). Although straight-chain saturated fatty acids with six carbon atoms or more are readily adducted, the presence of double bonds in the carbon chain increases the bulk of the molecule and reduces the likelihood of its complexation with urea (160). Monoenes are more readily complexed as compared with dienes, which, in turn, are more readily complexed than trienes. Therefore, the stability of fatty acid-urea adducts parallels the geometry of the molecules involved. Any deviation from a straight chain arrangement weakens the stability of the formed adduct. Therefore, formation of urea inclusion compounds depends on the degree of unsaturation of the fatty acids involved.

For UIC formation, first the oil (acylglycerol) is split into its fatty acid constituents using alcoholic KOH or NaOH. The unsaponifiable matter, such as sterols, Vitamins A, D, and E, and xenobiotics (e.g., PCB), as well as other undesirable components, are removed from oil during the splitting process. The free fatty acids which are mixed with an alcoholic (methanol or ethanol) solution of urea are then allowed to cool to a particular temperature, depending on the degree of concentration desired. The saturated fatty acids, monoenes, and, to a lesser extent, dienes are crystallized with urea and noncrystallized fatty acids in the solution can be separated by filtration. The liquid or nonurea complexing fraction (NUCF) is enriched with  $\omega$ 3-PUFA. Alternatively, this procedure can be carried out using methyl or ethyl esters of fatty acids rather than free fatty acids. There are advantages and drawbacks for each of these options. For example, fatty acids are more soluble in alcohol than their corresponding esters hence they require a much smaller volume of alcohol for processing. If the ester form of fatty acids is chosen, the re-esterification step of the concentrates is eliminated. Han et al. (161) have tested a series of solvents (ethanol, methanol, water, formamide, and acetonitrile) as wetting agents for urea. They reported that all solvents tested served as appropriate wetting agents, but water was the solvent of choice because of its low cost and lack of toxicity. Presence of phospholipids, di- and triacylglycerols greatly reduces the amount of UIC formed (162), thus refining of FFA mixtures prior to UIC-based fractionation is necessary. It is hypothesized that such compounds may enhance dissolution of FFA in the liquid phase, thus making entrapment of UIC less energetically favored (159).

Fractionation of FFA or alkyl esters of FA based on UIC is widely employed to enrich PUFA content in nonurea inclusion compounds (NUIC). It has been reported that complete removal of saturated fatty acids by urea complexation may be impossible because some of the shorter chain saturated fatty acids do not complex with urea during the crystallization process (79, 163, 164). Long-chain monounsaturated fatty acids (MUFA), especially those of the C20 and C22, form complexes with urea more readily than those of the shorter chain saturated fatty acids (C14 and C16), thus the amount of MUFA in UCF was increased depending on the reaction conditions (164). The overall recovery, varied inversely with increasing urea-to-fatty acid ratio as well as crystallization at lower temperatures as the extent of crystallization depends on the concentration of urea and the temperature of crystallization. Wille et al. (165) reported that C18:4 $\omega$ 3 and DHA are concentrated more efficiently in the filtrate when crystallization occurs at  $-5^{\circ}\text{C}$ , whereas total  $\omega$ 3-PUFA and EPA contents were maximized at  $10^{\circ}\text{C}$  and  $15^{\circ}\text{C}$ , respectively. Qualitatively, solubilities of fatty acids in the presence of urea are parallel, but the values are much lower than when they are used alone. Without urea, polyenoic compounds do not normally crystallize even at the temperature of dry ice, but they will do so in high concentrations of urea above  $0^{\circ}\text{C}$ . Successive crystallization with increasing concentrations of fatty acids and urea at decreasing temperatures down to  $13^{\circ}\text{C}$  allows separation of DHA from other components of the oil. Interestingly, urea complexation protects these  $\omega$ 3-PUFA from autoxidation (166). Complex formation is exothermic, but requires dissolution of both urea and fatty acids. Methanol and ethanol are preferred solvents for small-scale fractionation, but recently Han et al. (161) found that water may offer the best choice of solvent for large-scale operations. Swern (167) reviewed urea fractionation of fatty acids of many natural oils and Patokina et al. (168) studied the thermodynamics and other aspects of urea adduct formation.

Many publications have described the application of urea complexation in both analytical and preparative applications. Iverson and Weik (169) and Strocchi and Bonaga (170) correlated fatty acid structures with their preferential order of complexation with urea. Other publications have described application of this technique for concentration of specific fatty acids such as furanoid fatty acids (171), isoprenoid acids (172), *cis-trans*-isomers (173), an unusual fatty acid bearing a methyl branch and a double bond on the same carbon atom, such as 7-methyl-7-hexadecenoic acid (174), as well as preparation of  $\omega$ 3-PUFA concentrates from other marine oils (174–177). It should also be noted that in urea complexation, the complexed crystals are very stable, and hence, filtration at the very low temperatures used for solvent crystallization of fatty acids is not required (178). This method is also favored by many researchers because the complexation is based on the configuration of the fatty acid moieties because of the presence of multiple double bonds rather than pure physical properties such as melting point or solubility. Combined use of methods described above is common practice in order to achieve a high degree of separation of fatty acids. Use of urea complexation in combination with chromatographic separation allows preparation of fatty acids with a high degree of purity.

### 3. SEPARATION OF BYPRODUCT COMPONENTS

#### 3.1. Lecithin and Individual Phospholipids from Degumming Process

Lecithin is the widely obtained edible byproduct from oil processing. The definition of lecithin varies considerably however, lecithin represents a family of products based on naturally occurring phospholipids and other polar lipids. Most of the commercially available lecithins are obtained from various oilseeds or egg (179). In the scientific literature, lecithin stands for a special phospholipid, 1,2-diacyl-*sn*-glycero-3-phosphatidylcholine or phosphatidylcholine.

From a commercial point of view, soybean is the most important source for lecithin however, lecithin production from sunflower (180) and rapeseed (181–183) wet gum has also been successful. Wet gum is removed during the first refining step of crude oil that is the degumming process. Traditionally, crude oil obtained from solvent-extraction process is agitated with 1–3% water at elevated temperatures (70–80°C). Under these conditions, phospholipids and glycolipids start to swell and become insoluble in the oil. The hydrated mass is removed via centrifugation, and the dehydration is carried out under vacuum until the residual moisture is below 1%.

As lecithin and component phospholipids have become lucrative byproducts of edible oil processing industry, several improvements are included in their traditional methods of recovery. The yield and composition of phospholipids in lecithin can be altered by changes in the conditioning of seeds prior to oil extraction and also by changing the composition of the oil-extraction medium. The *Alcon* process uses heat treatment of the full-fat oilseed flakes prior to extraction, which inactivates phospholipases, thus minimizing nonhydratable phosphorus (NHP) in crude oil. This process, in turn, helps to enrich the content of phosphatidylcholine in lecithin. According to Short and others (184), phospholipid composition of lecithin that is from soy processing can be changed by altering the extraction medium. Full-fat flakes are extracted with a ternary mixture made of hexane, a lower alcohol, and water. This process results in a considerable increase in phosphatidylcholine content in lecithin, total yield of lecithin, and a more hydrophilic character of lecithin (because of the alteration of phospholipid composition). Van Nieuwenhuyzen (185) has shown that the modification of lecithin should be based on the fact that it is a mixture of different emulsifiers. Materials of widely different functionalities can be obtained from crude lecithin. The most widely practiced method is to remove the triacylglycerols (de-oiling) by contacting crude lecithin with acetone. When acetone is used for de-oiling, insoluble phospholipids, glycolipids, and associated compounds are left behind in an easily isolated form and subsequent desolventization yields a powdered or granular product. The de-oiling process not only improves the color, odor, and flavor, but also converts the water-in-oil (w/o) emulsifier of the starting material to an oil-in-water (o/w) type emulsifier. Use of supercritical CO<sub>2</sub> makes the de-oiling process without solvent consumption possible and helps to solubilize triacylglycerols leaving phospholipids insoluble (186–188). Extraction with lower alcohols, especially ethanol, could be employed to yield a

water dispersible phosphatidylcholine “soluble fraction” and an “insoluble fraction” rich in phosphatidylinositol and phosphatidic acid. Further fractionation and purification could be done by using adsorbents, such as alumina, silica, or by manipulating solvent polarity and temperature of extraction (179).

Lecithin, obtained from oil processing operations, is modified by different methods in order to increase water dispersibility and improved oil-in-water emulsifying properties. Among the methods of modification, acylation of lecithin by acetic anhydride is in practice. Conversion of phosphatidylethanolamine to the N-acetyl form (50–100%) renders crude lecithin as an o/w type emulsifier (179, 189). Introduction of hydroxyl groups at the points of unsaturation of the fatty acids in the phospholipid molecule is another way of modification. Blending of lecithin with high levels of hydrogen peroxide in the presence of an organic acid (e.g., lactic or acetic acid) at elevated temperatures performs the hydroxylation reaction, which improves the hydrophilicity of lecithin (179, 190). Use of phospholipase A (fungal or mammalian origin), which specifically cleaves the fatty acids at the second position of the phospholipid, is currently in commercial practice. Lecithin, so obtained, has highly improved o/w emulsification properties because of the formed lysophospholipids (190, 191). Application of lecithin is widespread and ranges from food, industrial, and pharmaceutical uses mainly because of the unique surface-active properties of the constituent phospholipids. Table 5 provides a list of applications of lecithin.

### 3.2. Tocopherols and (phyto) Sterols from Deodorizer Distillate

Deodorizer distillate (DOD) is another byproduct of edible oil processing and has a wide range of chemical compositions. This is produced during the refining step, in which the steam distillation under reduced pressure is carried out to obtain oil with desired flavor and stability characteristics. Deodorizer distillate/sludge contains a multitude of compounds, and generally, the unsaponifiable matter is about 10–30%, which may differ in composition depending on the type of oil being deodorized. In general, the unsaponifiable matter in DOD is composed of hydrocarbons (25%), terpenic and aliphatic alcohols (10%), phytosterols (40%), tocopherols (15%), and pigments (10%). Table 6 provides the composition of unsaponifiable matter of a few oilseeds. The distillates from unsaturated vegetable oils are generally high in unsaponifiable matter, such as stigmaterol and tocopherols, and suitable as a source of phytosterols and tocopherols. The distillates that are low in unsaponifiables (e.g., animal fats, palm oil) are mainly a source of fatty acids and contain low levels of tocopherols and sterols. There are intermediate-type distillates also.

**3.2.1. Tocopherols** Traditionally, tocopherols are purified by a combination of molecular distillation, ethanol fractionation, chemical alcoholysis, and ion-exchange chromatography. Molecular distillation may not produce a high purity tocopherol because of sterol contamination, as both molecules have very similar molecular weights. Industrial purification of tocopherol achieves about 75% purity by ethanol fraction; sterols are insoluble, whereas tocopherols are soluble in ethanol. Barnicki

**TABLE 5. Application of Lecithin in Relation to the Characteristics of Preparation (191).**

HLB Value	Applications			Performance Criteria <sup>a</sup>								
	Food	Industrial	Physical Properties	1	2	3	4	5	6	7	8	9
2.0	Chocolate, margarine	Ink, paints and coatings, textiles	Oil soluble	–	–	–	x	–	x	–	x	–
4.0	Baked goods, cheese products, confections, dairy products, dietetic and infant foods, margarine and shortenings, meats and poultry processing and coatings	Adhesives, adsorbents, detergents, dust control, dyes, fertilizers, inks, masonry, asphalt, paints and coatings, paper, pesticides, polymers/rubber, textiles	Oil soluble	–	–	–	x	–	x	x	x	–
4.0	Oil-based aerosol spray release applications	–	Easily sprayable, low viscosity, oil soluble	–	–	–	x	–	x	x	x	–
4.0	Chewing gum base	–	Low flavor	–	–	–	–	–	x	x	x	x
5.6	–	Paints and coatings, textiles	Water dispersible	–	–	–	x	x	–	–	–	–
7.0	Cake mixes, low-fat products, nondairy creamers, meat sauces, gravies, canned meat, frostings, frozen desserts	Animal feeds, ceramics and glass, cosmetics and soaps, dyes	Dry blendable, oil soluble, water dispersible, low flavor	x	x	x	x	x	–	x	x	x
7.0	–	Magnetic tapes, cosmetics, soaps, skincare	Dry blendable, oil soluble, water dispersible, low flavor	–	–	–	x	x	–	–	x	x
8.0	Functional food applications where higher choline content is desired	–	Higher choline content, low flavor, water dispersible	–	–	–	–	x	–	–	–	–
9.0	Baked goods, starch based sauces, puddings	–	Dry blendable, low flavor, oil soluble, water dispersible	x	x	x	x	x	–	–	–	x

**TABLE 5.** (Continued)

HLB Value	Applications		Physical Properties	Performance Criteria <sup>a</sup>									
	Food	Industrial		1	2	3	4	5	6	7	8	9	
9.0	Baked foods, instant foods, milk powders	Cosmetics and soaps, dyes, leather	Oil soluble, water dispersible	-	-	-	-	-	-	-	-	-	x
10.0	Salad dressings, release water-based flavor, colors	-	Oil soluble, water dispersible	-	-	-	x	x	-	x	-	-	-
12.0	Instant foods, Flavors	-	Easily sprayable, low viscosity, water dispersible	-	-	-	x	x	-	x	x	x	x

<sup>a</sup>1: Adds lubricity; 2: Crumb softener; 3: Dough conditioner; 4: Mixing/blending aid; 5: O/W emulsifier; 6: W/O emulsifier; 7: Release agent; 8: Viscosity modifier; 9: Wetting agent: - is no, x is yes.

**TABLE 6. Composition of Deodorizer Distillate from Various Oils (192).**

Component	Sunflower	Cottonseed	Soybean	Rapeseed
Unsaponifiables, %	39	42	33	35
Tocopherols, %	9.3	11.4	11.1	8.2
$\alpha$ -tocopherol, %	5.7	6.3	0.9	1.4
Sterols, %	18	20	18	14.8
Stigmasterol, %	2.9	0.3	4.4	1.8

et al. (193) have patented a process that involves a minimum number of processing steps to recover tocopherols without using solvents. This process is comprised of an esterification reaction where the more volatile alcohols are converted to their less volatile fatty acid esters, followed by a series of distillation steps, where components boiling higher and lower than tocopherols are separated from tocopherols and other similar boiling substances.

Shimada et al. (194) have shown that lipase from *Candida rugosa* recognizes sterols as a substrate for esterification reaction with free fatty acids in a deodorizer distillate containing fatty acids, sterols, and tocopherols. After this esterification, the high boiling point steryl esters (sterols with free fatty acids) could be removed by molecular distillation, and a tocopherol concentrate can be obtained. According to their work, soy deodorizer distillate containing 16% total tocopherol was stirred at 35°C for 24 h with 200 U of lipase per g reaction mixture. One-step esterification resulted in 80% esterification of sterols, which could be increased to 95% when repeated esterification steps were carried out. After the reaction, tocopherols and the remaining free fatty acids were recovered by molecular distillation of the oil layer. This solvent-free concentration resulted in a 65% tocopherol content in the concentrate (194). Use of supercritical fluid CO<sub>2</sub> extraction to selectively isolate tocopherols from deodorizer distillate has been described for sources such as rice bran, soy, palm, olive, and wheat germ (195–198).

**3.2.2. Phytosterols** Structurally related alicyclic alcohols found in the unsaponifiable fraction of plant lipids are collectively called “phytosterols.” Sterols are found in highest concentrations in the seeds and fruits of oleaginous plants and are also present in the leaves and seeds of legumes and vegetables that are non-oleaginous. The deodorizer distillates from vegetable oil refining and fatty residues from industrial processing of plant materials (e.g., tall oil, a byproduct of cellulose sulfate process used in paper making and derived from trees), including vegetable oils, are the major source of phytosterols. Phytosterols are used as starting materials in the synthesis of steroids for pharmaceutical uses, emulsifiers in the cosmetic and the food industries, starting material for pesticide manufacturing, and for liquid crystals in the optics industry. More recently, phytosterols have been used in ingredients for the development of functional foods and nutraceuticals (e.g., margarines containing phytosterols to lower blood cholesterol levels).



The unsaponifiable matter of soy, corn, canola/rapeseed, sunflower, cottonseed, peanut, and palm ranges from 10–30%, and it is composed of 40% phytosterols and 15% tocopherols (199–202). The temperature, duration, quantity of skimming vapor, and the extent of vacuum used for deodorization are the parameters that greatly influence the quality and quantity of DOD. The low content of tocopherols and sterols in the DOD often requires a concentration step; however, if the starting material is soybean, this is not an issue because of the high concentration of these compounds.

In the DOD, phytosterols are present in both the free and esterified forms with fatty acids. Therefore, the first step in the extraction of phytosterols is conversion of phytosterol fatty esters into free phytosterols. This is achieved either by hydrolysis or *trans*-esterification. Hydrolysis could be carried out under strong basic conditions (saponification with further acidulation), under strong acidic conditions, or under chemical or enzyme (specific or nonspecific) catalyzation. Re-esterification of phytosterols occurs during methyl ester distillation as a result of the high temperatures involved; therefore, a further *trans*-esterification step for free sterols is required. Esterification of phytosterols or *trans*-esterification of sterol fatty acid esters is the second step in this process. Methanol is the most commonly used alcohol, and it leads to methyl esters, which are characterized by a higher volatility, however, other C<sub>1</sub> to C<sub>4</sub> alcohols may also be used. Esterification and *trans*-esterification of fatty acids or phytosterols can be catalyzed by metal alcoholates, or hydroxide, by organic catalysts, or by enzymes (Table 7).

The recovery of phytosterols or their concentration is the third step in their isolation. Methyl esters of fatty acids are distilled to raise the phytosterol content up to 50% by weight. Molecular distillation can be used for phytosterols. Phytosterol concentrates are used as raw materials for steroid drug preparation via microbial fermentation, in which the pure form of phytosterols are not necessary. The final recovery of phytosterols can be achieved by crystallization (physical), solvent extraction (chemical), or crystallization with additives via adduct formation and separation (physicochemical). Organic solvents or solvent mixtures composed of low- and high-polarity solvents and water are used for crystallization of phytosterols

**TABLE 7. Phytosterol Liberation Methods (203, 204).**

Liberation Method	Catalyst	Duration of	
		Temperature, °C	Reaction, h
Chemical hydrolysis	Strong mineral acid	–	–
Enzymic hydrolysis	Lipases from microbial sources	35	5
Transesterification	Alkali metal hydroxides or alkoxides (alcoholates)	220	3
	Monoalkyltin or dialkyltin or phenyl phosphonic acid	200	4
	Zn oxides or hydroxides	150–240	3
Saponification and acidulation	Mineral bases	–	–

**TABLE 8. Additives and Solvents Used for Adduct Formation in Phytosterol Separation (207).**

Additive	Aprotic Solvent	Protic Solvent
Calcium chloride	Ligroin	Methanol
Metal salts	Toluene	Water
Dicarboxylic acids	Diethyl ketone	
Hydrochloric acids	Ethyl acetate	
Urea	1,2-dichloroethane	

(205, 206). The solid phase consists of either crystallized or precipitated phytosterols that can be filtered, centrifuged, or decanted from the liquid phase. The preferred high-polarity solvents for phytosterol crystallization are low-molecular-weight oxygenated hydrocarbons, lower alkanols, and low-polarity solvents, including alkanes, monoketones, monoaldehydes, monoesters, and monohydric alcohols. The solvent blend used for crystallization is generally comprised of at least 80% (by weight) low-polarity solvents and 20% or less high-polarity solvents. Water is included into the high-polarity solvent in the ratio ranging from 5 : 1 to 1 : 5 (by weight). Isolation of phytosterols from esterified and *trans*-esterified mixtures by adduct formation is performed in an aprotic solvent that contains a small amount of protic solvent. A combination of additives, protic, and aprotic solvents, listed in Table 8, are used for this task. After separation, the adduct is decomposed in a biphasic solvent system to liberate free sterols and additives. Recovering of tocopherols is achieved by different methods, such as molecular distillation and solvent extraction, which are carried out as the fourth and final step.

As a result of similar volatility of sterols, tocopherols, and fatty acids, it is quite difficult to separate sterols and tocopherols from fatty acids during fractional distillation and steam stripping under high vacuum. It has been shown that supercritical fluid extraction is promising and can concentrate both tocopherols and sterols (208). Lipase-assisted separation of sterols has been studied. The fatty acids in the deodorizer distillate are enzymatically hydrolyzed (lipases from microbial sources) and converted to methyl or butyl esters with the aid of another type of lipase. The esterified products are fractionally distilled to obtain fatty acid esters. The sterols and tocopherols are converted into the residue fraction. By employing these steps of reactions, Ramamurthy and McCurdy (209) were able to obtain a concentration of 17.5% sterols and 21.5% tocopherols from soybean deodorizer distillate. Using sunflower deodorizer distillate, Ghosh and Battacharyya (210) obtained a concentrate containing 30% tocopherols and 36% sterols.

### 3.3. Soapstock

Soapstock is the byproduct of caustic (dilute sodium hydroxide) washing of crude oil during refining. Free fatty acids, hydrolyzed phospholipids, and unsaponifiable matter are included in the soapstock. Free fatty acids are the most valuable compo-

nents that can be recovered from soapstock. Usually, soapstock is returned to the oilseed meal to increase its lipid content as well as the weight. For use in feed, the soapstock must be reasonably free of pesticide residues. As pesticide residues are concentrated in the deodorizer distillate, they are rarely combined with the soapstock (211). Acidulation with sulfuric acid stabilizes soapstock and also reduces the weight of the material to be handled; however, it forms a large amount of wastewater in the refinery. The patented method by Daniels (212) uses the acid water as a fertilizer ingredient. In this, KOH is used instead of NaOH for washing, and the acidulation with sulfuric acid is followed by neutralization with ammonia rather than NaOH (213).

**3.3.1. Soap** Raw or acidulated soapstock could be used to manufacture soap directly or subjected to distillation and hydrogenation to obtain fatty acids for soap making. Coconut and palm kernel soapstock is used in high-grade toilet soap making because of their sudsing and foaming properties. Palm oil soapstock is used in making low-quality, inexpensive soap. Distilled fatty acids from palm soapstock offer an alternative to tallow in toilet soap manufacture. Soybean and cottonseed soapstock that have high levels of unsaturation and pigmentation are suitable for medium grade soaps for industrial purposes, including powdered hand soaps, metal finishing compounds, laundry and dry cleaning, food plant cleaners, and low-temperature dye washes, but not for toilet soap.

**3.3.2. Fatty Acids** Soapstock is a low-cost raw material used for obtaining fatty acids, generally found in the source oil. Short- and medium-chain fatty acids are obtained from coconut and palm kernel soapstock. Soybean soapstock provides high-grade stearic acid when hydrogenated and linoleic and palmitic acids when fractionated. Multiple distillation steps may be required to obtain acceptable color, stability, and economical hydrogenation. Readers are advised to refer to Sonntag (214) for further details for the processing of soapstock.

## REFERENCES

1. G. G. Haraldsson, *J. Amer. Oil Chem. Soc.*, **61**, 219–222 (1984).
2. C. A. Guesserow, *Arch. Pharm.*, **27**, 153–244 (1828).
3. K. I. Zilch, *J. Amer. Oil Chem. Soc.*, **56**, 739–742A (1979).
4. H. Dubovitz, *Seifensieder Zeitung*, **37**, 1063–1064 (1911).
5. C. Greenfield, U.S. Patent 2,911,423, 1959.
6. A. W. Hixson and R. Miller, U.S. Patent 2,219,652, 1940.
7. A. W. Hixson and J. B. Bockelmann, *Trans Amer. Inst. Chem. Eng.*, **38**, 891–930 (1942).
8. D. A. Drew and A. N. Hixson, *Trans Amer. Inst. Chem. Eng.*, **40**, 675–694 (1944).
9. H. J. Passino, *J. Ind. Eng. Chem.*, **41**, 280–287 (1949).
10. S. W. Gloyer, *J. Amer. Oil Chem. Soc.*, **26**, 162–166 (1949).
11. E. A. Lawrence, *Chem. Eng. Prog.*, **49**, 627–634 (1953).

12. R. Kristler, V. J. Muckerheide, and L. D. Myers, *Oil Soap*, **23**, 146–150 (1946).
13. R. L. Demmerle, *J. Ind. Eng. Chem.*, **39**, 126–131 (1947).
14. L. D. Myers and W. J. Muckerheide, U.S. Patent 2,293,674, 1942.
15. L. D. Myers and W. J. Muckerheide, U.S. Patent 2,421,157, 1947.
16. W. P. Gee, U.S. Patent, 2,450,235, 1948.
17. W. P. Gee, U.S. Patent 2,520,801, 1950.
18. W. Stein, *J. Amer. Oil Chem. Soc.*, **45**, 471–474 (1968).
19. W. Stein and H. Hartman, Brit. Patent 925,674, 1955.
20. W. Stein and H. Hartman, U.S. Patent 2,800,493, 1957.
21. H. Waldman and W. Stein, U.S. Patent 3,052,700, 1962.
22. H. Hartman and W. Stein, U.S. Patent 3,733,343, 1973.
23. E. L. Smith, *Biochem. J.*, **36**, 22–30 (1942).
24. S. R. Elsdon, *Biochem. J.*, **40**, 252–256 (1946).
25. M. F. White and J. B. Brown, *J. Amer. Chem. Soc.*, **70**, 42–69 (1948).
26. S. F. Herb, *J. Amer. Oil Chem. Soc.*, **28**, 505–507 (1951).
27. J. Boldingh, *Experientia*, **4**, 270–271 (1948).
28. J. Boldingh, *Rec. Trav. Chém.*, **69**, 247–261 (1950).
29. A. T. James and A. J. P. Martin, *Biochem. J.*, **50**, 679–690 (1952).
30. C. A. Orr and J. E. Callen, *J. Amer. Chem. Soc.*, **80**, 249 (1958).
31. G. Üstün, *J. Amer. Oil Chem. Soc.*, **73**, 203–210 (1996).
32. T. J. Logan and D. C. Underwood, European Patent, 78,200,225.5, 1978.
33. M. T. Cleary and S. Kulprathipanja, European Patent, 82,305,310.3, 1982.
34. T. Nakahara, T. Yokochi, T. Higashihara, S. Tanaka, T. Yaguchi, and D. Honda, *J. Amer. Oil Chem. Soc.*, **73**, 1421–1426 (1996).
35. W. W. Christie, *Fat Sci. Tech.*, **93**, 65–66 (1993).
36. P. Manninen, P. Laasko, and H. Kallio, *Lipids*, **30**, 665–671 (1995).
37. S. Teshima, A. Kanazawa, and S. Tokiwa, *Bull. J. Soc. Sci. Fish.*, **44**, 927–930 (1978).
38. D. G. Corley, S. G. Zeller, P. James, and K. Duffin, International Patent, PCT/US00/04166, 2000.
39. K. Hayashi and H. Kishimura, *Nippon Suisan Gakkaishi*, **59**, 1429–1433 (1993).
40. R. O. Adlof and E. A. Emiken, *J. Amer. Oil Chem. Soc.*, **62**, 1592–1595 (1985).
41. S. Tokiwa, A. Kanazawa and S. Teshima, *Bull. Jap. Soc. Sci. Fish.*, **47**, 675–680 (1981).
42. M. Perrut, *LC-GC*, **6**, 914–920 (1988).
43. J. Krzynowek, D. L. D'Entremont, and R. S. Maney, *Proc. 12th Ann. Conf. Trop. Subtrop. Fish Technol. Soc.*, 74–77 (1988).
44. F. L. Goffic, *Lipid Technol.*, **9**, 148–150 (1997).
45. U. N. Wanasundara and P. Fedec, *Inform*, **13**, 726–730 (2002).
46. L. C. Craig and O. Post, *Anal. Chem.*, **21**, 500–504 (1949).
47. Y. Ito and F. E. Chou, *J. Chromat.*, **391**, 382–386 (1988).
48. Y. Ito, H. Oka, and J. L. Slemper, *J. Chromat.*, **475**, 219–227 (1989).
49. A. Marston and K. Hostettmann, *J. Chromat. A*, **658**, 315–341 (1994).

50. A. P. Foucault, in A. P. Foucault, ed., *Chromatographic Science Series*, Vol. 68, Marcel Dekker, New York, 1995, pp. 71–97.
51. Y. Ito, *Crit. Rev. Anal. Chem.*, **17**, 65–143 (1986).
52. K. Hostettman, *Planta Medica*, **39**, 1–8 (1980).
53. A. Marston, C. Borel, and K. Hosterttmann, *J. Chrom.*, **450**, 91–99 (1988).
54. J. M. Sorenson and W. Arlt, in D. Behrens and R. Eckermann, eds., *Chemical Data Series*, Dechema, Deutsche Gesellschaft für Chemisches Apparatewesen, Frankfurt, Germany, 1980.
55. O. Bousquet, N. Sellier, and F. L. Goffic, *Chrotographia*, **39**, 40–44 (1994).
56. O. Bousquet and F. L. Goffic, *J. Chromat. A*, **704**, 211–216 (1995).
57. W. Murayama, Y. Kosuge, and N. Nakaya, *J. Liquid Chrom.*, **11**, 283–300 (1988).
58. G. Boudimant, M. Maurice, A. Landrrein, G. Durand, and P. Durand, *J. Liq. Chromat. Rel. Technol.*, **19**, 1793–1804 (1996).
59. J. B. Brown and G. C. Stoner, *J. Amer. Chem. Soc.*, **59**, 3–6, (1932).
60. J. B. Brown and G. Y. Shinowara, *J. Amer. Chem. Soc.*, **59**, 6–8 (1937).
61. J. B. Brown and D. K. Kolb, *Prog. Chem. Fats Lipids*, **3**, 57–94 (1955).
62. P. Chawla and J. M. deMan, *J. Amer. Oil Chem. Soc.*, **76**, 329–332 (1990).
63. W. S. Singleton, in K. S. Markley, ed., *Fatty acids*, Interscience Publishers, New York, 1960, pp. 609–678.
64. V. F. Stout, W. B. Nilsson, J. Krzynowek, and H. Schlenk, in M. E. Stansby, ed., *Fish Oils in Nutrition*, Van Nostrand Reinhold, New York, 1990, pp. 73–119.
65. E. Deffense, Society of Chemical Industry Lecture and Paper Series. No. 0036, Society of Chemical Industry, London, 1994.
66. T. Willner, W. Sitzmann, and K. Weber, in V. J. S. Shukla and F. D. Gunstone, eds., *Oils and Fats in the Nineties*, International Food Science Centre, A/S, Lystruo, Denmark, 1992, pp 162–175.
67. W. Hamm, *Trends Food Sci. Technol.*, **6**, 121–126 (1995).
68. P. H. J. van Dam, W. Hogervorst, and F. Kamp, U.S. Patent 5,959,129, 1996.
69. M. Koji, Japanese Patent, JP5125389, 1993.
70. M. Koji, Japanese Patent, JP6181686, 1994.
71. K. F. Mattil, U.S. Patent 2,610,915, 1950.
72. F. J. Baur, U.S. Patent 3,059,008, 1962.
73. T. P. Hilditch and J. P. Riley, *J. Soc. Chem. Ind.*, **64**, 204–207 (1945).
74. A. W. Hixson and J. B. Bockelmann, *Trans. Amer. Inst. Chem. Eng.*, **38**, 891–930 (1942).
75. T. Yokochi, M. T. Usita, Y. Kamisaka, T. Nakahara, and O. Suzuki, *J. Amer. Oil Chem. Soc.*, **67**, 846–851 (1990).
76. H. P. Kreulen, *J. Amer. Oil Chem. Soc.*, **53**, 393–395 (1976).
77. H. Schlenk, *J. Amer. Oil Chem. Soc.*, **38**, 728–736 (1961).
78. K. S. Markley, in K. S. Markley, ed., *Fatty Acids*, Interscience Publishers, New York, 1964, pp. 1983–2123.
79. U. N. Wanasundara, *Marine Oils: Stabilization, Structural Characterization and Omega-3 Fatty Acid Concentration*, Ph.D. thesis, Memorial University of Newfoundland, St. John's, Newfoundland, 1996.

80. M. E. Chevreul, *Recherches chimiques sur les corps gras d'origine animale*, Levrault, Paris, 1823.
81. M. Levey, in E. Faber, ed., *Great Chemists*, Interscience, New York, 1961, pp. 3–6.
82. F. Kraft, *Ber*, **36**, 4336–4344 (1903).
83. H. Kreiser and A. Hafner, *Ber*, **36**, 2766–2773 (1903).
84. A. Haller and A. Youssoufian, *Compt. Rend.*, **143**, 803–806 (1906).
85. H. Bull, *Ber*, **39**, 3570–3576 (1906).
86. G. D. Eldson, *Analyst*, **38**, 8–11 (1913).
87. G. D. Eldson, *Analyst*, **39**, 78–81 (1914).
88. G. S. Jamieson and W. F. Baughman, *J. Amer. Chem. Soc.*, **43**, 2696–2702 (1921).
89. G. S. Jamieson and W. F. Baughman, *J. Amer. Chem. Soc.*, **44**, 2952–2957 (1922).
90. G. S. Jamieson and W. F. Baughman, *J. Oil Fat Ind.*, **2**, 40–44 (1925).
91. G. S. Jamieson, R. M. Hann, and W. F. Baughmann, *J. Oil Fat Ind.*, **4**, 63–65 (1927).
92. W. F. Baughman, *J. Amer. Chem. Soc.*, **44**, 2947–2952 (1922).
93. J. B. Brown and G. D. Beal, *J. Amer. Chem. Soc.*, **45**, 1289–1303 (1923).
94. J. B. Brown and G. S. Jamieson, *J. Biol. Chem.*, **83**, 783–790 (1929).
95. J. Reilly, *Distillation*, Methuen, London, 1936.
96. T. P. Hilditch, *Biochem. J.*, **28**, 779–785 (1931).
97. W. J. Podbielniak, U.S. Patent 2,004,911, 1935.
98. R. H. Baker, C. Barkenbus, and C. A. Roswell, *Ind. Eng. Chem., Anal. Ed.*, **12**, 468–471 (1940).
99. O. S. Privett, R. P. Weber, and E. C. Nickell, *J. Amer. Oil Chem. Soc.*, **36**, 443–449 (1959).
100. C. E. Bills, O. N. Massengale, K.C. D. Hickman, and E. LeB Gray, *J. Biol. Chem.*, **126**, 241–244 (1938).
101. S. Komari, *J. Chem. Soc. Japan, Ind. Chem. Sect.*, **54**, 225–228 (1951).
102. S. Komari, *Chem. Abstr.*, **47**, 3010 (1953).
103. K. C. D. Hickman, *Ind. Eng. Chem.*, **32**, 1451–1453 (1940).
104. E. H. Farmer and F. A. Van den Heuval, *J. Soc. Chem. Ind. Lond.*, **57**, 24–31T (1938).
105. R. Berger and W. McPherson, *J. Amer. Oil Chem. Soc.*, **56**, 743–746A (1979).
106. A. W. Weitkamp, *J. Amer. Oil Chem. Soc.*, **32**, 640–646 (1955).
107. H. Brevik, *AOCS Short Courses, Modern Application of Marine Oils*, Toronto, Canada, May 7–8, 1992.
108. R. G. Ackman, *Chem. Ind.*, **3**, 139–145 (1988).
109. R. C. Wijesundara, W. M. N. Ratnayake, and R. G. Ackman, *J. Amer. Oil Chem. Soc.*, **66**, 1822–1830 (1989).
110. O. S. Privett, R. P. Weber, and E. C. Nickell, *J. Amer. Oil Chem. Soc.*, **36**, 443–449 (1959).
111. O. S. Privett, and E. C. Nickell, *J. Amer. Oil Chem. Soc.*, **40**, 189–193 (1963).
112. R. G. Ackman, P. J. Ke, and P. M. Jangaard, *J. Amer. Oil Chem. Soc.*, **50**, 1–8 (1973).
113. E. Twitchell, *Ind. Eng. Chem.*, **13**, 806–807 (1921).
114. D. S. Han, H. B. Ahn, and H. K. Shin, *Korean J. Food Sci. Technol.*, **19**, 430–434 (1987).

115. A. Marangoni and D. Rousseau, *Trends Food Sci. Technol.*, **6**, 329–335 (1995).
116. N. R. Bottino, G. A. Vandenberg, and R. Reiser, *Lipids*, **2**, 489–493 (1967).
117. Y. Tanaka, J. Hirano, and T. Funada, *J. Amer. Oil Chem. Soc.*, **69**, 1210–1214 (1992).
118. T. Hoshino, T. Yamane, and S. Shimuzu, *Agric. Biol. Chem.*, **54**, 1459–1467 (1990).
119. D. R. Tocher, A. Webster, and J. R. Sargent, *Biotechnol. Appl. Biochem.*, **8**, 675–679 (1986).
120. Y. Shimada, K. Murayama, S. Okazaki, M. Nakamura, A. Sugihara, and Y. Tominga, *J. Amer. Oil Chem. Soc.*, **71**, 951–954 (1994).
121. Y. Noguchi and H. Hibino, Japanese Patent, 59–14793, 1984.
122. J. Kotting and H. Elbl, in P. Woolley and S. B. Petersen, eds., *Lipases*, Cambridge University Press, Cambridge, United Kingdom, 1994, pp. 289–313.
123. U. N. Wanasundara and F. Shahidi, *J. Food Lipids*, **4**, 51–64 (1997).
124. U. N. Wanasundara and F. Shahidi, *J. Amer. Oil Chem. Soc.*, **75**, 945–951 (1998).
125. S. El-Boustani, C. Colette, L. Monnier, B. Descomps, C. A. Paulet, and F. Mendey, *Lipids*, **22**, 711–714 (1987).
126. L. D. Lawson and B. G. Hughes, *Biochem. Biophys. Res. Comm.*, **152**, 328–335 (1988).
127. L. Y. Yang, A. Kuksis, and J. J. Myher, *Biochem. Cell Biol.*, **67**, 192–204 (1989).
128. K. Osada, M. Nakamura, M. Nonaka, and M. Hatano, *J. Jpn. Oil Chem. Soc.*, **41**, 39–43 (1992).
129. Y. He and F. Shahidi, *J. Amer. Oil Chem. Soc.*, **74**, 1133–1136 (1997).
130. C. C. Akoh, B. H. Jennings, and D. A. Lillard, *J. Amer. Oil Chem. Soc.*, **72**, 1317–1321 (1995).
131. C. C. Akoh, B. H. Jennings, and D. A. Lillard, *J. Amer. Oil Chem. Soc.*, **73**, 1059–1062 (1996).
132. R. Sridar and G. Lakshminarayana, *J. Amer. Oil Chem. Soc.*, **69**, 1041–1042 (1992).
133. S. Basheer, K. Mogi, and M. Nakajuma, *J. Amer. Oil Chem. Soc.*, **72**, 511–518 (1995).
134. Y. Shimada, H. Murayama, A. Sugihara, T. Baba, S. Komemushi, S. Moriyama, and Y. Tominga, *J. Amer. Oil Chem. Soc.*, **75**, 1565–1571 (1998).
135. M. Schmitt-Rozieres, V. Deyris, and L. Comeau, *J. Amer. Oil Chem. Soc.*, **77**, 329–332 (2000).
136. G. A. M. Diepen and F. E. C. Scheffer, *J. Amer. Chem. Soc.*, **70**, 4085–4089 (1948).
137. J. B. Hanny and J. Hogarth, *Proc. Royal Soc. London*, **29**, 324 (1879).
138. J. B. Hanny and J. Hogarth, *Proc. Royal Soc. London*, **30**, 178 (1880).
139. J. Chrastil, *J. Phys. Chem.*, **86**, 3016–3021 (1982).
140. W. B. Nilsson, G. J. Gauglitz, Jr., J. K. Hudson, V. F. Stout, and J. Spinelli, *J. Amer. Oil Chem. Soc.*, **65**, 109–117 (1988).
141. V. K. Mishra, F. Temelli, and B. Ooraikul, *Food Res. Int.*, **26**, 217–226 (1993).
142. P. M. F. Paul and W. S. Wise, *The Principles of Gas Extraction*, Mills and Boon, London, 1971.
143. M. A. McHugh and V. J. Krukoni, *Supercritical Fluid Extraction: Principles and Practices*, 2nd ed., Butterworth-Heinemann, Stoneham, Massachusetts, 1986.
144. J. P. Friedrich and G. R. List, *J. Agric. Food Chem.*, **30**, 192–195 (1982).

145. S. S. H. Rizvi, J. A. Daniels, A. L. Bernado, and J. A. Zollweg, *Food Technol.*, **40**, 57–64 (1986).
146. T. Görner and M. Perrut, *LC-GC Int.*, **2**, 36–39 (1989).
147. D. Pyo and D. Ju, *Chromatographia*, **38**, 79–82 (1994).
148. M. Demirbükler, I. Hägglund, and L. G. Blomberg, *J. Chrom.*, **605**, 263–267 (1992).
149. J. A. Merkle and D. K. Larick, *J. Food Sci.*, **58**, 1237–1240 (1993).
150. A-I. Yeh, J. H. Liang, and L. S. Hwang, *J. Amer. Oil Chem. Soc.*, **68**, 224–229 (1991).
151. W. Eisenbach, *Ber. Bunsenges. Phys. Chem.*, **88**, 882–887 (1984).
152. W. B. Nilsson, E. J. Gauglitz, and J. K. Hudson, *J. Amer. Oil Chem. Soc.*, **66**, 1596–1600 (1989).
153. S. Peter and G. Brunner, *Angew. Chem. Int. Engl.*, **17**, 746–750 (1978).
154. G. Brunner and S. Peter, *Sep. Sci. Technol.*, **17**, 199–214 (1982).
155. K. Zosel, *Angew. Chem. Int. Engl.*, **17**, 702–709 (1978).
156. V. F. Stout and J. Spinelli, U.S. Patent 4,675,132, 1987.
157. M. Pinton, *Practical Short Course on Processing of Nutraceuticals/Functional Foods*, Food Protein Research and Development Center, The Texas A & M University, College Station, Texas, Chapter 16, 1998.
158. A. E. Smith, *Acta Cryst.*, **5**, 224–235 (1952).
159. D. G. Hayes, *Inform*, **13**, 832–835 (2002).
160. H. Schlenk, in R. T. Holman, W. O. Lundberg, and T. Malkin, eds., *Progress in the Chemistry of Fats and Other Lipids*, Pergamon Press, New York, 1954, pp. 243–267.
161. D. Han, H. K. Shin, and S. H. Yoon, in F. Shahidi and K. R. Cadwallader, eds., *Flavor and Lipid Chemistry of Seafoods*, ACS Symposium Series 674, American Chemical Society, Washington, D. C. 1997, pp. 255–263.
162. D. G. Hayes, J. M. van Alstine, and F. N. Setterwall, *J. Amer. Oil Chem. Soc.*, **77**, 207–215 (2000).
163. G. M. Pigott and B. W. Tucker, *Food Rev. Inter.*, **3**, 105–138 (1987).
164. W. M. N. Ratnayake, B. Olsson, D. Matthews, and R. G. Ackman, *Fat Sci. Technol.*, **90**, 381–386 (1988).
165. H. J. Wille, H. Traitler, and M. Kelly, *Rev. Fr. Corps Gras.*, **34**, 69–71 (1987).
166. H. Schlenk and R. T. Holman, *J. Amer. Oil Chem. Soc.*, **27**, 5001–5004 (1950).
167. D. Swern, in K. S. Markley, ed., *Fatty Acids*, Interscience Publishers, New York, 1964, pp. 2309–2358.
168. E. V. Patokina, S. M. Alekseev, I. K. Sarycheya, G. V. Karpova, and R. P. Evstigneeva, *Khim. Farm. Zh*, **22**, 1129–1132 (1985).
169. J. L. Iverson and R. W. Weik, *J. Assoc. Off. Anal. Chem.*, **50**, 1111–1118 (1967).
170. A. Strocchi and G. Bonaga, *Chem. Phys. Lipids*, **15**, 87–94 (1975).
171. F. D. Gunstone, R. C. Wijesundara, and C. M. Schrimgeour, *J. Sci. Food Agric.*, **29**, 539–550 (1978).
172. R. G. Ackman, J. C. Sipos, S. N. Hooper, and G. Dube, *J. Amer. Oil Chem. Soc.*, **54**, 199–201 (1977).
173. A. Piconneaux, A. Grandgirard, and J. Sebedio, *Competes Rendus de Academie des Sci., Series 3, Sci. de la Vie*, **300**, 353–358 (1985).



174. N. Haagsma, C. M. Gent, J. B. Luten, R. W. Jong and E. Doorn, *J. Amer. Oil Chem. Soc.*, **59**, 117–118 (1980).
175. M. A. Grompone, *Fat Sci. Technol.*, **94**, 388–394 (1992).
176. A. R. Medina, A. G. Gimenez, F. G. Camacho, J. A. S. Perez, E. M. Grima, and A. C. Gomez, *J. Amer. Oil Chem. Soc.*, **72**, 575–583 (1995).
177. D. G. Hayes, J. M. Van Alstine, and F. Setterwall, *J. Amer. Oil Chem. Soc.*, **77**, 207–213 (2000).
178. Anonymous, *Fish oil production and potentials in Atlantic Canada*, Project Report No. 115, Fisheries Development Branch, Halifax, Nova Scotia 1996.
179. G. L. Dashiell, in D. R. Erickson, ed., *World Conference Proceedings: Edible Fats and Oils Processing, Basic Principles and Modern Practices*, AOCS Press, Champaign, Illinois, 1990, pp. 396–401.
180. J. Hollo, J. Peredi, A. Ruzics, M. Jeranek, and A. Erdely, *J. Amer. Oil Chem. Soc.*, **70**, 997–1001 (1993).
181. M. Sosada, B. Pasker, and K. Kot, *Fat Sci. Technol.*, **94**, 233–236 (1992).
182. M. Sosada, *J. Amer. Oil Chem. Soc.*, **70**, 405–410 (1993).
183. M. Sosada, *J. Amer. Oil Chem. Soc.*, **73**, 367–370 (1996).
184. R. G. Short and F. T. Orthofer, U.S. Patent, 4, 221,731, 1978.
185. W. van Nieuwenhuyzen, *J. Amer. Oil Chem. Soc.*, **58**, 866–888 (1981).
186. W. Heigel and R. Huschens, Danish Patent DE3011185, 1981.
187. E. Stahl, A. Hubgen, and K. Quirin, Danish Patent DE3329249, 1985.
188. R. Kirchner-Reinhardt, S. Peters, and E. Weidner, Danish patent DE3630676, 1988.
189. P. Davis, U.S. Patent 3,301,881, 1967.
190. P. Julian, U.S. Patent 2,629,662, 1953.
191. American Lecithin Company, Oxford, CT. (2003). Available: <http://www.american-lecithin.com>.
192. R. I. Winters, in D. R. Erickson, ed., *World Conference Proceedings: Edible Fats and Oils Processing, Basic Principles and Modern Practices*, AOCS Press, Champaign, Illinois, 1990, pp. 402–405.
193. S. D. Barnicki, C. E. Sumner, Jr., and H. C. Williams, U.S. Patent 5,512,691, 1996.
194. Y. Shimada, S. Nakai, M. Suenaga, A. Sugihara, M. Kitano, and Y. Yominag, *J. Amer. Oil Chem. Soc.*, **77**, 1009–1013 (2000).
195. T. S. Shin and J. S. Godber, *J. Chromat. A*, **678**, 49–58 (1994).
196. M. Saito and Y. Yamauchi, *J. Chromat.*, **505**, 257–271 (1990).
197. E. Ibáñez, J. Palacios, F. J. Señoráns, G. Santa-Maria, J. Tabera, and G. Reglero, *J. Amer. Oil Chem. Soc.*, **77**, 187–190 (2000).
198. Y. M. Choo, A. N. Ma, H. Yahaya, Y. Yamaguchi, M. Bounoshita, and M. Saito, *J. Amer. Oil Chem. Soc.*, **73**, 523–525 (1996).
199. T. Gutfinger and A. Letan, *J. Sci. Food Agric.*, **25**, 1143–1147 (1974).
200. D. Daguet, *Lipid Technol.*, **12**, 77–80 (2000).
201. T. Verleyen, R. Verhe, L. Garcia, K. Dewittinck, A. Huyghebaert, and W. De Greyt, *J. Chrom. A.*, **921**, 271–285 (2001).

202. T. Verleyen, R. Verhe, L. Garcia, K. Dewittinck, A. Huyghebaert, and W. De Greyt, *J. Amer. Oil Chem. Soc.*, **79**, 117–122 (2002).
203. A. Sturve, F. F. Hill, and F. Schindler, U.S. Patent 4,374,776, 1983.
204. D. Daguët, *Lipid Technol.*, **12**, 77–80 (1999).
205. A. Sturve, U.S. Patent, 4,148,810, 1979.
206. T. K. Hunt, WO PCT 97/21697, 1997.
207. D. Daguët and J-P. Coic, *Olaeaginaux Crops Gras Lipides*, **6**, 25–28 (1999).
208. H. Lee, B. H. Cheung, and Y. H. Park, *J. Amer. Oil Chem. Soc.*, **88**, 571–573 (1991).
209. S. Ramamurthy and A. R. McCurdy, *J. Amer. Oil Chem. Soc.*, **70**, 287–295 (1993).
210. S. Ghosh and D. K. Battacharya, *J. Amer. Oil Chem. Soc.*, 1271–1274 (1996).
211. J. B. Woerfel, in A. R. Baldwin, ed., *World Conference on Emerging Technologies in the Fats and Oil Industry*, AOCS Press, Champaign, Illinois, 1986, pp. 165–168.
212. R. S. Daniels, Canadian Patent 1,256,449, 1989.
213. R. S. Daniels, in D. R. Erickson, ed., *World Conference Proceedings: Edible Fats and Oils Processing, Basic Principles and Modern Practices*, AOCS Press, Champaign, Illinois, 1990, pp. 430–431.
214. N. O. V. Sonntag, in D. R. Erickson, ed., *World Conference Proceedings: Edible Fats and Oils Processing, Basic Principles and Modern Practices*, AOCS Press, Champaign, Illinois, 1990, pp. 406–412.

# *BAILEY'S INDUSTRIAL OIL AND FAT PRODUCTS*

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# 1

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## *Frying Oils*

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### **1. INTRODUCTION**

Fried foods have provided culinary delight to people worldwide for centuries. It is difficult to determine when and where frying was first used by mankind. However, there is evidence that man used fried products long before the modern civilization reinvented fried products. Modern day frying involves sophisticated equipment, techniques, ingredients, and packaging. This is because the industrial fried products require long shelf life for warehousing, distribution, and sale.

In the frying process, food, such as vegetables, meat, or seafood, is brought in direct contact with hot oil. The food surface becomes golden yellow to dark brown in color and develops a pleasant fried food flavor.

Frying is done in homes, restaurants (food services), and at large industrial operations. Pan frying or griddle frying is done mostly at homes or at the restaurants. In this process, a thin layer of oil is heated on a skillet or a griddle. The food is fried in a layer of oil and fried until completion.

Restaurants also use batch fryers, where the food is placed in a wire basket, which is lowered into a bed of hot oil. The basket is removed from the hot oil when the product is fully fried.

The restaurants follow their guidelines on the frying temperature and time of frying. Frying temperature and frying time vary with the products fried.

Large-scale production of snack food is done in deep fat fryers. These are either batch or continuous fryers. In a batch fryer, the food is added into a large pan of hot oil. The oil is heated either directly from under the fryer or in an external heater. In the latter case, the oil is continuously recirculated into the pan and it is stirred with a stirrer. Formerly, manual stirring was common but modern kettles are generally equipped with mechanical stirrers. Fried product is removed and spun through a centrifugal device to remove the excess oil from the surface. The product is seasoned and packaged. The recovered oil is reused.

In a continuous fryer, the food enters the fryer at one end, is fried and taken out from the other end. The product is submerged in a bed of hot oil for a specified time depending on the type of food being fried. The oil is heated directly or indirectly as described above for the batch fryers.

Products in the above procedures are fully fried and are ready for consumption. There is another industrial method of frying that is used quite extensively. This is known as the par-frying process. The food is partially dehydrated in an industrial fryer and flash frozen at  $-20^{\circ}\text{C}$ . The packaged par-fried food is stored at  $-5^{\circ}\text{F}$  ( $20.6^{\circ}\text{C}$ ) to  $-10^{\circ}\text{F}$  ( $23.3^{\circ}\text{C}$ ) and distributed in freezer trucks. The product remains in a freezer at the destination. It is taken out of the freezer and fried immediately without thawing. Most common par-fried products are French fries, potato wedges, breaded chicken, coated or uncoated vegetables, cheese-filled vegetables, coated cheese sticks, etc. This reduces the manpower and preparation times at the restaurants and provides a great deal of convenience and cost savings to the restaurants and food services.

Advancement in the packaging materials and packing methods has enabled the industrial frying operations to extend the shelf life of the fried products so they can be stored, distributed, and marketed over several weeks to several months, without losing freshness in the product. This has provided a tremendous boost to the growth of the packaged fried food industry.

Oil plays a great role in determining the storage stability quality of the fried product. However, oil is also prone to oxidation, which leads to rancidity of the product in storage. Use of packaging material with high oxygen, nitrogen, and moisture barrier properties can significantly reduce oil degradation and increase the shelf life of packaged fried food.

Frying oil has been available to man in various parts of the world. Most of the time a specific oil has been selected for frying because it is locally available. Man also has moved from the crude expelled oils to refined oils as the oil technology advanced. In addition, the availability of most oils across the world has also increased due to improved transportation and storage systems developed over the years. Consumers have been exposed to the taste of products fried in different types of oil for quite sometime. Production of other than the indigenous oils has also become common where the local climate, soil conditions, and overall agronomy have been favorable to a particular type of oilseed or oil palm trees.

In spite of the widespread distribution of various types of oils across the world, it is found that there are regional preferences for particular oils in fried foods. For example, cottonseed oil is considered as the “gold standard” for potato chips in

the United States. This is largely because cottonseed oil was the primary vegetable oil grown in the United States when potato chips were introduced 150 years ago at Saratoga Falls, New York (1).

Similarly, the Mexican consumers prefer sesame seed oil or safflower oil in fried snack foods. Consumers in the Indian sub-continent prefer peanut (groundnut) oil in fried snacks. Bias towards the original indigenous oil can be found in every oil-producing country. Availability and the necessity for sufficient supply of the oil have played a great role in local selection of oil for frying products. For example, the Mexican consumers have accepted palmolein for frying snack foods because the fried food has good flavor and taste, although they prefer safflower oil or sesame seed oil. Acceptance of palmolein in Mexico has been influenced by the fact that sesame seed and safflower oils are in short supply and more expensive and palmolein produces good fried food at reduced cost.

## 2. ROLE OF OIL OR FAT IN FRYING

Oil provides several important attributes to the fried product that makes the fried food palatable and desirable to the consumers, these include:

- Texture
- Fried food flavor
- Mouthfeel
- Aftertaste

Fortunately, oil has also been an excellent heat transfer medium for dehydration of the food during frying. Some mechanical engineers in the frying industry tend to treat the oil as a true heat transfer medium. Subsequent discussions in this chapter will show that oil plays a much greater role than just being a heat transfer medium in frying.

## 3. APPLICATIONS OF FRYING OIL

As previously mentioned, frying oil is used in homes, restaurants (food services), and industrial frying operations. Home fried food is consumed almost immediately after preparation. At restaurants, the fried food is generally made to order and consumed within minutes of its preparation. Frying oil is always considered acceptable at homes or restaurants when it produces good flavor and texture in the food. There is little or no concern regarding the shelf life of the fried product at either of these locations.

Industrial products, on the other hand, are packaged and distributed for sale. Some of these products may require weeks or months for their distribution and sale. Therefore, these products must maintain good flavor and texture in order to

be acceptable to consumers when they are purchased. The oils (fats) used for industrial frying must have good oxidative and flavor stability in order to achieve good shelf life for the products. In this chapter, one will be able to understand the requirements that are critical for industrial frying oil. All subsequent discussions on oils in this chapter will be pertaining to industrial frying, although, the same criteria apply in restaurant frying.

#### 4. SELECTION OF FRYING OIL

The following criteria (2) are applied for the selection of oil for industrial frying:

1. Product flavor
2. Product texture
3. Product appearance
4. Mouthfeel
5. Aftertaste
6. Shelf life of the product
7. Availability of the oil
8. Cost
9. Nutritional requirements

Flavor, aroma, and appearance are generally the first three attributes that the consumer looks for in the fried food. Subsequently, the consumer judges the fried food for texture, mouthfeel, and aftertaste. Thus, the first five items from the above list are important for consumer acceptance of the product.

Product shelf life is important for quality and economic reasons. All products require a certain number of weeks or even months for distribution and sales. The product flavor and texture must be acceptable to the consumer at the time it is used. The texture of the product (staleness) is caused by moisture pickup during storage. This can be corrected through proper initial moisture control and the use of appropriate packaging with a good moisture barrier property.

Oil quality and oil flavor stability greatly influence the flavor stability of the product in storage.

Availability and cost of oil are important economic factors. Even the best performing frying oil is not beneficial to the business if it is not available in sufficient quantities. The cost of oil is extremely critical for the industry. Most fried snack foods contain 20–40% oil. Therefore, the snack food company has to minimize the delivered cost of oil at the plant. Sometimes, the procurement department purchases oil from a supplier that does not have good control over their operation. This ends up costing money and goodwill for the snack food company in the long run.

Nutritional value of oil in the snack food has become important. To meet today's consumer desire's, the frying oil must have the following attributes:

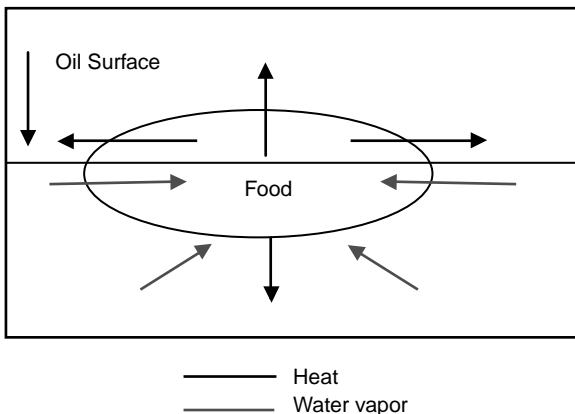
1. Low in saturated fat
2. Low in linolenic acid
3. High oxidative and flavor stability
4. Not hydrogenated (*trans-fat* free)

This is a difficult challenge for the snack food industry to meet because the modified composition oils are in very limited supply. Palmolein has no *trans-fat* but it is high in saturated fat. Soybean oil and canola oil must be hydrogenated for industrial frying. Thus, they will have *trans-fat*. Moreover, it is important to recognize the fact that the joint supply of palm oil and soybean oil constitutes almost 80% of the world's oil consumption (2). There is not enough of either of these two oils to supply the world's total oil needs. Corn oil, cottonseed, modified sunflower, and modified canola oils are in limited supply. They are grown in limited geographic areas where they are facing stiff competition against other cash crops. Therefore, nutritional needs in fried snack foods can be met in limited geographic areas and at a significantly higher cost.

## 5. THE FRYING PROCESS

Frying is a complex process where simultaneous heat and mass transfer as well as chemical reactions take place (3). In this process, the hot oil supplies the heat to the product being fried. Heat turns the internal moisture of the food product into water vapor. The water vapor comes out of the product through the outer surface (see Figure 1). This is why one can always see bubbles around the food being fried. Bubbling is vigorous at the beginning when the food is added into the hot oil and stops when the moisture in the product drops to a low level.

The food product undergoes dehydration. At the same time, several physical changes and chemical reactions occur in the food as well as the frying oil, as described below:



**Figure 1.** Conceptual heat and mass transfer during frying.



## 5.1. The Changes Occurring in the Food

- The food loses moisture
- The food surface develops a darker color (sometimes, hard crust)
- The fried food develops a firmer texture (or crust)
- The food also develops fried flavor and aroma

## 5.2. The Changes Occurring in the Oil

- The fresh oil passes through a breaking-in period during which the fried food appears quite bland
- Fried food flavor develops as the frying process continues
- Along with flavor development, the oil undergoes the following chemical reactions:
  - 1) Hydrolysis
  - 2) Autoxidation
  - 3) Oxidative Polymerization, and
  - 4) Thermal Polymerization
- The oil in the fryer becomes darker

The oil quality and the fried food flavor go through an optimum stage. Thereafter, both oil quality and product flavor decline (4). All of the above chemical reactions alter the chemical structure of the oil molecules. The unsaturated fatty acids are mostly affected. Some desirable as well as undesirable chemical compounds are formed in the oil during frying (5). Oil in the freshly fried foods contains the same compounds that are present in the fryer oil. The desirable compounds help provide good flavor to the freshly fried product. Sometimes, the undesirable oil components can affect the fresh product flavor. In many instances, a fried product with good initial flavor may develop oxidized or rancid flavor during storage. This is because the products of oil oxidation are strong catalysts and cause further degradation of the oil (contained in the product) during storage. This phenomenon is quite pronounced when the oil is abused in the frying process. This is even more evident in products fried in oil with poor fresh oil quality. Therefore, oxidative stability of the oil in packaged fried foods is critical for achieving the desired shelf life for the product.

Darkening of the product surface, also called the browning reaction, is produced by the chemical reaction between the frying oil or oil present in the food (lipids in general) and proteins, and saccharides present in the food. This reaction is known as Maillard reaction (6, 7), which is responsible for the following:

1. Brown or dark brown surface appearance of the fried product
2. Fried flavor of the product

Browning reaction also provides some protection against photooxidation (8, 9), which will be discussed later.

## 6. CHEMICAL REACTIONS OCCURRING IN OIL DURING FRYING

It has been mentioned earlier that several chemical reactions take place in the oil during frying (10, 11). These include hydrolysis, autoxidation, oxidative polymerization, and thermal polymerization, as explained below.

### 6.1. Hydrolysis

In this process, an oil (triacylglycerol, also known as triglyceride) molecule reacts with a molecule of water, releasing a molecule of fatty acid (12), commonly known as free fatty acid (FFA), and a molecule of diacylglycerol (DG, also called diglyceride). The reaction scheme is shown below:

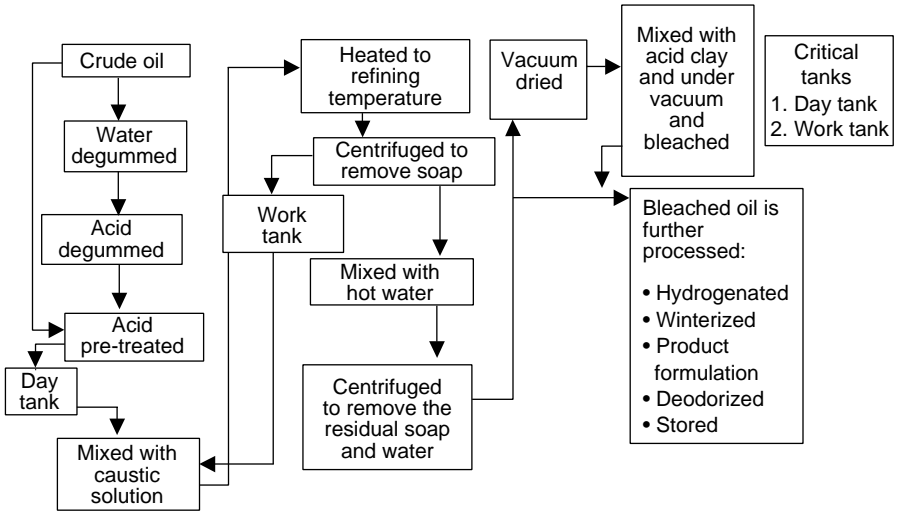


Although, it is common for the oil to undergo this reaction during frying, presence of a surfactant is required for hydrolysis to occur. Hydrolysis cannot occur unless oil and water form a solution (13). Oil and water do not mix except at very high temperatures under high pressure at 500°F (260°C) or higher, water boils at 212°F (100°C), at sea level. Therefore, one can expect that very little oil and water solution should result at frying temperatures (300–415°F or 149–213°C), unless there is a small amount of surfactant present in the fryer (14, 15). A surfactant can facilitate the formation of an oil/water solution during frying. This is primarily responsible for generating the FFA in the fryer oil. Several sources of surfactants are listed below.

**6.1.1. Fresh Oil.** Fresh frying oil is obtained by refining palm oil or seed oils. It would be appropriate to briefly discuss the vegetable oil refining process for the readers to understand how various processing steps impact the quality of freshly refined oil. Vegetable oils are refined principally by:

1. Physical refining method
2. Chemical refining method

Palm oil and coconut oil are refined by the physical refining method. The crude oil is bleached with acid-activated clay and citric acid at elevated temperatures under vacuum. The objective is to remove phosphorus (phospholipids), trace metals, oil decomposition products, and some of the color bodies from the crude oil. The volatile impurities in the bleached oil are then removed via steam distillation under very low absolute pressure and high temperature in a deodorizer.



**Figure 2.** Chemical refining process.

Physical refining process is fairly simple and is environmentally friendly and more economical for palm oil, coconut oil, and palm kernel oil. The process may not remove the trace impurities if the bleaching step is not done properly.

Seed oils are refined mostly by the chemical method where the crude oil is intimately mixed with a caustic (sodium hydroxide) solution under controlled conditions. The caustic primarily reacts with the free fatty acids to form soap (in this case, sodium soap of fatty acids). The soap is removed from the refined oil using a centrifuge. Aside from free fatty acids, some of the phospholipids, trace metals, and some of the color bodies from the crude oil are also removed and they appear in the soap phase. The soap is processed further to regenerate fatty acids. The refined oil is water washed to reduce the soap and then bleached with acid-activated clay and citric acid at elevated temperatures under vacuum. The bleached oil is either deodorized to make liquid oil or is hydrogenated to make shortening and margarine and the formulated product is then deodorized. The schematic flow diagram for the chemical refining process is shown in Figure 2.

Various processing steps are involved in vegetable oil refining. Each step is controlled under specific operating and oil quality standards in order to produce the best quality oil. Oil-refining procedures have been discussed in another chapter in this series. However, the processing steps, and their impact on the hydrolytic and oxidative stability of fresh oil, have been discussed briefly. The impacts of improper processing on hydrolytic stability of the freshly refined vegetable oil are listed below.

1. Higher than the normal level of phospholipids, calcium, or magnesium left in the fresh oil (16, 17) as a result of:

- Poor quality crude oil
  - Incomplete refining that leaves high levels of phosphorus, calcium, and magnesium
  - Poor bleaching condition, leaving high levels of phosphorus, calcium, and magnesium
  - Poor harvest and storage conditions for the seeds before crushing, producing crude oil that is hard to refine
2. Soap left in the oil as a result of poor water washing and bleaching (18).
  3. High levels of diacylglycerol and monoacylglycerol in the refined oil as a result of the (19) factors listed below:
    - Poor-quality crude oil that requires excess caustic treatment
    - Repeated refining of the crude oil because of poor crude oil quality
    - Caustic overdose in the refining step as a result of poor process control
    - High temperature in refining

**6.1.2. Food.** Surfactants can originate from the food itself. Food products contain many naturally occurring components that have surface-active properties. The metals, naturally present in the food, can form soap by reacting with the free fatty acids in the oil. The coating materials on breaded products contain acid-phosphates of sodium or calcium. These metal ions react with free fatty acids in the oil to form soap. Soap, thus formed in the oil, can produce an oil/water solution in the fryer and promote hydrolysis in the oil.

**6.1.3. Oil Decomposition Products.** Some of the oil decomposition products have surface-active properties. These compounds can promote hydrolysis of the fryer oil somewhat similar to soap.

**6.1.4. Poor Rinsing of the Fryer After Sanitation.** Soap is left in the fryer when it is not completely rinsed after sanitation, leaving residual caustic or soap in the system. This can promote hydrolysis in the fryer oil.

## 6.2. Autoxidation

Oil is oxidized during frying. Oxidized fatty acids develop flavor in the fried food (20). The compounds known to impart good fried food flavor are lactones and certain aldehydes. Most of these compounds originate from linoleic acid. Autoxidation is one of the major reactions taking place during frying (20–24) and, subsequently, in the oil present in the packaged product in storage.

Autoxidation of unsaturated fatty acids is initiated by a free radical, which is formed in the oil when an unsaturated fatty acid is exposed to oxygen in the

presence of a metal initiator, such as iron, nickel, or copper. The fatty acid could be a fatty acid moiety on a triacylglycerol molecule or it could be a free fatty acid molecule that is either present in the fresh oil or produced in the oil from hydrolysis during frying. The reaction mechanism for autoxidation involves several steps as described below:

**6.2.1. Step 1: Initiation.** Metal initiator produces a free alkyl radical from an unsaturated fatty acid molecule. The following conditions are required for this reaction step:

- There must be a metal initiator (iron, nickel, copper) in contact with the unsaturated fatty acid
- Heat generally accelerates the process of free radical formation and the subsequent reaction steps
- Phospholipids, monacyloglycerol, and diacylglycerol can reduce the interfacial tension between oil and air. This increases the amount of contact between the oil and oxygen in frying, promoting autoxidation
- Calcium and magnesium form soap in reaction with the free fatty acid in the fryer oil, forming soaps. These can behave in the same way as the phospholipids, causing increased autoxidation in the fryer oil.

**6.2.2. Step 2: Reaction with Oxygen.** The free radical reacts with a molecule of oxygen, forming a peroxy (alkoxy) radical. Presence of oxygen is absolutely necessary. This is why oil does not oxidize when it is stored under vacuum or saturated with nitrogen.

**6.2.3. Step 3: Propagation.** In this step, the peroxy radical reacts with a molecule of unsaturated fatty acid, forming a molecule of hydroperoxide and releasing another free alkyl radical, which can then react with an oxygen molecule to form a peroxy (alkoxy) radical. This step becomes rapid and more complicated when the oil contains linolenic acid.

Hydroperoxides are very unstable and decompose into a series of aldehydes, ketones, hydrocarbons, alcohols, and many more reaction products as the oil-oxidation process continues. In reality, these reactions can continue during storage of the packaged product, as the oil in the product continues to break down via autoxidation and develops oxidized or rancid flavor in the product.

**6.2.4. Step 4: Termination.** Free radicals can react with each other. This occurs when:

1. There are no more unsaturated fatty acids left in the system, or
2. When there is no more oxygen present in the system

## 7. SOURCES OF FREE RADICALS

Free radicals are formed whenever oil containing unsaturated fatty acids is heated in the presence of a metal initiator, (25) such as iron, nickel, or copper. Free radicals are formed in the oil during frying. The metal initiator in the frying process can come from several sources as given below:

1. The food being fried
2. The oil itself

Trace metals are present in crude vegetable oils at parts per million (ppm) levels. Researchers have shown that soybean oil flavor can deteriorate from autoxidation, even at an iron content as low as 0.3 ppm (26) in the deodorized oil. Metal initiators initiate autoxidation in all vegetable oils and animal fats.

Trace metals in the crude oil are removed in the refining and, primarily, in the bleaching steps (27–32). Inadequate bleaching of the oil can leave trace metals at high concentration in the oil. This can promote autoxidation in the fryer. In addition, atmospheric bleaching, poor vacuum in the vacuum bleacher, high temperature in the bleacher, or poor vacuum in the deodorizer can produce free radicals in the fresh oil (33). These free radicals can rapidly oxidize the oil in a fryer.

## 8. POLYMERIZATION

There are two types of polymers formed in the fryer oil (25). These include:

- Oxidative polymers
- Thermal polymers

### 8.1. Oxidative Polymers

Oxidative polymers are formed in autoxidation when the free radicals terminate each other as under autoxidation. When a triacylglycerol molecule breaks down during autoxidation, the partial triacylglycerol molecules are not removed in the deodorization process and can react with each other, forming dimers, trimers, or polymers.

These oxidative polymers do not always impart an off flavor to the freshly fried food. However, an off flavor in the packaged product might be observed within a few days after production and may exhibit oxidized or rancid flavor in the product before expiration of the code date on the package. This is because of the following events that might occur:

- The oxidative polymers are strong free radicals and can decompose while the fried product is in storage

- Some of oxidative polymer molecules may contain a higher amount of oxygen than the triacylglycerol molecule. When these oxidative polymers decompose, they produce free radicals and release some oxygen (34)
- The free radicals and the released oxygen can continue the autoxidation process in the product during storage
- This phenomenon can be observed in packaged fried products, even when the package is nitrogen flushed, using a film that has high nitrogen barrier property
- This reaction can progress even when the above product is stored in a freezer
- Some researchers have called this reaction “hidden oxidation” (35)

Improperly processed oil can have high concentrations of free radicals even after deodorization (34). This can compound the situation, producing rapid oxidation of oil during frying.

## 8.2. Thermal Polymers

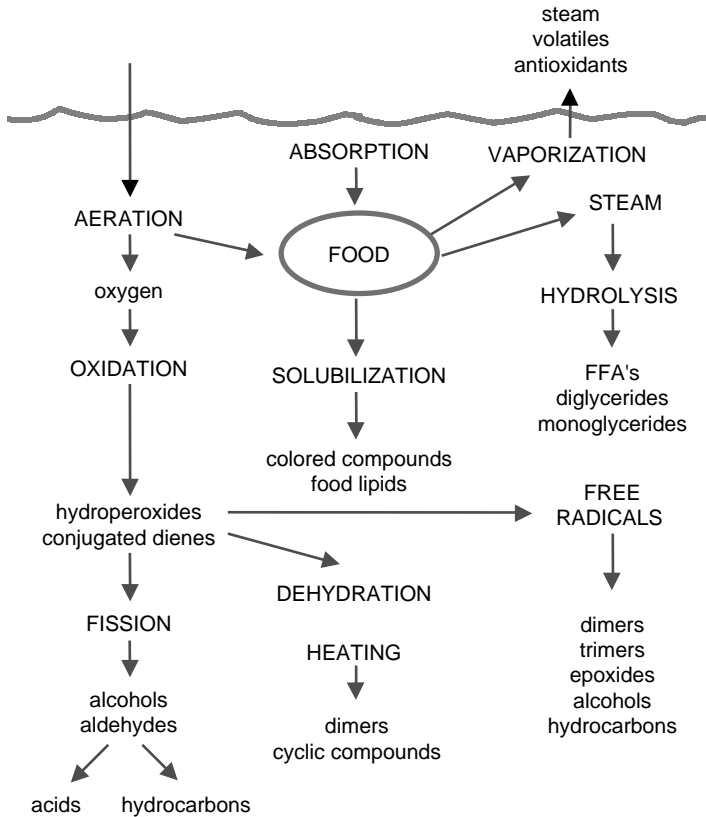
Polymerization of oil occurs under heat with or without the presence of oxygen. Heat can cleave the oil molecule or fatty acid. These cleaved compounds can then react with each other, forming large molecules. These polymers are referred to as thermal polymers. In the frying process, excessive fryer heat and excessive fryer down time can produce high levels of thermal polymers. Thermal polymers can be detected in the fresh product by expert panelists because they generally impart a bitter aftertaste to the fried food.

## 9. COMPLEXITY OF OIL REACTIONS IN FRYING

The reactions in the oil in the frying process are very complex. Figure 3, published by Carl W. Fritsch, illustrates the various pathways for oil reaction in the fryer (10).

Figure 3 shows that the autoxidation reaction produces alcohols and acids, among others. Some of these are dibasic acids, i.e., they contain two carboxylic acid groups (-COOH). This is why oil expelled from a highly oxidized or rancid fried product can exhibit a higher free fatty acid content than the fryer oil sampled at the same time as the product. This does not mean that the oil in the product was hydrolyzed in storage. It means the oil was abused at the time the product was packaged. The above phenomenon is observed in fried food even if the product is packaged under nitrogen flush.

Figure 3 indicates that there are several reactions that take place simultaneously in the fryer. Also, the degradation of product flavor is associated with oxidation and other reactions in the oil. Therefore, it is important to note that measurement of free fatty acid in the fryer oil does not necessarily represent the absolute quality of the oil. This is because the free fatty acid content in the fryer oil ranges from 0.25% to 0.40% for most snack food products. In many frying operations, a portion of the fryer oil is replaced by fresh oil when the free fatty acid level exceeds 0.35%. At this low level of free fatty acid, there is little correlation between the free fatty acid



**Figure 3.** Reactions in oil during frying.

and the degree of oxidation of the fryer oil. Free fatty acids in the fryer oil could be considered as a valid oil quality indicator, where the free fatty acids are allowed to go up to  $>0.5\%$  in the fryer.

In fact, the author conducted the following experiment on a commercial potato chip fryer to prove that the high level of free fatty acid in the oil was not a determinant for poor flavor in the fresh product. The test procedure and the observations are listed as follows:

- The fryer was filled with fresh oil
- Pure oleic acid at 2% level was added into the fryer oil and circulated for complete mixing
- The make-up oil also contained 2% of added oleic acid
- The fryer was operated under normal operating conditions
- The fresh product did not have any objectionable flavor from high free fatty acid content of the fryer oil
- The flavor of the product was affected only after the oil began to oxidize



It was mentioned earlier that the standard procedure in the snack food industry is to discard oil when the free fatty acid in the fryer oil reaches or exceeds 0.5%. The experimental oil contained 2% free fatty acids, and yet the product flavor was unaffected until the oil began to oxidize.

Referring to Figure 3, one can see that a multitude of reactions occur in the fryer oil simultaneously. This includes hydrolysis, autoxidation, polymerization, and many others. Therefore, the fryer oil should also be analyzed for the state of oxidative and polymeric degradation aside from free fatty acids. These analyses will not be the same for all types of fried food. The specific analysis needs to be established through shelf life study and consumer acceptability tests on the product.

It is a difficult and lengthy process to identify and quantify the specific compound or compounds in the fried food that makes the consumer reject it for poor flavor.

The following techniques are generally applied by the snack food companies to identify and quantify the compound or compounds (markers) that are responsible for consumer rejection of the product for unacceptable flavor:

- A well-designed shelf life test protocol including oil and corresponding product sampling procedures are outlined
- Highly skilled analysts to analyze oil breakdown products in the fryer oil and in the product (fresh and stored)
- A well-designed consumer test or multiple tests to determine the point at which the product flavor is found to be unacceptable
- Identification and quantification of the oil-degradation compounds that are responsible for the rejection of the product for unacceptable flavor
- Statistical correlation are established between the oil-quality parameters (also called markers) and consumer test results to establish the threshold value for the marker/markers responsible for product failure.
- The data are correlated with the initial oil quality in the product to establish the standards for the fryer oil quality in the process.

The markers and the threshold values are expected to be different for different products.

## 10. ANALYTICAL REQUIREMENTS FOR FRESH FRYING OIL

Based on the prior discussions, it can be stated that frying oil must meet the following quality criteria:

1. Good oxidative stability
2. Low levels of trace impurities in the fresh oil to prevent rapid hydrolysis and oxidation during frying

Oxidative stability of the oil depends primarily on its polyunsaturated fatty acid content. This includes linolenic and linoleic acids. Linolenic acid-containing

three-double bonds is most prone to oxidation; linoleic acid is less reactive than linolenic acid. As the number of double bonds increase in the fatty acid, the relative rate of oxidation increases at higher than a linear rate (36).

Fatty Acid	Relative Rates of Oxidation
Stearic acid	1
Oleic acid	10
Linoleic acid	100
Linolenic acid	150

Linolenic acid content must be low in order to provide maximum oxidative stability to the oil. This is why soybean and canola oil, which contain about 8% linolenic acid in the natural state, are hydrogenated to reduce their linolenic acid content to less than 2% determined by the capillary GC Method (2). Poor frying stability in sunflower oil comes primarily from the high level of linoleic acid. Therefore, sunflower oil must also be hydrogenated to reduce its linoleic acid content to 35% or lower for industrial frying. Table 1 lists the analyses of the most commonly used industrial frying oils.

**TABLE 1. Analyses of Common Industrial Frying Oils and Fats.**

Analysis	PH Soy	PH Canola	Corn	Cottonseed	PH Sun	Palm Olein	Frying Shtg.
FFA%	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
PV	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
IV	100	90	118-130	98-118	100	55-58	75-78
	+/- 4	+/- 2			+/- 4		
M.Pt °F (Max)	75	70	—	—	70	75	105-109
AOM hrs (minimum)	35	70	16	16	25	60	>100
FAC (%)							
C-14	—	—	—	—	—	1.0-1.5	—
C-16	10	4	11	22	7	39-43	—
C-18	5	2	2	2	4	4-5	—
C-18:1	55	75	20	19	53	40-44	—
C-18:2	28	12	60	53	35	12-14	—
C-18:3	<1.5	<1.5	<1.0	<1.0	<1.0	<0.5	—
C-20	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	—
C-22	—	—	—	—	—	—	—
C-22.1	—	<0.5	—	—	—	—	—
% Trans	25	25	—	—	25	—	35
SFI @ °F							
50°F	<10	<10	—	—	<10	—	20-26
70°F	<1	<1	—	—	<1	—	18-24
82°F	—	—	—	—	—	—	14-18
92°F	—	—	—	—	—	—	12-16
104°F	—	—	—	—	—	—	4-8

PH—Partially hydrogenated; Frying Shtg—Frying shortening; FFA—Free fatty acid; PV—Peroxide value; IV—Iodine Value; M.Pt.—Melting Point; AOM—Active oxygen method; FAC—Fatty acid composition; SFI Solid fat index (50°F = 10°C; 70°F = 21.1°C; 80°F = 26.7°C; 92°F = 33.3°C; 104°F = 40°C).

It has been mentioned earlier that linoleic acid is more stable than linolenic acid, but far less stable than oleic acid. This is why regular sunflower oil, which contains as much as 65% linoleic acid, is not suitable for frying shelf-stable industrial products. However, Table 1 indicates that liquid cottonseed and corn oil are used for industrial frying, although both oils contain over 50% linoleic acid.

Sunflower oil contains 10–12% higher linoleic acid than corn or cottonseed oil. However, this does not fully explain why corn or cottonseed oil has much higher frying stability than sunflower oil. The value of AOM does not fully explain why corn oil and cottonseed oil have higher frying stability than sunflower oil. This is discussed further in the following section.

## 11. IMPACT OF TOCOPHEROLS AND TOCOTRIENOLS IN FRYING OIL

Tocopherols are natural antioxidants present in all vegetable oils (30). The seed oils contain four types of tocopherols at varying amounts as listed in Table 2.

It is important to note the difference between the seed oil and palm oil. Seed oils contain tocopherols. Palm oil contains tocotrienols in addition to tocopherols, and also a small amount of coenzyme Q10. Both tocotrienols and coenzyme Q10 high antioxidant properties. Gamma- and delta-tocopherols, and their corresponding tocotrienols, are very effective in protecting oils from autoxidation (37).

Alfa-tocopherol is a good antioxidant against photooxidation (38). Table 2 shows that corn and cottonseed oils are rich in gamma-tocopherol, while sunflower oil contains very little gamma-tocopherol. This may explain why cottonseed oil and corn oil have superior frying stability than the sunflower oil even without hydrogenation.

Palm oil (also its fraction, palmolein) has good frying stability because of its high contents of gamma- and delta-tocotrienols, aside from the corresponding tocopherols. Tocotrienols contain three unsaturated double bonds as compared with the

**TABLE 2. Tocol Content in Typical Oils (Source: Food Codex).**

Tocols (ppm)	Sunflower	Cottonseed	Soybean	Canola	Palm Oil <sup>+</sup>
Tocopherols:					
Alpha	403–935	136–674	9–352	100–386	130–260
Beta	ND-45	ND-29	ND-36	ND-140	22–45
Gamma	ND-34	138–746	89–2306	189–753	19–20
Delta	ND-7	ND-21	154–932	ND-22	10–20
Tocotrienols:					
Alpha	ND*	ND	ND	ND	44–90
Beta	ND	ND	ND	ND	44–90
Gamma	ND	ND	ND	ND	260–525
Delta	ND	ND	ND	ND	70–140
Total Tocol (ppm)	440–1520	380–1200	600–3370	430–2680	600–1200

ND—Nondetectable.

corresponding tocopherols. This makes tocotrienols more effective as antioxidants against autoxidation in the frying process.

## 12. FACTORS AFFECTING FRYING OIL QUALITY

Table 3 lists the desired quality attributes for frying oils. It indicates that the oil must be low in free fatty acids, peroxide value, conjugated dienes, anisidine value, monoacylglycerols, diacylglycerols, and trace impurities, such as iron, phosphorus, calcium, and magnesium. Each of these quality parameters has specific significance in influencing the performance of the frying oil.

Quality of the oil starts from the seeds (39) for seed oils and from fruit palm (for palm oil and palmolein). Seeds that are physically damaged, insect-infested, moldy, too dry, or too moist produce poor-quality crude oil (40–43). Crude oil derived from such seeds can have high free fatty acids and oil oxidation products that inherently make the oil less stable, even after complete refining.

**TABLE 3. Recommended Analytical Parameters for Fresh Frying Oil (RBD oil).**

Analysis	Desired Level	Maximum Level	AOCS Method
Free fatty acid, FFA (%)	0.03	0.05	Ca 5a-40 (97)
Peroxide value, PV, meq/kg	<0.5	1.0	Cd- 8b-90 (97)
Para anisidine value, pAV, AVU Unit	<4.0	6.0	Cd-18-90 (97)
Conjugated dienes, (%)	Trace	<0.5	Th-1a-64 (97)
Polar compounds, (%)	<2.0	<4.0	Cd-20-91(97)
Polymers, (%)	<0.5	<1.0	Cd-22-91(97)
Phosphorus, PPM	<0.5	<1.0	Ca-12b-92 (97)
Iron, PPM	<0.2	<0.5	Ca-17-01 (01)
Calcium, PPM	<0.2	<0.5	Ca-17-01 (01)
Magnesium, PPM	<0.2	<0.5	Ca-17-01(01)
Monoacylglycerol, (%)	ND	Trace	Cd-11b-91(97) Cd-11b-96 (97)
Diacylglycerol*, (%)	<0.5	<1.0	Cd-11b-91 Cd-11b-96 Cc-13b-45 (97)
Lovibond Red Color:			
Soybean oil	<1.0	<1.5	
Canola oil	<1.0	<1.5	
Sunflower oil	<1.0	<1.5	
Cottonseed oil	<3.0	<3.5	
Corn oil	<3.0	<3.5	
Palmolein	<2.5	<3.0	
Peanut (Groundnut) oil	<1.5	<2.5	
Smoke point, **F (minimum)	460	—	Cc-9a-43 (97)
Soap, PPM	—	0.0	Cc-17-95 (97)
Flavor grade	8	—	Cg-2-83 (97)
Chlorophyll (ppb)	<30	<30	Cc-13d-55 (97)

\*Commercial palm oil may contain 5–11% diacylglycerol.

\*\*420°F for commercial palm oil or palmolein.

Excessive rain before harvest can increase chlorophyll content of the crude oil. There are two types of chlorophylls found in oilseed: Chlorophyll-A and Chlorophyll-B. Chlorophylls are oil soluble and produce green color in the oil. At normal concentrations, they are easily reduced in the bleaching process. It is very difficult to reduce the chlorophylls from the crude oil if it is obtained from seeds that are not mature or have developed higher chlorophylls because of excessive rain before the harvest. The normal practice is to use an excess amount of bleaching clay to reduce chlorophylls. This reduces the natural antioxidants in the oil, produces higher amounts of free radicals in the oil, and reduces its stability.

A high concentration of chlorophyll catalyzes photosensitized reaction (photo-oxidation) in the product fried and stored in clear packages. In addition, extra bleaching of the oil can decompose chlorophylls into pheophytines, pheophorbides, and pyropheophorbides, which do not have visual green color but are ten times more active photosensitizers than their parent compounds (44–46).

Seeds must be dried before storage. Failure to do so can increase enzyme activity in the seeds and damage the oil inside. Moisture content, the drying process, and the storage condition of the seeds impact the quality of the crude oil obtained from the seeds. In extreme cases, it becomes very difficult to obtain refined oil with high stability and good color from poor quality or poorly handled seeds.

Damaged seeds, whether resulting from the harvest, subsequent handling, insect infestation, mold infestation, or weather conditions can trigger the activities of several enzymes in the seeds (47). These are summarized below.

### **12.1. Lipase Reaction**

Lipase hydrolyzes the triacylglycerol molecules in presence of moisture and form free fatty acids, diacylglycerols, or even monoacylglycerols. Monoacylglycerols are mostly removed from the oil during deodorization, but a great majority of the diacylglycerols remain in the processed oil after deodorization. It has been mentioned earlier, high concentration of diacylglycerols in the fresh oil can accelerate the process of hydrolysis in the fryer.

### **12.2. Lipoxygenase Reaction**

Lipoxygenase oxidizes the unsaturated fatty acids in the oil in the seeds during storage under the conditions described above (48). There are different types of lipoxygenases in oilseeds. Lipoxygenase- Type-II and Type-IV are more reactive than the others (48).

### **12.3. Phospholipase-D Reaction**

This enzyme converts the hydratable phospholipids present in the seeds into non-hydratable phospholipids (42), when the seeds contain 14% moisture or higher and stored at 115°F (45°C) or higher. It is undesirable to have a high level of nonhydratable phospholipids in the crude oil because extra caustic treatment or longer

contact time between caustic and crude oil is required to reduce the nonhydratable phospholipids in crude oil. This increases the potential for increased diacylglycerol content in the refined oil.

The crude oil with high nonhydratable phospholipids requires acid pretreatment before refining. This may increase the chlorophyll breakdown, forming pheophytines, pheophorbides, and pyropheophorbides and make the finished oil more susceptible to photooxidation (44).

High temperature storage of seeds can also produce darker color crude oil as a result of color fixation (49) (this is also true for oil storage). This crude oil requires extra caustic or stronger caustic treatment as well as extra bleaching treatment to reduce the red color. Sometimes, it is difficult to obtain a light bleached color in color-fixed oil even after heavy bleaching. Extra refining and bleaching not only increases the potential to have higher concentration of diacylglycerols in the refined oil but also removes tocopherols from the refined oil. This reduces oxidative stability of the refined oil (50).

Storage of crude oil is also critical. Prolonged storage or high temperature storage of crude oil can greatly affect the quality of the refined oil (51). Long-term storage of crude oil increases oxidation, free fatty acids, and nonhydratable phospholipids in the crude oil, resulting in poor-quality refined oil. Crude oil with a high peroxide value can be refined to meet the fresh oil analytical standards but can oxidize very rapidly during frying.

Aside from free fatty acid, peroxide, aldehydes (by para-anisidine, pAV test), and conjugated dienes, the oil must also be low in trace components as listed in Table 3.

### 13. QUALITY STANDARDS OF OILS (TABLE 3)

1. The oil is either improperly processed or it is derived from poor-quality crude if the fresh oil analysis indicates the following:
  - FFA >0.05%
  - Lovibond Red color is higher than normal (see Table 3 above)
  - Tocopherol content is lower than normal (see Table 4)
  
2. The FFA in the oil rises rapidly in storage if it contains high levels of (47):
  - Phosphorus >1 ppm
  - Calcium >0.3 ppm
  - Magnesium >0.3 ppm
  - Sodium >0.2 ppm
  - Diacylglycerols >1.5%
  - Monoacylglycerols >0.4%

**TABLE 4. Typical Values of Tocopherols in Refined Seed Oils (ppm).\***

Tocopherol (ppm)	Cottonseed	Corn	Soybean	Palm*	Sunflower
Alfa-tocopherol	320	134	75	256	487
Beta-tocopherol	—	18	15	—	—
Gamma-tocopherol	313	412	797	316	51
Delta-tocopherol	—	39	266	70	8
Total tocopherol	633	603	1153	642	546

Source: *Bailey's Industrial Oils and Fat Products*, 5th ed., pp. 133 and 194 (52). 2nd ed., p. 131 (53) *The Lipid Handbook*, (54)

\* 150 ppm of tocotrienols.

3. The oil will oxidize rapidly in the fryer if the oil contains high levels of (47):

- PV >1.0
- pAV >6.0
- Iron >0.3 ppm
- Conj. Dienes >0.5%
- Polars >4.0%
- Polymers >2.0%
- Iron >0.3%

Frying oil needs certain amounts of solid fat to fry foods that require crunchy texture or crispness. Table 1 lists the typical industrial frying fats and their analyses.

## 14. COMMENTS ON PALM OIL

Palm oil is the second largest source of oil in the world, next to soybean oil (55). Worldwide production of palm oil is increasing at a rapid rate and the volume of palm oil in the world may surpass that of soybean oil in the near future. Palm oil and palm olein (the liquid fraction) show excellent frying performance. In addition, both palm oil and palm olein produce a highly desirable fried food flavor (56, 57).

Sometimes the commercial palm oil and palm olein contain high amounts of diacylglycerols and phospholipids. This makes the oils prone to rapid hydrolysis in frying.

Diacylglycerols are produced in the fruit palm after harvest. The fruits are required to be processed within 24 hours after the harvest in order to control hydrolysis of the oil in the fruit by lipase. This guideline cannot be followed by all palm oil processors during the peak harvest season. As the trading rule for palm oil allows 5% free fatty acids in crude palm oil and 0.25% free fatty acid in neutralized palm olein (58), there is no incentive for the common palm oil processors in producing oils with lower free fatty acids. Commercially available palm oil and palm

olein may contain higher than 1 ppm of phosphorus after refining, which may make the free fatty acids go up rapidly in the oil during frying.

It is possible to produce crude palm oil with less than 1.5% free fatty acid and less than 4% diacylglycerols through careful separation of the damaged fruits from the bulk and by exercising some other process precautions.

Some palm oil processing companies in Malaysia are producing and exporting fully processed palm oil and palm olein with low free fatty acids, diacylglycerols, phosphorus, and trace metals. They guarantee the delivered quality for the oil. These oils have shown excellent frying stability.

## 15. ENHANCEMENT OF FRYING OIL PERFORMANCE

Natural as well as synthetic antioxidants can improve the oxidative stability of edible oils. Most common natural antioxidants are mixed tocopherols. Tertiary-butylhydroquinone (TBHQ) is one of the most common synthetic antioxidants used in commercial frying oils. This also costs only a fraction of the natural antioxidants.

Tocopherols are already present in the oil. The amounts and the types of tocopherols vary with the type of oil. Most common types in seed oils are alpha-, gamma-, and delta-tocopherols. These compounds protect the oil from oxidation during processing, storage, transportation, and, also, later in the fryer. Tocopherols terminate the free radicals by reacting with them in the autoxidation reaction. This is why they are considered as free radical scavengers/quenchers.

For various reasons, as described earlier, the freshly refined oil may contain lower than the desired level of tocopherols. In those cases, supplementing the oil with gamma- and delta-tocopherols or gamma- and delta-tocotrienols can significantly improve oxidative stability of the oil. This is not commercially practiced because of the high cost of the antioxidants.

As antioxidants are free radical quenchers, the level of the added antioxidants in the refined oil decreases while the oil is in storage or in transit. However, one must recognize the fact that, because of the presence of antioxidants, the free radical content of the oil is going to be low at the time it is added to the fryer (provided the oil is not stored for too long or oxidized as a result of mishandling before use).

A large portion of the TBHQ is lost during frying as a result of high heat and agitation. Therefore, some suggest that addition of TBHQ in frying oil is not beneficial. However, it is known that, despite the loss of TBHQ in the fryer, the finished product will have a better chance to retain good oil flavor during storage and distribution because of the lower concentration of free radicals in the fresh oil used in frying. It must be emphasized that the added antioxidant does not necessarily protect the oil against abusive treatment during storage, handling, or frying.

There are various synthetic antioxidants, such as butylated hydroxytoluene (BHA), propyl gallate (PG), ethylenediaminetetraacetic acid (EDTA), etc. that are used in commercial oils; TBHQ is known to produce the best results in frying applications.



Many countries have regulations regarding the type of antioxidants permitted for use and also on the maximum allowable limits in foods. The snack food companies must be aware of local regulations regarding the use of synthetic and natural antioxidants in foods.

Added tocopherols, tocotrienols, or synthetic antioxidants must be declared on the label, according to the USFDA (United States Food & Drug Administration), if they are added to the oil after deodorization.

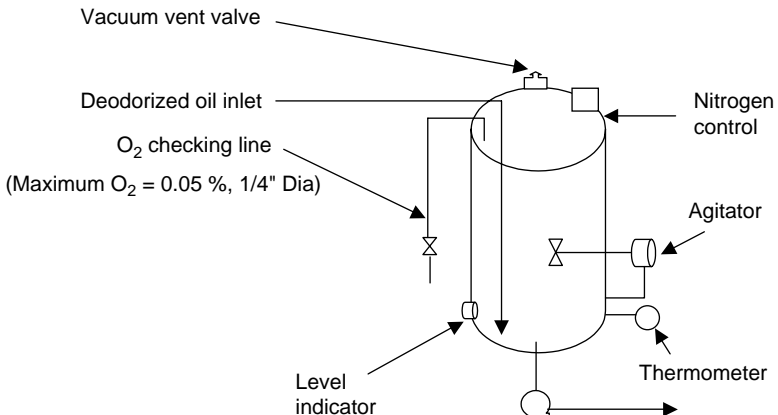
## 16. STORAGE AND TRANSPORTATION OF FRYING OIL

Deodorized oil must be cooled, saturated with nitrogen, and stored in nitrogen-blanketed tanks (59). The storage tanks can be made of carbon steel. It is not necessary to have stainless steel tanks for freshly deodorized oil with a low free fatty acid content as shown in Table 3. The following guidelines are recommended for oil storage:

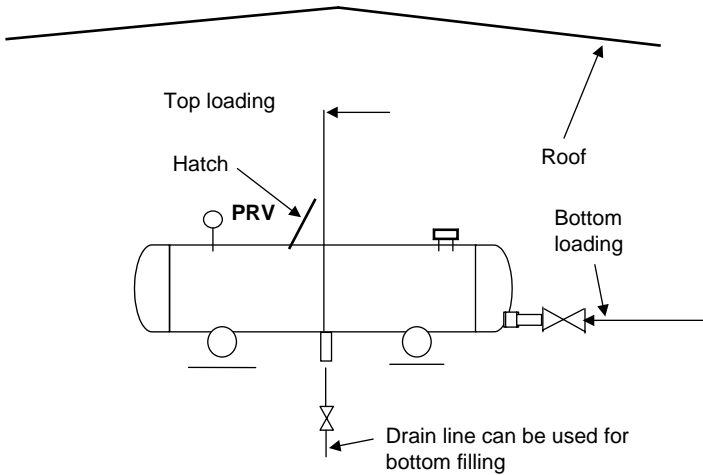
- Liquid oils should be stored under 86°F (30°C) and should not be stored at a temperature higher than 104°F (40°C)
- The storage temperature of hydrogenated fats must not exceed its melt point by 10°F (5.6°C)

The best results are obtained when the deodorized oil is cooled to the above recommended temperatures, saturated with nitrogen as it leaves the deodorizer, and stored in nitrogen-blanketed tanks. The headspace in the storage tank should have maximum oxygen content of 0.5% (see Figure 4).

Loading trucks or rail cars are also critical for the quality of the oil delivered at the point of destination. The oil can pick up air (oxygen) at the loading station, which can initiate autoxidation in the oil during transit. The clearest indication



**Figure 4.** Schematic for deodorizer oil storage.  
(Copyrighted by: MG Edible Oil Consulting, Int'l.).



**Figure 5.** Schematic diagram for truck loading.  
(Copyrighted by: MG Edible Oil Consulting, Int'l.).

of such a situation is found when the oil, at delivery, shows a higher peroxide value than the certificate of analysis (written at the time of loading and sent with the oil shipment). In addition, partially loaded trucks or rail cars have more air space above the oil. This causes higher oxygen absorption by the oil during transit. The following steps are recommended to minimize the oxygen absorption by oil during loading and transit:

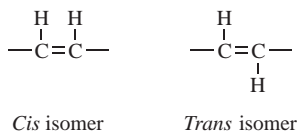
- Follow the guidelines provided above on oil temperature
- Saturate the oil with nitrogen at the discharge of the loading pump
- Bottom load the truck or rail car
- Extend the loading boom to the floor of the truck or rail car to avoid splashing of the oil and air entrainment during loading
- Fill the truck or rail car to the maximum limit to minimize air space at the top

See Figure 5 for truck or rail car loading.

Unloading and storage of the oil is equally critical at the snack food company. Care must be taken not to overheat any solidified hydrogenated oil. The oil must be saturated with nitrogen and stored under nitrogen as described for the deodorized oil.

## 17. HYDROGENATION AND TRANS-FAT

Sabatier and Senderens (59) conducted the first hydrogenation reaction in the vapor phase. In 1903, Normann (60) patented the liquid-phase hydrogenation process for oils. Later, Crossfield and Sons, a British company, purchased the patent.



**Figure 6.** *Cis-* and *trans-*isomers of unsaturated fatty acids.

Procter & Gamble Company of USA acquired the patent right from Crossfield and Sons (59) in 1909 and started to produce Crisco shortening from cottonseed oil in 1911. The American patent on hydrogenation was under Burchenal (61) patent, which was declared null and void in the country to clear the way to allow an all out expansion of hydrogenation by every American oil company.

In the hydrogenation process, unsaturated fatty acids are reacted with hydrogen gas with the help of nickel catalyst. In this process, two hydrogen atoms are attached to the double bond in unsaturated fatty acids. Complete hydrogenation of vegetable oils converts essentially all of the unsaturated fatty acids to stearic acid.

During the course of transformation of the unsaturated fatty acids to saturated fatty acids, positional isomers of unsaturated fatty acids are produced. These unsaturated fatty acid isomers are known as *trans*-fatty acids (62), as shown in Figure 6.

Scientists recognized the benefits of the hydrogenation process, which revolutionized the fats and oils industry for a century. Hydrogenation of oils provides the following benefits to the oils:

- Increased oxidative stability due to reduced level of unsaturation
- Higher concentration of saturated fatty acids
- Higher melting point
- Unique functional products like shortening and margarine

Improved oxidative stability of selected hydrogenated oil is demonstrated in Table 5.

Liquid soybean oil and liquid canola oil contain 8% linolenic acid. This makes both oils very prone to oxidation, especially in the frying application. Light hydrogenation can reduce the linolenic acid to less than 2% (measured by the capillary GC method). This makes the oils suitable for industrial frying of shelf-stable products.

Table 4 shows that hydrogenation of soybean oil to a linolenic acid content of less than 2% increased the AOM value from 15 hours to over 30 hours. Similarly, the AOM value rose from 20 hours to over 75 hours for canola oil after hydrogenation. However, hydrogenation also produced significant levels of *trans*-fats in both oils (2).

During the past century, while *trans*-fats were *not* on the “unwanted list” in diet, the oil technologists found that these fatty acids possessed some unique properties and used this to achieve the following:

**TABLE 5. Effect of Hydrogenation on AOM.**

Analysis	Soybean Oil Before Hydrogenation	Soybean Oil After Hydrogenation	Canola Oil Before Hydrogenation	Canola Oil After Hydrogenation
Iodine Value (Typical)	130	100	115	90
M.P.(°F)	—	<75	—	<75
AOM, hr (minimum)	12–20	>30	12–20	>70
Palmitic acid (%)	10	10	4	4
Stearic acid (%)	5	5	2	2
Oleic acid (%)	25	55	62	75
Linoleic acid (%)	50	28	21	12
Linolenic acid (%)	8	1.5	8	1.5
Erucic acid (%)	—	—	<2.0	<0.5
Gadeloic acid (%)	—	—	1	0
<i>Trans</i> -fat (%)	—	>30	—	>20

- Formulate margarine with sharp melting curve, similar to butter
- Beta-prime stable crystals in the shortening, which is desirable in the baking industry

Without hydrogenation, one could not make these products using only seed oils.

In recent years, *trans*-fats have been under the scrutiny of nutritionists and regulatory agencies in various countries. Multiple clinical studies have revealed *trans*-fats elevate of the low-density lipoprotein (LDL), depression of high-density lipoprotein (HDL), and elevation of triacylglycerol (TG) in human blood serum (63–68). In this respect, *trans*-fats behave like saturated fats. The U.S. Food & Drug Administration has released a mandatory labeling requirement that shows that, beginning January of 2006, all food packages must indicate the level of *trans*-fats on the food packaging (69). This is to be shown as a separate line item. The FDA has not recommended daily value for *trans*-fat intake. Some European countries and Canada have also issued labeling requirements on *trans*-fat in all food packages.

## 18. ALTERNATIVES TO TRANS-FATS

Home frying does not require any shelf life for the fried food. Therefore, use of any liquid oil is acceptable for home frying. High oxidative stability is required for the

frying oil used at the restaurants or food service operations as well as for manufacturing shelf-stable products that are packaged and distributed. The standard seed oils, such as those from soybean or canola, must be hydrogenated to different degrees depending primarily on the type of fried product and their required texture, flavor, and mouthfeel.

Three basic alternatives are available to make *trans*-free oils for industrial frying as well as other uses. These are described below.

### 18.1. Modified Composition Oils

These oilseeds have been developed over the past two decades. These oils are very low in linolenic acid (except high oleic canola). All of these oils can be used for industrial frying without hydrogenation. Fatty acid compositions of these oils are listed in Table 6. These oils are in limited supply and they are expensive. Corn oil, cottonseed oil, and palmolein have been included along with the others for comparison.

### 18.2. Pourable Shortening

This is made from a mixture of 90–98% liquid oil and 2–10% fully hydrogenated soybean oil. The blend is specially processed to obtain the pourable consistency. The fully hydrogenated component can also be made from canola or corn oil.

Standard liquid shortening sold in the market contains lightly hydrogenated soybean or canola oil. These contain approximately 20–30% *trans*-fats. Substitution of this lightly hydrogenated oil with one of the oils listed in Table 6 would produce *trans*-free pourable shortening.

### 18.3. Alternative Catalysts

Platinum, palladium, or copper catalyst can produce hydrogenated oils with a lower *trans*-fat content (70–72). The hydrogenated oils produced by these catalysts

**TABLE 6. Typical Fatty Acid Composition of Modified Composition Oils (weight %).**

Analysis	NuSun	HO Sun	HO Canola	LL Canola	Cotton-Seed	Corn	Palm Olein
C-14	<1	<1	<1	<1	—	—	1.0–1.5
C-16	5	6	6	4	22	11	39–43
C-18	4	2	2	3	2	2	4–5
C-18:1	50–65	83	76	65	19	20	40–44
C-18:2	20–35	11	13	22	53	60	12–14
C-18:3	<1	<2	4.5	3	<1	<1	<0.5
C-20	—	—	—	<1	<1	<1	<1.0
C-22	—	—	—	—	—	—	—
C-22:1	—	—	—	—	—	—	—
% Trans	—	—	—	—	—	—	—

NuSun—Mid-oleic sunflower oil; HO Sun—High oleic sunflower oil; HO Canola—High oleic canola oil; LL Canola—Low linolenic canola oil.

contain higher stearic acid and higher solids compared with nickel catalysts. One could choose to use either platinum or palladium catalysts to make shortenings or frying fat with less *trans*-fat contents. Platinum and palladium catalysts are very costly and not commercially competitive with the nickel catalysts.

Copper catalysts are cheaper and work just as well as the precious metal catalysts. However, complete removal of catalyst from the hydrogenated oil is never possible. Copper, present in the oil even at very low concentrations (less than 0.05 ppm) can cause rapid hydrolysis in the oil during frying.

#### 18.4. Hydrogenation Under High Pressure and High Agitation

Use of high reactor pressure (10–20 bars) with a high rate of agitation can produce similar results as the precious metal catalysts (62). However, this process cannot be carried out in the United States, because the reactors are designed to operate at a maximum pressure of 5 bars. There is only one oil processor in the United States that owns a high-pressure reactor, but it has not been used to any great extent. There are high-pressure hydrogenation reactors in Europe.

#### 18.5. Interesterification for Frying Shortening

In this process, two oils are reacted under special conditions to exchange the fatty acids from one another (73–76). A fully hydrogenated oil, such as cottonseed, palm, soybean, canola, or corn oil, can be interesterified with any of the liquid oils in Table 5 to produce *trans*-free shortening to suit the need for frying, baking, margarine, etc. Fully hydrogenated cottonseed or palm oil will produce beta-prime shortening, which is desirable for baking applications.

Intesterification is done by two methods: chemical and enzymatic. The cost for making interesterified products is very similar for both processes. The enzymatic interesterification process is becoming more popular because it is environmentally friendly.

#### 18.6. Fractionation

This is commonly done with palm oil because of the higher concentration of the tri- and disaturated acylglycerol molecules. This technique is highly developed and used in Europe, South America, Far East, Mexico, and Canada. The seed oils are not suitable for this process because of the lack of natural solids in them. This is why seed oils require hydrogenation to create solids to make functional products.

### 19. FRYING SHORTENING VERSUS FRYING OILS

Oils are used mostly for frying snack foods. Some snack foods use a topical coating on the product to hold the seasoning on the surface. In most cases, the spray oil needs a certain amount of solid fat for proper adherence of the topical seasoning

and also to reduce the oily surface appearance. Coating oil or spray oil, made from seed oils, is partially hydrogenated and contains *trans*-fats. It is possible to produce coating oil with no *trans*-fats by using a blend of liquid oil, such as listed in Table 6, mixed with a very small amount of fully hydrogenated fat. This small amount of saturated triacylglycerol provides the solids needed to hold the seasoning and reduce the oily appearance of the product.

Fried products with harder texture, such as French fries, extra crunchy fried chicken, and like products require solid shortening. The standard frying shortening in the United States is made from hydrogenated oils. It is possible to make *trans*-free shortening using either liquid shortening or interesterified technology, as described above.

It is unfortunate that palm oil is not acceptable to some consumers in the United States. Supply of modified composition oil is also very low. This makes it difficult to produce *trans*-free shortening in large volumes in the country at present.

## 20. SUMMARY

Frying is a mature industry. There are two major sectors of industrial frying, namely restaurant (food services) and large-scale manufacturing of shelf-stable products. Most large-scale manufacturers use continuous fryers, although there are some products that are strictly fried in batch (or kettle fryers) to achieve the desired product texture and appearance. Products made in either type of fryer are packaged in appropriate packaging material and distributed.

Par-fried products, such as French fries, chicken, coated vegetables, etc., have become extremely important to the fried food industry. These products are partially dehydrated, packaged, and shipped in the frozen state. The end users store these products in the freezers and fry them without thawing.

The longer shipping and distribution time required for the fried products has increased the need for oils with higher oxidative stability in order to meet the shelf life for all fried products. Soybean, sunflower, or canola oil needs to be hydrogenated for industrial frying. Heavy-duty frying shortening, used for frying French fries, donuts, and chicken, require a high degree of hydrogenation. Hydrogenation of oil produces *trans*-fatty acids. These are unsaturated fatty acids that behave like saturated fatty acids in terms of promoting coronary heart disease (CHD). Therefore, several countries, including the United States, have focussed their attention on the *trans*-fatty acid content in the prepared foods.

Palm oil and palm oil fractions contain natural solid triacylglycerols and no *trans*-fat. Palm oil is used extensively by many countries to make *trans*-free shortening and margarine (except the United States). Interesterification is also applied in Europe, the Far East, and South America. Some modified composition oils have been introduced in the United States, but they are in very limited supply. Several of these oilseeds are grown in specific geographic areas where there is stiff competition from other cash crops. This will continue to be a challenge for the United States in producing sufficient amounts of *trans*-free frying fats, unless palm oil

or palm olein is accepted by the consumers in the country. On the other hand, palm oil will be in extremely short supply, if the United States begins to use it because there is not enough surplus palm oil in the world at present (55).

The Better Bean Initiative from the United Soybean Board of USA is developing the mid-oleic soybean oil, which will not require hydrogenation for industrial frying applications. Full commercialization of this oil will take at least six to ten years. In the meantime, the food industry in the United States will have to be very creative to reduce *trans*-fats in fried foods as well as in other products.

## REFERENCES

1. America Snack Food Association Web Site.
2. M. K. Gupta, in Proceedings of the World Conference of Oilseed Technology and Utilization, American Oil Chemists Society Publication, Champaign, Illinois, 1992, p. 204.
3. M. K. Gupta, in Proceedings of the World Conference of Oilseed Technology and Utilization, American Oil Chemists Society Publication, Champaign, Illinois, 1992.
4. M. M. Blumenthal, *Optimum Frying: Theory and Practice*, Libra Laboratories, Inc., Piscataway, New Jersey, 1987.
5. J. Pokorny, *Flavor Chemistry of Deep Fat Frying, Flavor Chemistry of Lipid Foods*, American Oil Chemists Society Publication, Champaign, Illinois, 1989.
6. L. C. Maillard, *Compt. Rend.*, **154**, 66 (1912).
7. L. C. Maillard, *Ann. Chim.*, **9**(5) 258 (1916).
8. J. S. Smith and M. Alfawaz, *J. Food Sci.*, **60**(2), 234 (1995).
9. J. P. Eiserich, C. Macku, and T. Shibamoto, *J. Agric. Food. Chem.*, **40**(10), 1982 (1992).
10. C. W. Fritsch, *J. Amer. Oil Chem. Soc.*, **58**, 272 (1981).
11. J. Pokorny, *Flavor Chemistry of Deep Fat Frying, Flavor Chemistry of Fats and Oils*, American Oil Chemists Publication, Champaign, Illinois, 1989.
12. K. S. Markley, *Fatty Acids*, Interscience, New York, 1968.
13. L. L. Seifensieder, *Zig.*, **64**, 122 (1937).
14. E. Y. Yuki, Ishikawa, and T. Okabe, *Yukagaku*, **23**, 489 (1974).
15. M. M. Blumenthal, *Food Technol.*, **45**, 68 (1990).
16. M. K. Gupta, *Oil Quality Improvement through Processing, Introduction to Fats and Oils Technology*, 2nd ed., American Oil Chemists Society Publication, Peoria, Illinois, 2000.
17. T. L. Mounts, Effects of Oil Processing Conditions on Flavor Stability—Degumming, Refining, Hydrogenation and Deodorization, Flavor Chemistry of Fats and Oils, American Oil Chemists Society Publication, Champaign, Illinois, 1985.
18. M. M. Blumenthal and J. R. Stockler, *J. Amer. Oil Chem. Soc.*, **63**, 687 (1986).
19. M. K. Gupta, in Proceedings of the World Conference on Oilseed Processing and Utilization, American Oil Chemists Society Publication, Champaign, Illinois, 2000.
20. E. N. Frankel, Chemistry of Autoxidation, Mechanism, Products and Flavor Significance, Flavor Chemistry of Fats and Oils, American Oil Chemists Society Publication, Peoria, Illinois, 1985.



21. T. H. Smouse, Flavor Reversion in Soybean Oil, American Oil Chemists Society Publication, Peoria, Illinois, 1985.
22. A. J. Shin and D. H. Kim, *Korean J. Food Sci. Technol.*, **17**, 71 (1985).
23. E. N. Frankel, C. D. Evans, D. G. McConnel, and H. J. Dutton, *J. Org. Chem.*, **26**, 4663 (1961).
24. H. W. S. Chan and J. Levitt, *Lipids*, **15**, 837 (1977).
25. W. W. Nawar, Flavor Reversion in Soybean Oil, American Oil Chemists Society Publication, Peoria, Illinois, 1985.
26. G. R. List and D. R. Ericson, Storage, Stabilization and Handling, Handbook of Soy Oil Processing and Utilization, 1st ed., American Oil Chemists Society Publication, Peoria, Illinois, 1985.
27. O. L. Brekke, Bleaching, Handbook of Soy Oil Processing, 1st ed., American Oil Chemists Society Publication, Peoria, Illinois, 1985.
28. H. J. Dutton, *J. Amer. Oil Chem. Soc.*, **58**, 234 (1981).
29. L. H. Weideman, *J. Amer. Oil Chem. Soc.*, **58**, 159 (1981).
30. D. R. Ericson and L. H. Weideman, *INFORM*, **2**, 201 (1991).
31. M. K. Gupta, *INFORM*, **4**, 1267 (1993).
32. W. Zschau, Bleaching, Introduction to Fats & Oils Technology, Second Edition, American Oil Chemists Society Publication, Peoria, Illinois, 2000.
33. M. K. Gupta, Presentation at the Short Course on Vegetable Oil Processing, Texas A&M University, 1998.
34. S. S. Chang and F. A. Kummerow, *J. Amer. Oil Chem. Soc.*, **31**, 324 (1954).
35. E. N. Frankel, Soybean Oil Flavor Stability, Handbook of Soy Oil Processing and Utilization, American Oil Chemists Society Publication, Peoria, Illinois, 1985.
36. H. J. Beckman, *J. Amer. Oil Chem. Soc.*, **60**, 282 (1983).
37. Y. Basiron, Palm Oil, Bailey's Industrial Oils and Fats, 5th ed., Wiley, New York, 1996.
38. D. B. Min, S. H. Lee, and E. C. Lee, Singlet Oxygen Oxidation of Vegetable Oils, Flavor Chemistry of Lipid Foods, American Oil Chemists Society Publication, Peoria, Illinois, 1989.
39. J. A. Robertson, W. H. Morrison, and O. Burdick, *J. Amer. Oil Chem. Soc.*, **50**, 443 (1973).
40. C. D. Evans, G. R. List, R. E. Beal, and L. T. Black, *J. Amer. Oil Chem. Soc.*, **51**, 544 (1974).
41. G. R. List, T. L. Mounts, and A. J. Heakin, *J. Amer. Oil Chem. Soc.*, **56**, 883 (1979).
42. G. R. List, T. L. Mounts, and A. C. Lancer, *J. Amer. Oil Chem. Soc.*, **69**, 443 (1992).
43. E. N. Frankel, Chemistry of Autoxidation: Mechanism, Products and Flavor Significance, Flavor Chemistry of Fats and Oils, American Oil Chemists Society Publication, Peoria, Illinois, 1985.
44. K. R. Ward, J. K. Daunand, and T. K. Thorstainson, *INFORM*, **4**, 519 (1993).
45. R. Usuki, Y. Endo, T. Suzuki, and T. Kanada, Proceedings of the 16th ISF Congress, Budapest, Hungary, 1983.
46. R. Usuki, Y. Endo, and T. Kanada, *Agric. Biol. Chem.*, **48**, 99 (1984).
47. G. R. List, Special Processing for Off Specification Oil, Handbook of Soy Processing and Utilization, American Oil Chemists Society publication, Peoria, Illinois 1992.

48. D. Hildebrand, Genetics of Soybean Lipoxygenase, Lipoxygenase and Lipoxygenase Pathways, American Oil Chemists Society Publication, Peoria, Illinois, 1996.
49. G. R. List and D. R. Ericson, Storage, Stability and Handling, Handbook of Soy Oil Processing and Utilization, American Oil Chemists Society Publication, Peoria, Illinois, 1985.
50. B. Mistry and D. B. Min, *J. Amer. Oil Chem. Soc.*, **65**, 528 (1988).
51. C. D. Evans, E. N. Frankel, P. M. Cooney, and H. A. Moser, *J. Amer. Oil Chem. Soc.*, **37**, 452 (1960).
52. in *Bailey's Industrial Oils and Fat Products*, 5th ed., Wiley, New York, 1995.
53. in *Bailey's Industrial Oils and Fat Products*, 5th ed., Wiley, New York, 1995.
54. in *The Lipid Handbook*, 2nd ed., 1994.
55. Oil World Annual Report, 2002.
56. A. Fauziah, R. Ismail, and S. Nor. Anini, Frying Performance of Palm Olein and High Oleic Sunflower Oil During Batch Frying of Potato Chips Proceedings of the Porim International Palm Oil Congress, Kuala Lumpur, 1999.
57. S. Masashi, Y. Takashashi, and M. Sonehera, *J. Amer. Oil Chem. Soc.*, **62**, 449 (1985).
58. Specification set by the PORLA.
59. P. Sabatier, Catalysis in Organic Chemistry, Translated by E. E. Reid, Van Nostrand, New York, 1922.
60. W. Normann, British Patent 1,515, 1903.
61. J. J. Burchenal, U.S. Patent 1,315,351, 1915.
62. R. R. Allen, in *Bailey's Industrial Oils and fats*, 4th ed., Wiley, New York, 1981.
63. J. T. Judd, B. A. Clevidence, R. A. Muesing, J. Wittes, M. E. Sunkin, and J. J. Podczasy, *Am. J. Clin. Nutr.*, **59**, 861 (1994).
64. J. Judd, et al., *FASEB J.*, **12**, 1339 (1998).
65. M. B. Katan, R. P. Mensink, and P. L. Zock, *Annu. Rev. Nutr.*, **15**, 473 (1995).
66. R. P. M. Mensink and M. B. Katan, *N. Engl. J. Med.*, **323**, 439 (1990).
67. K. Sundram, A. Ismail, K. C. Hayes, R. Jeyamalar, and R. Pathmanathan, *J. Nutr.*, **127**, 514S (1997).
68. R. P. Mensink and M. B. Katan, *Arterioscl. Thromb.*, **12**, 911 (1992).
69. Rules and Regulations, *Fed. Register*, **68**, 41433–41506 (2003).
70. S. Koritala, *J. Amer. Oil Chem. Soc.*, **45**, 197 (1968).
71. S. Koritala, *J. Amer. Oil Chem. Soc.*, **49**, 83 (1972).
72. M. Zajcew, *J. Amer. Oil Chem. Soc.*, **37**, 11 (1960).
73. N. O. V. Sonntag, Fat Splitting, Esterification, and Interesterification, *Bailey's Industrial Oils and Fats*, 4th ed., Wiley, New York.
74. G. R. List, K. R. Steidley, and W. R. Neff, *INFORM*, **11**, 980 (2000).
75. P. Quinlan and S. Moore, *INFORM*, **4**, 580 (1993).
76. Morten Würtz Christensen Technical Service Oils & Fats Industry, Novozymes North America Inc., Personal Communication.

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## *Margarines and Spreads*

Michael M. Chrysan

This chapter discusses margarine as well as vegetable oil margarine and butter substitutes containing less than 80% fat. These products are generically referred to as table spreads. The table spread category in the United States has undergone great changes in recent years and continues to change rapidly even today. One hundred years ago, annual butter consumption was 8.6 kg (19 lb) per person. Now it is less than 2.2 kg (5 lb), and per capita table spread consumption is about 4.5 kg (10 lb) (1). Historical production and consumption data for table spreads and butter are shown in Table 1. Although consumption appears to have peaked around 1980 and the average amount of fat in these products has declined in recent years, table spreads still make a significant contribution to the fat content of the American diet. Approximately 75% of production is sold at retail, with 19% going to food service and 6% for bakery and other industrial uses.

1. In 2001, according to the information of IMACE Member Associations, the margarine and fat spreads production in the EU amounted to 2,191,301 MT (metric tons). The biggest producing countries are Germany (573,973 MT), United Kingdom, Belgium (278,789 MT), and the Netherlands (262,006 MT).
2. Other big margarine-producing countries in the world other than the United States, are Brazil (485,900 MT), Turkey (266,465 MT), and Japan 254,200 MT.

TABLE 1. Production and Consumption of Margarine and Butter.<sup>a</sup>

Year	Annual Production, million kg (million lb)		Average Per Capita Consumption, kg (lb)			
	Margarine <sup>b</sup>		Butter			
1930	147.7	(325.7)	737.5	(1,625.9)	1.2 (2.6)	8.0 (17.6)
1935	173.1	(381.6)	758.2	(1,671.5)	1.4 (3.0)	8.0 (17.6)
1940	145.3	(320.4)	833.2	(1,836.8)	1.1 (2.4)	7.7 (17.0)
1945	278.5	(614.0)	618.6	(1,363.7)	1.9 (4.1)	4.9 (10.9)
1950	425.0	(937.0)	628.9	(1,386.4)	2.8 (6.1)	4.8 (10.7)
1955	596.4	(1,314.9)	628.8	(1,386.2)	3.7 (8.2)	4.1 (9.0)
1960	768.9	(1,695.2)	622.8	(1,372.9)	4.3 (9.4)	3.4 (7.5)
1965	863.6	(1,903.9)	599.1	(1,320.7)	4.5 (9.9)	3.0 (6.5)
1970	1011.7	(2,230.5)	517.1	(1,140.0)	5.0 (11.0)	2.4 (5.3)
1975	1093.1	(2,409.8)	446.3	(984.0)	5.0 (11.0)	2.1 (4.7)
1980	1158.1	(2,553.2)	519.4	(1,145.0)	5.1 (11.2)	2.0 (4.5)
1985	1180.9	(2,603.3)	566.1	(1,248.0)	4.9 (10.8)	2.2 (4.9)
1990	1255.2	(2,767.1)	590.6	(1,302.0)	4.9 (10.9)	2.0 (4.4)
1995	1,128	(2,490)	538	(1,186)	4.1 (9.1)	2.0 (4.5)
1996	1,124	(2,480)	520	(1,148)	4.1 (9.0)	1.9 (4.3)
1997	1073	(2367)	505	(1,114)	3.8 (8.4)	1.9 (4.2)
1998	1045	(2311)	547	(1,208)	3.7 (8.2)	2.0 (4.5)
1999	1029	(2274)	591	(1,305)	3.6 (7.9)	2.2 (4.8)
2000	1086	(2398)	—	—	3.8 (8.3)	—

<sup>a</sup>Data courtesy of the National Association of Margarine Manufacturers. One kilogram equals 2.2 lb and the USDA.

<sup>b</sup>Includes spread products beginning in 1975.

3. By way of comparison, total production in 1999 of butter and dairy spreads amounted to 1,691,900 MT.
4. According to IMACE information, the per-capita consumption of margarine and spreads in the EU is 5.02 kg, down from 5.18 kg in 1999, 5.45 kg in 1998, and 6.16 kg in 1993.

Fig. 1 shows a comparison of production of margarine versus butter in EU-15.

The market for table spreads, which was originally driven primarily by cost relative to butter, has, in the past 40 years, been greatly impacted by health claims relative to content of cholesterol, polyunsaturated fat, saturated fat, and including most recently, total fat and *trans*-fatty acids. Therefore, as composition, as well as price and taste, is of major importance to the consumer, regulations regarding labeling and advertising have become a driving force in formulation of these products and the oil blends used therein. The resulting proliferation of table spreads available today is somewhat over-whelming and often confusing to the consumer.

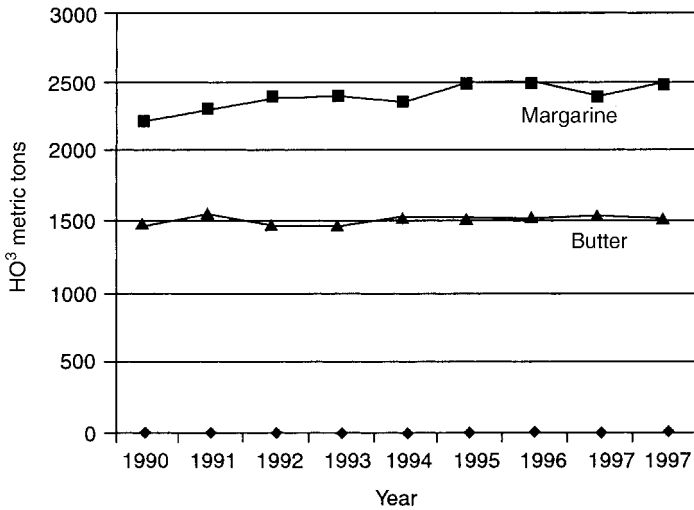


Figure 1. Comparison between the production of margarine and butter in the EU-15 (3).

## 1. HISTORICAL DEVELOPMENT OF MARGARINE (4-7)

Margarine was patented and first manufactured in 1869 by Hippolyte Mege Mouriés, a French chemist. The product was developed to meet butter shortages caused by the increasing urban population during the Industrial Revolution, as well as the need for a table spread with satisfactory keeping quality for the armed forces. The original process was designed to imitate production of butter fat by the cow. Fresh tallow was subjected to a low-temperature rendering with artificial gastric juice and slowly cooled to approximately 26°C (80°F) to partially crystallize the fat. The olein, a soft yellow semifluid fraction obtained in about 60% yield, was then dispersed in skim milk along with cow udder tissue. The emulsion was agitated for several hours and cold water was added to the churn, causing the fat to solidify. The water was drained, and the granular mass that remained was kneaded and salted.

During the late nineteenth century, some margarines were prepared from lard or unfractionated beef suet to which liquid oils such as cottonseed or peanut were added to reduce the melting point of the blend. In the early 1900s, some 100% vegetable oil margarines were formulated with coconut and palm-kernel oils. Examples of these blends and the early margarine manufacturing processes are described by Clayton (8). Although hydrogenation came into practice around 1910, particularly in Europe, it was not used extensively in U.S. margarine manufacture until the 1930s. At that time the use of lauric oils became politically unpopular and tariffs were imposed.

In addition to the use of new oils, significant advances were made in the manufacturing process. Peptic digestion of fat and the use of udder extracts soon were

abandoned. Pasteurization allowed the milk to be cultured in order to develop a more buttery flavor. The invention of dry chilling using a metal drum containing circulating brine resulted in improved cleanliness and reduced fat and milk losses. Regardless of whether ice or a chill roll was used, however, the product still had to be worked subsequently to achieve consistency. It was not until about 1940 that the first closed continuous systems were used for margarine manufacture.

Legislation has played an important role in the evolution of margarine. When the product was introduced in the United States in 1874, dairy farming was expanding more rapidly than the needs of the population. Hostility from the large number of dairy farmers set the tone for almost a century of antimargarine legislation and taxation of the lower priced spread. Adulteration of butter with margarine, and later the use of imported tropical oils, also added to the call for government intervention. The first tax was levied in 1886, and in 1902 a much more severe tax and producer-licensing fees were imposed on colored margarine. Many states also passed laws prohibiting the sale of colored margarine. In 1941, margarine gained recognition as a food in itself with the adoption of a Federal Standard of Identity that defined the product and provided for vitamin fortification. In response to both public pressure generated by high butter prices following World War II and expanding soybean and cottonseed oil farming interests, federal taxes were abolished by the Margarine Act of 1950. This legislation also stipulated that the product could not be sold at retail in units greater than 0.4536 kg (1 lb). Most states were quick to follow the lead of the federal government. However, it was 1967 before Wisconsin became the last state to repeal a law prohibiting colored margarine, and the final state margarine tax was repealed by Minnesota in 1975.

## 2. U.S. TRENDS

In the early 1950s almost all consumer margarine was a stick variety usually sold in packages of 113.4 g (quarter-pound) prints. With most restrictions on colored margarine removed, sales continued to rise and per-capita consumption surpassed that of butter in 1957. Sales increases stimulated important technological progress and new product development (Table 2). Spreadable margarine, polyunsaturated margarine, and low-fat products were developed to satisfy the desire for convenience, nutritional awareness, and weight consciousness of the consumer.

The first major departure from the past was the introduction of soft margarine spreadable from the refrigerator and packaged in tubs. This soft, full-fat product captured one-fourth of the margarine market by 1973. Today stick table spreads account for slightly less than half of the total table spread market. The most significant recent trend is away from margarine (80% fat) to spreads containing lower fat levels. Originally introduced at 60% fat, since 1980 spreads containing from 75% to less than 5% fat have appeared in the marketplace. The products are sold in soft, stick, liquid, and soft whipped forms. Market share increased from under 5% in 1976 to more than 15% in 1983, and in the early 1990s, this trend has accelerated to the point that there are very few full-fat margarine products available in the

**TABLE 2. New Table Spread Products Introduced in the United States Since 1950.**

Product	Year Introduced
Spreadable stick margarine	1952
Whipped margarine	1957
Corn oil margarine (high polyunsaturates)	1958
Soft margarine	1962
Liquid margarine	1963
Diet margarine (40% fat)	1964
Spread (60% fat)	1975
Whipped spread	1978
Butter blends (widespread distribution)	1981
Improved 40% fat spreads <sup>a</sup>	1986
Lower fat spreads (20% fat)	1989
Nonfat spread	1993
Balanced spreads	2003

<sup>a</sup>Containing gelling agents.

United States. Reasons for the growth in spread production are (1) low price, (2) availability in sizes greater than 0.4536 kg (1 lb), and (3) fewer calories and lower fat than margarine. Much of the recent rapid shift from margarine to spread production is attributable to manufacturers' cost reduction efforts in times of rising vegetable oil prices.

The table spread market is composed of several segments differentiated by image and price. At the high end of the price range are products perceived either as being healthy or as having a superior buttery taste. The healthy image products generally contain liquid oils such as corn, sunflower, or canola and have been promoted with statements relating to cholesterol, total fat, or saturated fat contents. For the last 10 years this segment has held at 16–17% of the market. Unsalted products are not popular and account for less than 1.5% of table spread volume. The buttery image or blend segment has sharply increased during the last decade and consists of spreads that, by their name or positioning, merely convey a strong dairy taste perception to the consumer, as well as spreads that actually contain various amounts of butter. The branded value grouping, which accounts for about 50% of the retail market, includes brands that are nationally advertised and priced lower than the premium segments discussed. The remaining products, which usually have the lowest everyday price, consist of regional and private label store brands.

### 3. REGULATORY STATUS IN THE UNITED STATES

The following section contains a description of the legal requirements for composition and labeling of margarine and table spreads. Such information is essential for formulating products to meet specific claims; however, because it contains

considerable detail, the reader wanting only an overview may wish to skip all or part of this section.

### 3.1. Standard of Identity

There are two standards of identity for margarine in the United States. Vegetable oil margarines are regulated by the Food and Drug Administration (FDA), and animal fat and animal-vegetable margarines are subject to federal meat inspection regulations of the United States Department of Agriculture (USDA). The standards are similar but not identical. The FDA standard was revised in 1973 (9) and the USDA standard in 1983 (10) to conform more closely to the international standard as adopted by the Food and Agriculture Organization/World Health Organization Codex Alimentarius Commission. In 1993, a new Codex standard for fat spreads was proposed by the commission. Finalization of this standard, which includes margarine, butter, and lower fat spreads, is not expected for several years. The following is the current FDA standard (11) including recent revisions allowing the use of marine oils (12) and removing previous emulsifier restrictions (13):

- (a) Margarine (or oleomargarine) is the food in plastic form or liquid emulsion, containing not less than 80 percent fat determined by the method prescribed in "Official Methods of Analysis of the Association of Official Analytical Chemists," 13th Ed. (1980), section 16.206, "Indirect Method," under the heading "Fat [47]—Official Final Action," which is incorporated by reference. Copies may be obtained from the Association of Official Analytical Chemists, 2200 Wilson Blvd., Suite 400, Arlington, VA 22201-3301, or may be examined at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC 2001. Margarine contains only safe and suitable ingredients as defined in section 130.3(d) of this chapter. It is produced from one or more of the optional ingredients in paragraph (a)(1) of this section, and one or more of the optional ingredients in paragraph (a)(2) of this section, to which may be added one or more of the optional ingredients in paragraph (b) of this section. Margarine contains vitamin A as provided for in paragraph (a)(3) of this section.
  - (1) Edible fats and/or oils, or mixtures of these, whose origin is vegetable or rendered animal carcass fats or any form of oil from a marine species that has been affirmed as GRAS or listed as a food additive for this use, any or all of which may have been subjected to an accepted process of physico-chemical modification. They may contain small amounts of other lipids such as phosphatides, or unsaponifiable constituents and of free fatty acids naturally present in the fat or oil.
  - (2) One or more of the following aqueous phase ingredients:
    - (i) Water and/or milk and/or milk products.
    - (ii) Suitable edible protein including, but not limited to, the liquid, condensed, or dry form of whey, whey modified by the reduction of



lactose and/or minerals, nonlactose containing whey components, albumin, casein, caseinate, vegetable proteins, or soy protein isolate, in amounts not greater than reasonably required to accomplish the desired effect.

- (iii) Any mixture of two or more of the articles named under paragraphs (a)(2) (i) and (ii) of this section.
  - (iv) The ingredients in paragraphs (a)(2) (i), (ii), and (iii) of this section shall be pasteurized and then may be subjected to the action of harmless bacterial starters. One or more of the articles designated in paragraphs (a)(2) (i), (ii), and (iii) of this section is intimately mixed with the edible fat and/or ingredients to form a solidified or liquid emulsion.
- (3) Vitamin A in such quantity that the finished margarine contains not less than 15,000 international units per pound.
- (b) Optional ingredients:
- (1) Vitamin D in such quantity that the finished oleomargarine contains not less than 1,500 international units of vitamin D per pound.
  - (2) Salt (sodium chloride); potassium chloride for dietary margarine or oleomargarine.
  - (3) Nutritive carbohydrate sweeteners.
  - (4) Emulsifiers.
  - (5) Preservatives including but not limited to the following within these maximum amounts in percent by weight of the finished food: Sorbic acid, benzoic acid and their sodium, potassium, and calcium salts, individually, 0.1 percent, or in combination, 0.2 percent, expressed as the acids; calcium disodium EDTA, 0.0075 percent; propyl, octyl, and dodecyl gallates, BHT, BHA, ascorbyl palmitate, ascorbyl stearate, all individually or in combination, 0.02 percent; stearyl citrate, 0.15 percent; isopropyl citrate mixture, 0.02 percent.
  - (6) Color additives. For the purpose of this subparagraph, provitamin A (beta-carotene) shall be deemed to be a color additive.
  - (7) Flavoring substances. If the flavoring ingredients impart to the food a flavor other than in semblance of butter, the characterizing flavor shall be declared as part of the name of the food in accordance with section 101.22 of this chapter.
  - (8) Acidulants.
  - (9) Alkalizers.
- (c) The name of the food for which a definition and standard of identity are prescribed in this section is “margarine”, or “oleomargarine”.
- (d) Label declaration. Each of the ingredients used in the food shall be declared on the label as required by the applicable sections of parts 101 and 130 of this chapter. For the purposes of this section the use of the term “milk”

unqualified means milk from cows. If any milk other than cow's milk is used in whole or in part, the animal source shall be identified in conjunction with the word milk in the ingredient statement. Colored margarine shall be subject to the provisions of section 407 of the Federal Food, Drug, and Cosmetic Act as amended.

### 3.2. Label Requirements

Detailed labeling regulations for margarine are set forth in the *Code of Federal Regulations* (14). In general, the label must include the product name, net weight, name and address of the manufacturer or distributor, an ingredient statement, serving size, number of servings per package, and nutritional information. The name *margarine* or *oleomargarine* must appear in lettering at least as large as any other on the label. For products resembling margarine but that contain less than 80% fat, the product name should include the term *spread*, the total percentage of fat, and a listing of each fat ingredient in order of predominance or a generic term such as *vegetable oil*, e.g., "60% vegetable oil spread." This nomenclature, proposed in 1976 (15), generally has been followed by the industry and a final regulation was not published. In January 1993 the FDA promulgated extensive labeling regulations implementing the Nutrition Labeling and Education Act of 1990 (16). Clerical corrections (17) and technical amendments (18) to these regulations have also been published, and all of these will be incorporated in subsequent editions of the *Code of Federal Regulations*. All food products labeled on or after May 8, 1994, must be in compliance. Included in these regulations are provisions for use of a standardized term such as *margarine* to describe foods that do not comply with the standard of identity because of a deviation described by an expressed nutrient content claim that has been defined by FDA regulation (e.g., reduced fat margarine; see next section). Other than the deviation described by the claim, the product must comply with the margarine standard in all respects with the exception that safe and suitable ingredients not provided for in the standard may be added to improve functional characteristics so that these are not inferior to those of margarine.

A complete listing of all ingredients in order of predominance is required. If nonstandard ingredients are contained in a product labeled as margarine described by a nutrient claim, these ingredients must be so noted in the ingredient statement. Fats must be listed according to type and must be declared as hydrogenated or partially hydrogenated if applicable. The individual fats may be placed in order of predominance in the listing, or they may be grouped together in parenthesis and prefixed by "vegetable oil blend." The listing of fats may not be preceded by "and/or" or "may contain" if, by weight, fat constitutes the predominant ingredient. Dry or condensed dairy ingredients (e.g., whey powder, dry milk, or buttermilk) may be declared as such or as the reconstituted ingredient. Any water in excess of that required for standard reconstitution also must be declared at the appropriate place in the ingredient statement. The specific form of added vitamins must be listed. If colors or flavors are listed by specific name such as "colored with beta-carotene," the term "artificial" is not required. Flavors usually are not labeled

specifically, and “artificially flavored” appears in the ingredient statement. Preservatives must be declared by their common names together with a statement of intended use, for example, “sodium benzoate as a preservative” or “potassium sorbate as a mold inhibitor.”

All products must be labeled with a nutrition panel. Details of the content and format are prescribed (16, 18). All information in the nutrition panel is based on a single serving of 15 mL (one tablespoon) rounded to the nearest gram. For unwhipped 80% fat margarine, this amount is 14 g; however, this weight may vary for whipped products and some low-fat spreads if the density is significantly different than that of margarine.

As regards fat labeling, the nutrition panel must include total fat, calories from fat, saturated fat, and cholesterol. Declaration of polyunsaturated or monounsaturated fat content are mandatory only if the other is declared or if a claim about fatty acids or cholesterol is made, unless the product meets the criteria for a *fat-free* claim. All fat amounts are expressed to the nearest 0.5-g increment below 5 g, to the nearest gram above 5 g, and as zero if a serving contains less than 0.5 g. Saturated fat includes all fatty acids that do not contain a double bond. Polyunsaturated fat includes only *cis*, *cis*-methylene-interrupted fatty acids, and monounsaturated fat is defined as *cis*-monounsaturated fatty acids. Saturated, polyunsaturated, or monounsaturated fat is declared as weight of the fatty acids, while fat is expressed as the weight of triglyceride based on the total amount of lipid fatty acids.

### 3.3. Label Claims

*Nutrient Content Claims.* The following is a brief description of nutrient content claims of most relevance to the positioning of margarine and spread products. For a more complete discussion, the reader is referred to the regulations (16, 18). Use of the terms *fat free*, *low fat*, and *reduced fat* or their equivalents have been fully defined by the new regulations. A product may be called *fat free* or *no fat* if it contains less than 0.5 g of fat per serving. If the food contains an ingredient generally understood by consumers to contain fat, that ingredient must be asterisked in the ingredient statement with a following explanation that the ingredient contributes a dietarily insignificant amount of fat. A claim of 100% fat free may only be made if the product contains no added fat. FDA has indicated (18) that the terms “fat free” and “margarine” cannot be used together because standardized foods must contain a significant level of characterizing ingredients, in this case, fat. Such a product can be called a fat-free spread. A margarine or spread may be called *low fat* if it contains 3 g or less of fat per serving and per 50 g, thus limiting its use to spreads containing 6% or less fat. The term *saturated fat free* may be used if the margarine or spread contains less than 0.5 g of saturated fatty acids and less than 0.5 g of *trans*-fatty acids per serving. To make the claim *low in saturated fat* the food must contain 1 g or less of saturated fat per serving and derive not more than 15% of its calories from saturated fat. A *reduced fat* or *reduced saturated fat* claim may be made provided the reduction is at least 25%. The term *light* or *lite* may be used only if the fat content is reduced by at least 50% compared to an appropriate

reference product if the spread derives 50% or more of its calories from fat. The minimum reduction is 33% if the spread derives less than 50% of its calories from fat. For *reduced*, *light*, or comparative claims, the identity of the reference food and the percent reduction must be declared in immediate proximity to the most prominent claim. Quantitative information comparing the grams of fat or saturated fat per serving with that of an appropriate reference food that it replaces also must be declared in proximity to the claim or on the information panel.

A *no cholesterol* or equivalent claim may be made if the product contains less than 2 mg of cholesterol and 2 g or less of saturated fat per serving. If the spread contains more than 13 g of fat per 50 g, the amount of total fat per serving must be stated in immediate proximity to the most prominent claim on each panel except the panel that bears nutrition labeling. If the food contains an ingredient understood by consumers to contain cholesterol, this ingredient must be asterisked in the ingredient list followed by a statement that this ingredient contributes a negligible amount of cholesterol. The requirements for a *low-cholesterol* claim are similar with the exception that the spread must contain less than 20 mg of cholesterol and less than 2 g of saturated fat per serving. *Cholesterol reduced* or comparative claims require declaration of the identity of the reference food, the percent reduction, and quantitative information regarding the reduction in immediate proximity to the most prominent label claim, e.g.: “Cholesterol free margarine, contains 100% less cholesterol than butter, no cholesterol compared with 30 mg in one serving of butter. Contains 11 g of fat per serving.”

**Health Claims.** Approved health claims with potential applicability to margarines or spreads are the relationships between fat and cancer, saturated fat and cholesterol and risk of heart disease, and sodium and hypertension. A food that contains more than 13 g of fat, 4 g of saturated fat, 60 mg of cholesterol, or 480 mg of sodium per serving or per 50 g is disqualified from making any health claim. In addition the product must qualify as “low fat” for the cancer claim, “low fat, low saturated fat,” and “low cholesterol” for the coronary heart disease claim, and “low sodium” for the hypertension claim. The first two claims, therefore, would be limited to spreads containing 6% or less fat, and the hypertension claim would require the spread to contain less than 140 mg of sodium per 50 g (approximately 0.7% salt).

## 4. PRODUCT CHARACTERISTICS

The consumer-directed functional aspects of spreads and margarines, which primarily depend on fat level, type of fat, and stability of the emulsion, are spreadability, oiliness, and melting properties. Spattering, which is a concern for products intended for pan frying, is discussed in Section 6.2.

### 4.1. Spreadability

Spreadability is one of the most highly regarded attributes of margarine, perhaps second only to flavor. Products with a solid fat index (SFI) of 10–20 at serving

temperature were found to be optimal on a consumer panel (19). The standard method (20) for evaluation of hardness of fatty materials uses a cone penetrometer. For some products, hardness measurements may not correlate well with SFI because, in addition to the amount of solid fat, the fat crystal network, which is processing dependent, is also important rheologically (21). The penetration is the distance, in units of 0.1 mm, traveled by a standard cone in 5 s after its release on the surface of the product. The values may be converted mathematically to a hardness index (22) or to a yield value (23) that are independent of cone weight. In an assessment of hedonic spreadability preferences of butter, margarines, and spreads as a function of temperature, correlation with penetration measurements indicated optimum spreadability in the yield value range of 30–60 kPa (24). Dynamic techniques involving motor-driven penetrometers (25–27), extrusion devices (28, 29), viscometers (30, 31), and a mechanical spectrometer (32) also have been used to evaluate margarine consistency. Although the action of a penetrometer may seem different from assessment of the spreadability with a knife, both techniques evaluate the force required to bring about a significant deformation, and cone penetrometer readings have been found, in general, to correlate with spreadability (33). This method has the advantage of low equipment cost, minimal sample preparation, and reproducible results. Smoothness and brittleness, which depend on the crystal network and are not measured with the cone penetrometer, were measured by means of an Instron compression test (34). In a study of North American stick margarines (35), compression of cylindrical samples was found to be the most sensitive method for detecting differences in textural attributes. Constant speed penetration was the next most sensitive, whereas the cone penetrometer was least sensitive. These methods were also used to evaluate a series of North American soft margarines (36). Consistency measurements have been reviewed by deMan (22).

Instrumental methods are used by manufacturers to ensure product uniformity and for comparison with competitive products. The sample is generally evaluated at a single temperature, most often in the 4.4–10°C (40–50°F) range. The results are meaningful only at the measurement temperature. For example, two margarines with the same solids content at 7.2°C (45°F) may have much different solids–temperature slopes at that point; one has significantly greater solids at 4.4°C (40°F) and less solids at 10°C (50°F) than the other. One must consider this when attempting to relate spreadability measurements to consumer perception in the home, where storage and use conditions are variable.

## 4.2. Oil Separation

Oil-off occurs in margarine when the matrix of fine fat crystals is no longer of sufficient size or character to be able to enmesh all of the liquid oil. The problem is most serious for stick products as the outside of the inner wrappers may become oil soaked, and if severe, oil will leak out of the package. Prints are also the most susceptible to oil-off because of the pressures to which the product is subjected when pallets or individual packages are stacked. If the margarine is to be merchandised outside of dairy cases, the 21.1°C (70°F) SFI should be specified as high as

possible, consistent with 10°C and 33.3°C (50°F and 92°F) requirements. Soft margarines in bowls are not as much a problem because the package supports and contains the product. Emulsion damage, that is, coalescence and settling of the milk phase, seldom will be significant as long as 3–4% solid fat remains. Oil-off testing is most often conducted by placing a margarine sample of defined geometry and weight on a wire screen (37) or on filter paper (38) at a temperature of 26.7°C (80°F), or sometimes greater, for a period of 24–48 h. The oil exuded into the filter paper or through the screen is then measured. Another test used for determining structural stability, most often for stick margarine, is slump. This involves placement of a standard size cube of margarine at 23.9–29.4°C (75–85°F) for several hours. Deformation of the cube is graded according to visual reference to a standard slump chart (39). Results of these types of test are only directional; products are more appropriately evaluated in the package under actual distribution conditions. The harshest of such conditions would generally be a stacked, out-of-dairy-case display as can be observed in some markets.

### 4.3. Melting

A high-quality table margarine melts quickly with a cooling sensation on the palate. Flavor and salt components of the aqueous phase are immediately perceptible by the taste buds, and there is no lingering greasiness or waxiness. The factors affecting these qualities are the melting profile of the fat, the tightness of the emulsion, and the storage conditions of the finished product. In order for a margarine to melt cleanly without seeming gummy or waxy, it should be completely melted at body temperature and contain less than 3.5% solid fat at 33.3°C (92°F). A cool tasting product, typified by butter, results from the almost instantaneous absorption of the heat of crystallization due to a steep melting profile between 10°C and 26.7°C (50°F and 80°F). The cooling sensation, as measured by differential scanning calorimetry, is significant only for butter and high-fat stick margarines and spreads (40). When margarine is produced, quick chilling results in solid solutions of high- and low-melting glycerides. If the product subsequently is stored at higher temperatures for several days, the recrystallization of the higher melting portions of the melted solid solutions may result in a product with greater waxiness and a slower flavor release (41).

Emulsion tightness is a function of processing, emulsifier content, and formulation of the aqueous phase. If the aqueous droplets are uniformly small or heavily stabilized with emulsifiers, the flavor and salt release will be delayed. A margarine in which about 95% of the droplets have a diameter of 1–5  $\mu\text{m}$ , 4% of 5–10  $\mu\text{m}$ , and 1% of 10–20  $\mu\text{m}$  is described as light on the palate (1). Droplet size also affects the microbiological susceptibility of the product and, to some extent, the consistency. A pulsed nuclear magnetic resonance (NMR) method for determining droplet size based on restricted diffusion in droplets has been reported (42–44). Zschaler (45) has described a method for microscopically evaluating the size distribution of the aqueous phase in margarine. For low-fat spreads the rheological changes generally associated with melting may be more a function of the stabilizers employed and the

degree of emulsification than the melting behavior of the fat blend (40). This is also true of some of the oil-in-water emulsion-type products containing very little fat or no solid fat that have recently appeared in the marketplace.

Melting quality usually is assessed by oral response. Such evaluations are run under standard conditions using an established rating scale (46). Empirical methods also have been used in attempts to quantify melt-in-the-mouth properties that depend on both emulsion tightness and the melting profile of the fat blend. Moran (47) describes a "phase instability temperature," which is the temperature at which the product shows a marked increase in electrical conductivity under shear conditions similar to those that occur on the palate. A viscometer is used in the determination, which is intended for low-calorie products. Cooling sensation on the palate has been estimated by recording the temperature drop in a 35°C (95°F) metal sensing head when placed in contact with the product for 6 s (39). The rate of salt release has been evaluated by determining the chloride ion increase in water at 36°C (97°F) in which a margarine sample is suspended (48). Softening point, which may relate to how a product will melt in hot food applications, can be measured using a Mettler dropping point apparatus. The softening points of a series of North American soft (36) and stick (49) margarines have been determined.

## 5. OILS USED IN VEGETABLE OIL MARGARINES AND SPREADS

### 5.1. Source Oils

Usage of fats and oils in table spreads in the United States is shown in Table 3 (2). Most table spreads are formulated with soybean oil with the exception of the healthy image category in which corn, sunflower, or safflower oil is usually the primary ingredient. Canola oil (low-erucic-acid rapeseed oil, erucic acid content <2%) was approved for use in foods in the United States in 1985 (50) and recently has been used in some table spreads that are primarily positioned in the healthy image category. Lard and tallow, of which lard constitutes the predominant portion, are blended mostly in very low-cost products. In Europe, use of palm oil, lauric fats, and hydrogenated marine oils is common. The tropical oils, palm, palm kernel, and coconut, are not used currently in table spreads in the United States, in part because of their high saturate content and the intense negative public opinion generated by consumer advocate groups in the late 1980s. Marine oils, particularly California sardine oil, were at one time used in American margarines, usage reaching a peak of 18 million kg (40 million lb) in 1936 (51). By 1951, these fish had almost completely disappeared, and no provision had been made for the use of marine oils in food. Hydrogenated and partially hydrogenated menhaden oil (iodine value 10–85) was affirmed as a Generally Recognized as Safe (GRAS) food ingredient in 1989 (52), but to date it has not been used for table spreads in the United States. The GRAS status has not yet been affirmed for liquid (unhydrogenated) marine oil. A European study of margarine containing 10–20% liquid fish oil indicated that there are shelf-life problems that would need to be overcome (53). Several methods

**TABLE 3. Fats and Oils Used in U.S. Manufacturing Margarine 1980–2002,  $\times 10^6$  lb.**

Calendar Year	Soybean	Cottonseed <sup>a</sup>	Corn <sup>a</sup>	Animal Fats <sup>b</sup>	Total <sup>c</sup>
1980	1,653	25	223	104	2,039
1981	1,685	25	213	78	2,017
1982	1,718	22	220	29	1,997
1983	1,549	34	212	41	1,850
1984	1,544	26	196	38	1,842
1985	1,628	8	220	65	1,946
1986	1,741	24	204	48	2,041
1987	1,615	28	248	22	1,931
1988	1,619	D	210	35	1,894
1989	1,573	D	214	32	1,875
1990	1,749	D	208	35	2,102
1991	1,853	25	196	43	2,160
1992	1,926	24	176	37	2,174
1993	2,013	26	161	31	2,239
1994	1,793	D	D	42	2,003
1995	1,684	D	D	41	1,847
1996	1,694	D	77	28	1,816
1997	1,650	D	61	14	1,733
1998	1,606	D	55	22	1,692
1999	1,574	D	D	21	1,664
2000	1,465	D	56	12	1,547
2001	1,298	D	D	7	1,394
2002	1,212	D	D	16	1,300

<sup>a</sup>D = Data withheld by Census to avoid disclosure.

<sup>b</sup>Includes lard and edible tallow.

<sup>c</sup>Includes small quantities of other fats and oils.

Source: Bureau of the Census.

for purification of fish oil to remove flavors (54–56) or to stabilize the flavor in foods (57–60) have been patented. Today, fish oil in the United States, predominantly menhaden oil, is used for manufacture of protective coatings and fatty acids and derivatives. Much of the oil is exported for use in margarines and shortenings abroad (61). In the future, other oils may be used in table spreads as a result of positive comments in the popular press based on epidemiological and dietary studies reported in the scientific literature. Spreads containing olive oil are being marketed in Europe and Canada. Rice bran oil is expected to become available in commercial quantities in the United States in 1995 (62). Margarines prepared from rice bran oil have been described (63).

## 5.2. Fat Crystallization

*Solid Fat.* The consistency and the emulsion stability of margarine and most other table spreads depends on crystallized fat. Freeze-fracture electron microscopy of deoiled margarine shows the crystalline nature of the water droplet interface as well as a continuous fat matrix that appears to be an interconnected network



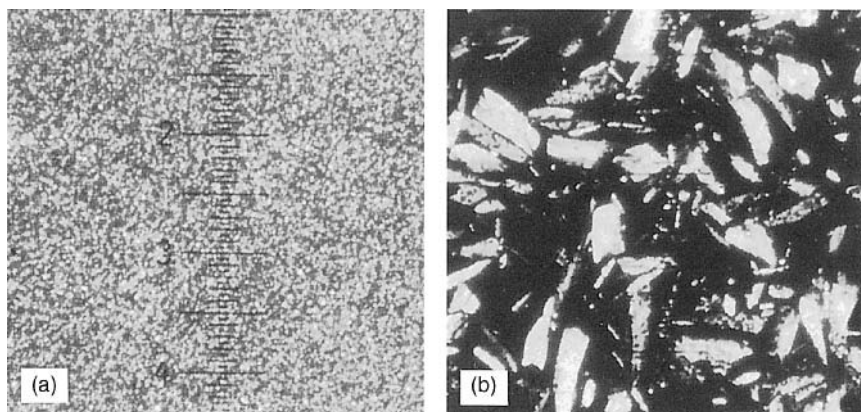
TABLE 4. Typical Solid Fat Indices of U.S. Table Spreads.

Product	Solid Fat Index				
	10°C (50°F)	21.1°C (70°F)	26.7°C (80°F)	33.3°C (92°F)	37.7°C (100°F)
Stick	28	16	10	2	0
Soft stick	20	13	9	2.5	0
Soft tub	11	7	5	2	0.5
Liquid	3	2.5	2.5	2	1.5
Butter	32	12	7	2	0

structure composed of single crystals and sheetlike crystal aggregates (64). The microstructure of margarine and shortenings has been reviewed (65). Two factors predominantly determine the influence of the margarine oil on the textural properties of the finished product: the amount of solid fat present and the conditions under which the margarine is processed. For identical processing conditions of products containing oil blends with similar crystallization characteristics, there is a direct relationship between solids content and consistency (66, 67). There are two primary methods in use today for measuring the amount of solid fat in an oil blend. The SFI (68) is an empirical method based on density variations between solid and liquid fat. The more recent NMR procedure (69) yields an absolute percent solids and depends on differences in the magnetic environment of protons in the liquid and solid phases. The results of the NMR determination, referred to as solid fat content (SFC), are similar but not directly comparable with the SFI; however, the two methods can be correlated (70). In the United States, SFI is still in wide use for commercial specification, whereas in Europe, SFC has found greater acceptance.

Typical SFI values of U.S. margarine oils are given in Table 4. The numbers are representative of commercial products. For a given type of margarine, however, specifications may vary considerably between manufacturers depending on (1) the organoleptic characteristics desired, (2) compositional requirements to meet nutrient content claims or other information on the nutrition panel, (3) whether the product will be marketed using unrefrigerated display, and (4) the type of packaging equipment available. The values at 10°C, 21.1°C, and 33.3°C (50°F, 70°F, and 92°F) are used by most, if not all manufacturers in the United States and are considered to be critical specifications. These solids values are indicative of finished product spreadability at refrigerator temperatures, resistance to oil-off at room temperature, and melt-in-the-mouth qualities, respectively.

*Polymorphism.* The structural stability of margarine is influenced by the properties of the crystal lattice and by the actual amount of solid fat present. Many organic compounds or mixtures such as fats can solidify in more than one crystalline pattern. The primary crystal forms of triglycerides are designated  $\alpha$ ,  $\beta'$ , and  $\beta$ , which correspond to three principal cross-sectional arrangements of the fatty acid chains (71). These may be differentiated by characteristic x-ray diffraction patterns (72) and heats of transition observed in calorimetric studies (73). Phase behavior of



**Figure 2.** Crystallization of sunflower oil margarine. (a) Normal  $\beta'$  form, (b) sandy  $\beta$  form. (Courtesy of Grindsted Products, Inc., Industrial Airport, Kansas.).

triglycerides is not completely understood. Studies using Raman spectroscopy, x-ray crystallography, and differential scanning calorimetry (DSC) indicate that there are two closely related  $\beta'$  polymorphs of both triundecanoin (74) and tristearin (75). However, the data of Kellens and Reynaers (76) do not support the existence of two distinct  $\beta'$  forms. The observed differences are attributed to the degree of crystal perfection of a single  $\beta'$  polymorph.

The  $\alpha$  phase, the least stable and lowest melting polymorph, is initially formed during the rapid chilling conditions used in margarine manufacture; however, it quickly transforms to the  $\beta'$  state (41). Riiner (77) studied production of margarines containing hydrogenated marine oil and found no significant influence of the  $\alpha$  phase on consistency. The  $\beta'$  structure of margarine, which may be relatively stable, consists of a very fine network that, because of its great surface area, is capable of immobilizing a large amount of liquid oil and aqueous-phase droplets. Although produced in the  $\beta'$  form, if a margarine oil has strong  $\beta$  tendencies, it may, under certain storage conditions, transform to  $\beta$ , the highest melting and most stable crystalline state. This is usually accompanied by development of a coarse, sandy texture consisting of large crystals (Figure 2). A storage study of commercial canola oil margarines indicates the crystal size threshold for consumer detection of sandiness to be about 22  $\mu\text{m}$  (78). In severe cases, transformation to the  $\beta$  form also may result in exudation of liquid oil from the product and partial coalescence of the aqueous phase, which increases the microbiological susceptibility.

Some triglycerides such as 1,3-dipalmitoyl-2-stearoylglycerol are very stable in the  $\beta'$  form, but tristearin has strong  $\beta$ -crystal forming tendencies (71). Similarly, fats, which are complex triglyceride mixtures, have characteristic polymorphic preferences. Wiedermann (79) has grouped some common fats according to their crystal habits (Table 5). In general, the more diverse the triglyceride structure of the highest melting portion of a fat, the lower the  $\beta$  forming tendencies. Therefore, fats such as sunflower, safflower, and canola (low-erucic rapeseed) are most likely

**TABLE 5. Classification of Fats and Oils According to Crystal Habit (79).**

$\beta$ Type	$\beta'$ Type
Soybean	Cottonseed
Safflower	Palm
Sunflower	Tallow
Sesame	Herring
Peanut	Menhaden
Corn	Whale
Canola	Rapeseed (high erucic)
Olive	Milk fat (butter oil)
Coconut	Modified lard (interesterified)
Palm kernel	
Lard	
Cocoa butter	

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to undergo this transformation because the palmitic acid contents are very low, and when hydrogenated, the solid component consists of a series of closely related homologues. In a study of the 1:1 interesterification products of completely hydrogenated soybean oil with each of nine common vegetable oils, it was found that the  $\beta'$  form was favored by higher palmitic acid content in the liquid oil (80). The effect of some surfactants on the kinetics of polymorphic transitions in saturated mono-acid triglycerides has been studied by Aronhime et al. (81). Heertje (64) has used scanning electron microscopy and light microscopy to detail the microstructural features of graininess.

Problems with sandiness in 100% canola oil margarines have been reported (82). In a study of low- and medium-erucic-acid content hydrogenated rapeseed oils, the  $\beta' - \beta$  transition occurred more readily at low-erucic-acid content and with a higher degree of hydrogenation (83). The stability of the  $\beta'$  polymorph of hydrogenated canola oil as it relates to iodine value and *trans*-fatty acid content has been studied by deMan (84). The fat crystals of commercial soft (36) and stick (49) canola oil margarines were found to be in the  $\beta$  form. Hernqvist and Anjou (85) found that in canola oil margarines, the relatively rapid development of graininess could be significantly retarded by the addition of 0.5–5% diglycerides. The transition is much more retarded by 1,2-diglycerides than by 1,3-diglycerides, and it appears that diglycerides containing saturated fatty acids of the same chain length as the fat to be stabilized are most effective (86). The effect of diglycerides on the crystallization properties of fats has been reviewed (87). Interesterification has been employed to reduce the development of sandiness in sunflower oil margarine (88, 89). Sorbitan tristearate has been extensively studied and has been found to retard the  $\beta' - \beta$  transformation (90–93) and is permitted by the Canadian margarine standard for use as a crystal inhibitor. Addition of 10% of a hard palm fraction or 15% hydrogenated palm oil was effective in delaying polymorphic transformation in a

canola oil stick margarine formulation (94). Interesterified palm stearine and hydrogenated canola oil, when used as a hard stock in soft margarine oil blends, was found to reduce the tendency of the blend to crystallize in the  $\beta$  form relative to blends containing the uninteresterified hard stocks (95). Processing techniques to minimize problems with stick canola margarines have been suggested by Ward (96). Graininess was at one time found to be a problem in 100% soybean oil margarines, and cottonseed oil was added to modify the product (41). Today, with the development of more spreadable stick margarines and the use of multiple base stocks, formulation of 100% soybean or corn oil margarines with stable textural characteristics is achieved readily.

Sandiness develops rapidly when margarine is stored under conditions where melting and recrystallization of the fat can occur. By reducing the storage temperature to 0°C (32°F), undesirable polymorphic transitions can be decreased substantially (97). Accelerated procedures for evaluation of sandiness development involve cycling the margarine between 7.2°C and 23.8°C (45°F and 75°F) or storage at 21.1–23.8°C (70–75°F) for several weeks. Madsen (98) found that a sunflower oil margarine transformed to the  $\beta$  form after 4 weeks at 18.3°C (65°F) or after 2 weeks at 25°C (77°F). Van der Hock (99) has described a rapid method for estimating structural stability using the solid fat that is isolated from margarine by use of a high-pressure press. The high-melting fraction thus obtained is evaluated both by differential thermal analysis and by observing recrystallization microscopically. The polymorphic behavior of some commercial margarines has been related to the composition of the high-melting glyceride fractions obtained by crystallization from acetone (100, 101) or the solid fat obtained by extraction with isobutanol (101).

*Crystallization Rate.* The rate at which crystals form and undergo polymorphic transitions is of critical importance to the processing of margarine. The  $\alpha - \beta'$  transition rate is accelerated by agitation. Haighton (66) has used metal dilatometers with scraper blades to study crystallization rates under dynamic conditions. Blanc (102) has evaluated the speed of crystallization of several fats under static, super-cooled conditions. Crystallization times range from 3 min for coconut oil to 27 min for palm oil and 45 min for shea butter. Riiner (103) has studied polymorphic transition times and found that palm oil has a long  $\alpha$  lifetime; that is, the transition for  $\alpha$  to  $\beta'$  occurs very slowly. It was also found that the degree of hydrolysis of shea nut oil influences the rate of polymorphic transition (104). Berger (105) observed that the  $\alpha$  lifetime of a palm oil sample was reduced from 30 to 4 min after partial glycerides and other nontriglyceride components were removed by column chromatography. Palm oil from unbruised fruit has been found to contain almost 6% diglycerides (106). Okiy (107) demonstrated that the  $\alpha$  lifetime of pure palm oil triglycerides increases when diglycerides isolated from palm oil are added back in concentrations from 2% to 15%. The melting point of the  $\beta'$  form and the heat of fusion of the palm oil also decreased with increasing amounts of added diglyceride. Yap et al. (108) have studied the crystallization rates of palm oil, palm oil fractions, and hydrogenated palm oil.

Monoglycerides have been found to hasten the onset of crystallization of various fats under agitation in the presence of 16% water (109), but lecithin had the

opposite effect (110). In a study of mixtures of margarine oil components, Chikany (111) found that the crystallization rate is accelerated by increasing the amount of coconut oil or decreasing the amount of palm oil in the blend. Crystallization problems associated with blends containing predominantly palm oil can be alleviated by interesterifying a portion of the palm oil with other fats (112). The crystallization rate of a margarine-type oil blend was found to be increased by the addition of palmitate sucrose polyester (SPE, average degree of esterification ca. 5.4), stearate SPE, and sorbitan tristearate (113). The rate was decreased by laurate SPE, and oleate SPE had little effect. Van Meeteren and Wesdrop (114) have found that 1,3-saturated-2-*trans*-unsaturated glycerides, particularly 1,3-dipalmitoyl-2-elaidoylglycerol, accelerate fat crystallization from a supercooled melt, increasing the processability of spreads formulated with a slowly crystallizing fat.

### 5.3. Oil Blending

In the United States, most margarines are formulated using hydrogenation as the only means of oil modification. For the most part soybean and corn oils are used, although some healthy image products contain sunflower, canola, or safflower oil as the liquid portion of the blend. High-quality margarines can be prepared using selectively hydrogenated base stocks that are characterized by steep SFI slopes resulting from high *trans*-isomer development with minimal saturate formation. Although interesterification can be used in preparation of soybean margarine oils (115, 116), there has been no necessity or incentive for the use of either fractionated or interesterified components unless such components are byproducts of another operation such as winterization. The type of margarine oil blends used in the preponderance of table spread products in the United States have changed very little in the last 15 years, with the notable exception that in many soybean oil products, the manufacturer has replaced the softest (lightly hydrogenated) base stocks with liquid soybean oil. However, public awareness of the results of recent studies on the effects of various fatty acid classes on serum lipids and possible changes in fatty acid labeling regulations may lead to significant changes in the near future.

Latondress (117) has described some typical soybean oil base stocks and the margarine oils prepared therefrom (Tables 6 and 7). The percentages of each stock may be adjusted somewhat to take into account batch variability in hydrogenation. The greater the number of base stocks, the greater the flexibility in allowing a slightly out-of-specification stock to be used to meet final blend requirements. The 66-IV stock (number 4 in Table 6) is the most difficult to control because, at the endpoint of hydrogenation, the saturate content is increasing rapidly. Cottonseed or corn oil can be used to prepare base stocks similar to those shown in Table 6. A refiner may have as many as eight base stocks with which to formulate blends. As different refiners may have different specifications for their base stocks, the margarine producer must realize that the composition of blends received from alternate suppliers may vary somewhat in crystallization characteristics even though the blends meet the same solid fat profile. Moustafa (118) has described the composition, solid fat characteristics, and polyunsaturate content of oil blends found in soft

**TABLE 6. Typical Base Stocks Used in Soybean Oil Margarine (117).**

Stock Number	1	2	3	4
Hydrogenation conditions				
Initial temperature, °C (°F)	148.8 (300)	148.8 (300)	148.8 (300)	148.8 (300)
Hydrogenation temperature, °C (°F)	165.5 (330)	176.6 (350)	218.3 (425)	218.3 (425)
Pressure, kPa (psig)	103 (15)	103 (15)	103 (15)	35 (5)
Nickel (%)	0.02	0.02	0.02	0.02
Characteristics				
Iodine value	80–82	106–108	73–76	64–68
Congeval point, °C (°F)	—	—	23.8–25 (75–77)	33–33.5 (91.4–92.3)
SFI at 10°C (50°F)	19–21	4 max.	36–38	58–61
SFI at 21.1°C (70°F)	11–13	2 max.	19–21	42–46
SFI at 33.3°C (92°F)	0	0	2 max.	2 max.

Source: Adapted from the *Journal of the American Oil Chemists' Society*, with permission.

margarines available in the United States. Low-calorie soft and stick spreads containing 40–75% fat are usually formulated from the same oil blends as those used for the manufacture of soft and stick margarines, respectively.

Oil blends for liquid margarine consist of a liquid oil and a highly hydrogenated fat (e.g., liquid and 5-IV soybean oil). Pichel (119) used 0.75–7.5% hard fat depending on the melting point. Too much of the hard fraction gives too viscous a product, whereas too little results in oil-off and water separation. Melnick and Josefowicz (120) describe the SFI ranges of a similar liquid product to be 1.5–4.0 at 10°C (50°F) and 1.0–3.0 at 33.3°C (92°F). If an oil such as base stock 2 from Table 6 is used in place of liquid oil, a product with improved oxidative flavor stability and greater temperature dependence of the viscosity will result.

Because of the greater variety of competitively priced oils and because labeling of source oils in order of predominance is not generally legislated in Europe, oil

**TABLE 7. Typical Soybean Margarine Oil Formulas (117).**

Type	Soft Stick	Stick	Stick	Soft Tub
Composition (%)				
Stock 1 (Table 2.7)	—	—	60	—
Stock 2	—	42	—	80
Stock 3	—	20	25	—
Stock 4	50	38	15	20
Liquid soybean oil	50	—	—	—
SFI at 10°C (50°F)	20–24	27–30	28–32	10–14
SFI at 21.1°C (70°F)	12–15	17.5 min	16–18	6–9
SFI at 33.3°C (92°F)	2–4	2.5–3.5	1–2	2–4

Source: Adapted from the *Journal of the American Oil Chemists' Society*, with permission.

blends are often more complex and interesterified oils are common. When prices permit, low percentages of lauric fats sometimes are added for their sharp melting characteristics (121). Frequently, oil formulation changes may be dictated by economics and availability. Blends that are optimum in terms of solid content and cost can be calculated according to the method of statistical dilatation equivalents (66). These calculations, which are based on measurements taken on a large number of mixtures of available components, assume that each component yields a linear contribution to the solids content of the blend. The contribution factors, which are calculated by multiple regression analysis, must be determined at each temperature of interest. For example, coconut oil would have a strong positive solids contribution at 10°C (50°F) and a negative coefficient at 21.1°C (70°F). The validity of the relationships are bound by the range of mixtures studied and the linearity of contributions in that range. Cho et al. (122) used an experimental design to identify suitable margarine and shortening blends formulated from four component oils, two of which were interesterified. Besides solids requirements, least-cost computer programs can be written to take into account other constraints such as minimum essential fatty acid content required and limits on individual components due to cost, crystallization behavior, availability, or production capacity (123).

Wieske (123) also has described a more general calculation method in which fatty acids are assigned to one of four classes: high-melting saturated C<sub>16</sub> or greater; *trans*-monoenes and dienes; medium melting (C<sub>12</sub> and C<sub>14</sub>) saturated; and low melting saturated and *cis*-unsaturated. If triglyceride structures based on these four fatty acid types are subgrouped into types with similar melting characteristics, a model can be generated containing 16 glyceride classes that contribute to the solids content. The triglyceride types in any oil blend are the sums of the values found by analysis of the triglyceride structures of the components. The effect of interesterification of any part or all of the blend can then be calculated mathematically. A margarine oil patent specification based on these fatty acid and triglyceride classifications has been issued (124). As more knowledge is gained of mutual solubility relationships and melting behavior of triglyceride classes, and as economical methods of achieving specific rearrangement of triglyceride structures are developed, it may become possible to formulate optimum oil blends with knowledge of only the fatty acid composition of the components available.

*High-Liquid-Oil Blends.* From the late 1950s to the 1980s, considerable effort was devoted to the development of margarines containing high levels of linoleic acid. Although polyunsaturate content is less emphasized in the United States today, oils such as corn, sunflower, and safflower continue to be perceived as healthy by the consumer, as are olive oil with its high monounsaturates and canola oil with its low saturated fat content. Therefore, it appears advantageous to market a product containing 100% of one of these oils. When the selectively hydrogenated liquid oils are used as hard stocks, the upper limit on the liquid oil content of such a stick margarine blend is about 60–65% for formation of a satisfactory print. For a high-liquid-oil blend, the SFI at 10°C (50°F) is limited on the high side by the percentage of *trans*-fatty acids that can be developed in the hard stock without increasing the saturate content to such an extent that the 33.3°C (92°F) solids are too high.

These high-*trans*-hard stocks can be achieved most consistently by use of a sulfur-poisoned catalyst (125). A *trans*-free hard structural fat for margarine and spreads reported in the patent literature (126). Soft margarine blends containing as much as 85% liquid oil are feasible. As discussed in Section 5.2, 100% sunflower, safflower, or canola margarines may become grainy during storage. Randomization of the hard component (127) or interesterification of the hard stock with a portion of the liquid oil (128) has been used to alleviate texture problems. Soft margarines containing more than 80% of a highly polyunsaturated liquid oil have been found to develop a granular, lumpy appearance accompanied by texture breakdown when stored at less than  $-23.3^{\circ}\text{C}$  ( $-10^{\circ}\text{F}$ ). McNaught (129) discovered that randomization of at least part of the liquid oil alleviates this problem.

*Low-Trans-Oil Blends.* Based on recently published studies (130–138) that indicate that *trans*-fatty acids may not be neutral in their effect on serum lipids, negative press has heightened consumer awareness of these fatty acids, particularly in North America. A consumer advocate group has petitioned the U.S. FDA that *trans*-acids be labeled (139). At the present time, it is unknown whether these acids will eventually be classed as cholesterol-raising fatty acids like saturates or whether health implications more or less negative than saturates will be attributed to them. Over the last decade, such concerns have led to considerable research in the development of low- and no-*trans*-oil blends for margarines and spreads. Most of these formulations also contain a high level of liquid oil. Of course, for very low fat spreads, the *trans*-content of the oil blend is less significant, because only the amount of *trans*-fatty acids per serving would be labeled. The approaches that some companies are taking to reduce *trans*-levels and new product introductions in the area of no-*trans*-margarines and spreads has been discussed by Haumann (140).

Margarines high in polyunsaturates and low in *trans*-fatty acids are available in Canada and Europe. These products can be formulated from tropical oils, with no hydrogenated fats or with fats that are completely hydrogenated. Tropical oils are viewed negatively by the consumer in the United States, and therefore, they are not used to any significant extent. Palm oil and lauric fats such as coconut, palm-kernel, and babassu oils are rich in saturated fatty acids. However, because of eutectic formation and steep melting profiles, satisfactory stick margarine oil blends cannot be achieved with these oils as the sole hard stock without some additional modification. Ward has found that interesterification of a completely hydrogenated mixture of palm and babassu oil yields hard stocks that can be used in very high polyunsaturated soft (141) and stick (142) margarines capable of being processed on standard equipment. Similar soft margarines containing approximately 90% liquid oil have been formulated from fully hydrogenated interesterified palm oil and palm-kernel oil (143) or palm-kernel olein (144). Soft and stick margarines were prepared with an unhydrogenated hard fraction consisting of an interesterified mixture of coconut oil, palm oil, and palm stearine (145). By using fractionation or cofractionation of palm and lauric oils, soft (146) and stick (147) margarines were prepared without addition of hydrogenated or interesterified fats. A process for producing hard stocks from high-palmitic oils, high-lauric oils, and hydrogenated oils having



a high-behenic-acid content by 1,3-specific enzymatic interesterification has been described (148). Spreads have been prepared from a blend containing liquid oil and 3–10% of a fully hydrogenated fat composed of at least 25% palmitic acid, less than 3% *trans*-acids, and less than 10% glycerol tristearate (149). Suitable hard stocks are fully hydrogenated fish oils or palm oils. Hard stocks produced by interesterification of high palmitic and/or stearic acid fat and high behenic acid fat were used at low levels to prepare a margarine containing low saturated and *trans*-fatty acids (150). The characteristics of hard stocks obtained by interesterification of fully hardened soybean oil and nine common vegetable oils (1:1) have been described (80). Margarine oils containing very low levels of *trans* and C<sub>8</sub>–C<sub>16</sub> fatty acids can be prepared by enzymatic transesterification of a stearic acid source and a liquid oil (151).

Formulation of zero-*trans*-margarines from single-source high-linoleic oils also has been reported. The random interesterification of an 80/20 blend of liquid soybean oil and completely hydrogenated soybean oil resulted in SFI values of 8, 3.5, and 2.2 at 10°C, 21.1°C, and 33.3°C (50°F, 70°F, and 92°F), respectively. The margarine had a stable crystal structure and good oxidative stability (152). The enzymatic interesterification of blends of liquid and 10–15% fully hydrogenated soybean oil has been reported to result in a fat base suitable for preparing table spreads (153). Low-*trans*-margarines also were prepared from 100% sunflower, soybean, or corn oils by fractionating an interesterified mixture of liquid and completely hydrogenated oil (154). The olein thus obtained was blended with additional liquid oil to obtain fats suitable for stick margarine. A spread formulated using a single unmodified fat has also been characterized (155). The fat is preferably high-stearic soybean oil containing at least 30% disaturated triglycerides.

An alternative method for preparing low-*trans*-margarines makes use of directed interesterification to prepare fats from liquid oil without the aid of hydrogenation. Sreenivasan (156) prepared a 100% sunflower oil with solids of 10.7 at 0°C (32°F), 6.0 at 21.1°C (70°F), 5.2 at 26.7°C (80°F), and 2.1 at 33.3°C (92°F) by directed interesterification in an aprotic solvent for 6 days at –9.4–0°C (15–32°F). At these temperatures the di- and trisaturated glycerides are precipitated from the reaction mixture as they are formed. The reaction is then stopped at low temperature and the oil blend has the same fatty acid composition as the original liquid oil. Directed interesterification of liquid corn oil using temperature cycling between 0.6°C (33°F) and 10°C (50°F) in the absence of solvent was accomplished in 6 h (157). The resulting solids were 13.1, 10.3, 7.2, 4, 8, and 2.2 at 0°C, 10°C, 20°C, 30°C, and 40°C (32°F, 50°F, 68°F, 86°F, and 104°F), respectively. In order to increase the amount of solids attainable by directed interesterification, the liquid oil can first be enriched with a small percentage of the completely hydrogenated oil (158). Alternatively, the solids may be enriched without hydrogenation by removing a portion of the saturate-reduced liquid oil during the reaction and adding additional starting oil (159). Although soft zero-*trans*-margarines can be prepared from 100% liquid oils, it is unlikely that satisfactory stick margarine oils will be obtained through directed interesterification alone. The limited melting ranges and high

melting points of triglycerides composed of stearic and palmitic acid would appear to preclude this possibility.

A novel alternative to the use of hydrogenated or saturated fats for structural stability in oil-continuous emulsions is the addition of oil-soluble polymers as thickening or texturizing agents (160). These polymers are condensation products of hydroxyacids or polyhydric alcohols and polybasic acids. Currently they are not approved for food use. Another option to hydrogenated oils is to base the product on an oil-in-water emulsion. Such a product, which contains 80% liquid canola oil, has been introduced in the United States (140).

*Special Dietary Oils.* Some reports have been published regarding special health margarines that contain nontraditional ingredients. Stahl (161) has described a low-calorie margarine in which the oleaginous phases consist of monoglycerides with no appreciable quantities of di- or triglycerides. The product may be used for nutritional supplementation where certain digestive disorders exist. Margarines also have been prepared using medium-chain triglycerides based on caprylic and capric acids (162–164). Such fats are rapidly absorbed and are useful where fat metabolism is impaired. Formulation of low-calorie margarines and spreads using sucrose octaoleate as a fat replacer has been reported (165, 166). Improved margarine hard stocks composed of sucrose polyesters of short- and long-chain fatty acids (167) as well as palm oil fatty acids (168) also have been described. These sucrose polyesters are nonabsorbable fat substitutes that have been found to be useful in reducing calorie intake and plasma cholesterol in clinical trials (169). In the future there may be a market for spreads formulated from other oils perceived to be healthy such as rice bran oil (170) or oils containing long-chain highly unsaturated  $\omega$ -3 acids (171) or  $\gamma$ -linolenic acid (172). The use of the latter two types of oil in the United States will depend not only on their approval for food use but also on whether sufficient scientific evidence is generated to support approval of a health claim. The food uses and properties of fish oils, which contain high levels of  $\omega$ -3 fatty acids, have been discussed (53, 173, 174). Plant sources of  $\gamma$ -linolenic acid such as borage, black currant, and evening primrose oils, as well as microbial sources have been reviewed by Gunstone (175).

## 5.4. Oil Specifications

Patel (176) has listed the parameters usually included in specifications for a margarine oil blend (Table 8). In addition to those listed, specifications sometimes include anisidine value, totox value, and limits on heavy metals and microorganisms. The oil used in margarine should be of the highest quality and as bland as possible. Flavors are evaluated organoleptically upon receipt of the oil using a standard scoring system such as that recommended by the American Oil Chemists' Society (177). Some manufacturers may confirm organoleptic results using volatiles analysis. Physical properties are described by SFI. Some specifications also include SFI at 26.7°C and 37.8°C (80°F and 100°F) as well as a melting point or Mettler dropping point. Characteristics of the base stocks used to formulate the blend generally are left to the discretion of the oil supplier. As Wiedermann

**TABLE 8. Typical Margarine Oil Specification.**

Parameter (139)	Example
<b>Composition</b>	
Source oil(s)	100% corn
Blend	50% liquid oil, min.
Additives (permitted/required)	Citric acid permitted
<b>Quality</b>	
Flavor (organoleptic score)	7, min.
Color (Lovibond red)	3, max.
Peroxide value (mEq/kg)	1.0, max.
Free fatty acids (% as oleic)	0.05, max.
Moisture (%)	0.05, max.
Stability (active oxygen method), 8-h AOM	Peroxide values less than 10
<b>Physical</b>	
SFI at 10°C (50°F)	22–26
SFI at 21.1°C (70°F)	14–17
SFI at 33.3°C (92°F)	1.5–3
<b>Nutritional</b>	
Percent polyunsaturates (enzymatic)	28, min.
Percent saturates	19, max.
<b>Shipping</b>	
Mode	Truck
Nitrogen blanketing	Yes
Loading temperature	57.2°C (135°F) max.
Arrival temperature	48.8 ± 15°C (120 ± 5°F)

(178) has pointed out, this may, at times, lead to difficulties in the manufacture of a margarine product for which process control is critical. The SFI profile should not be viewed merely as three separate specification ranges. In the example in Table 8, oils with SFIs of 26-15-1.5 and 22-16-3 would both meet specification but could result in noticeably different finished product characteristics. If the SFI-temperature curve for a given oil blend is routinely steeper or flatter than the slope through the midpoints of the SFI ranges, the base stocks being used may not be suited to the specifications or the specifications may not be realistic. The specification of polyunsaturates or saturates is usually included only if these are necessary to meet specific label claims on the margarine product. If labeling of *trans*-fatty acids becomes mandatory in the future, the *trans*-content may become part of many manufacturers' specifications.

As many margarine plants in the United States are located at considerable distance from their suppliers, truck or rail shipment is required. Loading temperatures and whether the oil must be nitrogen blanketed are specified. For truck deliveries, the temperature on arrival often is specified to ensure that the oil is completely liquid. In order to minimize the possibilities for oil degradation, it is optimal to process and deodorize the oil blend at the margarine production location.

## 6. OTHER COMMON INGREDIENTS

The following section discusses the functionality of some of the ingredients, other than the fat blend, that are commonly used in table spreads. There is considerable latitude in the choice of which ingredients to use and the levels of these ingredients. The recipe is generally decided by consumer preference testing and process considerations. Some typical formulations are listed in Table 9.

### 6.1. Milk Products and Protein

In the margarine standard of identity, milk products have been interpreted (179) by the FDA to include butter or butter fat in any percentage as long as some vegetable oil is used to meet the 80% minimum fat requirement. Therefore, butter blends fall under the FDA regulations governing margarines and spreads. In the past, cultured milk was used in almost all margarines. Because of the time and space required for culturing, this practice was largely abandoned in favor of using skim milk together with starter distillate, diacetyl, or other flavors. Skim milk, although still used by some manufacturers in the United States, for the most part has now been replaced by spray-dried whey, which is sometimes supplemented with potassium caseinate to a standardized protein content. Soy protein can be used for products where dietary considerations preclude dairy ingredients.

**TABLE 9. Typical Margarine and Spread Formulations.**

Ingredient	Percent in Finished Product		
	80% Fat	60% Fat	40% Fat
Oil phase			
Liquid and partially hydrogenated Soybean oil blend	79.884	59.584	39.384
Soybean lecithin	0.100	0.100	0.100
Soybean oil mono- and diglycerides (IV 5, max.)	0.200	0.300	—
Soybean oil monoglyceride (IV 60)	—	—	0.500
Vitamin A palmitate- $\beta$ -carotene blend <sup>a</sup>	0.001	0.001	0.001
Oil-soluble flavor	0.015	0.015	0.015
Aqueous phase			
Water	16.200	37.360	54.860
Gelatin (250 bloom)	—	—	2.500
Spray-dried whey	1.600	1.000	1.000
Salt	2.000	1.500	1.500
Sodium benzoate	0.090	—	—
Potassium sorbate	—	0.130	0.130
Lactic acid	—	to pH 5	to pH 4.8
Water-soluble flavor	0.010	0.010	0.010

<sup>a</sup>Custom blended for correct vitamin content and color; suspended in corn oil.

Protein affects margarine products in several ways. In addition to flavor, dairy solids undergo the Maillard reaction and brown during frying. Low-lactose milk or whey proteins can be used to control or eliminate the browning effect while retaining desirable flavor characteristics (180). Milk solids also act as a preservative by sequestering metals that promote oil oxidation (181). Protein exerts a destabilizing effect on water-in-oil emulsions. If protein is removed from a margarine formulation without changing the processing or the fat/emulsifier system, flavor and salt release will be impaired because the aqueous-phase droplets are smaller and the emulsion is more resistant to breaking. Linteris (182) found that the addition of 0.01–0.1% sodium caseinate enhanced the salt sensation of milk-free margarine. In very low fat spreads where the aqueous phase contained only water, preservative, salt, acid, and flavor, the incorporation of 5–10 ppm of protein caused a significant enhancement of flavor release (183). The emulsion instability caused by the presence of protein is a particularly important concern in formulating products that contain less than 50% fat. In the past, all 40% fat diet margarines in the United States contained no protein. Today, however, many 40% and lower fat spreads contain gelling agents or other water-binding ingredients that afford sufficient stability to allow addition of milk protein.

## 6.2. Emulsifiers

Emulsifiers are multifunctional in margarine. They reduce surface tension between the aqueous and oil phases so that the emulsion forms with minimal work. Emulsifiers stabilize the finished product during storage to prevent leakage or coalescence of the aqueous phase. They also act as antispatting agents by preventing coalescence and violent eruption of steam during frying. Common emulsifiers and their uses are listed by Dziezak (184). The role of emulsifiers in spreads and shortenings has been discussed by Madsen (185). The effects of emulsifiers and their interactions are complex and become more critical at lower fat levels. Gaonkar and Borwankar (186) have reported the influence of lecithin, monoglyceride, and surface-active impurities present in the oil on the vegetable oil–water interface. Using microscopy, Heertje (64) found that saturated monoglycerides appear to be more effective in displacing proteins at the interface than unsaturated monoglycerides, and that phospholipids are much more surface active than monoacylglycerols.

Crude lecithin is used at levels of 0.1–0.5% in almost all margarines because of its antispatting properties; however, in very low fat spreads, it may lead to decreased emulsion stability and increased tendency to oil-off. The production, properties, and food uses of lecithin have been reported by Schneider (187). Lecithin may be unbleached or single or double bleached with hydrogen peroxide. In addition to producing an even, stable foam during frying, lecithin contributes to a fine dispersion of the protein sediment, interacts with protein to form a brown gravy, and results in a quicker salt release (188). Enzymatic hydrolysis of lecithin yields  $\alpha$ -monoacylglycerophosphatides that improve frying performance and resistance to oil-off in liquid margarines (189). Fractionation and partial hydrolysis of the alcohol-insoluble fraction is claimed to afford improved antispatting effects in

margarine (190). Antispattering properties have also been claimed for proteose-peptone-enriched milk protein (191), finely divided metal or metalloid oxides (192), citric acid esters of monoglycerides (193), sodium sulfoacetate derivatives of monoglycerides (194), and polyglycerol esters (195). Incorporation of finely dispersed gases such as nitrogen, carbon dioxide, or air also reduces spattering (196). Nitrogen is preferred and is most often used in whipped margarines and spreads. The use of helium or its admixtures with other gases is reported to result in a very fine dispersion that can be attained relatively easily (197).

In stick margarines where the high percentage of solid fat is capable of stabilizing the crystallized emulsion, only lecithin is necessary. However, most margarines also contain mono- and diglycerides of low or intermediate iodine value (IV) for added protection against weeping. Very high IV monoglycerides such as those produced from sunflower or safflower oil have been found to function well in low-fat products (198). The rate of coalescence of a 50% water-in-vegetable-oil emulsion as a function of concentration of monolinolein has been studied (199). Some very low fat spreads contain polyglycerol esters. A combination of monoglycerides and polyglycerol esters is effective in allowing production of very low fat spreads containing a significant amount of milk protein, especially if the spread also contains a gelling agent (200). Although polyglycerol polyricinoleate is said to be particularly effective (201, 202), and is used in Europe, this specific polyglycerol has not been approved in the United States. Erucic acid esters of polyglycerols also were found to stabilize high internal phase water-in-oil emulsions (203). The use of sucrose esters to form a stable liquid margarine with only unhydrogenated liquid oil has been described (204). It has also been reported (205) that a high liquid oil containing fluid margarine with low viscosity, and which is not susceptible to oiling-out, can be formulated using a distilled behenic acid monoglyceride.

In addition to emulsifiers, low-fat spreads may contain aqueous-phase gelling and/or thickening agents such as gelatin, pectin, carrageenans, agar, xanthan, gellan, starch or starch derivatives, alginates, or methylcellulose derivatives. Commercially, the most important of these is gelatin. High-quality gelatin is a costly ingredient and a process for removal of off-flavors from the less expensive grades of gelatin by membrane filtration has been patented (206). The functionality of gelling agents is discussed later in this chapter. The microstructural nature of the emulsion in margarine and low-fat spreads has been elucidated by Heertje (64) using microscopical techniques.

### 6.3. Preservatives

The preservatives that can be added to margarine fall into three categories: antioxidants, metal scavengers, and antimicrobial agents. Because of hygienic manufacture of both raw materials and the finished table spreads, lipolytic microorganisms are not found and hydrolytic rancidity is not a problem, even in products formulated with lauric oils. Antioxidants may be necessary for keeping quality of spreads formulated with significant amounts of animal fat, but they are not added to most vegetable oil margarines. However, in the future, these may become necessary in order

to incorporate even low levels of highly unsaturated fats such as unhydrogenated fish oils. Vegetable oil margarines containing milk protein have been shown to be stable for 6 months at 4.4°C (40°F) with no added antioxidant (207). Residual tocopherol levels in vegetable oils are said to be near optimum for protection (208); however, excess tocopherol may have a pro-oxidant effect (209). Lecithin and ascorbic acid act as antioxidant synergists (210). The addition of nonlipolytic, non-proteolytic, oxygen-consuming yeasts has been suggested as a means of preventing autoxidation of the finished product (211). Salt-tolerant lactobacillus that convert aldehydes to alcohols are said to improve margarine shelf life by removing the oxidation products responsible for off-flavors (212).

The presence of heavy metals can cause serious metallic off-flavors to develop in margarine within days. Copper has the strongest pro-oxidant effect. The maximum amount of copper that can be tolerated is indicated to be on the order of 0.02 ppm (213). Citric acid, citrates, and salts of ethylenediaminetetraacetic acid (EDTA) act as sequestering agents to inactivate metals that may be present. EDTA has been found to be very effective in preventing off-flavors due to copper-induced degradation and often is added to milk-free margarines in the form of calcium disodium salt. Melnick (214) has patented a process of crystallizing salt in the presence of EDTA to reduce the heavy-metal content. Such high-purity salt is available for use in margarine.

Microbiologically, water-in-oil emulsions are more stable than the aqueous phase itself because only a small fraction of the droplets are occupied by microorganisms. The droplet size limits growth provided that the organism does not excrete lipases (215). As droplet size depends to a great extent on processing parameters, process control is critical, particularly in the manufacture of low-fat spreads. The median and largest diameters of the droplets, pH, available nutrients, and degree of inoculation play essential roles in determining the fate of contaminating microorganisms (216). The concentration of salt in the aqueous phase of a 2% salt, 80% fat margarine is also very effective; however, in the absence of additional preservatives or acidulents, molds occasionally do proliferate. Sorbic acid, benzoic acid, and their salts are used as preservatives, particularly in low-fat and low-salt products. The undissociated acids are primarily responsible for the preservative effect, and therefore, the lower the pH, the greater their effectiveness. However, the acids are more soluble in the oil than in the aqueous phase, where protection is needed, and sorbic acid has the more favorable partition coefficient. Although salt acts synergistically with these preservatives in aqueous solution, in an emulsion it has a negative effect on the partition coefficient, driving more of the free acid into the oil (217). Studies have been conducted on the effectiveness of benzoic acid (218) and sorbic acid (219) in margarine as a function of concentration. Castenon and Inigo (220) recommend the addition of 0.05% sorbic or benzoic acid and a pH of 4–5 for unsalted margarine and a pH of 5–6 for salted margarine. Lactic acid is said to be the most effective acidulent for use as a preservative as long as at least 0.2% is present (216); however, citric and phosphoric acids are also used. Demineralization of acidified milk can reduce its sour taste by lessening its buffering capacity and hence the acidity required to achieve a given pH (221). The use of two aqueous phases

formulated such that the preservatives and the nutrients are concentrated in the same phase has been suggested (222). Klapwijk (223) has discussed the hygienic production of low-fat spreads and has outlined a predictive modeling approach to microbiological hazard analysis.

#### 6.4. Flavors

Many synthetic butter flavors are available for use in margarine. These are based generally on mixtures of compounds that have been identified as contributing to the flavor of butter, such as lactones, ethyl esters of short-chain fatty acids, ketones, and aldehydes (224). Diacetyl is a primary volatile constituent of many margarine flavorings and contributes significantly to a buttery aroma. The concentration in butter varies from 1 to 4 ppm (225). It is formed from citric acid present in milk during the culturing process. If the milk is not cultured, synthetic diacetyl or starter distillate can be added. Flavors obtained by lipolysis of butterfat also are available, and the use of a combination of starter distillate and heat-treated butterfat has been described (226). The tightness of the emulsion and the melting characteristics of the fat will affect the rate and the order in which flavors are perceived. Salt concentration and pH also affect flavor balance because they may influence the partition coefficients of various flavor components. As the fat content of spreads is reduced to very low levels, the challenge of formulating flavors whose oral response is similar to that of high-fat spreads is increased considerably.

#### 6.5. Vitamins and Colors

The mandatory fortification of margarine with Vitamin A is accomplished by the addition of  $\beta$ -carotene (pro-Vitamin A) and/or Vitamin A esters. The carotene level is adjusted for the desired color and the colorless esters (acetate, palmitate, etc.) are used to standardize the vitamin content. Addition of Vitamin D is optional. Fortification with Vitamin E is not permitted by the U.S. margarine standard, but recently some spreads fortified with Vitamin E have appeared in the marketplace in the United States, and fortification of both margarines and spreads has recently been done in Europe. The naturally occurring Vitamin E content of vegetable oil margarines available in the United States has been reported (227).

Margarine is colored with carotenoids, and synthetic  $\beta$ -carotene is by far the most widely used. Carotene dissolves very slowly in oil. Therefore, the compound is pulverized to a particle size of 2–5  $\mu\text{m}$  and the microcrystals are suspended in oil to retard oxidation (228). Natural extracts containing carotenoids, for example, annatto (bixin), carrot oil, and red palm oil also have been used. Annatto, which is used in butter, is somewhat sensitive to light and may have an orange or slightly pink hue, particularly when the aqueous phase is acid (4). Mixtures of annatto and turmeric extracts result in a more typical color than annatto alone (229). Many margarine manufacturers purchase blends of colors and vitamins customized for their specific products.



## 7. PROCESSING

There is a wide range of formulations used in the margarine industry today. Just as the fat must be tailored to suit the product, in order to attain the desired finished product characteristics, processing parameters must be established that are appropriate for level, solids content, and crystallization rate of the fat used in the formulation. The basic process consists of five operations: emulsification, cooling, working, resting, and packaging. The following is a brief general description of these operations and their usage for different product types together with some published process modifications. Detailed descriptions of the equipment, process, packaging, and plant layout, as well as the production of puff pastry margarine, are given elsewhere in this work. Some aspects of low-fat spread processing will be discussed later in this Chapter.

### 7.1. Processing Operations

*Emulsification.* The formation of the coarse initial emulsion may be a strict batch process in which the warm oil and the oil-soluble ingredients are individually weighed or melted into an agitated tank, which, in the past, was referred to as a churn. The pasteurized, aqueous phase is then weighed in with agitation. In many plants the aqueous phase is held at 4.4–10°C (40–50°F) after pasteurization, so that heat may be necessary to maintain the emulsion at a temperature above the melting point of the fat. Usually the emulsion is held at about 43.3–42.8°C (110–120°F). If the temperature is not sufficiently high, crystal nuclei and precrystalline structures may be formed that can affect the consistency of the finished product (230). Spattering properties of the margarine may be affected by the aqueous-phase temperature (231). The emulsion at this point is very unstable. Without agitation the milk-phase droplets would immediately begin to coalesce and settle out. After the emulsion is well mixed to ensure uniformity, it is pumped to an agitated holding tank that feeds the processing line.

Alternatively, the emulsion may be formed on a continuous basis. If all oil-phase ingredients are added to the primary oil storage tank, the two phases can be metered into the line feed tank or simply mixed in-line using metering pumps or mass flow meters. Whether this is feasible depends on the oil holding tanks available and the formulation and production scheduling of products in which the base oil is used. The ultimate in flexibility consists of a multihead pump capable of metering individual components such as brine, water, whey concentrate, flavor, and preservative solutions to compose the aqueous phase, and also the individual oil base stocks, emulsifiers, and colors to form the oil phase. In-line static mixers are used to blend the separate phases, which are then joined in-line and emulsified through another static mixer.

Prechilling the emulsion just enough to form a low level of solid fat (0.5–2.5%) followed by homogenization prior to normal processing is said to result in a very fine dispersion of the aqueous phase that obviates the need for preservatives (232). A novel process for forming stable low-fat emulsions of uniform droplet size has

been recently described (233). The method consists of dispersing an aqueous-phase or an oil-in-water emulsion at low pressure into the oil phase through a hydrophilic microporous membrane that has been pretreated with the oil phase. The process is said to be applicable for fat levels as low as 20 and 25% to water-in-oil and oil-in-water-in-oil emulsions, respectively. Recently, a 25% fat spread, which is believed to have been manufactured by this process, and which contains no preservatives and has a 6-month shelf-life, has been introduced in Japan.

*Chilling.* When the emulsion is formed, it is fed via a high-pressure positive pump to a tubular swept-surface heat exchanger, usually referred to as an A unit. Examples of the tube chillers available are the Votator (Cherry-Burrell, United States), the Chemetator (Crown Chemtech Limited, United Kingdom), the Perfector (Gerstenberg and Agger, Denmark), and the Kombinator (Shroeder, Germany). In these devices the product passes through the annulus between a rotating shaft and an insulated outer jacket containing a refrigerant, usually liquid ammonia. Temperature control is achieved by regulating the suction pressure on the refrigerant. The tube often is fabricated from chromeplated nickel or steel, which have high heat transfer coefficients. Free-floating blades attached to the mutator shaft are caused by centrifugal force to scrape the jacket inner wall continuously to achieve maximum cooling. In general, shaft speeds range from 300 to 700 rpm, scraping the surface clean as many as 1500 times per minute (234). The high internal pressures and shearing forces generated by the blades and their holding pins cause rapid crystal nucleation. High rotational speeds in the A unit result in a much finer emulsion than low speeds (235). Warm water is circulated through the shaft to prevent buildup of solid fat. The A units are available in a range of sizes and commonly are used in series, which allows for processing flexibility.

*Working.* When the margarine emerges from the cooling tubes, it is only partially crystallized. In many processes it then goes to a working unit or blender; sometimes called a B unit. In some patent literature it is referred to as a C unit or crystallizer; however, an eccentric swept-surface heat exchanger following a blender has also been described as a C unit. The working unit has pins arranged in a helical pattern on a variable-speed shaft. These pins intermesh with stationary pins positioned on the cylinder wall. In the blender, crystallization is intense and a temperature rise results both from the heat of crystallization and mechanical work. Agitation in the crystallizer facilitates free diffusion of crystals to the surface of the aqueous-phase droplets, forming a crystalline shell (the so-called Pickering stabilization). Due to the increased viscosity, some coarsening of the emulsion may occur during passage through the working unit (235). Intermediate crystallizers, which are driven by the mutator shaft of the A unit are available from some manufacturers. The temperature rise across the blender is an indication of the amount of crystallization achieved. The degree of crystallization in the working unit depends on the residence time (volume and throughput) and the rotational speed of the shaft as well as the crystallization rate of the fat. An experimentally derived relationship between these variables and an automated process for controlling the degree of crystallization in the crystallizer has been described (236).

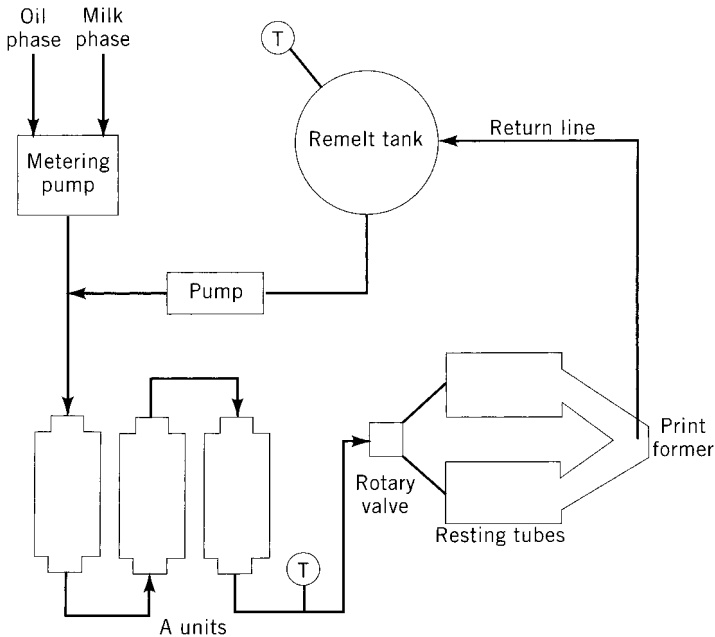
*Resting.* If the product requires a stiffer consistency for packaging, this is accomplished through the use of a static B unit or quiescent tube. This is a warm-water-jacketed cylinder that sometimes contains baffles or perforated plates to keep product from channeling through the middle of the cylinder. The static B unit usually consists of flanged sections so that the length can be varied to suit the product. Additional resting time is often achieved with two quiescent tubes in parallel, with the use of a timed, rotary valve that alternates flow to the two units.

*Packaging.* Two basic types of stick packaging machinery are in use in the United States. The first of these forms a molded print that is then wrapped. This may be an open or closed system. With an open system the product exits the quiescent tube through a perforated plate, forming “noodles” that drop into a hopper. Screw impellers feed the margarine to a mold where the print is formed prior to wrapping and cartoning. The closed system is similar except that the margarine is not extruded but filled directly into the mold cavity with line pressure. On the second type of machine, the filled print, margarine from the holding tube is still in a semi-fluid state. It is filled directly into a cavity that is prelined with the inner wrap. The wrapper is then folded and the print ejected from the mold. This equipment is more suitable for filling a soft stick because the product is not molded before wrapping. Soft tub margarines are packaged in a fluid or semifluid state on straight-line or rotary-head fillers.

## 7.2. Specific Processes

*Stick Margarine.* A typical equipment configuration for closed-system stick margarine production is illustrated in Figure 3. The emulsion is proportioned together, cooled and allowed crystallization time prior to packaging. For *molded print* equipment, the product passes from the A units directly to the quiescent tubes. For *filled print* packaging, a small blender may be used prior to the resting tubes to achieve the proper consistency for packaging and a slightly softer finished product. The remelt line is necessary because, in a closed system, some overfeeding must be present in order to maintain sufficient product to the filler for adequate weight control. The excess product is returned to a tank where it is remelted and pumped back into the product stream to be reprocessed. In the event of a packaging equipment malfunction, all the product goes back to the return tank. If an emulsion tank is used to feed the line, the excess product can be remelted in-line and returned to the emulsion tank.

The crystallization rate of the fat blend affects the equipment requirements and the processing parameters necessary to achieve a satisfactory product. If the fat blend is prone to extreme supercooling, the crystal lattice will not be formed in sufficient time for the blender to be effective. In this case the product may set up too firm in the quiescent tubes, or severe postpackaging hardening may occur. In order to process a blend containing a high level of palm oil, Kattenberg and Verburg (237) used a slowly agitated working unit between the A units to provide a residence time of 2–3 min for crystallization to occur.



**Figure 3.** Continuous stick margarine production.

Several processing variations have been claimed to improve the organoleptic quality of margarines. Controlled precrystallization is used to achieve early crystallization and crystal enlargement of the higher melting glycerides. This is achieved by feeding all or part of the oil blend to a precooler prior to mixing it with the aqueous phases. Alternatively, part of the emulsion leaving the A units is recycled and mixed with the emulsion at the inlet of the cooling tubes. The greater distribution of crystal sizes is said to improve consistency and flavor release (238). In another modification of the process, the oil blend and 25% of the aqueous phase are chilled and the remaining 75% of the cold milk added to the emulsion just prior to the blender (239). This milk injection results in a greater range of droplet sizes, some quite large, which improves flavor and salt release. Part of the milk is added prior to cooling so as to obtain a uniform opaqueness, with no regions of pure fat. Flavor improvement also is claimed for margarine composed of a double emulsion (oil-in-water-in-oil). The emulsion is formed by first preparing an emulsion of a portion of the fat in the aqueous phase and then emulsifying this into the remainder of the oil blend (240–242). Advantages of this process are said to include (1) improved flavor release due to the greater surface area of the aqueous phase, (2) the potential for increase of the liquid oil content by using this as the internal oil phase while the consistency depends on the external phase, and (3) the ability to incorporate high flavor levels for baking and cooking into the internal oil phase and at the same time retain acceptable flavor levels if the product is used as a spread. Phase inversion processing of high-fat table spreads is claimed to impart some of the textural and

flavor release properties of butter. The margarine is prepared by churning a filled vegetable oil cream (243, 244). Injection of some of the fat, preferably a liquid oil, into the cream as it is being churned is reported to facilitate the process (245).

*Whipped Margarine.* Whipped margarines usually contain 33% nitrogen by volume (50% overrun). The gas is introduced in-line through a flow meter before or between the A units. A backpressure valve is placed after the A units to maintain a constant line pressure against which the nitrogen is injected. The nitrogen also may be introduced at low pressure between two pumps with the second operating at a higher throughput rate. A high-speed whipper is used just prior to the filler to ensure a fine dispersion of the nitrogen. In order to control overrun, a heat exchanger in the return line completely melts the product to eliminate nitrogen from the recycled emulsion. Closed-system packaging equipment needs some modification to run whipped sticks because of the expansion when line pressure is released at the fill head. Line pressures are critical in producing a product of uniform texture and appearance (246). Equipment especially designed for packaging six sticks to a 1-lb carton generally is used.

*Soft Margarine.* In order to fill the container properly, soft margarine must be quite fluid. Usually there are no quiescent tubes. A large blender is used for work softening so that the product does not become brittle as a result of excess crystallization in the bowl (Figure 4). For a low-solids oil blend the blender can be placed between the A units or replaced with an extrusion valve to avoid

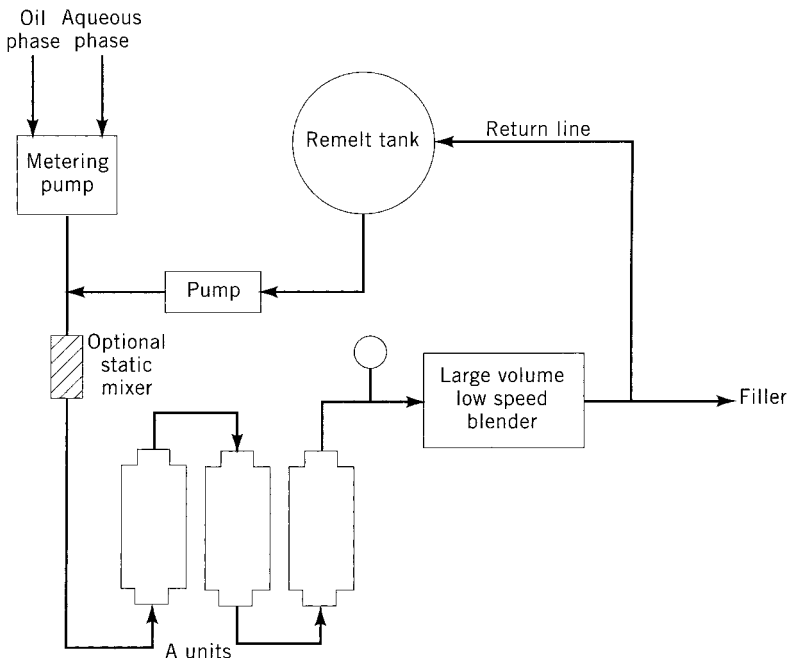


Figure 4. Continuous soft margarine production.

overworking the margarine. Bouffard (247) has described precrystallization techniques for controlling the consistency of spreadable margarines. Faur (248) has demonstrated that, for an A unit–blender–A unit configuration, by increasing the relative amount of cooling in the first A unit or by adding another blender after the second A unit, the spreadability of products packed at the same temperature is enhanced significantly.

*Liquid Margarine.* Liquid or “squeeze” margarine can be prepared by use of the same equipment to chill and work the product as that used for soft margarine (119). Improved resistance to oil separation is claimed for a process in which the oil blend is cooled and held in a quiescent state for at least 5 h prior to adding the aqueous phase, rechilling, and whipping (120). Stability also is said to be improved by finely dispersing about 5% nitrogen in the emulsion (249). Improved resistance to oil-off may also be attainable by use of suitable emulsifiers (see Section 6.2). A process for high liquid oil margarine has been reported (250).

## 8. LOW-CALORIE SPREADS

Low-fat spreads, as originally introduced in the 1960s, contained 40% fat and only water, salt, and preservatives in the aqueous phase. The spreads were labeled “diet margarine” and usually consisted of very tight water-in-oil emulsions that had poor melting and flavor release properties. Some such products continue to be marketed today. The first 40% fat products containing dairy solids to be marketed in the United States in the early 1980s were in fact stabilized by the water binding ability of the very high level of milk protein employed. These spreads exhibited poor microbiological stability, did not have the 4- to 6-month shelf life of other spreads, and were withdrawn from the marketplace. Today, lower fat, higher quality spreads, usually containing milk protein and various stabilizing agents, are being produced. Formulation and processing of modern low-fat spreads has been summarized by Moran (251), and production of low-calorie spreads and butters in Europe has been discussed by Madsen (252). In Europe these products are understood by the consumer to be spreads and not as replacements for full-fat margarine. In the United States there remains considerable consumer confusion about the distinction between margarine and low-fat spreads, perhaps because of the multitude of fat levels available between 20 and 75% in the spread category. However, products containing 40% or less fat usually have a statement on the package that the spread is not intended for baking or frying.

Oil-continuous products are favored because of their lubricity, release of oil-soluble buttery flavors, microbiological stability, and reduced tendency for moisture loss. It is preferred that the product melts in the mouth and on hot foods, and that it does not exude water when being spread. Common tests for emulsion stability are electrical resistance, absence of staining with a water-soluble dye on a microscope slide, failure to disperse easily when stirred in water at room temperature, and negative or only slight reaction with paper impregnated with a water-indicating dye, such as bromophenol-blue, after spreading.

The poor organoleptic response of the original diet margarines, which contain only fat, water, salt, emulsifier, flavor, vitamins, and preservatives, is due to the fact that the products are highly emulsified in order to achieve the stability required. Some of these products consist of such tight emulsions that even when held at temperatures high enough to melt all of the fat, phase separation does not occur. This results in a greasy sensation on the palate and little flavor and salt delivery. Processing is similar to that described in the preceding section, except that the lower the fat content, the more critical the manufacturing parameters as a result of emulsion instability. In forming the emulsion, the aqueous phase and oil should be similar in temperature and should be combined slowly. Stronger agitation is required to ensure homogeneity because of the inherent viscosity of a high internal phase emulsion. Care is also taken to avoid incorporation of air during emulsification. To prepare a low-fat spread with protein, Altrock and Ritums (253) used deaerated water and circulated the fat phase through the system to precoat the equipment and displace air. Then the phases were fed to the chiller in concentric streams with the oil blend on the outside. Low-fat emulsions have been found to be sensitive to line pressures and cooling rate (254). Fill temperatures are higher than corresponding 80% fat products because the emulsion is more viscous. If the fill temperature is too low, the product will mound in the bowl with excessive lid contact and may become crumbly with water leakage as it is packaged (255). If too much crystallization takes place early in the process, the shearing forces of processing become so great that they break down the emulsion (251). Therefore, low-fat products are more easily prepared by use of high-liquid-oil and low-SFI blends (256). Norton (257) has prepared oil-continuous spreads with less than 14% fat utilizing a high-melting mono-, di-, or triglyceride seeding component. The seeding component allows the use of higher crystallization temperatures, where the crystallization in the  $\alpha$  form is avoided and crystallization directly into the  $\beta$  or  $\beta'$  form is slower and more controllable.

This poor oral response and processing difficulties encountered with these low-fat spreads can be overcome by using gelling agents. Gelatin is particularly suitable since the gel melts in the mouth allowing dairy solids, or other oil-in-water-promoting ingredients incorporated in the oil or in the aqueous phase, to disrupt the water-in-oil emulsion. Thus, the spread melts cleanly with good flavor and salt release. The aqueous phase can be cooled to below the gelation temperature and worked to form gelled beads that are then mixed with the fat. Alternatively, the fat can be dispersed in the heated aqueous phase as an oil-in-water emulsion, which upon cooling to below gelation temperature and working, inverts to a water-in-oil emulsion (258). For very low fat spreads the use of a combination of monoglyceride and polyglycerol ester emulsifiers is claimed to result in wider processing latitude and enhanced finished product stability (195). In practice the oil-in-water emulsion can be cooled by standard swept-surface heat exchangers, or even by static heat exchangers (259), and inversion accomplished in a high-shear working unit (260). It is critical that the cooling times and temperatures employed allow gelation to occur before exiting the working unit. Inclusion of low levels of starch or starch derivatives was found to be beneficial for increasing the gelation rate of the gelatin.

Spreads containing less than 27% fat have been prepared in this manner (261). It can be difficult to maintain inversion at high throughput rates or if fats with a high solid fat content such as butter oil are used. These difficulties may be overcome by injecting a small amount of the molten fat into the process stream just prior to the inversion unit after the bulk of the emulsion has been cooled (262). Spreads containing 20% fat and high levels of milk protein and gelling agents have been reported (263). Stability was enhanced by homogenization of the aqueous phase prior to forming a water-in-oil emulsion.

Some of the alternatives to gelatin that have been proposed are low-melting carrageenan gels (264), agar and/or pectin (265), gellan gum blends (266), and gelling starch derivatives (267). Oil-continuous spreads containing 15–35% fat and high levels of a nongelling, low-dextrose equivalent starch hydrolyzate have also been prepared by an inversion process (268). The use of nongelling proteinaceous aqueous phases, which contain starch and/or sodium alginate and which have specific viscosity characteristics, has been reported (269). It has been suggested that finished product stability problems may be encountered if the aqueous phase contains amino acid residues in excess of 200 ppm, and such amino acid residues may be components of commercial gums (270).

Very low fat spreads have also been prepared by homogenizing and chilling oil-in-water emulsions containing substantial amounts of gelling and/or thickening agents (270–274). The finished products may, in a sense, be described as “bicontinuous” because the nature of the fat phase, although essentially dispersed, contributes significantly to the plasticity and organoleptic properties. Water-continuous spreads containing as little as 5% or less fat have also been disclosed. These products consist of at least two gelled aqueous phases, one or more of which is continuous, and at least one of which contains an aggregate-forming gelling material such as modified starch, denatured protein, or microcrystalline cellulose (275). Some quick-setting purified starches have been found to be particularly applicable in terms of flavor, texture, and stability in this application (276). Preferably, besides the aggregate gel, the dispersion also includes a network-forming gelling agent such as gelatin, carrageenan, alginate, agar, gellan, pectin, or mixtures of these. The properties of such a spread were enhanced by the addition of high levels of a casein source (277). Preferably the dairy ingredient is demineralized to reduce the acidity required in order to attain the pH necessary for microbiological preservation. Extremely low fat spreads have also been prepared from a gelled or thickened aqueous phase and a mesomorphic phase consisting of edible surfactants and water (278).

## 9. BALANCED SPREADS

A method of increasing the HDL concentration and the HDL/LDL concentration ratio in human serum by providing a balance between a sufficient and required proportion of cholesterol-free saturated fatty acids in the daily dietary fat of the human and a sufficient and required, but not excessive, proportion of polyunsaturated fatty acids comprising linoleic acid in dietary fat, while the remaining proportion of fatty



acids and energy from the dietary fat is provided by monounsaturated fatty acids comprising oleic acid was patented by Brands University. The patent also describes compositions, including fat compositions and filled dairy products, containing such balanced fatty acid proportions (279).

In 2004, Smart Balance was licensed by Brandeis to produce a blend of natural vegetable oils to improve cholesterol levels (280).

Smart Balance 67% Buttery Spread is ideal for all-purpose use, ie, cooking, pan-frying, recipes, and table use. It offers four important benefits.

- (1) No hydrogenated oil and no trans-fatty acids. Trans-fats can raise LDL “bad” cholesterol and lower HDL “good” cholesterol.
- (2) Optimum balance of the three principal fatty acids: polyunsaturates, mono-unsaturates, and saturates. The patented oil blend enhances the good to bad cholesterol ratio when fat intake is balanced in the diet with total fat limited to 30% of calories and dietary cholesterol to under 300 mg per day.
- (3) Favorable ratio of Omega-6 to Omega-3 fatty acids.
- (4) The taste and texture resembles butter. The light 37% buttery spread contains similar health benefits as the 67% buttery spread but, being lower in fat, can only be used for light frying, sautéing, and table use. It is not recommended for baking.

## 10. DETERIORATION AND SHELF LIFE

In the United States, the “sell by” or “best when used by” date stamped on most margarines is 6–12 months from the date of manufacture. Some lower fat products are given only a 4-month shelf life. Table spreads generally are distributed and merchandised under refrigeration. At higher temperatures the product is more susceptible to oiling out, discoloration, off-flavor development, flavor loss, texture problems, and mold. In a sensory evaluation of polyunsaturated margarines, McBride and Richardson (281) found the high-quality shelf life to be approximately 8 months with storage at 5°C (41°F) and 6 months at 10°C (50°F). Naudet and Biasini (282) evaluated the organoleptic and chemical characteristics of three types of margarine over a 12-month period. Animal–vegetable margarine was acceptable for 20 weeks at 5°C (41°F) but became unacceptable after 12 weeks at ambient temperature. Vegetable oil stick and soft margarines were both acceptable for 6 months in the refrigerator or for 3 months at ambient temperature. Deterioration in flavor score was accompanied by increases in peroxide value and Kreis number. Shelf life of two commercial margarine samples was estimated by calculating induction times based on peroxide value measurements during long-term storage (283). The temperature at which margarine is stored may influence its texture. When fresh production samples of commercial margarine were stored at either 4 or 13°C (39.2 or 55.4°F) these products became softer and harder, respectively, when rheological measurements were conducted at 15°C (59°F) (284). In the

United States it is not uncommon that margarine products are "end-aisle" displayed outside of the refrigerator case during promotional events. This can sometimes lead to recrystallization of higher melting glycerides and an increase in melting point. Organoleptically this results in a heavier sensation on the palate and slower flavor and salt release. If an oil has a significant tendency to form  $\beta$  crystals, this type of abuse will accelerate the development of sandiness in the margarine.

Packaging plays a significant role in maintaining the quality of the product. If margarine is packed in plastic tubs or bottles that transmit light, storage under fluorescent lighting in the dairy case can cause development of off-flavors within days. Products containing liquid soybean oil, liquid canola oil, or other oils with a high linolenic acid content are particularly susceptible to developing a fishy, "light-struck" flavor and aroma. Care also must be exercised in the selection of resins, pigments, adhesives, and other additives used in the manufacture of plastic containers and laminated wrappers for margarine. The package must allow no unapproved components to enter the food and should be free of solventlike odors and metals such as copper. Margarine readily picks up off-flavors and odors from its surroundings, so that care must be exercised during shipping and storage. In a study of volatile halocarbons in margarines, Entz and Diachenko (285) generally found less than 100 ppb, probably contributed by the oil and water as well as packaging materials. In those instances where a high level (1–5 ppm) was found, the products had been obtained from a supermarket adjacent to a dry cleaning establishment.

Discoloration of the margarine surface is another package-related defect observed frequently, particularly in oil-continuous spreads containing low fat and/or salt. Surface darkening occurs when moisture evaporates, leaving an outer layer enriched in the fat phase that contains the coloring matter. This reportedly is alleviated by dispersing a material such as titanium dioxide in the product, which minimizes the change in optical properties as the moisture evaporates (286). The use of a thin, imperceptible, edible fat layer on the surface of tub-type spreads has been suggested as a means of reducing mold susceptibility and discoloration (287). Poot and Verburg (288) have derived a mathematical equation correlating increased water content and lower solid fat content of a margarine to greater moisture loss. The drying rate also is affected by temperature and relative humidity. Discoloration of margarine packed in bowls is not a problem if the container is heat sealed or has a tight-fitting lid. Stick margarine wrapped in laminated foil sometimes is observed to form dark yellow streaks on the surface in the region of overlapping flaps or on product surfaces not in contact with the foil due to irregularities on the surface of the product. Moisture loss is considerably greater for parchment wrapped prints; however, discoloration may be less apparent than when foil is used, because evaporation occurs from the whole surface rather than from highly localized areas (289). Moisture loss during shelf life must also be considered in determining the fill weight required to ensure that the product remains in compliance with existing net weight regulations. One advantage of moisture loss is that it provides a surface that is less prone to proliferation of microorganisms.

Margarine has an excellent health record in regard to food-borne disease of microbial origin. An implication in the British press in 1989 that a case of listeriosis

was caused by contaminated margarine was later concluded to be false (290). Mossel (291) has reported the results of an extensive study of microorganisms found in margarine and has recommended quality control procedures and microbiological specifications. Molds and yeasts, especially lipolytic yeasts, occasionally cause spoilage of margarine. The use of salt and preservatives and the control of the aqueous-phase droplet size can prevent the growth of organisms in an 80% fat product. However, increased vigilance must be exercised in product formulation and inspection of raw materials, packaging, and equipment when lower salt and higher moisture spreads are produced.

The effect of adverse storage and transportation conditions has been reviewed by Faur (292), and Muys (216) has discussed the microbiological storage qualities of margarine. Dritschel (293) has detailed a rating system for evaluation of finished product quality based on appearance, texture, flavor, and packaging.

## REFERENCES

1. *Food and agriculture: Consumer Trends and Opportunities, Fats, Oils, and Sweets*, Cooperative Extension Service, University of Kentucky. (July 2004). Available: [www.ca.uky.edu](http://www.ca.uky.edu).
2. *Oil Crops Situation and Outlook*, Economic Research Server, USDA (Oct. 2000) (updated 2004).
3. International Margarine Association of the Countries of Europe. (2001). Available: <http://www.imacl.org>.
4. A. J. C. Andersen and P. N. Williams, *Margarine*, 2nd ed., Pergamon Press, New York, 1965.
5. S. F. Riepma, *The Story of Margarine*, Public Affairs Press, Washington, D.C., 1970.
6. S. C. Miksta, *J. Amer. Oil Chem. Soc.*, **48**, 169A (1971).
7. K. Snodgrass, *Margarine as a Butter Substitute*, Food Research Institute, Stanford University, 1930.
8. W. Clayton, *Margarine*, Longmans Green, New York, 1920.
9. *Fed. Reg.*, **38**, 25672 (1973).
10. *Fed. Reg.*, **48**, 52692 (1983).
11. *Code of Federal Regulations*, Title 21, Sec. 166.10, 1993.
12. *Fed. Reg.*, **58**, 43580 (1993).
13. *Fed. Reg.*, **58**, 21648 (1993).
14. *Code of Federal Regulations*, Title 21, Sec. 166.40, 1993.
15. *Fed. Reg.*, **41**, 36509 (1976).
16. *Fed. Reg.*, **58**, 2066 (1993).
17. *Fed. Reg.*, **58**, 17096, 17171, 17328 (1993).
18. *Fed. Reg.*, **58**, 44020 (1993).
19. J. M. deMan, J. E. Dobbs, and P. Sherman, in P. Sherman, ed., *Food Texture and Rheology*, Academic Press, New York, 1979, p. 43.

20. American Oil Chemists' Society, *Official Methods and Recommended Practices*, 4th ed., AOCS Press, Champaign, Illinois, Method Cc 16-60, Reapproved 1989.
21. J. M. deMan and A. M. Beers, *J. Texture Stud.*, **18**, 303 (1987).
22. J. M. deMan, *J. Amer. Oil Chem. Soc.*, **60** (1983).
23. A. J. Haighton, *J. Amer. Oil Chem. Soc.*, **36**, 345 (1959).
24. H. Rohm and S. Raaber, *J. Sensory Stud.*, **8**, 81 (1991).
25. B. S. Kamel and J. M. deMan, *Can. Inst. Food Sci. Technol. J.*, **8**, 117 (1975).
26. P. W. Board, K. Aichen, and A. Kuskis, *J. Food Technol.*, **15**, 277 (1980).
27. M. Tanaka, J. M. deMan, and P. W. Voisey, *J. Texture Stud.*, **2**, 306 (1971).
28. J. H. Prentice, *Lab. Pract.*, **3**, 186 (1954).
29. I. Vasic and J. M. deMan, *J. Amer. Oil Chem. Soc.*, **44**, 225 (1967).
30. F. Shama and P. Sherman, *J. Texture Stud.*, **1**, 196 (1970).
31. P. Stern and J. Cmolik, *J. Amer. Oil Chem. Soc.*, **53**, 644 (1976).
32. J. L. Kokini and A. Dickie, *J. Texture Stud.*, **13**, 211 (1982).
33. A. J. Haighton, *J. Amer. Oil Chem. Soc.*, **46**, 570 (1969).
34. L. deMan, J. M. deMan, and B. Blackman, *J. Amer. Oil Chem. Soc.*, **66**, 128 (1989).
35. L. deMan, E. Postmus, and J. M. deMan, *J. Amer. Oil Chem. Soc.*, **67**, 323 (1990).
36. L. deMan, C. F. Chen, and J. M. deMan, *J. Amer. Oil Chem. Soc.*, **68**, 70 (1991).
37. P. Seiden (to Procter and Gamble Ltd.), Canadian Patent 718,372, 1965.
38. J. M. deMan and F. W. Wood, *J. Dairy Sci.*, **41**, 369 (1958).
39. R. B. Lomneth, D. R. Blair, G. L. Parnell, and B. Y. Tao (to Procter and Gamble), U.S. Patent 4,388,339, 1983.
40. R. P. Borwankar, L. A. Frye, A. E. Blaurock, and F. J. Sasevich, *J. Food Eng.*, **16**, 55 (1992).
41. E. S. Lutton, *5th Margarine Research Symposium*, October 17, 1958.
42. B. Balinov, O. Söderman, and T. Wörnheim, *J. Amer. Oil Chem. Soc.* **71**, 513 (1994).
43. P. T. Callaghan, K. W. Jolley, and R. S. Humphrey, *J. Coll. Interface Sci.*, **93**, 521 (1983).
44. K. J. Packer and C. Rees, *J. Coll. Interface Sci.*, **40**, 206 (1972).
45. R. Zschaler, *Fette Seifen Anstrichm.*, **79**, 107 (1977).
46. C. Poot, C. Verburg, D. Kirton, and A. MacNeill (to Lever Brothers), U.S. Patent 4,087,564, 1978.
47. D. P. Moran (to Lever Brothers), U.S. Patent 4,115,598, 1978.
48. J. Henricus, M. Rek, and E. Baas (to Unilever), British Patent 1,358,260, 1974.
49. E. Postmus, L. deMan, and J. M. deMan, *Can. Inst. Food Sci. Technol. J.*, **22**, 481 (1989).
50. *Fed. Reg.*, **50**, 3745 (1985).
51. M. E. Stansby, *J. Amer. Oil Chem. Soc.*, **50**, 220A, 222A, 224A (1973).
52. *Fed. Reg.*, **54**, 38223 (1989).
53. V. Young, *Lipid Technol.*, **2**, 7 (1990).
54. S. S. Chang (to Vitrum AB), U.S. Patent 4,101,673, 1978.
55. M. Takso (to Q.P. Corporation), U.S. Patent 4,623,488, 1986.
56. I. P. Freeman, G. J. van Lookeren, F. B. Padley, and R. G. Polman (to Unilever), European Patent Application 0,304,115 A2, 1989.

57. L. R. Schroeder and D. J. Muffett (to General Mills), U.S. Patent 4,913,921, 1990.
58. R. L. Antrim and J. B. Taylor (to Nabisco Brands), U.S. Patent 4,961,939, 1990.
59. R. L. Antrim, N. E. Lloyd, and J. B. Taylor (to Nabisco Brands), U.S. Patent 4,963,368, 1990.
60. R. L. Antrim and J. B. Taylor (to Nabisco Brands), U.S. Patent 4,963,385, 1990.
61. M. E. Stansby, *J. Amer. Oil Chem. Soc.*, **55**, 238 (1978).
62. F. Orthofer, private communication, 1994.
63. A. Joshi, V. V. R. Subrahmanyam, and S. A. Momin, *J. Oil Technol. Assoc. India*, **25**, 7 (1993).
64. I. Heertje, *Food Struct.*, **12**, 77 (1993).
65. A. C. Juriaanse and I. Heertje, *Food Microstruct.*, **7**, 181 (1988).
66. A. J. Haighton, *J. Amer. Oil Chem. Soc.*, **53**, 397 (1976).
67. J. Lefebvre, *J. Amer. Oil Chem. Soc.*, **60**, 295 (1983).
68. American Oil Chemists' Society, in Ref. 17, Method Cd 10–57, Reapproved 1989.
69. American Oil Chemists' Society, in Ref. 17, Method Cd 16–81, Reapproved 1989.
70. J. C. van den Enden, A. J. Haighton, K. van Putte, L. F. Vermaas, and D. Waddington, *Fette Seifen Anstrichm.*, **80**, 180 (1978).
71. E. S. Lutton, *J. Amer. Oil Chem. Soc.*, **49**, 1 (1972).
72. C. W. Hoerr, *J. Amer. Oil Chem. Soc.*, **41**, 4, 22, 32, 34 (1964).
73. I. Wilton and G. Wode, *J. Amer. Oil Chem. Soc.*, **40**, 707 (1963).
74. T. D. Simpson and J. W. Hagemann, *J. Amer. Oil Chem. Soc.*, **59**, 169 (1982).
75. L. Hernqvist and K. Larsson, *Fette Seifen Anstrichm.*, **84**, 349 (1982).
76. M. Kellens and H. Reynaers, *Fett Wiss. Technol.*, **94**, 94 (1992).
77. U. Riiner, *Lebensm. Wiss. Technol.*, **4**, 175 (1971).
78. M. Vaisey-Genser, B. K. Vane, and S. Johnson, *J. Texture Stud.*, **20**, 347 (1989).
79. L. H. Wiedermann, *J. Amer. Oil Chem. Soc.*, **55**, 823 (1978).
80. M. A. M. Zeitoun, W. E. Neff, G. R. List, and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **70**, 467 (1993).
81. J. S. Aronhime, S. Sarig, and N. Garti, *J. Amer. Oil Chem. Soc.*, **65**, 1144 (1988).
82. B. Weinberg, *Can. Inst. Food Sci. Technol. J.*, **5**, A57 (1972).
83. V. Persmark and L. Bengtsson, *Riv. Ital. Sostanze Grasse*, **3**, 307 (1976).
84. J. M. deMan, *J. Inst. Can. Sci. Technol. Aliment.*, **11**, 194 (1978).
85. L. Hernqvist and K. Anjou, *Fette Seifen Anstrichm.*, **85**, 64 (1983).
86. L. Hernqvist, B. Herslöf, K. Larsson, and O. Podlaha, *J. Sci. Food Agric.*, **32**, 1197 (1981).
87. V. S. Wähnelt, D. Meusel, and M. Tülsner, *Fat Sci. Technol.*, **93**, 117 (1991).
88. K. F. Gander, J. Hannewijk, and A. J. Haighton (to Unilever), Canadian Patent 830,938, 1969.
89. B. Freier, O. Popescu, A. M. Ille, and H. Antoni, *Ind. Aliment.*, **24**, 61, 75 (1973).
90. N. Krog, *J. Amer. Oil Chem. Soc.*, **54**, 124 (1977).
91. S. Lee and J. M. deMan, *Fette Seifen Anstrichm.*, **86**, 460 (1984).
92. J. Hojerová, S. Schmidt, and J. Krempasky, *Food Struct.*, **11**, 147 (1992).

93. K. Sato and T. Kuroda, *J. Amer. Oil Chem. Soc.*, **64**, 124 (1987).
94. C. F. Shen, L. deMan, and J. M. deMan, *Elaeis*, **2**, 143 (1990).
95. F. Cho and J. M. deMan, *J. Food Lipids*, **1**, 53 (1993).
96. J. Ward, *J. Amer. Oil Chem. Soc.*, **65**, 1731 (1988).
97. G. K. Belyaeva, N. I. Kozin, V. S. Golosava, and E. Fal'k, *Maslozhirovaya Promyshlennost*, **38**, 15–16 (1969); through *Food Sci. Technol. Abstr.*, No. 3, N0096 (1970).
98. J. Madsen, *Zesz. Probl. Postepow Nauk Roln.*, No. 136, 147 (1973).
99. W. van der Hoek, *Rev. Fr. Corps Gras*, **16**, 761 (1969).
100. V. D'Souza, L. deMan, and J. M. deMan, *J. Amer. Oil Chem. Soc.*, **68**, 153 (1991).
101. V. D'Souza, L. deMan, and J. M. deMan, *J. Amer. Oil Chem. Soc.*, **69**, 1198 (1992).
102. I. Blanc, *Rev. Fr. Corps Gras*, **16**, 457 (1969).
103. U. Riiner, *Lebensm. Wiss. Technol.*, **3**, 101 (1970).
104. U. Riiner, *Lebensm. Wiss. Technol.*, **4**, 76 (1971).
105. K. G. Berger, *Chem. Ind. (London)*, 910–913 (1975).
106. B. Jacobsberg and Oh Chuan Ho, *J. Amer. Oil Chem. Soc.*, **53**, 609 (1976).
107. D. A. Okiy, *Oleagineaux*, **33**, 625 (1978).
108. P. H. Yap, J. M. deMan, and L. deMan, *Fat Sci. Technol.*, **91**, 178 (1989).
109. E. Sambuc, Z. Dirik, G. Reymond, and M. Naudet, *Rev. Fr. Corps Gras*, **27**, 505 (1980).
110. E. Sambuc, Z. Dirik, G. Reymond, and M. Naudet, *Rev. Fr. Corps Gras*, **28**, 13 (1981).
111. B. Chikany, *Olaj Szappan Kosmet.*, **31**, 107 (1982); through *Food Sci. Technol. Abstr.*, No. 10, N0472 (1983).
112. H. R. Kattenberg and C. Poot (to Lever Brothers), U.S. Patent 4,016,302, 1977.
113. A. Yuki, K. Matsuda, and A. Nishimura, *J. Jpn. Oil Chem. Soc.*, **39**, 236 (1990).
114. J. A. Van Meeteren and L. H. Wesdorp (to Unilever), European Patent Application 0,498,487 A1, 1992.
115. W. Heimann and J. Baltes, *Fette Seifen Anstrichm.*, **73**, 113 (1971).
116. A. Yaron, B. Turzynski, C. Shmulinzon, and A. Letan, *Fette Seifen Anstrichm.*, **75**, 533 (1973).
117. E. G. Latondress, *J. Amer. Oil Chem. Soc.*, **58**, 185 (1981).
118. A. Moustafa, *Rev. Fr. Corps Gras*, **26**, 485 (1979).
119. M. J. Pichel (to Swift), U.S. Patent 3,338,720, 1967.
120. D. Melnick and E. L. Josefowicz (to Corn Products Company), U.S. Patent 3,472,661, 1969.
121. F. V. K. Young, *J. Amer. Oil Chem. Soc.*, **60**, 374 (1983).
122. F. Cho, J. M. deMan, and O. B. Allen, *J. Food Lipids*, **1**, 25 (1993).
123. T. Wieske, in 7th European Symposium, 1977, pp. 214–224.
124. T. Wieske (to Unilever), British Patent 1,479,287, 1977.
125. J. Baltes (to Harburger Oelwerke Brinckman & Mergell), Canadian Patent 846,842, 1970.
126. M. S. Sabasranamam, U.S. Patent Application 2002001662, January 3, 2002.
127. T. A. Pelloso and L. Kogan (to Nabisco Brands), U.S. Patent 4,316,919, 1982.
128. H. Heider and T. Wieske (to Lever Brothers), U.S. Patent 4,230,737, 1980.

129. J. P. McNaught (to Lever Brothers), U.S. Patent 3,900,503, 1975.
130. R. P. Mensink and M. B. Katan, *N. Engl. J. Med.*, **323**, 439 (1990).
131. P. L. Zock and M. B. Katan, *J. Lipid Res.*, **33**, 399 (1992).
132. R. P. Mensink, P. L. Zock, M. B. Katan, and G. Hornstra, *J. Lipid Res.*, **33**, 1493 (1992).
133. A. H. Lichtenstein, L. M. Ausman, W. Carrasco, J. L. Jenner, J. M. Ordovas, and E. J. Schaefer, *Arteriosclerosis Thrombosis*, **13**, 154 (1993).
134. R. Wood, K. Kubena, B. O'Brien, S. Tseng, and G. Martin, *J. Lipid Res.*, **34**, 1 (1993).
135. E. N. Siguel and R. H. Lerman, *Am. J. Cardiol*, **71**, 916 (1993).
136. R. Troisi, W. C. Willett, and S. T. Weiss, *Am. J. Clin. Nutr.*, **56**, 1019 (1992).
137. W. C. Willett et al., *Lancet*, **341**, 581 (1993).
138. J. T. Judd et al., *Am. J. Clin. Nutr.*, **59**, 861 (1994).
139. *Food Labeling News*, **2**(21), 15 (1994).
140. B. F. Haumann, *INFORM*, **5**, 346, 350, 352, 354, 356, 358 (1994).
141. J. Ward (to Nabisco Brands), U.S. Patent 4,341,812, 1982.
142. J. Ward (to Nabisco Brands), U.S. Patent 4,341,813, 1982.
143. H. A. Graffelman (to Lever Brothers), U.S. Patent, 3,617,308, 1971.
144. J. Van Heteren, J. N. Pronk, W. J. Smeenk, and L. F. Vermaas (to Lever Brothers), U.S. Patent 4,386,111, 1983.
145. M. Fondu and M. Willens (to Lever Brothers), U.S. Patent 3,634,100, 1972.
146. R. Keuning, A. J. Haighton, W. Dijkshoorn, and H. Huizinga (to Lever Brothers), U.S. Patent 4,360,536, 1982.
147. W. Dijkshoorn, H. Huizinga, and J. Pronk (to Lever Brothers), U.S. Patent 4,366,181, 1982.
148. A. Nagoh, O. Kaizuka-shi, and M. Miyabe (to Fuji Oil Company), European Patent Application 0,526,980 A1, 1993.
149. R. Schijf and V. K. Muller (to Unilever), European Patent Application 0,470,658 A1, 1992.
150. A. J. Lansbergen and R. Schijf (to Unilever), European Patent Application 0,455,278 A2, 1991.
151. D. K. Yayashi, R. C. Dinwoodie, M. T. Dueber, R. G. Krishnamurthy, and J. J. Myrick (to Kraft General Foods, Inc.), U.K. Patent Application 2,239,256 A, 1991.
152. G. R. List, E. A. Emken, W. F. Kwolek, T. D. Simpson, and H. J. Dutton, *J. Amer. Oil Chem. Soc.*, **54**, 408 (1977).
153. M. A. M. Zeitoun, W. E. Neff, and T. L. Mounts, *Rev. Fr. Corps Gras*, **39**, 85 (1992).
154. W. Stratmann and L. F. Vermaas (to Lever Brothers), U.S. Patent 4,425,371, 1984.
155. P. W. Eilott, M. R. J. Greep, J. A. Van Meeteren, and L. H. Wesdorp (to Unilever), European Patent Application 0,369,519 A2, 1990.
156. B. Sreenivasan (to Lever Brothers), U.S. Patent 3,859,447, 1975.
157. R. DeLathauwer, M. Van Opstal, and A. J. Dijkstra (to Safinco), U.S. Patent 4,419,291, 1983.
158. J. Boot, A. Rozendaal, and R. Schijf (to Unilever) European Patent Application 0,060,139 A2, 1982.
159. L. Kogan and T. Pelloso (to Nabisco Brands), U.S. Patent 4,335,156, 1982.

160. A. Zaks, R. D. Feeney, and A. Gross (to Opta Food Ingredients, Inc.), International Patent Application WO 92/03937, 1992.
161. M. Stahl, German Federal Republic Patent Application 2,935,572, 1981.
162. H. Menz, J. Rost, and T. Wieske (to Lever Brothers), U.S. Patent 3,658,555, 1972.
163. T. Weiske and H. Menz, *Fette Seifen Anstrichm.*, **74**, 133 (1972).
164. G. Von Rappard and W. Kretschner (to Walter Rau), German Federal Republic Patent Application 2,832,636, 1980.
165. B. A. Roberts (to Procter and Gamble), U.S. Patent 4,446,165, 1984.
166. P. D. Orphanos and co-workers, (to Procter and Gamble), U.S. Patent 4,940,601, 1990.
167. R. J. Jandacek and J. C. Letton (to Procter and Gamble), U.S. Patent 5,017,398, 1991.
168. F. W. Cain, F. R. De Jong, A. J. Lanting Marijs, and J. J. Verschuren (to Unilever), European Patent Application 0,415,468 A2, 1991.
169. C. J. Glueck and co-workers, *Am. J. Clin. Nutr.*, **35**, 1352 (1982).
170. R. Nicolosi, *INFORM*, **1**, 831 (1990).
171. A. P. Simopoulos, *Am. J. Clin. Nutr.*, **54**, 438 (1991).
172. D. F. Horrobin, *Prog. Lipid Res.*, **31**, 163 (1992).
173. A. P. Bimbo, *J. Am. Oil Chem. Soc.*, **66**, 1717 (1989).
174. F. V. K. Young, "The Chemical and Physical Properties of Crude Fish Oil for Refiners and Hydrogenators," *International Association of Fish Meal Manufacturers Fish Oil Bull.*, No. 18, Hertfordshire, U.K., 1986.
175. F. D. Gunstone, *Prog. Lipid Res.*, **31**, 145 (1992).
176. S. Patel, "Quality Considerations in Margarine Oil Manufacturing," presented at ISF AOCS World Congress, New York, 1980.
177. American Oil Chemists' Society, in Ref. 17, Method Cg 2-83, Reapproved 1989.
178. L. H. Wiedermann, *J. Amer. Oil Chem. Soc.*, **49**, 478 (1972).
179. *Fed. Reg.*, **38**, 25671 (1973).
180. T. H. Smouse, J. K. Maines, and R. R. Allen (to Anderson Clayton), U.S. Patent 4,038,436, 1977.
181. C. E. Eriksson, *Food Chem.*, **9**, 3 (1982).
182. L. L. Linteris (to Lever Brothers), U.S. Patent 3,721,570, 1973.
183. C. F. Cain, I. J. Day, M. G. Jones, and I. T. Norton (to Unilever), European Patent Application 0,279,499 A2, 1988.
184. J. D. Dziezak, *Food Technol.*, **42**, 172, 174 (1988).
185. J. Madsen, *Fett Wiss. Technol.*, **89**, 165 (1987).
186. A. G. Gaonkar and R. P. Borwankar, *Colloids and Surfaces*, **59**, 331 (1991).
187. M. Schneider, *Fett Wiss. Technol.*, **94**, 524 (1992).
188. W. van Nieuwenhuyzen, *J. Amer. Oil Chem. Soc.*, **58**, 886 (1981).
189. H. W. Lincklaen and J. H. M. Rek (to Lever Brothers), U.S. Patent 3,796,815, 1974.
190. T. Wieske, K. H. Todt, J. A. De Feÿter, and W. A. Castenmiller (to Van den Bergh Foods), U.S. Patent 5,079,028, 1992.
191. H. J. Duin, A. F. van Dam, and J. H. M. Rek (to Lever Brothers), U.S. Patent 4,148,930, 1979.
192. J. H. M. Rek and P. M. J. Holemans (to Lever Brothers), U.S. Patent 4,325,980, 1982.



193. J. Madsen, *Res. Discl.*, No. 238, 91 (1984).
194. B. R. Harris, U.S. Patent 1,917,255, 1933.
195. M. F. Stewart and E. J. Hughes, *Process Biochem.*, **7**, 27 (1972).
196. W. A. Gorman, R. G. Christie, and G. H. Kraft (to National Dairy Products Corporation), U.S. Patent 2,937,093, 1960.
197. W. A. M. Castenmiller, A. K. Chesters, and P. B. Ernsting (to Lever Brothers), U.S. Patent 4,874,626, 1989.
198. K. Brammer and T. Wieske (to Lever Brothers), U.S. Patent 3,889,005, 1975.
199. F. Groeneweg, F. van Voorst Vader, and W. G. M. Agterof, *Chem. Eng. Sci.*, **48**, 229 (1993).
200. J. Van Heteren, T. R. Kelly, R. M. Livingston, and A. B. MacNeill (to Unilever), European Patent Application 0,420,314 A2, 1991.
201. Annon., *Res. Discl.*, No. 329, 689 (1991).
202. Annon., *Res. Discl.*, No. 352, 512 (1993).
203. K. Matsuda and M. Kitao (to Mitsubishi Kasei Corporation), European Patent Application 0,430,180 A2, 1991.
204. K. Terada, S. Fujita, and N. Yoshida (to Asahi Denka), U.S. Patent 3,914,458, 1975.
205. P. F. Pedersen, *Res. Discl.*, No. 321, 64 (1991).
206. B. Barmantlo and N. K. Slater (to Van den Bergh Foods), U.S. Patent 5,145,704, 1992.
207. H. Kanematsu, E. Morise, I. Niiya, M. Imamura, A. Matsumoto, and G. Katsui, *J. Jpn. Soc. Food Nutr.*, **25**, 343 (1972); through *Food Sci. Technol. Abstr.*, No. 12, N0647 (1973).
208. E. R. Sherwin, *J. Amer. Oil Chem. Soc.*, **53**, 430 (1976).
209. J. Cillard, P. Cillard, and M. Cormier, *J. Amer. Oil Chem. Soc.*, **57**, 255 (1980).
210. K. Klaui, *Flavours*, **7**, 165 (1976).
211. G. T. Muys, C. T. Verrips, and R. T. S. van Gorp (to Lever Brothers), U.S. Patent 3,995,066, 1976.
212. C. T. Verrips and H. Vonkeman (to Lever Brothers), U.S. Patent 3,904,767, 1975.
213. W. G. Mertens, C. E. Swindells, and B. F. Teasdale, *J. Amer. Oil Chem. Soc.*, **48**, 544 (1971).
214. D. Melnick (to Corn Products), U.S. Patent 3,243,302, 1966.
215. C. T. Verrips and J. Zaalberg, *Eur. J. Appl. Microbiol. Biotechnol.*, **10**, 187 (1980).
216. G. T. Muys, *Process Biochem.*, **4**, 31 (1969).
217. E. Lueck, *Antimicrobial Food Additives*, Springer-Verlag, New York, 1980.
218. F. Kapp and B. Mittag, *Lebensmittelindustrie*, **29**, 160 (1982).
219. N. E. Harris and D. Rosenfield, *Food Process. Ind.*, **43**, 23 (1974).
220. M. Castenon and B. Inigo, *Lebensmittel Wiss. Technol.*, **6**, 70 (1973).
221. J. Bodor, A. W. Schoenmakers, and W. M. Verhue (to Van den Bergh Foods), U.S. Patent 5,013,573, 1991.
222. Anon. (to Unilever), Australian Patent Application 52102, 1990.
223. P. M. Klapwijk, *Food Control*, **3**, 183 (1992).
224. T. J. Siek and R. C. Lindsay, *J. Dairy Sci.*, **53**, 700 (1970).
225. J. G. Keppler, *J. Agric. Food Chem.*, **18**, 998 (1970).

226. P. G. M. Haring, J. G. van Pelt, and C. F. Andreae (to Unilever), European Patent Application 0,478,036 A2 (1992).
227. H. T. Slover, R. H. Thompson Jr., C. S. Davis, and G. V. Merola, *J. Amer. Oil Chem. Soc.*, **62**, 775 (1985).
228. H. Klaui and O. Raunhardt, *Alimenta*, **15**, 37 (1976).
229. P. H. Todd (to Kalamazoo Spice Extraction Company), U.S. Patent 3,162,538, 1964.
230. E. Sambuc and M. Naudet, *Rev. Fr. Corps Gras*, **12**, 239 (1965).
231. R. Presse, H. Quendt, and H. Raeuber, *Lebensmittelindustrie*, **22**, 34 (1975).
232. G. Gabriel, M. Havenstein, P. M. J. Holemans, and B. E. Kapellen (to Unilever), European Patent Application, 0,422,712 A2, 0,422,713 A2, and 0,422,714 A2 (1991).
233. S. Okonogi et al. (to Morinaga Milk Industry Company, Ltd.), U.S. Patent 5,279,847, 1994.
234. N. T. Joyner, *J. Amer. Oil Chem. Soc.*, **30**, 526 (1953).
235. I. Heertje, J. Van Eendenburg, J. M. Cornelissen, and A. C. Juriaanse, *Food Microstruct.*, **7**, 189 (1988).
236. P. De Bruijne, J. Van Eendenburg, and H. J. Human (to Unilever), European Patent Application 0,341,771 A2, 1989.
237. H. R. Kattenberg and C. C. Verburg (to Lever Brothers), U.S. Patent 4,055,679, 1977.
238. A. D. Wilson, H. B. Oakley, and J. Rourke (to Lever Brothers), U.S. Patent 2,592,224, 1952.
239. B. D. Miller, P. Phelps, and H. W. Bevarly (to Girdler Corp.), U.S. Patent 2,330,986, 1943.
240. K. Terada, S. Fujita, H. Kohno, and H. Sugiyama (to Asahi Denka), U.S. Patent 3,917,859, 1975.
241. K. F. Gander (to Lever Brothers), U.S. Patent 3,488,199, 1970.
242. D. P. J. Moran (to Lever Brothers), U.S. Patent 3,490,919, 1970.
243. G. C. Cramer (to Madison Creamery), U.S. Patent 4,315,955, 1982.
244. R. D. Price and W. L. Sledzieski (to Nabisco Brands), European Patent Specifications 0,139,398 B1, 1988.
245. J. J. Brockhus, D. Schnell, and K. T. Vermaat (to Unilever), European Patent Application 0,505,007 A2, 1992.
246. W. A. Gorman, R. G. Christie, and G. H. Kraft (to National Dairy Products Corporation), U.S. Patent 2,937,093, 1960.
247. C. Bouffard, *Rev. Fr. Corps Gras*, **21**, 351 (1974).
248. L. Faur, *Rev. Fr. Corps Gras*, **27**, 319 (1980).
249. I. Wilton and K. Bauren (to Margarinbolaget), U.S. Patent 3,682,656, 1972.
250. Y. Kahuda et al., U.S. Patent Application 2003016193, August 28, 2003.
251. D. P. J. Moran, *PORIM Technology*, Publication Number 15, Palm Oil Research Institute of Malaysia, 1993.
252. J. Madsen, in D. Erickson, ed., *World Conference Proceedings, Edible Fats and Oils Processing: Basic Principles and Modern Practices*, American Oil Chemists' Society, Champaign, Illinois, 1990, pp. 221–227.
253. W. Altrock and J. A. Ritums (to Lever Brothers), U.S. Patent 4,366,180, 1982.

254. E. L. Josefowicz and D. Melnick (to Corn Products Company), U.S. Patent 3,457,086, 1969.
255. J. G. Spritzer, J. J. Kearns, and O. Cooper, U.S. Patent 3,360,377, 1967.
256. L. L. Linteris (to Unilever), Canadian Patent 871,647, 1971.
257. I. T. Norton (to Van den Bergh Foods), U.S. Patent 5,244,688, 1993.
258. I. T. Norton and J. Underdown (to Van den Bergh Foods), U.S. Patent 5,306,517, 1994.
259. P. B. Ernsting (to Lever Brothers), U.S. Patent 4,883,681, 1989.
260. B. Sreenivasan (to Lever Brothers), U.S. Patent 4,849,243, 1989.
261. I. T. Norton and J. Underdown (to Van den Bergh Foods), U.S. Patent 5,151,290, 1992.
262. B. Milo and R. Ochmann (to Unilever), European Patent Application 0,396,170 A2, 1990.
263. S. Madsen, *Res. Discl.*, No. 330, 774 (1991).
264. I. T. Norton and C. R. T. Brown (to Unilever), European Patent Application 0,271,132 A2, 1987.
265. I. T. Norton (to Unilever), European Patent Application 0,474,299 A1, 1991.
266. D. J. Pettitt, W. Gibson, and I. A. Challen, *Res. Discl.*, No. 301, 338 (1989).
267. F. W. Cain, M. G. Jones, and I. T. Norton (to Lever Brothers), U.S. Patent 4,917,915, 1990.
268. A. L. Morehouse and C. J. Lewis (to Grain Processing Corporation), U.S. Patent 4,536,408, 1985.
269. I. T. Norton and R. M. Livingston (to Unilever) European Patent Application 0,496,466 A2, 1992.
270. F. W. Cain, M. G. Jones, and I. T. Norton (to Unilever), European Patent Application 0,279,499 A2, 1988.
271. M. G. Jones and I. T. Norton (to Van den Bergh Foods), U.S. Patent 5,217,742, 1993.
272. P. M. Bosco and W. L. Sledzieski (to Standard Brands), U.S. Patent 4,279,941 and 4,292,333, 1981.
273. D. E. Miller and C. E. Werstak (to SCM Corporation), U.S. Patent 4,238,520, 1980.
274. D. F. Darling (to Lever Brothers), U.S. Patent 4,443,487, 1984.
275. F. W. Cain et al., (to Lever Brothers), U.S. Patent 4,956,193, 1990.
276. L. H. Wesdorp, R. A. Madsen, J. Kasica, and M. Kowblansky (to Van den Bergh Foods), U.S. Patent 5,279,844, 1994.
277. G. Banach, L. H. Wesdorp, and F. S. Fiori (to Van den Bergh Foods), U.S. Patent 5,252,352, 1993.
278. I. Heertje and L. H. Wesdorp (to Unilever), European Patent Application 0,547,647 A1, 1993.
279. K. Sundram, D. Perlman, and K. C. Hayes (to Brandeis University), U.S. Patent 6,630,192, October 7, 2003.
280. *www.Smartbalance.com*. (accessed July 2004).
281. R. L. McBride and K. C. Richardson, *Lebensm. Wiss. Technol.*, **16**, 198 (1983).
282. M. Naudet and S. Biasini, *Rev.Fr. Corps Gras*, **23**, 337 (1976).
283. M. Maskan, M. D. Öner, and A. K. Aya, *J. Food Qual.*, **16**, 175 (1993).
284. J. A. Segura, M. L. Herrera, and M. C. Añón, *J. Amer. Oil Chem. Soc.*, **67**, 989 (1990).

285. R. C. Entz and G. W. Diachenko, *Food Addit. Contam.*, **5**, 267 (1988).
286. H. M. Princen and M. P. Aronson (to Lever Brothers), U.S. Patent 4,176,200, 1979.
287. A. G. Havenstein, W. Kahle, and D. Schnell (to Unilever), European Patent Application 0,240,089 A1, 1987.
288. C. Poot and C. C. Verburg, *Fette Seifen Anstrichm.*, **76**, 178 (1974).
289. E. L. Josefowicz and D. Melnick (to Corn Products Company), U.S. Patent 3,148,993, 1964.
290. P. Barnes, *Lipid Technol.*, **1**, 46 (1989).
291. D. A. A. Mossel in *Margarine Today: Technological and Nutritional Aspects*, Symposium, Dijon, France, March 21, 1969, pp. 104–125.
292. L. Faur, *Rev. Fr. Corps Gras*, **27**, 371 (1980).
293. M. E. Dritschel, *Food Eng.*, **42**(10), 90 (1970).

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## *Shortenings: Science and Technology*

Douglas J. Metzroth

### 1. INTRODUCTION

#### 1.1. Definition and Characteristics

Shortening is a commercially prepared edible fat used in frying, cooking, baking, and as an ingredient in fillings, icings, and other confectionery items. It may have been so named because, when dough is mixed, water-insoluble fat prevents cohesion of gluten strands, literally “shortening” them and thus generating tender baked goods. Shortening is a typically 100% fat product formulated with animal and/or vegetable oils that have been carefully processed for functionality and to remove undesirable flavor and aroma. Overall, shortening improves the texture and palatability of food products while its calories provide heat and energy to fuel the body.

In its most recognized form, household shortening is a white, relatively soft, plastic solid with a bland flavor and no detectable odor. Some types have a butter-like color and flavor added. Household and industrial all-purpose shortenings are products formulated with properties permitting their use in both frying and baking. Pourable types include clear liquid or fluid (opaque) shortenings. Liquid shortenings are typically used as cooking or salad oils. Fluid or opaque shortenings are pourable products with a small amount of solid fat or emulsifier suspended in oil. Because they are convenient to use, pourable shortenings are increasing in

popularity especially for frying and baking. Shortening is also available in dry form as powder, pellets, or flakes encapsulated in a water-soluble material. Skim milk, cheese whey, corn syrup, soy protein isolate, and cellulose compounds have proven feasible as encapsulating materials (1).

## 1.2. Products with Characteristics Similar to Shortening

Lard, tallow, and ghee are traditional animal fats that have existed for centuries. Like most shortenings, all of these products are 100% fat. Vanaspati, another all-fat product now primarily vegetable-based, is popular in all Eastern countries but especially India and Pakistan. Other commercial shortening-like products are available with fat contents from 5% to 90%. Most of these contain an aqueous phase emulsified in the oil phase. Butter and margarine are water-in-oil emulsions manufactured worldwide, and most areas have legal labeling stipulations fixing their fat content at 80% minimum. Table spreads are formulated with intermediate fat levels generally from 40% to 80% and many popular brands fall within the 50–70% range. New low-fat or dietary products, spreads with fat levels of 5–40%, have recently been developed and are just entering the marketplace.

Animal fats were once the primary source oils in both North America and Europe; however, shortening, margarine, spread, and low-fat, dietary table products are now usually formulated from vegetable oils. Blends containing animal fats are still available and popular in certain areas. In fact, new “blends” of butter and vegetable oil are gaining acceptance. Animal fats and marine oils are important fat sources in many areas of the world, and quality products are available in Latin America, Australia, and Asia based exclusively on or containing significant levels of these oils.

## 1.3. Production of Shortening

Shortening is generally considered an American invention. Table 1 gives U.S. supply and utilization of shortening for the period 1986–2000 (2).

The focus of this chapter is shortening; brief information regarding margarine and other similar products is offered due to similarities in raw materials, usage, production methods, and equipment.

## 1.4. Functionality

*Functionality* is a term food technologists employ to describe how well a product performs in a specific application. Shortening and margarine are often characterized as highly functional products. In baking, margarine and shortening contribute to the quality of the finished product by imparting a creamy texture and rich flavor, tenderness, and uniform aeration for moisture retention and size expansion. Liquid and fluid shortenings are used in salad oils and for restaurant and industrial deep-fat and pan frying. In frying, shortening functions as more than a heat transfer medium; it also reacts with components in the food to develop unique, savory flavors and

**TABLE 1. U.S Supply and Utilization of Shortening (in million lb) (2).**

Year	Supply			Utilization				
	Production			Total Supply <sup>a</sup>	Shipments to		Food Disappearance <sup>a</sup>	
	Vegetable Oil	Animal Fat	Total		Exports	U.S. Territories	Total	Per Capita
1986	4,238	1,136	5,374	5,500	36	10	5,318	22.1
1987	4,233	1,005	5,237	5,374	31	10	5,195	21.4
1988	4,241	1,087	5,328	5,467	40	12	5,270	21.5
1989	4,288	1,027	5,315	5,460	19	13	5,309	21.5
1990	4,729	860	5,589	5,708	21	13	5,558	22.2
1991	5,004	720	5,724	5,841	31	8	5,654	22.3
1992	4,988	731	5,719	5,866	33	10	5,722	22.3
1993	5,818	706	6,524	6,626	37	7	6,488	24.9
1994	5,658	676	6,334	6,427	32	14	6,291	23.9
1995	5,316	659	5,975	6,065	33	12	5,914	22.2
1996	5,327	603	5,929	6,035	40	3	5,911	21.9
1997	5,034	622	5,656	5,737	39	3	5,603	20.5
1998	5,208	516	5,724	5,815	54	2	5,668	20.5
1999	5,447	498	5,945	6,037	65	1	5,886	21.1
2000	6,105	488	6,593	6,679	69	1	6,512	23.1
2001								

<sup>a</sup>Computed from unrounded data.

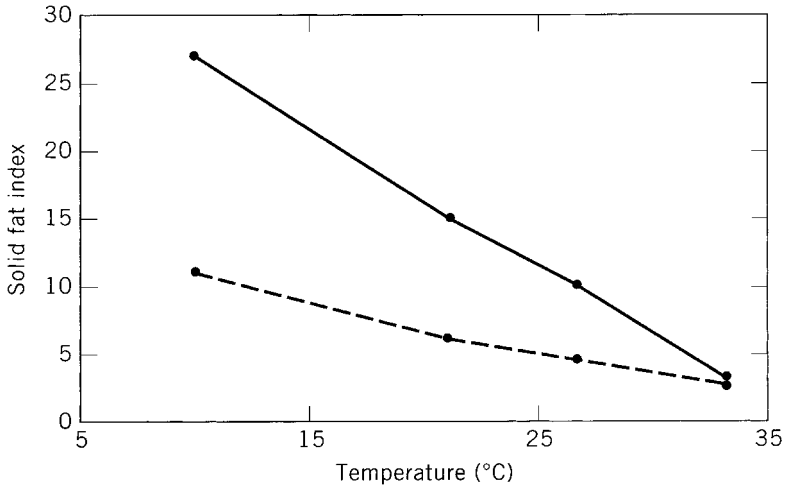
Source: USDA/Economic Research Service.

odors. Dry shortenings are convenient to store and use. Grease will not soak packaging materials, and although expensive, it can be used in prepackaged cake, biscuit, and pie crust mixes, which are free-flowing at room temperature. Those fat-based products formulated and processed for plasticity spread readily and disperse thoroughly and uniformly in dough, batter, icing, and so on over a wide temperature range.

### 1.5. Solid Fat Profiles for Margarine

The fat in shortening and margarine products exists in both liquid and solid form. The solid fat index (SFI) is an analytical measure approximating the solid fat content. It is always less than the actual solid content and, to be meaningful, must be determined at several standard temperatures, usually 10°C (50°F), 21.1°C (70°F), 26.7°C (80°F), 33.3°C (92°F), 37.8°C (100°F), and sometimes 40°C (104°F).

The SFI measurements for table margarine are usually determined at 10°C (50°F) as an indication of consistency during crystallization and refrigeration, at 21.1°C (70°F) to simulate room conditions during use, and at 33.3°C (92°F) to approximate “mouth feel” or eating quality. If the 33.3°C (92°F) SFI level is too high, the margarine will melt slowly in the mouth, often creating a “waxy” sensation. SFI curves for stick table-grade margarine are generally steep with solids



**Figure 1.** Typical solid fat indices for margarine oils. (Courtesy of Kraft Food Ingredients, Memphis, Tennessee.) —, Table-grade stick; ----, soft tub.

levels from about 30% at 10°C (50°F) to less than 5% at 33.3°C (92°F) (3). Soft tub margarine oils have less steep SFI curves for a smooth, more plastic consistency. The SFI curves in Figure 1 are typical for U.S. tub- and stick-type margarine.

### 1.6. Solid Fat Profiles for Shortening

The SFI profile is a good indicator of the plastic range of a fat formulated for shortening. *High-stability* shortenings have a steep SFI profile and a narrow plastic range. Typical all-purpose plastic shortenings retain much of their solid fat content over a wider temperature range than high-stability types and consequently possess much flatter profiles. *Liquid* pourable shortenings include clear oils as well as *fluid* or *opaque* types. Pourable shortenings contain low solids levels with very flat SFI profiles. Specialty shortenings have been formulated for specific applications including cakes, dry cake mixes, bread, Danish and puff pastry, pie crust, cookies, crackers, icing, creams and fillings, coating fat, nondairy products, and frying. Specialty shortenings may be of any general type depending on the requirements for that specific application, and their SFI profiles will be characteristic of that particular type.

*High-Stability Shortenings.* A steep SFI profile is indicative of a narrow plastic range. Products with this type of profile are often referred to as high-stability shortenings. The SFI values may be 50 or greater at 10°C (50°F) but usually less than 10 at 40°C (104°F). As their SFI profiles indicate, these shortenings are not intended to be workable over a wide temperature range. They tend to be hard and brittle below 18.3°C (65°F) and soft above 32.2°C (90°F). High-stability shortenings are used for deep frying, as center fat for confectionery and bakery items, replacements for butter and coating fats, in vegetable/dairy systems, and for crackers and hard cookies.



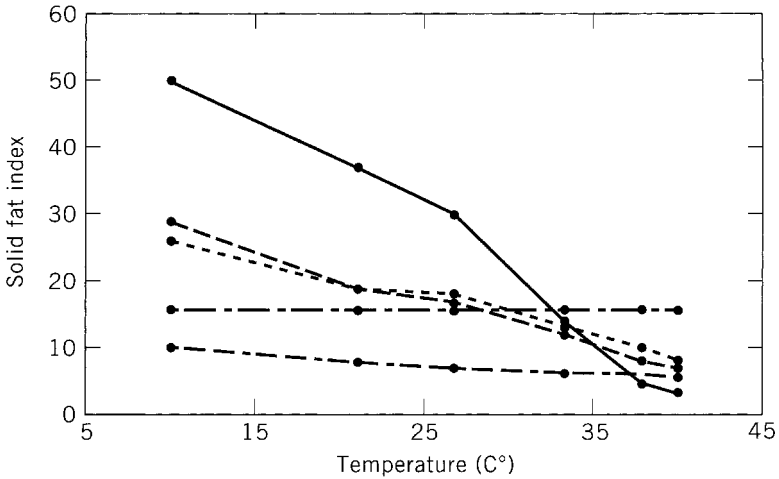
*All-Purpose Shortenings.* All-purpose shortenings were developed for household use and to allow production of a wide variety of baked goods by firms that cannot stock individual types formulated for every specialty item. Typical all-purpose plastic shortenings contain 15–30% solid (crystalline) fat and retain many of these solids over their intended temperature usage range of 16–32°C (60–90°F) (4). A wide plastic range is essential as these products must resist breakdown during creaming and are subject to wide temperature variations in the workplace and during shipping and storage. All-purpose shortenings for baking contain emulsifiers to enhance creaming ability and to improve air retention. As they reduce the smoke point, emulsifiers should be omitted from the formulation when this type shortening is used for deep frying. All-purpose shortenings are formulated as a compromise of individual properties but yet to possess capabilities making them suitable for frying, baking, and confectionery uses.

*Pourable Shortenings.* It is not unusual to classify an edible fat or oil as shortening simply to differentiate it from products such as margarine that contain moisture and other nonfat materials. Liquid shortenings include clear oils as well as fluid, opaque pourable products. The SFI profiles for clear oils are very flat as they normally contain very low levels of oil-soluble emulsifiers or hard fat. Clear oils can be used in household grilling and frying and in institutional deep frying provided the turnover rate is high enough (15–25%) so that stability is not a concern. The flavor and oxidative stability of such oils is greatly improved by partially hydrogenating soybean, safflower, corn, sunflower, or other source oils. After hydrogenation, the oil is fractionated and the clear liquid oil is separated from solid portion. Above 16°C (60°F), these oils are usually free of suspended solids.

Fluid shortenings can be distinguished from liquid shortenings by their opacity resulting from the suspension of high-melting emulsifiers or fully hydrogenated fats. The total amount of suspended solids ranges between 5% and 15%. These products are usually fluid between 18.3°C (65°F) and 32.2°C (90°F); outside of this range, opaque shortenings may lose their pourability or become more fluid depending on how temperature has altered their solid content. Fluid shortenings are widely used in commercial frying but have also been formulated and marketed successfully for baking cakes, bread, buns, rolls, and pie crust.

*Specialty Shortenings.* *Roll-in* shortenings are specialty products used almost exclusively for baking. Their primary use is as an ingredient in puff pastry. Puff pastry is prepared by placing a layer of shortening on a layer of dough. This is folded and sheeted until there are more than 700 fat–dough layers. When baked, the shortening melts, liberating moisture that becomes steam “puffing” the thin dough layers into a very delicate flaky structure. The SFI profile for puff pastry is fairly flat with solid levels of 40% or higher at 10°C (50°F) to about 20% at 33.3°C (92°F).

Dry shortenings are fats that have been encapsulated in a water-soluble coating material; their fat content is generally between 75% and 80%. Dry shortenings are used in ready-to-use mixes where only water is added to form a batter ready for baking.



**Figure 2.** Typical solid fat indices for shortening. (Courtesy of Kraft Food Ingredients, Memphis, Tennessee.) —, High stability frying; ---, all-purpose; ····, all-purpose emulsified; -.-.-, bread; - - - -, liquid (fluid) frying.

Ambient temperatures, source oils, performance requirements, and storage conditions change throughout the world, requiring SFI profile adjustment to meet varying product needs. Typical SFI shortening profiles are shown in Figure 2.

## 2. PLASTIC THEORY

### 2.1. Plastic Solids

Modern edible fats are blends of one or more of about a dozen common oils. Those with higher fat levels such as shortening, margarine, and spreads are formulated to possess special physical characteristics. These products appear to be solid yet, when subjected to a shearing force great enough to cause a permanent deformation, all assume the rheological flow characteristics of a viscous liquid. Such solids are referred to as *plastic solids*. Their plastic nature enables them to spread readily and combine thoroughly with other solids or liquids without cracking, breaking, or liquid oil separating from the crystalline fat. These solids are usually relatively soft at ambient temperature and may actually contain as little as 5% solidified fat; assuming that fat crystals are uniform spheres packed in a close cubical pattern, the theoretical maximum solid content is slightly more than 52%.

### 2.2. Process Definition for Shortening

Shortening is a classic example of a plastic solid. In fact, from a process view, shortening can be defined as a highly functional plastic solid commercially

prepared by carefully cooling, plasticizing, and tempering correctly formulated blends of molten, edible fats and oils.

### 2.3. Conditions Essential for Plasticity

Plastic solids derive their functionality from their unique plastic nature. Three conditions are essential for plasticity (5): (1) both liquid and solid phases must be present; (2) the solid phase must be so finely dispersed that the entire solid-liquid matrix can be effectively bound together by internal cohesive forces; and (3) proper proportions must exist between the phases. Incorrect phase ratios adversely influence product rheology. For example, deficient solids content may result in oil separation, whereas excessive solids can cause hardness or brittleness instead of the desired viscous flow.

### 2.4. Influence of Crystal Size

Crystal size has a major influence on the rheological properties of plastic solids and is therefore a critical factor that must be considered in their formulation. Fats exist as a three-dimensional liquid-solid matrix in which the liquid oil must be contained. Statically cooled molten fats always form large crystals; crystal population is low and, when the total surface area is insufficient to bind the liquid phase within the crystalline matrix, oil separation occurs. Products become progressively firmer as the crystal size decreases. Rapidly chilling the same fat produces many more and much smaller crystals with a far greater combined surface area enabling the liquid phase to adhere more effectively to the crystal surface. A rapidly chilled shortening formulation will be more stable, much firmer, and possess a greater plastic range than a statically or slowly cooled fat. Typical commercially prepared shortenings, especially those formulated from vegetable or marine oils, usually have mean crystal sizes ranging from 5  $\mu\text{m}$  to 9  $\mu\text{m}$  (6).

## 3. FORMULATION

### 3.1. Crystalline Nature

Commercial fats solidify in several crystalline polymorphic forms. Two desirable stable forms are commonly designated by the Greek letters beta ( $\beta$ ) and beta-prime ( $\beta'$ ). Table 2 (7) lists many common fats and oils and their most commonly exhibited polymorphic crystalline form.

When the higher melting portion of a solidifying fat crystallizes in a stable  $\beta'$  form, the entire fat will crystallize in this same  $\beta'$  configuration. Plastic shortenings in this polymorphic form consist of small, uniform needlelike crystals, exhibit a smooth texture, aerate well, have excellent creaming properties, and make good cake and icing shortenings. Palm or cottonseed oils in their liquid and partially hydrogenated forms are often included in shortening and margarine formulations to promote  $\beta'$  crystallization ensuring these qualities.

**TABLE 2. Classification of Fats and Oils According to Crystal Habit (7).**

$\beta$ Type	$\beta'$ Type
Soybean	Cottonseed
Safflower	Palm
Sunflower	Tallow (beef)
Sesame	Herring
Peanut	Menhaden
Corn	Whale
Canola	Rapeseed (high erucic acid)
Olive	—
Coconut	Milkfat (butter oil)
Palm kernel	—
Lard	Modified lard
Cocoa butter	—

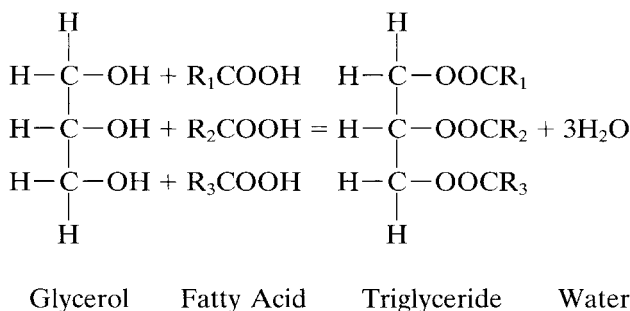
Fats that crystallize in the  $\beta$  polymorphic form tend to be more coarse textured with large granular crystals. They are poor aerators; yet they function well in pie crust applications. Lard crystals tend to be large and grainy, but pie crusts formulated from it have earned wide acceptance because of their flaky texture.

The degree to which these crystallization tendencies will be exhibited is influenced somewhat by blending but more significantly by the hardness of the fat in the formulation. For example, fluid margarine, stick products with 75–80% oil, and soft polyunsaturated tub types can be formulated by blending a high level of liquid oil with a low level of a high-melting-point fat. Stick and soft tub products containing 50% oil can be blends comprising an intermediate level of a liquid oil plus an intermediate level of a moderately hard fat. Base stock blending is the basis for the successful formulation of stick margarine using 100% soybean oil—an oil with a definite predisposition toward  $\beta$  crystallization (8).

### 3.2. Fatty Acid Distribution

Fats and oils are essentially triglycerides—glycerol molecules to which three fatty acids are attached. The symbolic representation of this chemical structure is shown in Figure 3.

Three different fatty acid chains are represented in Figure 3 by  $R_1$ ,  $R_2$ , and  $R_3$ . In actuality, either two or all three may be the same. However, each source oil has characteristic fatty acid compositions and distributions within their triglyceride molecules that influence the melting point and crystalline structure of the solidified fat in ways that are not always beneficial or desirable. For example, if all of the fatty acid chains represented by the letter R are different or  $R_1$  and  $R_2$  the same but different than  $R_3$ , the triglyceride is asymmetrical. Fat blends with a high proportion of asymmetrical triglycerides tend to develop a granular consistency on cooling that is objectionable for most shortening and margarine compositions. Palm or cottonseed oil is often included in blends because their  $\beta'$  behavior promotes small needlelike



**Figure 3.** Structure of a triglyceride.

crystals that result in smooth-textured products. One reason for this behavior may be that palm and cottonseed oils have a high ratio of symmetrical triglycerides (9).

*Fat* and *oil* are interchangeable terms. The distinguishing criterion is their physical state at ambient temperature; oils are usually thought of as liquids while fats are considered as solids. Table 3 (10) lists the melting point, titer, and iodine value (IV) of many oils commonly used to formulate shortening. Melting points for liquid oils are usually not measured, but titer and iodine value are general indicators of the relative fluidity of these oils. Titer is an analytical method for determining the congeal point of fats and for measuring the melting point of fatty acids. Although the melting point of a triglyceride is not the same as its titer, it generally approximates within a few degrees the actual melting point of harder fats. A high IV is a

**TABLE 3. Melting Point, Titer, and Iodine Value of Selected Oils (10).**

Fat or Oil	Melting Point (°C)	Titer (°C)	Iodine Value
Coconut	24–27	20–24	7.5–10.5
Palm kernel	24–26	21–27	14–22
American lard	36.5	36–42	46–70
Tallow	—	40–46	35–48
Butter oil	38	34	33–43
Palm oil	38–45	43–47	48–56
Sunflower	—	16–20	125–136
Sesame	—	20–25	103–116
Corn	—	14–20	103–128
Safflower	—	—	140–150
Rapeseed	—	11.5–15	97–108
Soybean	—	—	120–141
Cottonseed	—	30–37	99–113
Peanut	—	26–32	84–100
Cocoa butter	—	45–50	35–40
Herring	—	25	115–160
Menhaden	—	32	150–165
Whale	—	—	110–135
Olive	—	17–26	80–88

characteristic of a liquid oil while a low IV is indicative of a solid fat. Some fats such as lard, palm, and cocoa butter are solids only at the highest ambient temperatures while sesame, soybean, and peanut are clearly liquid except in cold climates.

To achieve shortening products with desirable physical and functional end properties, the melting and crystallization habits of commercial fats are manipulated through hydrogenation, fractionation, interesterification, or combinations of these processes. Each process is a legitimate topic for study. Without these processes, or more specifically, without the modified oil products obtained from them, margarine and shortening manufacture would be much more difficult. This chapter deals briefly and only in a general way with these processes and the role of each in the formulation of edible fat products. It is assumed that properly modified oil products are available and used correctly.

Producers normally rely on only a few source oils indigenous to their geographic area or that can be imported economically. Soybean is the primary oil used in the United States while very little palm is consumed and none is produced. Canada's major oil is canola (low-erucic-acid rapeseed). Malaysia, Indonesia, and Central America are the largest producers and users of palm oil. Eastern Europe, like Canada, relies on low-erucic-acid rapeseed (LEAR), sunflower, and soybean oils. It is apparent from Table 3 that it is virtually impossible to formulate products with controlled melting and crystalline properties using only one of these oils. Even in areas where conditions and economics justify a variety of types, modification methods other than blending are essential to adequately control rheologic properties.

### **3.3. Fractionation**

Gasoline, lubricating oil, fuel oil, diesel fuel, and various solvents are all familiar products obtained from petroleum. Several processes are employed in a petroleum oil refinery to separate and recover these useful "fractions" from the base oil.

Edible oils also contain liquid and solid fractions that can be separated by a fractionation process. Dry fractional crystallization is a process in which two or more components with different melting points are cooled and separated based on their solubility or crystallization at different temperatures. Fractional crystallization is frequently applied to palm oil to separate liquid palm olein from solid palm stearin.

Solvent fraction is a process in which the various fractions are separated by dissolving the triglyceride in a solvent. This solution is then carefully cooled until the desired fraction precipitates. The precipitate is recovered by filtration. Solvent fractionation can be applied to virtually any edible oil (11).

### **3.4. Hydrogenation**

Hydrogenation is a chemical process in which hydrogen gas is reacted with oils to increase their oxidative and thermal stability by converting liquid components to semisolid fractions. The melting and crystalline characteristics developed are essential for formulating shortenings with specific desirable physical and functional properties.

It is a catalyzed reaction dependent on catalyst type, temperature, time, pressure, agitation, and the starting oil. Platinum and palladium catalysts have been used but nickel, supported on an inert carrier, is now much more common. The catalyst must be removed after hydrogenation usually by filtration.

Each carbon atom in a fatty acid chain can be bonded to as many as four other atoms—two hydrogen and two carbon. When four bonds are present, they are referred to as single bonds and the fatty acid chain is saturated with hydrogen atoms. Naturally occurring triglycerides contain unsaturated fatty acid chains with carbon atoms interconnected by double bonds. In the hydrogenation reaction, hydrogen gas reacts with triglycerides at these selective points of unsaturation in their fatty acid chains.

Hydrogenation can be conducted in batch converters or continuous reactors. The reaction is controlled by stopping the flow of hydrogen gas. As hydrogen is added at the double-bond sites, the melting point of the original oil or fat gradually increases. If only a small amount of hydrogen is added to liquid oils such as soybean or cottonseed, the end product can still remain liquid. As more hydrogen reacts, more saturation is achieved, and soft base fats suitable for shortening formulations will be obtained. Hydrogenation can be continued until all of the double bonds have been saturated with hydrogen and the oil “fully” hardened. Fully hardened products are solids at room temperature and, although generally hard and brittle, are still useful formulation tools.

As the hydrogenation reaction is exothermic, it affords interesting possibilities for energy conservation. This heat of reaction can be used to preheat the feed oil, which in turn cools the hydrogenated fat. Systems are also available that use this heat to produce steam.

Hydrogenation is the most widely used and practical method of preparing fats and oils capable of imparting essential physical and functional properties to shortening. It is presently used to modify and stabilize marine, animal, and all types of vegetable oils. An excellent synopsis of batch and continuous hydrogenation processes is presented by Edvardsson and Irandoust (12).

### 3.5. Interesterification

Interesterification is an effective tool for raising and/or lowering the melting points of edible oils. Like hydrogenation, it is a catalyzed chemical reaction; however, it alters fats by rearranging the fatty acid distributions in their triglyceride molecules. This rearrangement can be effected in a random or directed manner. Total randomization is the most widely used practice but either randomization process results in profoundly different triglyceride compositions, which follow the laws of probability based on the composition of the starting triglycerides.

The random rearrangement reaction can be conducted in continuous or batch reactors. The batch reaction vessel is agitated and fitted with a nitrogen sparger and coils for heating and cooling. Moisture, which poisons the alkaline catalyst, is removed by heating the fat or oil blend under vacuum. After drying and cooling to the reaction temperature, the catalyst is added to the reaction vessel and the

oil-catalyst mixture vigorously agitated for 30–60 min. In the continuous reactor, the fats are flash dried and the fat-oil slurry is formed continuously by adding catalyst to the oil as it passes through coils sized to provide adequate time for randomization. When the reaction is completed, the catalyst is neutralized with water or acid and the salts formed or removed by filtration or centrifugation.

In directed rearrangement, the randomization process is interrupted by selectively removing one or more of the reaction products through continuous distillation or fractional crystallization. The remaining reactants continue to randomize promoting the formation of specific glycerides.

Palm oil production has increased significantly in the last 10 years and may soon exceed that of all other edible oils. Palm oil is also the only  $\beta'$  type whose crystal habit is not changed by interesterification. Interesterification has no effect on the oils in the  $\beta$  classification either; however, randomization in the presence of another oil can moderate their  $\beta$  tendency (13).

Interesterification is regularly used to process palm, palm-kernel, and coconut oils for use in various types of confectionery, margarine, cooking and frying fats, and as blends with lauric oils in reduced-calorie spreads. These three oils crystallize slowly, are often difficult to chill and package, and tend to become hard and grainy during storage. Interesterification often reduces or eliminates these undesirable characteristics (14).

## 4. MANUFACTURING PROCESSES AND EQUIPMENT

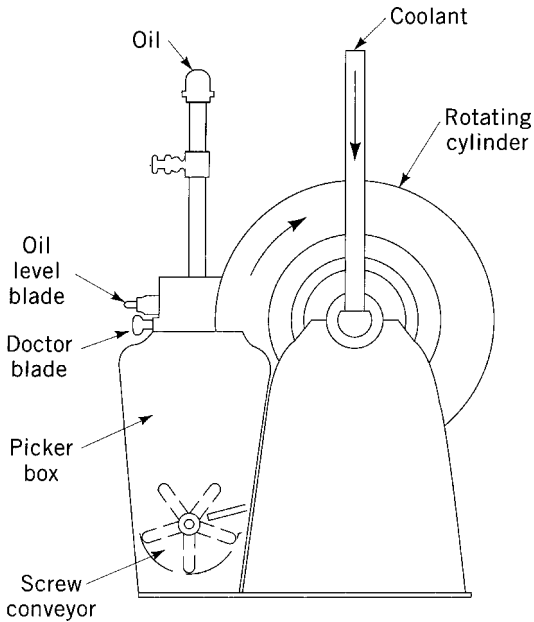
### 4.1. General

The ultimate consistency attainable depends on the fats and oils in the formulation, the processes to which these have been subjected, the equipment and conditions used to solidify them, and the conditions under which these products are stored prior to utilization. Properly formulated liquid blends can be converted to true plastic solids only when the apparatus employed provides controlled cooling, crystallization, and working techniques. The manner in which these plasticity and crystallization theories have been applied and employed in practice can be discerned by examining commercial production apparatus.

### 4.2. Anco Cooling Roll

The cooling roll is one of the earliest unions of equipment and theory actually applied to solidifying lard and shortening. Anco, with a commercial installation in 1881 in Chicago, was a pioneer supplier of this type of apparatus. As shown in the sketch in Figure 4, the device consisted of a hollow, internally refrigerated cast-iron cylinder rotating at 7–11 rpm in a trough containing molten fat slightly above its melting point. As this cylinder revolved, a thin film of fat solidified on its surface and was continuously removed by a doctor blade. The solidified fat film dropped into a special screw conveyor called a picker box. The flights of the

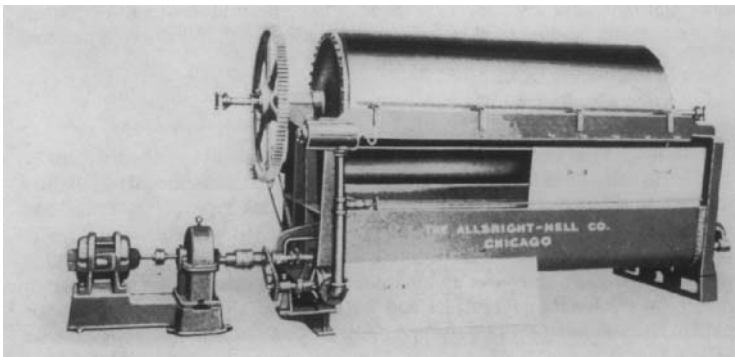




**Figure 4.** Anco cooling roll sketch. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)

conveyor were interspersed with blades that incorporated air while beating and working the fat. High-pressure pumps then forced the fat through orifices, slots, screens, and valves to break crystal aggregates and further disperse the entrapped air.

Figure 5 is a photograph of a roll with its ancillary equipment. Cylinder sizes ranged from 610 mm (2 ft) in diameter by 762 mm (2.5 ft) long to 1219 mm (4 ft) in diameter by 2743 mm (9 ft) long. Capacities for lard ranged from 454 kg/h to 6350 kg/h (1000–14,000 lb/h) and for shortening from 227 kg/h to 3175 kg/h



**Figure 5.** Anco cooling roll. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)

(500–7000 lb/h). Although still employed to produce fat flakes, the cooling roll is now virtually extinct having been replaced almost entirely by the Votator process for crystallizing and plasticizing shortening and margarine.

### **4.3. Votator Process**

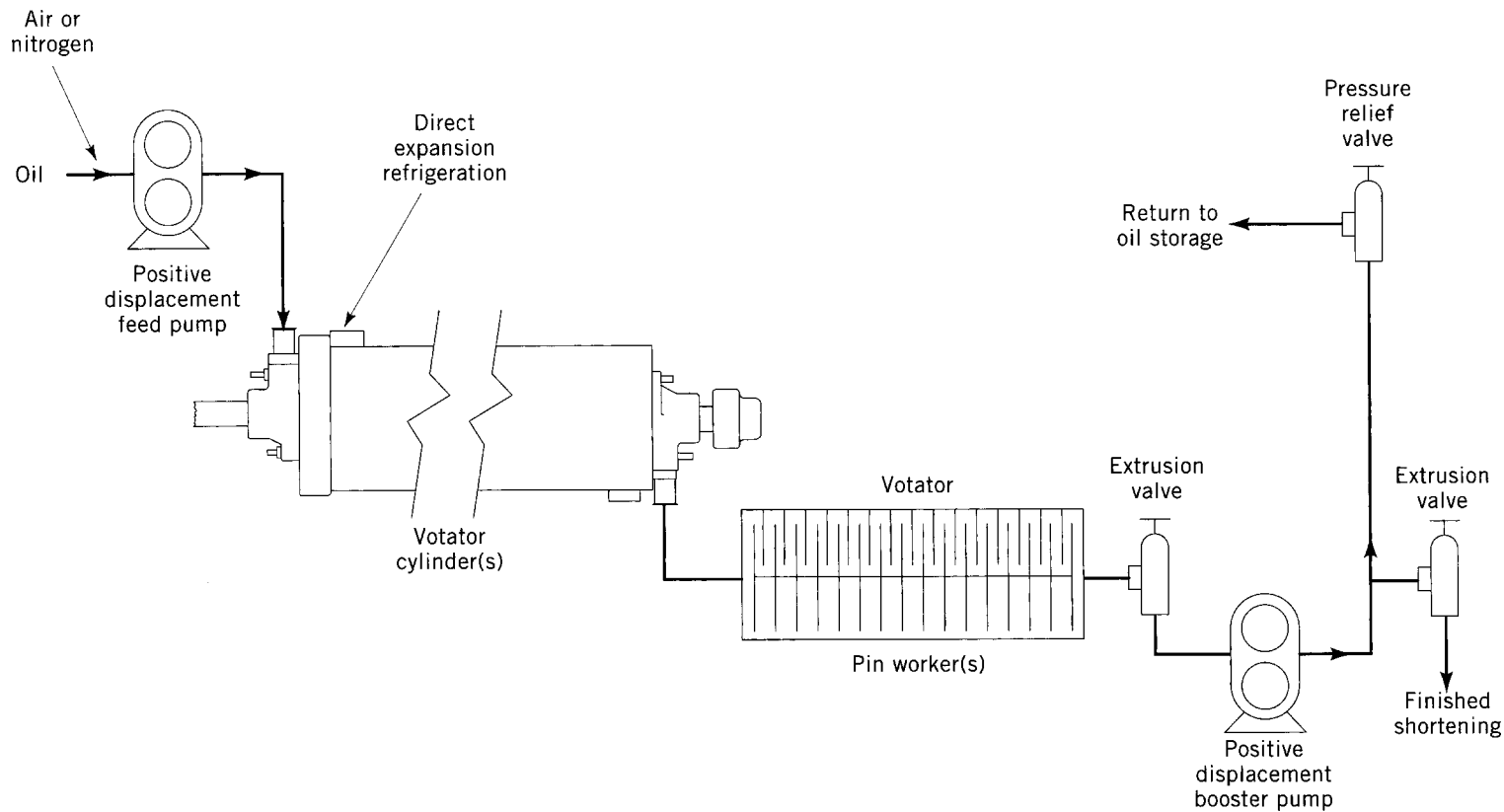
More than 60 years ago, Votator invented what is still the world's only closed continuous process for cooling, crystallizing, and plasticizing edible fat. A simplified diagram for this process is shown in Figure 6. This system employs Votator scraped-surface heat exchangers (A units) for cooling and agitated holding units (B units) for working and plasticizing the product as it crystallizes. Positive-displacement pumps develop high internal product pressure within the cooling and working units that, when combined with special extrusion valves, ensure that the crystallized shortening will be free of crystal aggregates, uniformly aerated, and possess the desired texture and plastic structure.

### **4.4. Votator Scraped-Surface Heat Exchanger**

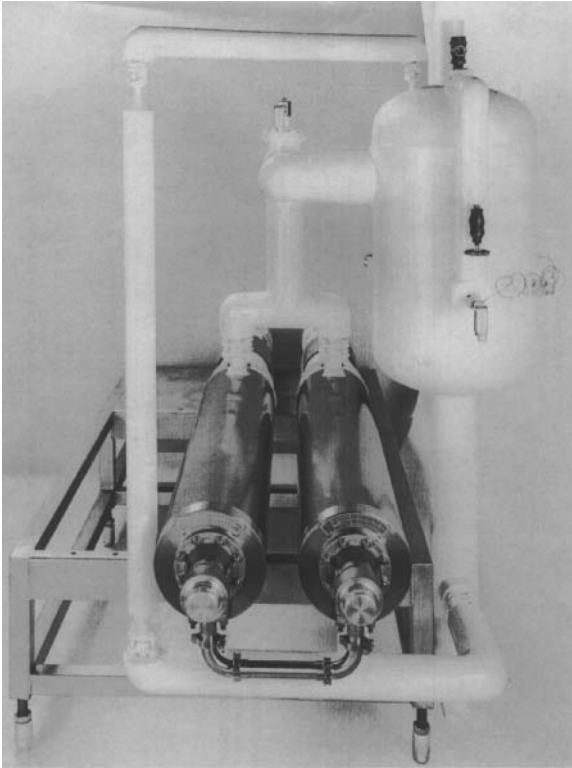
Scraped-surface heat exchangers are the most commonly used devices for chilling edible fats. Votator manufactured the first such heat exchanger in the early 1920s. The name has since become synonymous with the device and many scraped-surface heat exchangers are now commonly referred to as "Votators."

Figure 7 is a photograph of a two-cylinder Votator with a gravity ammonia refrigeration system. Its basic construction is shown in Figure 8, which is a cross-sectional view of a Votator scraped-surface heat exchanger. Each cylinder consists of a hollow cylindrical tube usually 152 mm (6 in.) in diameter by 1829 mm (72 in.) long. This tube is externally jacketed for cooling using brine or direct expansion refrigerants such as ammonia. As the molten oil formulation passes through the tube and cools, an electric motor rotates a shaft centrally located inside the product tube. This "mutator" shaft is fitted with mechanical seals at each end and floating blades that, as the shaft spins, constantly clean the heat transfer surface by scraping and removing the product film from the tube wall. Each mutator shaft has two effective rows of 152-mm (6-in.) long blades staggered along its entire length. This staggered blade arrangement provides improved mixing over the older conventional in-line blade mounting system. All margarine and shortening cooling units are equipped with a hollow mutator shaft and a rotary joint through which hot water can be circulated to prevent solids buildup on the shaft body. Standard shafts are 119 mm (4.6875 in.) in diameter and are rotated at about 400 rpm. Larger and smaller diameter shafts with three and four rows of in-line or staggered blades are available for special applications.

Shortening units are constructed of carbon steel. The water phase in margarine is corrosive, and sanitation procedures require that all of the equipment used to manufacture it must be chemically cleaned. Margarine processing units contain chromium-plated commercially pure nickel heat transfer tubes and stainless steel for all product-contacted metal surfaces.



**Figure 6.** Votator shortening process—block flow diagram. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)



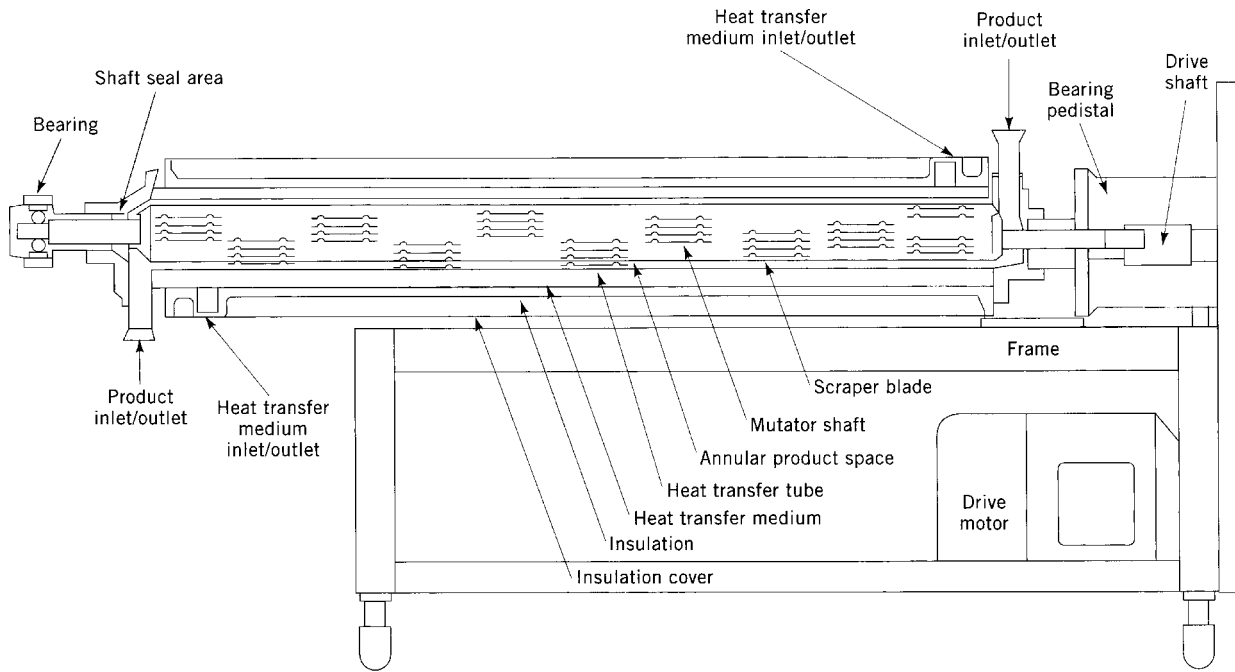
**Figure 7.** Votator scraped-surface heat exchanger. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)

#### 4.5. Supercooling and Direct Expansion Refrigeration

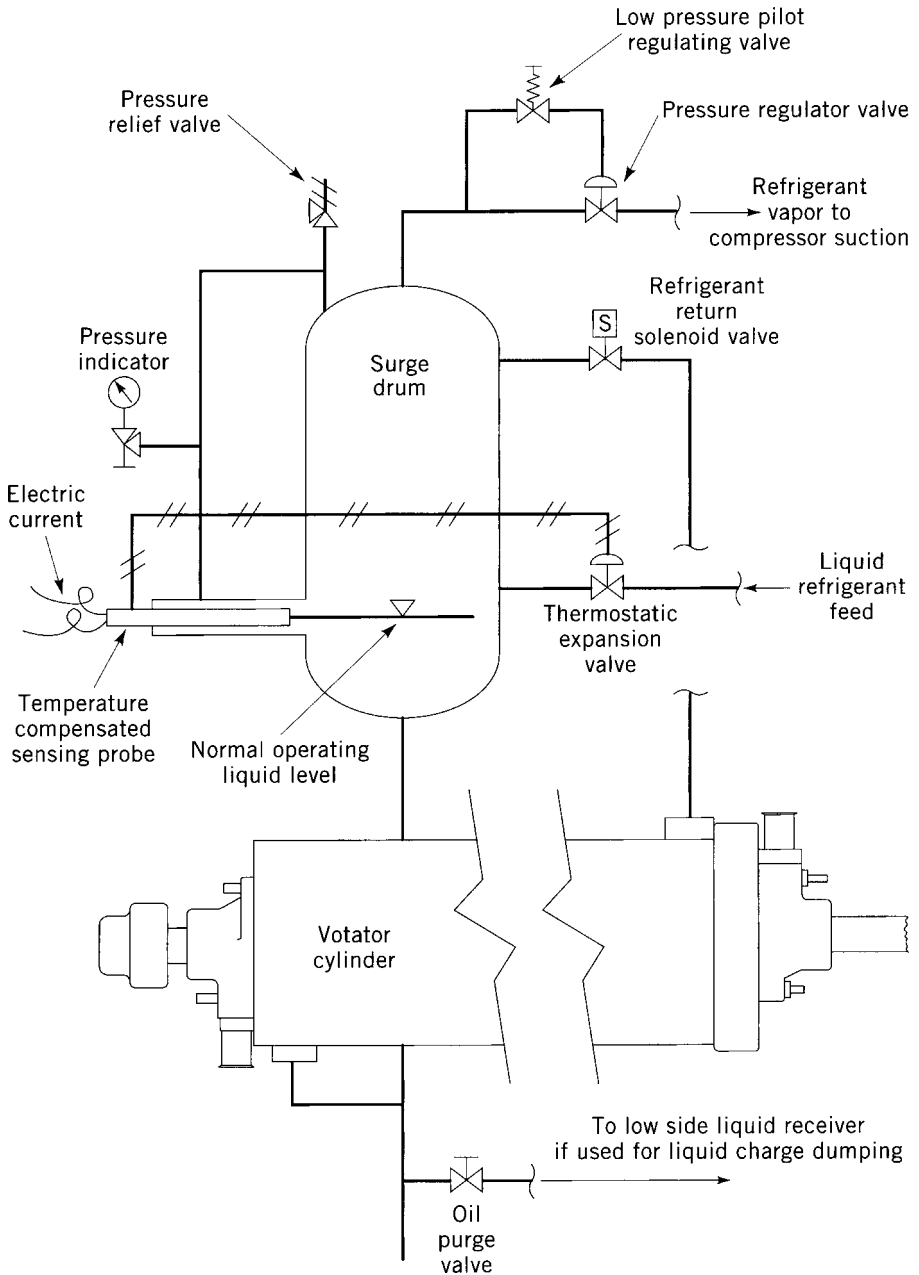
In order to form small crystals, shortening must be cooled very rapidly; so rapidly in fact that the flow at the exit of the Votator must contain virtually no crystals although cold enough for significant levels of solid fat to exist. In reality, the heat exchanger must be capable of supercooling the molten shortening formulation. Fortunately, all triglycerides exhibit a definite propensity for supercooling but, to achieve it, the residence time within the cooler must be limited to invariably less than 20 s. This mandates highly efficient heat exchangers and the use of refrigerants with favorable physical properties and effective heat transfer characteristics. Ammonia and chlorofluorocarbons fulfill these demands and are widely used as refrigerants in heat exchangers for cooling shortening.

This cooling concept is commonly called *direct expansion refrigeration*. Currently there are four basic variations employed: gravity, forced circulation, pool boiling, and liquid overfeed.

*Gravity Refrigeration System.* The gravity refrigeration system shown in Figure 9 is the simplest to comprehend. Liquid refrigerant flows from its receiver



**Figure 8.** Cross-sectional view of a Votator scraped-surface heat exchanger. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)



**Figure 9.** Direct expansion gravity refrigeration system. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)

in the compressor plant to a surge drum installed above the freezing cylinder(s). A modulating thermostatically controlled expansion valve automatically maintains the correct refrigerant level in this vessel. The temperature of the liquid refrigerant, even if subcooled, has no detrimental influence on the operation of this type of system. Gravity forces the refrigerant into the cooling jacket where product heat vaporizes a portion of the liquid while reducing the bulk density of the remainder. The flow of vapor and this density difference combine to create the classic *thermosiphon effect*, which forces liquid refrigerant to circulate from the surge drum to the cooler. Baffles in the surge drum effectively separate the vapor from the liquid. A pressure-regulating valve controls the pressure in the surge drum and, consequently, the temperature of the refrigerant in the cooling cylinder. Makeup liquid enters through the level control and the entire cycle continues. Individual surge drums can be provided for each cooling cylinder or a single drum can be used for as many as three cylinders.

The gravity system protects against freeze-up through an instantaneous current relay system. The current drawn by the heat exchanger drive motor is continuously monitored and, at a preset but higher than desired operating level, it closes a solenoid valve in the line from the cylinder to the surge drum. Refrigerant circulation is stopped and residual heat from hot metal surfaces and the product vaporizes enough refrigerant to build sufficient pressure to instantly force any remaining liquid refrigerant to return to the surge drum. When the current draw returns to normal, the solenoid valve opens and the heat exchanger automatically resumes cooling. Optional hot gas controls can be provided to assist in removing refrigerant for pump down.

*Forced Circulation Refrigeration Systems.* Forced circulation direct expansion refrigeration systems employ principles similar to gravity systems. Refrigerant must be physically transported to the cooling cylinder since the surge drum is generally located below the heat exchanger. Liquid level can be regulated with thermostatic controls or float valves. Some designs use a mechanical pump while others depend on pressure differential and gas pressure to force the liquid to flood the heat transfer tube. Injectors, based on Bernoulli's theorem, take advantage of the liquid supply pressure to transport refrigerant from the surge drum to the cylinder. Freeze-up protection devices sense motor load and either turn off the pump or stop the motive refrigerant flow to the injector. Since the surge drum is physically below the cylinder, refrigerant naturally drains to it. Hot gas systems can also be provided.

*Pool Boiling Refrigeration Systems.* In a pool boiling system, the jacket area surrounding the heat exchanger tube serves as the refrigerant surge drum. The heat exchanger tube is flooded with liquid refrigerant, and a level control device maintains this flooded condition by simply replacing the liquid as it vaporizes. A valve in the line returning vapor to the compressor plant regulates the pressure and temperature of the refrigerant in the jacket. This type of system is often provided with a "drop tank" to which the refrigerant charge is dumped during pump down or to prevent freeze-up. Hot gas systems are available.

*Liquid Overfeed Refrigeration Systems.* One commonality of all of the previous systems is that all of the liquid refrigerant entering is intended to be returned as vapor. Liquid overfeed (LOF) is a proven direct expansion concept in which only

25–35% of the liquid flowing to the heat exchanger is actually vaporized. A large low-pressure receiver replaces individual surge drums. This receiver, normally located in the compressor plant, is designed to separate the vapor from the circulated liquid (15). Overload protection against freezeup is included and hot gas systems can be provided.

Where LOF is already employed or when new installations require four or more surge drums, economics favor LOF. Advantages include:

1. One low-pressure receiver replaces multiple surge drums.
2. Surge drum controls and safety devices are eliminated.
3. Refrigerant volume in the operating area is reduced.
4. Oil does not accumulate in heat exchanger jackets.
5. The low-pressure receiver also serves as a suction trap.
6. Subcooled liquid can be used.
7. Liquid from one receiver can be returned to receivers at higher or lower pressures.
8. Has extremely rapid boil-off.
9. Refrigerant is immediately evacuated to the low-pressure receiver during pump down.

Only Votator is known to have designed scraped-surface heat exchangers for LOF refrigeration and to have plants actually operating successfully using this principle.

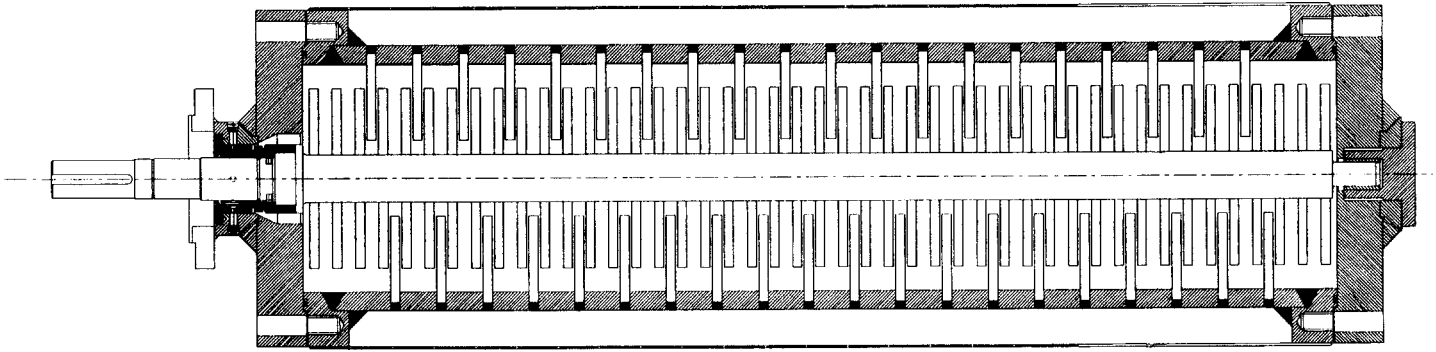
#### **4.6. Crystallization**

With efficient heat exchangers cooling through direct expansion refrigeration, the product delivered is supercooled significantly below its equilibrium temperature and primed for crystallization. A supercooled fat composition allowed to solidify without agitation and mechanical work will solidify to form an extremely strong crystal lattice and exhibit a narrow plastic range. This is a desirable characteristic for stick margarine formulations but, where specific body and plasticity is necessary, the plastic range can be altered and extended by mechanically working the fat while it is crystallizing from the supercooled state (16). In general, these fats require crystallization times with mechanical working of about 2–5 min. Votator developed a special device for this purpose—the agitated working unit.

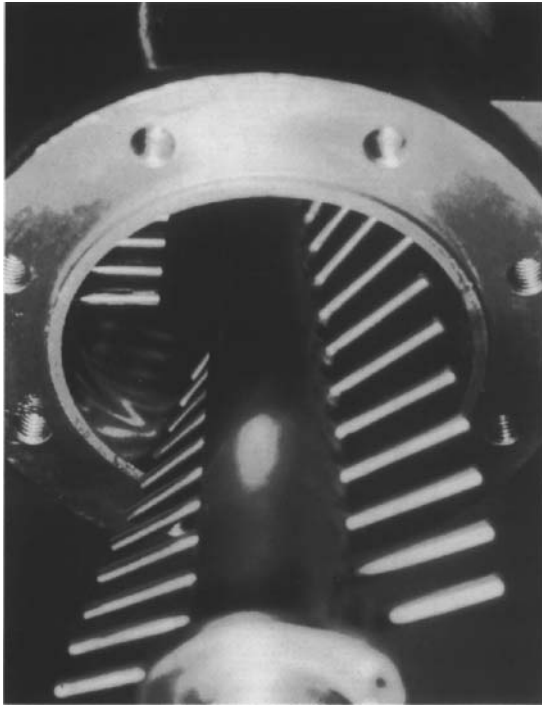
#### **4.7. Votator Agitated Working Unit**

Figure 10 is a cross-sectional, cutaway view of a Votator working unit often referred to as a B unit. Depending on the product and the required residence time, B units vary in size from 76 mm (3 in.) in diameter by 305 mm (12 in.) long to 457 mm (18 in.) in diameter by 1372 mm (54 in.) long. All sizes contain





**Figure 10.** Cross-sectional view of a Votator agitated working unit. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)



**Figure 11.** Votator agitated working unit. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)

relatively small diameter shafts with pins fixed throughout the length of the shaft. A photograph of a B unit is shown in Figure 11. Pins welded into the product cylinder intermesh with the shaft pins as the shaft rotates. The mechanical working accomplished by this device during the primary crystallization period distributes the latent heat evolved uniformly and forms fine discrete crystals throughout the crystallizing mass. For shortening, the residence time is normally 2–3 min with standard shaft speeds of 100–125 rpm. Margarine processing generally requires less residence time but variable and often higher agitation speeds. Although the product temperature rises within, B units are normally not jacketed for external cooling, but it is beneficial to jacket margarine B units with hot water to aid in melting and cleaning. Shortening worker units can be constructed of carbon steel, but stainless steel is required for margarine units.

#### 4.8. Tempering

*Definition.* Except for table margarine, plasticized edible fats are usually packaged and immediately transported to and stored in a constant temperature room for 24–72 h. This quiescent heat treatment is referred to as *tempering*. It is generally agreed that tempering should be conducted at about 27°C (80°F) and that it improves

plasticity and creaming properties and a plastic fat's ability to maintain a uniform consistency despite reasonable temperature fluctuations during storage.

*Theory.* Considerable confusion exists and theories abound as to exactly what happens during tempering. One contends that low-melting-point triglycerides dissolve and recrystallize to produce a stronger more homogeneous structure (17). Another proposes that all crystalline forms exist initially and that conversion to a stable polymorphic form for slowly transforming formulations requires quiescent holding at a temperature just below the melting point of the lowest melting polymorph with 29°C (85°F) being an acceptable temperature compromise (18). These products have complex triglyceride structures that can and do crystallize in multiple polymorphic forms. Since they pass from molten oils to packaged plastic solids in normally less than 5 min, all possible crystalline forms will be present. Most of these, however, are not stable but, given time and sufficient energy input, all will revert to stable structures. Whatever the mechanism, it is generally agreed that the rheologic properties most affected by crystal transformation are substantially stabilized during the first 48 h of tempering.

Fluid shortenings are finding increased acceptance in all segments of the bakery industry. While fluid products based on  $\beta'$  hard fats can be stabilized by normal quiescent tempering; products based on  $\beta$  hard fats require tempering under agitation to form a stable suspension. Votator's current recommendation for bakery shortening systems and fluid shortenings in general is that agitated tempering for at least 4 and preferably 6 h is necessary to form a crystalline dispersion with a viscosity low enough for pumping but high enough for prolonged suspension.

Constant-temperature rooms for tempering shortening are expensive to operate and create logistic problems maintaining and rotating inventories. While it cannot be eliminated, tempering time can be reduced in many cases by 50% or more. Obviously, the material leaving the B unit is not completely crystallized nor has it crystallized into a completely stable polymorphic form. Nevertheless, it must still conform to the laws of physical chemistry. Phase equilibria dictate that, if cooled further, additional liquid must solidify. Further cooling of this viscous crystalline mass is possible through the use of a special scraped-surface heat exchanger or "postcooler" often called a C unit.

*The Votator C Unit.* The Votator C unit is essentially an A unit with the mutator shaft installed 6 mm ( $\frac{1}{4}$  in.) off the centerline of the heat transfer tube. This "eccentric" design forces the scraper blades into a cam-type motion with each revolution of the mutator, and this continuous oscillation gently kneads the product while cleaning the tube surface. Consequently, sufficient mixing is developed to maintain efficient heat transfer even at very low shaft speeds. Since high shaft speeds are not required, mechanical power input is minimized. The net result is that the viscous crystalline material from the B unit can be cooled back to the temperature achieved in the A unit. Liquid fat, which in the quiescent state would ordinarily crystallize onto existing crystals increasing their size, is forced to crystallize creating more stable individual crystals. The C unit can reduce tempering time while also providing a means of controlling both viscosity and temperature at the filling station.

## 5. SHORTENING PRODUCTION SYSTEMS

### 5.1. Votator Carbon Steel Lard and Shortening Systems

Because shortening is a 100% oil product, expensive stainless steel equipment for a shortening is not required, and the Votator model LS182 shortening system is constructed entirely of mild steel. All of the equipment is cleaned simply by circulating oil until all of the fat has been melted and then purging with air or inert gas. No chemical cleaning is necessary.

The basic system components are an accumulator for storage of the direct expansion refrigerant and its necessary valves and controls, two  $6 \times 72$  Votators located directly below the accumulator and an  $18 \times 54$  agitated working unit. The accumulator is shown on the top right in Figure 12 immediately above the two cylinder scraped-surface heat exchanger. The working unit is installed on the same frame beneath the cooling cylinders. The pumping system on the left includes a raw material tank and two high-speed rotary gear pumps driven by a single, double-shafted motor. Special extrusion and backpressure valves are included in the interconnected product piping provided and installed at the factory.

Figure 12 is the process flow diagram for the Votator model LS182 shortening system described above. Prepared shortening formulations are normally stored at between  $49^{\circ}\text{C}$  ( $120^{\circ}\text{F}$ ) and  $60^{\circ}\text{C}$  ( $140^{\circ}\text{F}$ ). From storage, the molten oil is either pumped or gravity flows to a raw material tank located at the lower right-hand corner of the diagram. A float valve maintains a constant level in this tank as a high-speed positive displacement rotary gear pump draws oil from it. Air or inert gas is usually injected into the oil at the suction of this pump. The backpressure control valve maintains a constant pressure of approximately 24 bar (350 psig) at the pump discharge. With a controlled level in the raw material tank and a constant-speed positive-displacement pump, a fixed ratio of oil and air is maintained ensuring a constant product density. Shortening normally contains 10–15% air by volume although considerably more can be uniformly incorporated in proper formulations.

A water-jacketed shell-and-tube-type heat exchanger precools the molten oil to just above its melting point, usually  $43\text{--}46^{\circ}\text{C}$  ( $110\text{--}115^{\circ}\text{F}$ ). From this precooler, the oil flows directly to the Votator two-cylinder A unit. The primary function of the precooler is to reduce the heat load on the A units, thus maximizing their cooling capacity and ensuring that the greatest number of crystal nuclei are developed as the fat is supercooled. The A units chill the oil from the precooler to about  $18^{\circ}\text{C}$  ( $65^{\circ}\text{F}$ ) or to a previously determined temperature required to produce the desired plasticity. Very little crystallization actually occurs in the A unit although SFI profiles indicate that 25% or more solidification is possible at the A-unit exit temperature. The fat leaves the A unit in a semifluid supercooled state primed for crystallization and ideally prepared for plasticizing via the B unit.

The supercooled stream from the A unit flows directly to the worker unit. There is normally a  $5\text{--}8^{\circ}\text{C}$  ( $10\text{--}15^{\circ}\text{F}$ ) temperature rise across the B unit most of which results from latent heat of crystallization; mechanical power does not add significantly to the total heat input. The plasticized fat from the B unit is forced through a special extrusion valve that also maintains an internal pressure of 17–20 bar

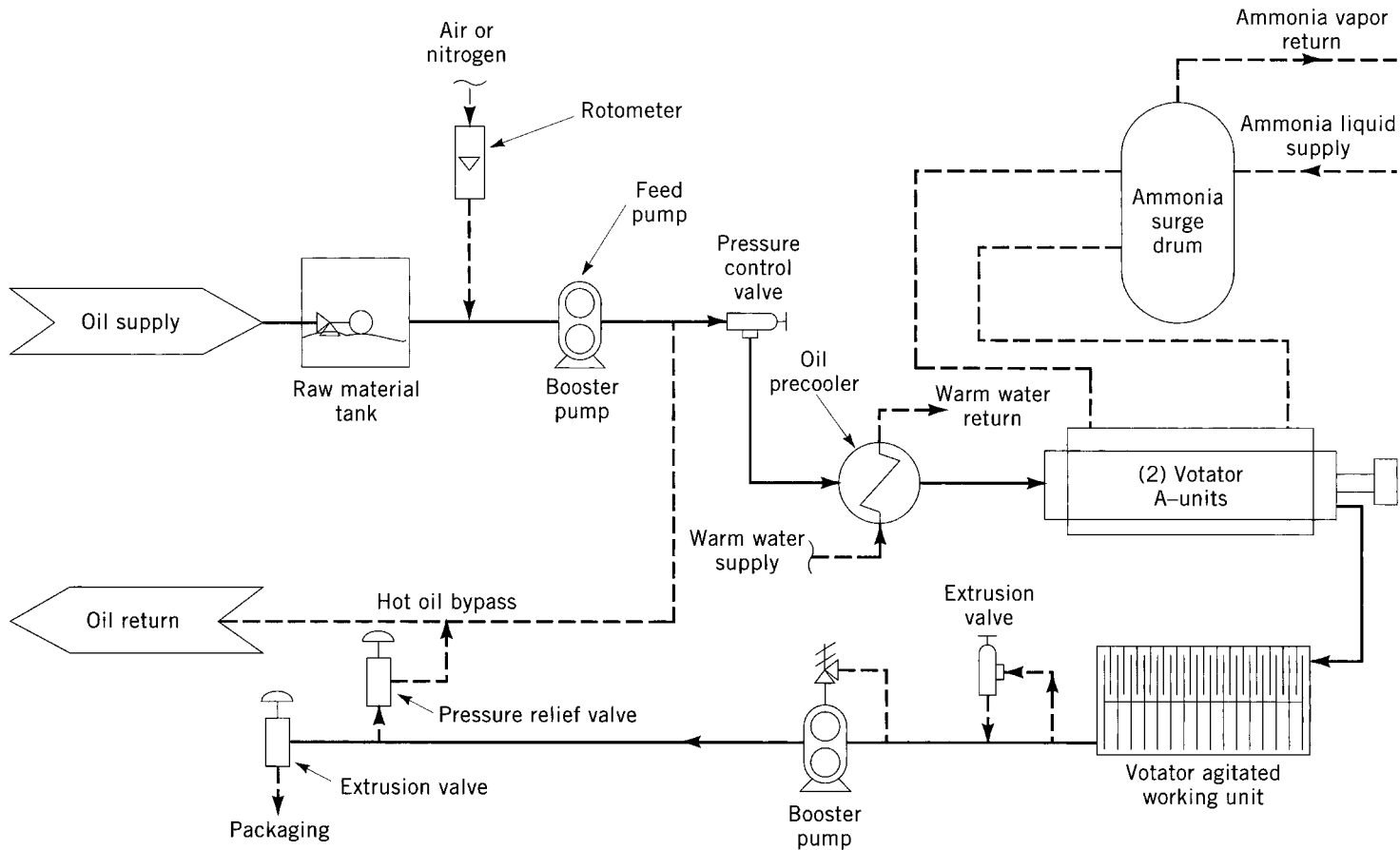


Figure 12. Process flow diagram for a Votator model LS182 shortening system. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)

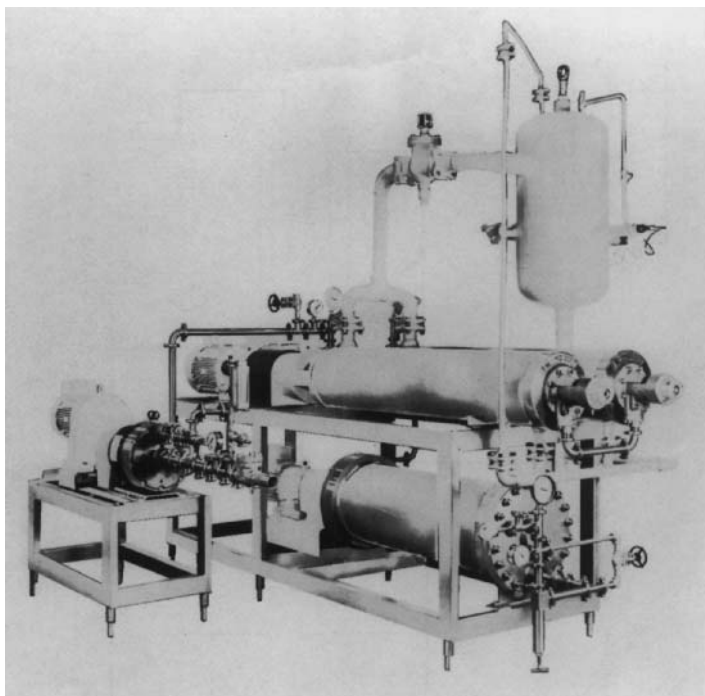
(250–300 psig) inside all the A and B units. This pressure combines with the action of the extrusion valve to ensure thorough gas dispersion and breaks any crystal aggregates that form. A second rotary gear pump develops the pressure necessary to transport the viscous crystalline mass to distant filling points while producing enough pressure for final texturization by a second extrusion valve located just prior to filling.

By varying the size and number of the coolers and working units, systems with virtually any desired capacity can be provided. Completely engineered and prepackaged shortening systems are available in capacities from 1361 kg/h to 9072 kg/h (3000–20,000 lb/h). Pilot plants for 91 kg/h (200 lb/h) are also available.

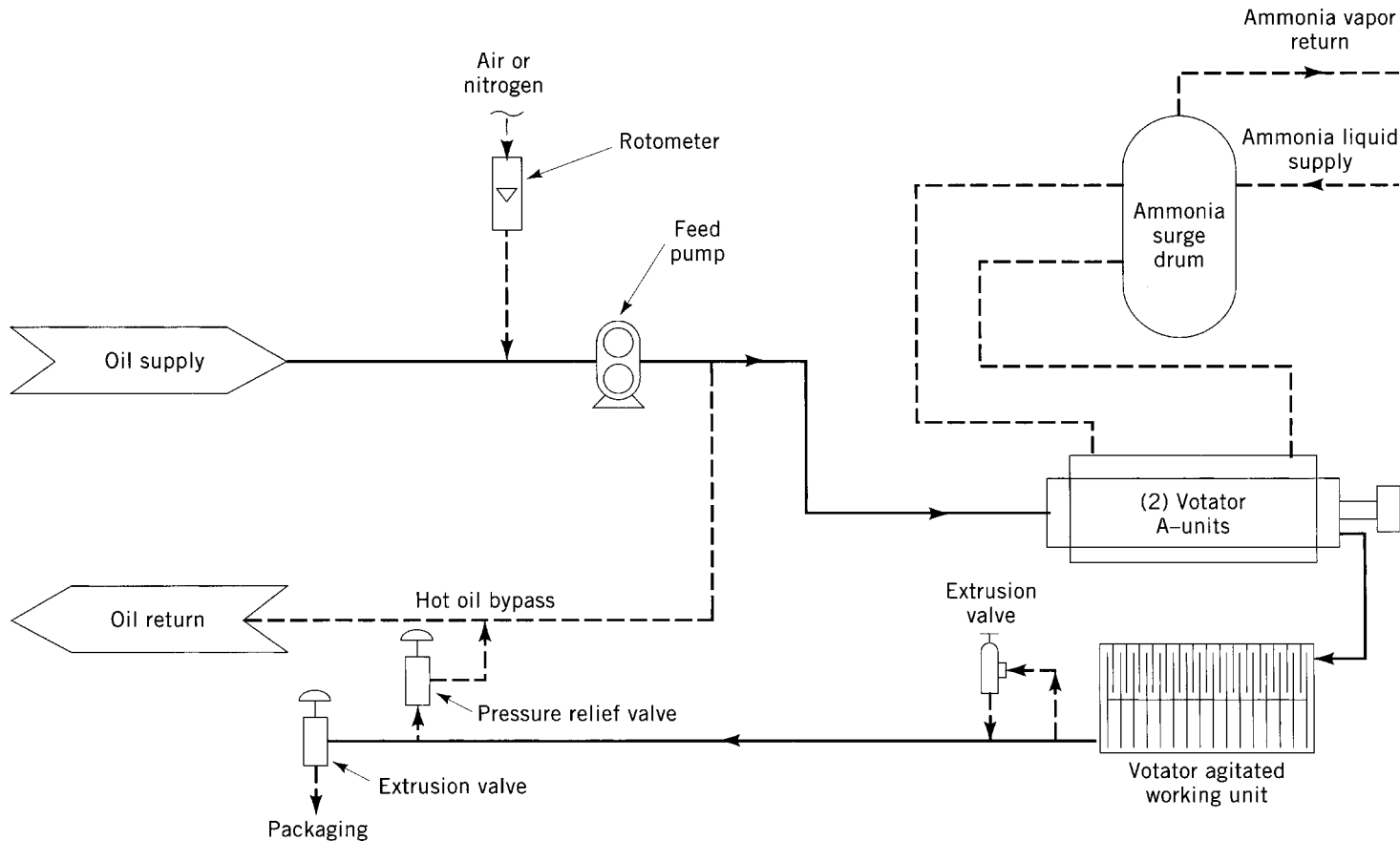
## 5.2. Votator Stainless Steel Margarine/Shortening Systems

While many manufacturers produce only shortening, there is an increasing demand for combination systems capable of processing margarine as well. In 1993, Votator introduced the stainless steel lard/shortening (SLS) series. Completely engineered, prepackaged, assembled, and factory-tested SLS combination margarine/shortening systems are now available with capacities from 1361 to 9072 kg/h (3000–20,000 lb/h) of shortening or 1043 to 6350 kg/h (2300–14,000 lb/h) of stick or tub-type margarine.

Figure 13 is a photograph of a system that has a capacity of 4536 kg/h (10,000 lb/h) for shortening and 3175 kg/h (7000 lb/h) for margarine. The metal



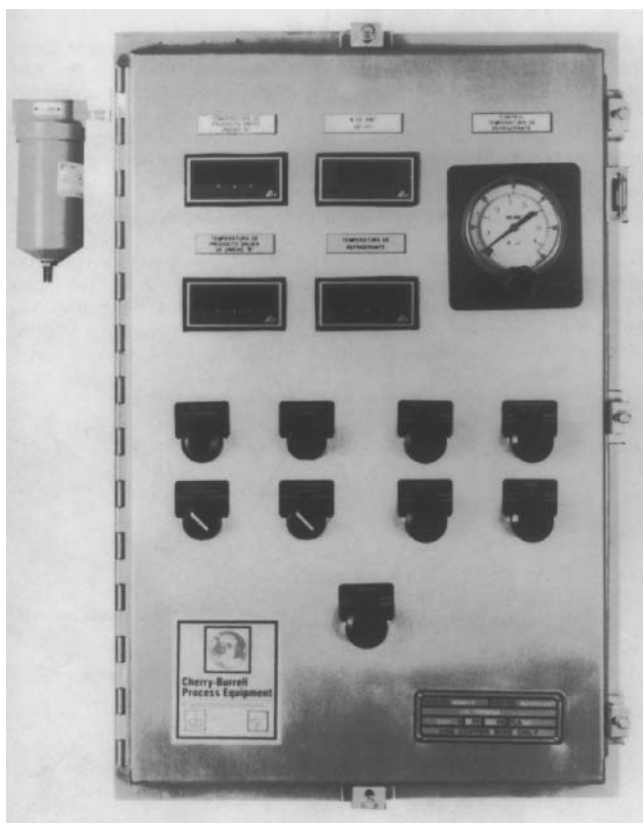
**Figure 13.** Votator model SLS182 margarine/shortening system. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)



**Figure 14.** Process flow diagram for a Votator model SLS182 margarine/shortening system. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)

surfaces in the feed pump, B unit, and all interconnecting valves and piping are stainless steel as is the mounting frame. All metal product-contacted surfaces in the Votator A units are also stainless steel except for the heat transfer product tube, which is commercially pure nickel with a hard chromium plating inside. Independent gear motor drives are provided for all A and B units.

The process flow diagram for the Votator model SLS182 margarine/shortening system in Figure 13 is shown in Figure 14. A units supercool the molten oil blend while B units work and texturize it as it crystallizes. A high-pressure stainless steel feed pump generates sufficient pressure to overcome the resistance created during supercooling and plasticizing and to transport this viscous product to the filling station. A units and B units experience higher internal pressures than with the two-pump arrangement and must be designed for at least 41 bar (600 psig). Newer designs are currently capable of operating at 68–102 bar (1000–1500 psig). The only option required to convert from shortening to soft margarine production are variable-speed drives for the feed pump and B units. Because of the elimination



**Figure 15.** Control panel for Votator SLS margarine/shortening systems. (Courtesy of Cherry Burrell, Louisville, Kentucky.)

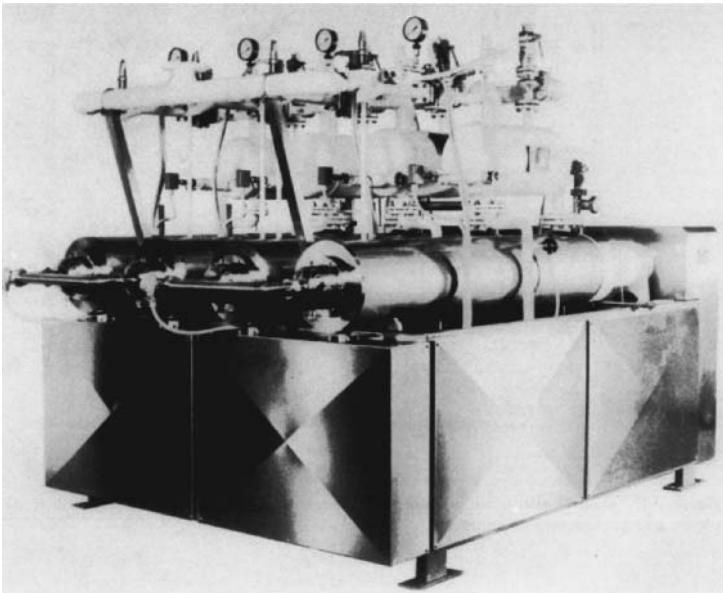


of the raw material tank, one feed pump and several control valves, a Votator SLS system is only about 20% more expensive than an all-carbon-steel Votator carbon steel lard/shortening (LS) system despite the upgrade to all stainless steel construction and individual gear motor drives.

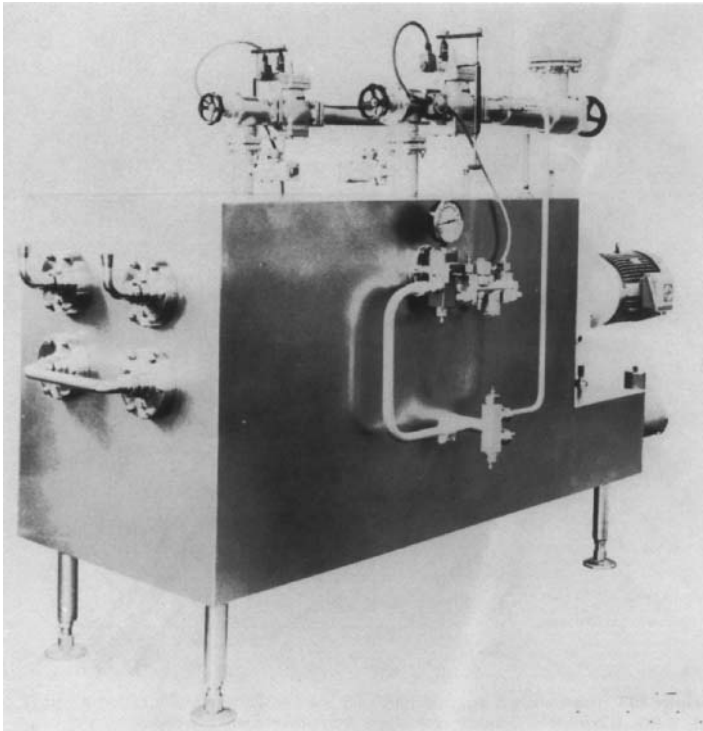
A completely redesigned manual control panel is shown in Figure 15. The operator panel, now a stainless steel washdown enclosure, also contains the necessary start/stop push buttons and running lights as well as digital indicators for product and refrigeration temperatures. The refrigeration temperature is adjustable from the operator panel. Circuit breakers, conforming to the International Electrical Code (IEC), have replaced fuse-protected motor starters. Motor current is sensed and indicated on an ammeter with a digital display. Freeze-up is prevented by stopping cooling at a factory preset current level. This power level can easily be reset in the field.

### 5.3. Chemetator Scraped-Surface Heat Exchanger

In 1993, Crown Iron Works Company of Roseville, Minnesota, acquired controlling interest in a new U.K.-based joint-venture operation. The new company, Crown Chemtech Ltd., provides processing systems for the manufacture of margarine and shortening. Figure 16 is a photograph of a Chemetator model 246-A4M with four cooling cylinders. Each cylinder has an individual ammonia refrigeration system and a separate drive motor for each mutator.



**Figure 16.** Chemoreactor scraped-surface heat exchanger unit. (Courtesy of Crown Chemtech Ltd., Reading, U.K.)

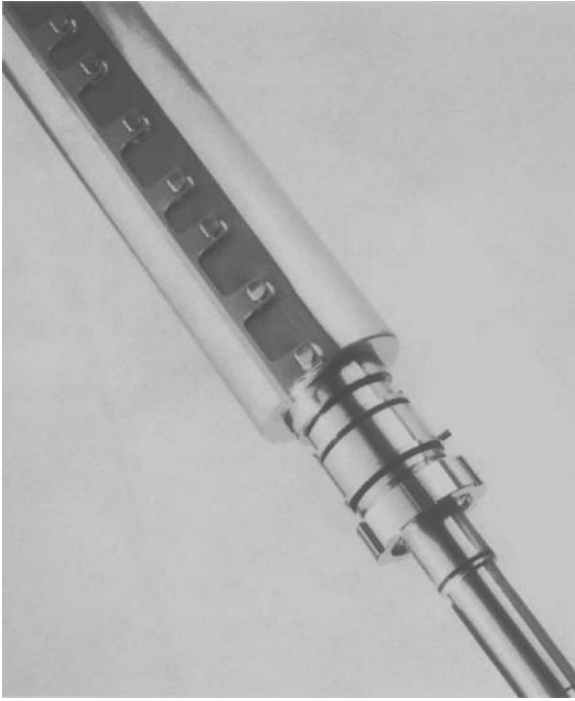


**Figure 17.** Gerstenberg & Agger Perfector type  $(2 + 2) \times 92R$ . (Courtesy of Gerstenberg & Agger A/S, Copenhagen, Denmark.)

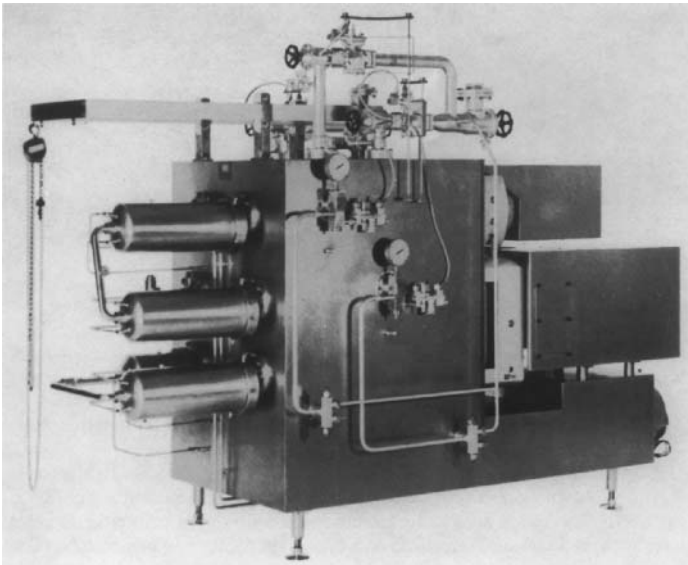
#### 5.4. Gerstenberg & Agger Margarine/Shortening Systems

Gerstenberg & Agger A/S also provides processing systems to manufacture shortening and margarine. Figure 17 is a photograph of a shortening system with a production capacity of 10,000 kg/h (22,000 lb/h). It has four chilling tubes, two independent cooling systems, and is also suitable for production of soft margarine. Special features include a unique drop tank system to ensure product does not freeze inside the chilling tubes in case of short production breaks, a maximum allowable product pressure rating of 80 bar (1176 psig), high-efficiency outside corrugated chilling tubes with chrome plating inside, tungsten carbide mechanical product seals, and optional separate cooling systems for each tube. The heat exchangers feature special floating knives as shown in Figure 18.

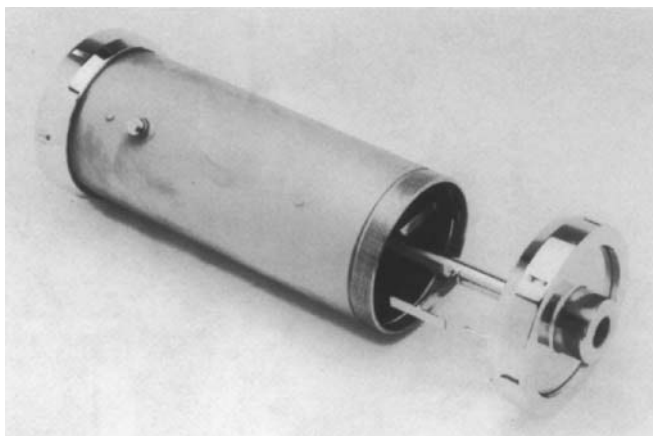
Figure 19 is a photograph of a puff pastry margarine system with a production capacity of 4000 kg/h (8800 lb/h). It has six chilling tubes, three independent cooling systems, a maximum product pressure rating of 180 bar (2646 psig) and a “bulldog” knife system. This unit also contains the special features described for the shortening Perfector type  $(2 + 2) \times 92R$  shown in Figure 18.



**Figure 18.** Gerstenberg & Agger floating knife mutator shaft system for Perfector type  $(2+2) \times 92R$ . (Courtesy of Gerstenberg & Agger A/S, Copenhagen, Denmark.)



**Figure 19.** Gerstenberg & Agger Perfector type  $(2+2) \times 180$ . (Courtesy of Gerstenberg & Agger A/S, Copenhagen, Denmark.)



**Figure 20.** Gerstenberg & Agger intermediate crystallizer type 15 Ltr. for Perfector type 125. (Courtesy of Gerstenberg & Agger A/S, Copenhagen, Denmark.)

Figure 20 is a photograph of an intermediate crystallizer that can be mounted directly on each chilling tube to ensure correct plasticity and crystal formation in all types of products such as table margarine, soft margarine, shortening, and cake and cream margarine. Intermediate crystallizers can be equipped with T pins that are especially suited for production of puff pastry.

## 6. ANALYTICAL EVALUATION AND QUALITY CONTROL

Control of the quality and consistency of the final product depends on understanding, adhering to, and applying the principles underlying cooling, crystallizing, and texturizing to production and selecting and controlling the ingredients that constitute the formulation. The *Official Methods and Recommended Practices of the American Oil Chemists' Society* (19) is the definitive analytical reference for evaluating raw materials and finished products in the fats and oils industry. Referred to in the industry as simply the AOCS Methods, it is an essential tool for every analytical laboratory. The fourth edition contains approximately 1200 pages in two heavy-duty loose-leaf ring binders, details more than 400 collaboratively tested and verified methods, and is currently used in more than 40 countries. Procedures and the apparatus required are described for all analyses required to properly characterize shortening. Typically, analytical methods are conducted to measure crystal size, color, solid fat index or content (SFI or SFC), iodine value, refractive index, Wiley melting point, dropping and softening point, oxidative stability, peroxide value, viscosity, penetration, consistency, and texture and color.

Sophisticated instruments have been developed to reduce the time needed to measure critical factors influencing shortening's rheologic properties. These include nuclear magnetic resonance (NMR), polarized light microscopes, x-ray scatterers, revolving laser beams, gas-liquid chromatography (GLC), high-performance liquid

chromatography (HPLC), thin-layer chromatography (TLC), and differential scanning calorimetry (DSC).

Crystal size and distribution was first measured using polarized light microscopy but the analysis is time consuming and inaccurate. Recently, light or x-ray scattering and sedimentation methods coupled with computer analysis have provided new and improved techniques for measuring mean crystal size and size distribution. A revolving laser beam generated by a laser particle counter can be coupled with a computer using special software to measure and record crystalline information. Crystal structure can be viewed by polarized light microscopy.

The SFC and SFI values can be determined by dilatometric methods or by pulsed NMR. The dilatometric method is still considered the most accurate, but NMR provides reliable information much more quickly.

Until the advent of chromatography, triglyceride and fatty acid analysis was difficult and time consuming. With GLC, HPLC, or TLC these analyses are now routine.

Differential scanning calorimetry is used to determine the melting behavior of shortenings. A small sample is rapidly chilled using liquid nitrogen and then reheated. The cooling and heating rates are accurately controlled at about 5°C per minute. Energy is graphed as a function of temperature. Exothermal crystallization appears as a peak in these curves. Curves for most shortenings contain a single major peak occurring at a temperature 4°C or 5°C (39.2°F or 41°F) higher than the softening point. Products crystallizing in the  $\beta'$  polymorphic form exhibit a single sharp melting peak while those in the  $\beta$  configuration have broader peaks. Hydrogenated fat may appear as additional peaks at higher melting temperatures. Differential scanning calorimetry analyses can be used to determine the polymorphic form of pure components, but these melting curves are not absolute indicators of the crystalline structure of compound shortenings (20).

Devices have also been developed to measure hardness and plasticity by compression. A curve is obtained comparing the compressing force applied to the resulting deformation. Initially, the curve is straight with the degree of deformation dependant on the formulation and the force applied. If the compressing force is increased further, a break will appear in most shortenings. Samples that are hard and brittle have a narrow plastic range and the breaking point occurs after very little deformation. Viscous flow and plasticity is indicated by curves that round off with long flat sections.

A cone penetrometer can also be used to determine hardness (AOCS Method Ce 16-60) (21). A cone of specified mass and dimensions is dropped into a prepared sample. The relative hardness of the sample is determined by dividing the mass of the cone by the depth of penetration. The cone penetrates farther into soft products and produces subsequently lower relative hardness values. A single temperature penetration value is not a true indication of overall relative plasticity. Narrow differences in penetration values at low and high temperatures indicate a wide plastic range while huge differences indicate a narrow range. Some products are formulated to be naturally firmer than others, depending on geographic area and intended usage.

Management support is the key to any quality assurance program. High-product quality standards must be established and line production supervision must have the technical training, authority, and support of upper management to enforce those controls and ensure the production of quality shortenings.

## 7. PACKAGING AND STORAGE

Semisolid plastic shortenings are usually packaged in 0.5-kg and 1.5-kg (1-lb and 3-lb) cans, cubes, 50-kg (110-lb) cartons, and in drums containing about 175 kg (380 lb). This type of plastic shortening is also available in “chubs,” prints, and sheets ready for direct end-use application.

Cubes, comprised of cardboard cartons with plastic liners, are probably the most popular foodservice and food processor packaging form. Systems equipped with optical sensors assure that an empty carton is in position on a scale before filling is initiated, and automatic controls then fill it to the correct weight. Most systems use two scales and switch to the second carton slightly before the first finishes filling. Cube sizes range from 10 kg to 25 kg (22 lb to 55 lb).

Chub packages are especially popular in Latin America. They are produced by machines that form plastic into cylindrical sheets and fill the cylinders with from 0.11 kg to 5 kg (0.25 lb to 10 lb) of shortening. The chubs are sealed by crimping both ends with metal clips.

Prints can be formed by extruding product directly into sticks or bricks with weights ranging between approximately 0.5 kg and 5 kg (1 lb and 10 lb). Prints can also be produced using rotary machines to fill plasticized shortening in paper-lined containers. The quick chilled shortening is fluid enough at filling to assume the shape of the container as it is filled but rapidly crystallizes into a rigid form.

Puff pastry shortening can be extruded in flat sheets or printed in 2-kg or 4.5-kg (5- or 10-lb) blocks. It is usually provided in corrugated boxes containing several sheets or slabs with paper sheets between the slabs to prevent them from sticking together.

Fluid and liquid shortenings are usually sold in 4-L, 8-L, and 20-L (1-gal, 2-gal, and 5-gal) containers or in bulk 200-L (50-gal) drums and 40,000-L (10,000-gal) or larger tanks. The 20-L (5-gal) size is available as a rigid package consisting of a soft plastic container inserted in a corrugated paper-board box. It is best to package liquid oil in dark containers since light catalyzes oxidation reactions in fats and oils. However, for household use, the consumer seems to prefer clear containers and some brands are packaged in clear glass or plastic. Polyethylene is permeable to oxygen and, because of low turnover rates, household oil stored in clear polyethylene containers frequently becomes rancid before it can be consumed. Other oxygen-impermeable plastics such as polyvinyl chloride, opaque polyethylene, and saran-coated polyethylene seem to be satisfactory packaging materials.

Pockets of free oil sometimes form in the package if the shortening has poor gas dispersion, a weak plastic structure, insufficient hard fat in the formulation, or the storage temperature is too high. If exposed to a sufficiently high temperature long enough for the lower melting fat fractions to liquify, plastic shortenings will

recrystallize improperly resulting in a loss of functionality. Products that have suffered heat damage during storage must be remelted and completely reprocessed to restore their plastic properties. In general, solid shortenings need not be refrigerated during storage but, since they will absorb odor, the storage area should be cool, dry, and free of odoriferous materials. Plastic shortenings that have been correctly formulated and properly prepared are stable and will tolerate considerable abuse during storage and transportation.

Clear liquid cooking and salad oils require no special storage considerations. They have no crystal structure or suspended solids and, if the storage temperature is low enough to cause some solids to form, these usually melt when the normal storage temperature is resumed. Should these solids not remelt, it is advisable to mix and suspend them before using the affected containers.

Fluid (opaque) shortenings are frequently used in applications where plastic crystallographic properties are not as important as pourability, homogeneity, and stability. Because they contain suspended solids, storage temperature is important. Most fluid products in the United States are formulated to be stored between 18.3°C and 35°C (65°F and 95°F). Storage below this temperature will cause the shortening to set up or lose fluidity. Warming normally reverses this condition. Storage at too high of a temperature results in partial or complete melting of the suspended solids. This situation is not reversible since reducing the storage temperature will result in the formation of large crystals that may settle to the bottom of the container. The loss of these solids may be of little consequence or it may have a disastrous effect on the functionality of the product.

Dry or powdered products are packaged in multiwalled paper bags or fiber drums. The bags usually contain 25 kg (50 lb) while drum capacities range from 45 kg to 90 kg (100–200 lb). As is recommended for semisolid plastic products, the storage area for these shortenings should also be cool, dry, and free of odoriferous materials.

All types of shortening have been formulated and prepared to possess essential functional properties. It is apparent that these properties can be impaired or totally destroyed by even limited exposure to excessively high temperatures during packing, storage, or shipment.

## **8. INNOVATIONS**

### **8.1. Automation and Computer Control**

With a proven process and time-tested reliable equipment, recent innovations have been directed toward improved process control and automation. Shortening lines are now available with semiautomated or completely automated screen-based control systems.

The process flow diagram for an automatic bakery shortening system is shown in Figure 21. The heart of the processing system is a standard A and B unit combination. A C unit can be added after the B unit for additional temperature control, crystal stabilization, and product flexibility. Shortening from the B unit is transferred to and held in agitated, jacketed tempering tanks until properly

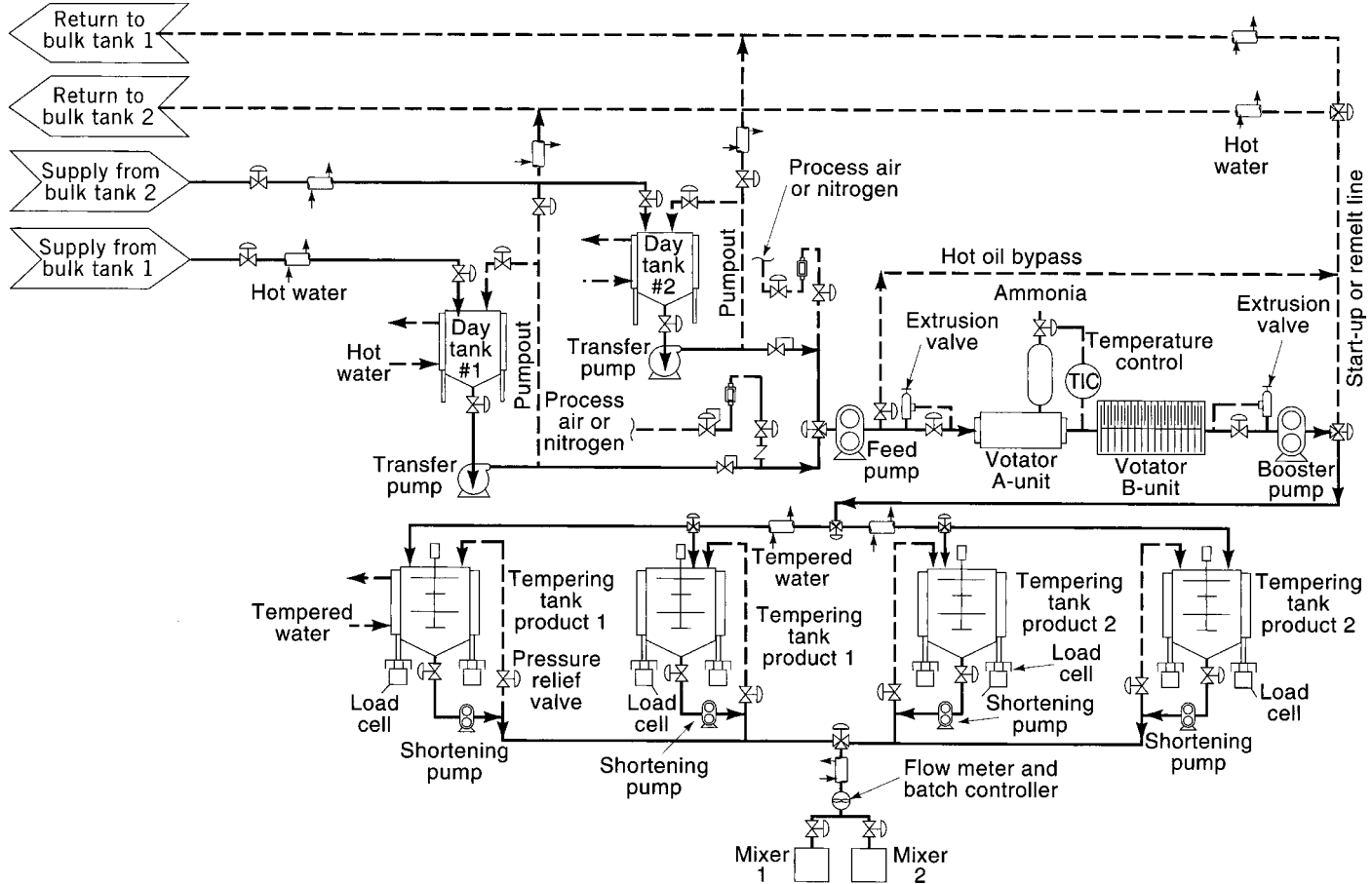
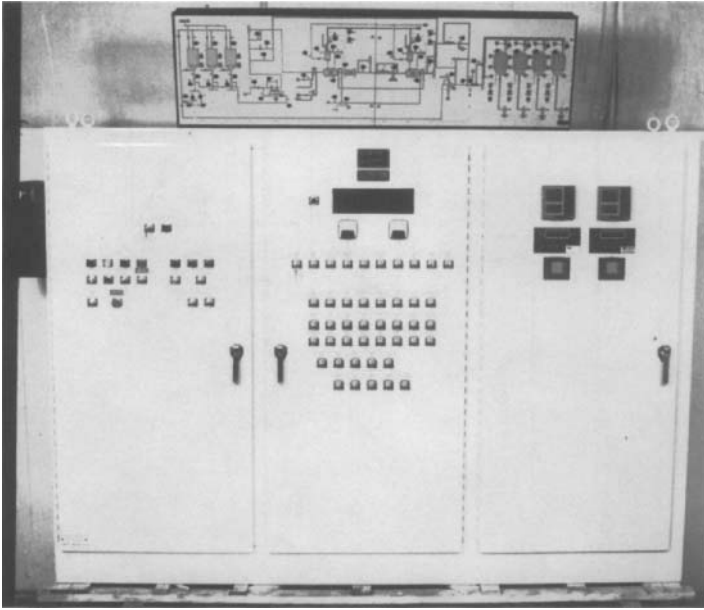
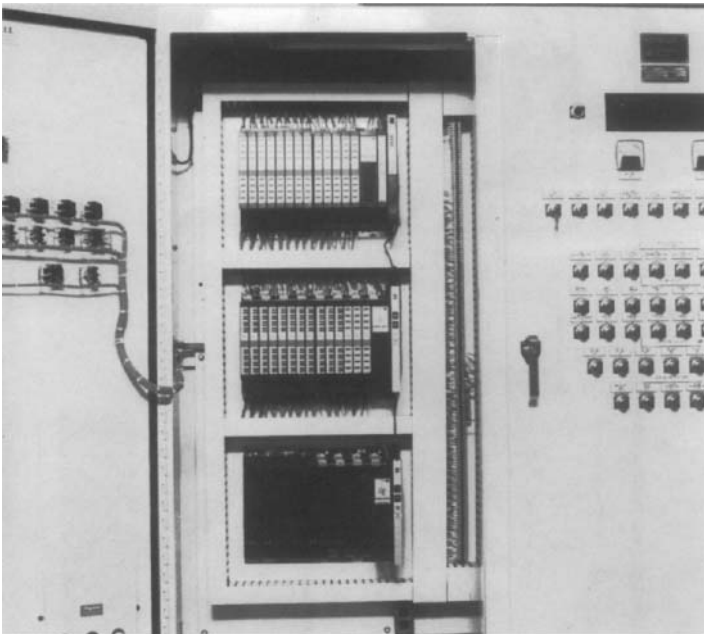


Figure 21. Process flow diagram for an automatic Votator bakery shortening system. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)

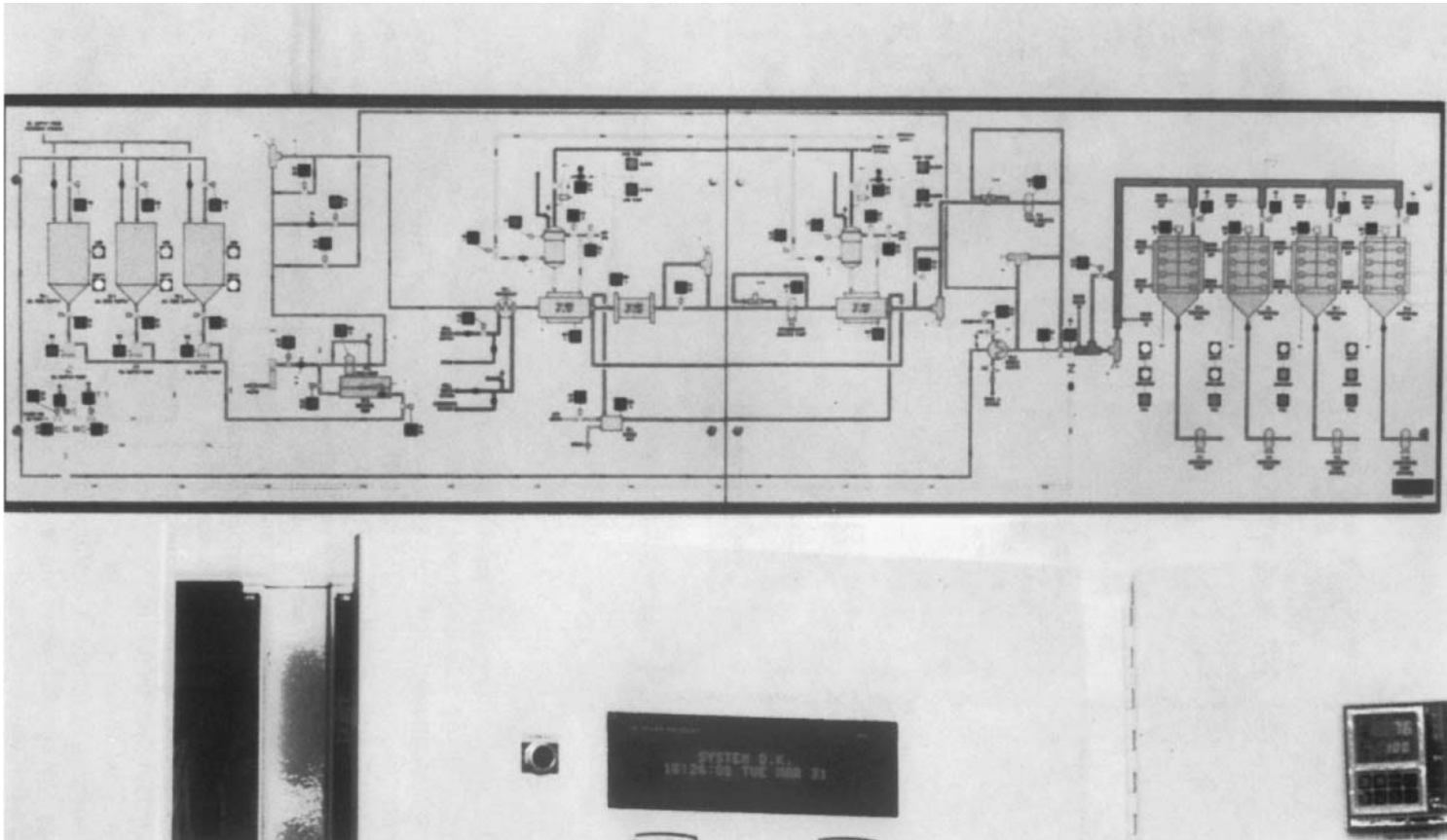




**Figure 22.** Control panel for an automatic Votator bakery shortening system. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)



**Figure 23.** PLC for an automatic Votator bakery shortening system. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)

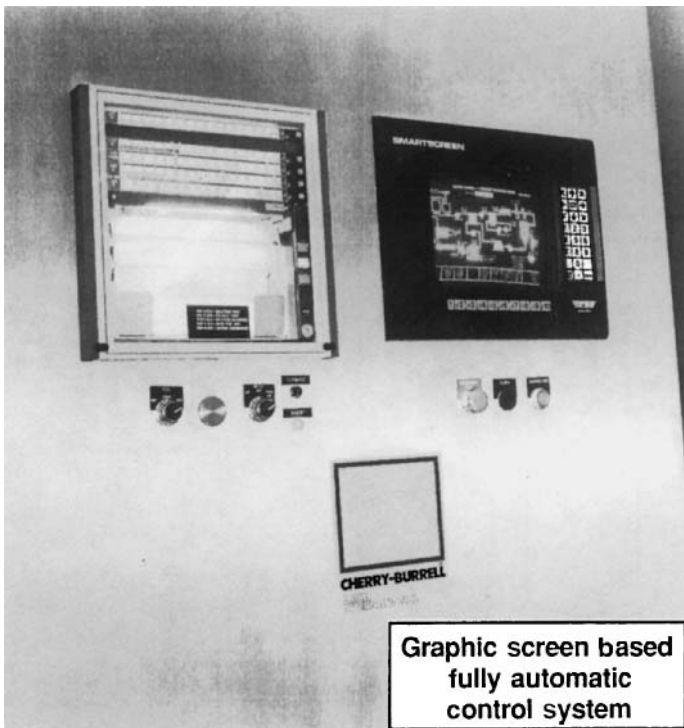


**Figure 24.** Graphic display for an automatic Votator bakery shortening system. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)

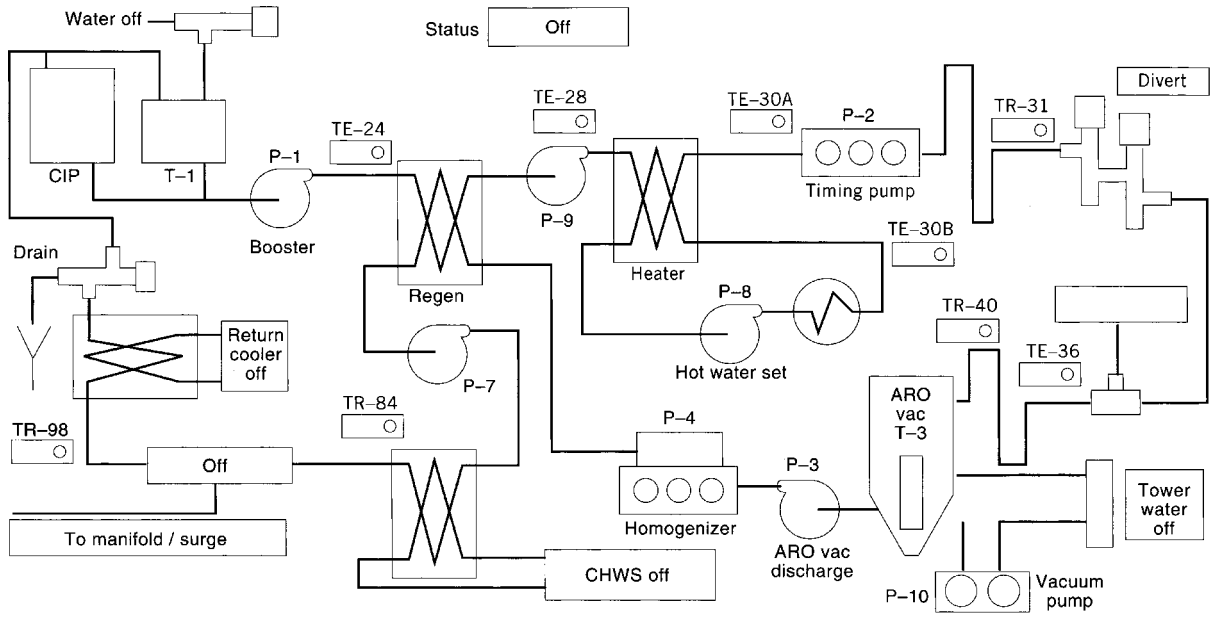
tempered and ready for use. Shortening is metered, on demand, from these tanks to end users. A programmable logic controller (PLC) continuously monitors and maintains product levels in the tempering tanks, filling each with the proper formulation as required. For each formulation change, the system is automatically purged to prevent intermixing. A user-friendly message center reports any anomaly and a graphic panel displays the current system status.

The next three figures are photographs of a typical control panel, PLC, and graphic display for the Votator bakery shortening system in Figure 21. Figure 22 contains all of the high-voltage switch gear, motor starters, as well as an operator control section with push button stations and running lights. The PLC is shown in the photograph in Figure 23. Figure 24 depicts the process flow diagram in Figure 22 and provides a visual indication of the process and equipment status.

Automation and control devices have been integrated into the system to pass information developed in the semiautomatic system to a full-color screen-based interface. This interface allows remote access to view, while in operation, process flow diagrams indicating product and utility status, valve positions, motor loading conditions, alarm status, and all other critical parameters with the ability to reset control variables simply by touching the display screen. A fully automatic screen-based control system is shown in Figure 25. Figure 26 is a typical screen display of a process and instrumentation diagram.



**Figure 25.** Graphic screen-based fully automatic control system. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)



HTST steam inject F1	System menu page F2	PID file page F3	Start in run CIP F4	CIP system page F5	Alarm page F6	ST-200 over view F7	ST-400 over view F8		System shut down F10
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Figure 26. Schematic of a process flow diagram graphic screen display. (Courtesy of Cherry-Burrell, Louisville, Kentucky.)

State-of-the-art control systems are also capable of accumulating, storing, and presenting production records. Many feature trending with records offered as options for formatted tables and histograms. Of course, all include screens for indicating and resetting control values and displaying alarm messages.

## REFERENCES

1. T. J. Weiss, *Food Oils and Their Uses*, AVI Publishing Co., Westport, Connecticut, 1983, p. 129.
2. *Oil Crops Situation and Outlook*, Economic Research Service, USDA, Oct. 2000, online update 2004.
3. E. G. Latondress, *J. Amer. Oil Chem. Soc.*, **58**, 187 (1981).
4. A. E. Thomas III, *J. Amer. Oil Chem. Soc.*, **55**, 831 (1978).
5. A. E. Bailey, *Industrial Oil and Fat Products*, 2nd ed., Wiley-Interscience, New York, 1951, pp. 211–212.
6. P. Chawla and J. M. deMan, *J. Amer. Oil Chem. Soc.*, **67**, 329 (1990).
7. L. H. Wiedermann, *J. Amer. Oil Chem. Soc.*, **55**, 825 (1978).
8. Ref. 7, p. 826.
9. M. S. A. Kheiri, *J. Amer. Oil Chem. Soc.*, **62**, 414 (1985).
10. N. O. V. Sonntag in *Bailey's Industrial Oil and Fat Products*, vol. 1, 4th ed., Wiley-Interscience, New York, 1979, pp. 292–448.
11. Institute of Shortening and Edible Oils, Inc., *Food Fats and Oils*, **13** (1988).
12. J. Edvardsson and S. Irandoust, *J. Amer. Oil Chem. Soc.*, **71**(3), 235 (1994).
13. Ref. 7, p. 825.
14. S. J. Laning, *J. Amer. Oil Chem. Soc.*, **62**, 403 (1985).
15. American Society of Heating, Refrigeration and Air-conditioning Engineers, *Refrigeration Handbook*, vol. 2, 1990, pp. 2.1–2.9.
16. Ref. 7, p. 827.
17. N. T. Joyner, *J. Amer. Oil Chem. Soc.*, **30**, 531 (1953).
18. Ref. 4, p. 832.
19. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, Champaign, Illinois.
20. L. deMan, J. M. deMan, and B. Blackman, *J. Amer. Oil Chem. Soc.*, **68**(2), 64 (1991).
21. W. E. Link, ed., *Official and Tentative Methods of the American Oil Chemists' Society*, American Oil Chemists' Society, Champaign, Illinois, 1974.
22. S. Mielke, *J. Amer. Oil Chem. Soc.*, **64**(3), 298 (1987).

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## *Shortenings: Types and Formulations*

**Richard D. O'Brien**

### **1. INTRODUCTION**

Shortening once was the reference to naturally occurring fats that are solid at room temperature and were used to “shorten” or tenderize baked foods. The material makeup of shortening has changed from a natural fat to blends of oils with hard fats to hydrogenated liquid oils to blends with additives like emulsifiers, antioxidants, antifoamers, metal scavengers, antispattering agents, etc. With all these changes, shortenings are still intended to tenderize, or shorten, baked products as well as to provide many other functional attributes for baked and other food preparations. Today’s shortenings are essential ingredients in virtually every type of prepared food product. Shortenings affect the structure, stability, flavor, storage quality, eating characteristics, and the eye appeal of the foods prepared.

#### **1.1. Historical**

The first fat and oil products used by humans were probably rendered from wild animal carcasses. Then, as animals were domesticated, their body fat became an important food source and was used for other things such as lubricants, illuminates, and soap. Lard or hog fat became the preferred animal fat for edible purposes, whereas the other animal fats were used for nonedible applications. The more

pleasing flavor of lard may have been one reason for choosing it for edible purposes. However, the principal reason undoubtedly was the plastic consistency of this fat. At room temperature, lard had a good consistency for incorporation into breads, cakes, pastries, and other baked products. The tallows were too firm for this purpose and the available marine oils were too fluid (1).

Plastic fat usage has been more prevalent in the Western Hemisphere, largely because of the meat-eating proclivities of the people and the availability of fats from the animals eaten. Vegetable shortening with all the physical characteristics of a plastic animal fat was an American invention; it was created by the cotton raising industry and perfected for soybean oil utilization. Cotton acreage expansion between the American Civil War and the close of the nineteenth century resulted in large quantities of cottonseed oil. At the same time, the pork industry could not satisfy all of the plasticized shortening requirements. Initially, cottonseed oil was blended with animal fats and called *lard compounds* or simply *compounds* and were marketed as substitutes for lard. Therefore, meatpackers had a prominent role in the development of the shortening industry due to the essential ingredient of the product: hard animal fat (2).

The introduction of the hydrogenation process into the United States about 1910 split the development of vegetable shortenings into two divergent courses. The meatpackers continued to process compounds or blended shortenings, employing the hydrogenation process only for the production of hardened oils to serve as occasional substitutes for oleostearine. Other manufacturers abandoned the lard substitute concept to create products with properties better than the traditional properties of lard. The terms *lard compound* and *compound* were replaced with proprietary names or brands not suggestive of meat fats.

The vegetable shortening manufacturers had to devise methods for improving their product to gain market share. It was necessary to more carefully refine and bleach the vegetable oils than had been the custom with the compounds. New deodorization methods with high temperatures and better vacuums were developed to remove all traces of odor and flavor to produce "bland" shortenings. Further improvements in the techniques of hydrogenation enabled the production of shortening products with increased oxidative stability, improved uniformity, and enhanced performance characteristics. Additionally, better processes for solidification, filling, packaging, and crystal development were devised to enhance the appearance and improve the keeping qualities and performance of the shortenings (3).

Pure vegetable shortening quickly assumed a position of preeminence and was accepted as a premium product both by the housewife and the commercial user or baker. The bland flavor, uniform white color, and smooth texture were probably the prime factors influencing acceptance by the consumer. Undoubtedly, these factors also influenced the bakers, but the deciding factors were the increased stability and the improved creaming properties (4).

The chemistry of natural fats was virtually an unexplored field; it did not attract many research chemists. However, a rapid expansion of interest in lipid chemistry began to be evident in the late 1920s. The obvious opportunities presented by the

advancements in the hydrogenation of liquid oils attracted new investigators to this field. This increased activity led toward the development of many new analytical tools and techniques plus new commercially important products to dispel the previous academic theory of the simplicity of natural fats and oils. It was found that these substances were capable of undergoing many of the classic organic chemistry reactions: isomerization, polymerization, oxidation, esterification, interesterification, condensation, addition, and substitution (5, 6).

High-ratio shortenings, introduced about 1933, brought about significant changes for the baker and the shortening industry. These shortenings contained mono- and diglycerides that contributed to a finer dispersion of fat particles causing a greater number of smaller sized fat globules, which strengthened cake batters. Emulsified shortenings allowed the baker to produce cakes with additional liquids, which permitted higher sugar levels. Additionally, the high-ratio shortenings had excellent creaming properties or the ability to incorporate air. Altogether, the superglycerinated shortenings produced more moist, higher volume cakes with a fine grain and a more even texture. Then, as a bonus, it was determined that lighter icings with higher moisture levels could be produced with these shortenings (7).

Development of emulsifiers and their ability to significantly improve bakery products added a new dimension to the fats and oils industry; it ushered in the era of tailor-made shortenings. New shortenings, specifically designed for a single application, such as layer cakes, pound cakes, cake mixes, creme fillings, icings, whipped toppings, breads, sweet doughs, and other baked products were developed and introduced at a rapid pace after World War II (8). Specialty shortening development fostered further improvements in all aspects of the fats and oils industry: processing techniques and equipment, quality control procedures and tools, processing aids, additives, analytical equipment and methods, packaging containers and equipment, plus others too numerous to list.

The advances in technology have increased the storehouse of fats and oils information allowing the introduction of even more sophisticated products for all aspects of the food industry: retail consumer, foodservice, and food processor. The specialty shortenings have been responsible for many of the recent advances within the foodservice and food processor industries. Likewise, new food developments have created the need for totally new shortening products. Currently, plasticized shortenings have been joined by three other shortening forms: liquid shortenings, shortening chips, and powdered shortenings. These additional shortening products were introduced to fill needs expressed by the food industry.

## 1.2. Source Oils

Raw material selection for shortenings has been influenced by availability and economics. These factors were the main reasons for the development of lard substitutes, introduction of pure vegetable shortenings, major processing improvements, the development of additives to fortify specific performance characteristics, and most of the other changes. Table 1 give U.S. information on fats and oils used



TABLE 1. Fats and Oils Used in U.S. Edible Products, for 2002–2003,  $\times 10^3$  lb.<sup>a</sup>

	Oct. 2002	Dec.	Feb.	Apr.	June	Aug.	Oct. 2002– Sept. 2003
Coconut oil:							
Total edible	27,706	23,207	24,436	24,645	27,201	25,892	280,461
Corn oil:							
Baking or frying fats	D	D	D	D	D	D	D
Margarine	D	D	D	D	D	D	D
Salad or cooking oil	D	D	D	D	D	D	D
Total edible	141,092	128,579	116,510	D	129,486	132,085	D
Cottonseed oil:							
Baking or frying fats	19,000	15,500	12,464	14,817	12,310	13,973	159,165
Margarine	D	D	D	D	D	D	D
Salad or cooking oil	20,100	20,800	27,364	23,198	D	23,479	227,311
Other edible	D	D	D	D	D	D	D
Total edible	41,400	37,700	41,257	D	36,192	39,067	386,983
Lard:							
Baking or frying fats						D	D
Margarine	1,821	1,805	782	695	702	477	11,843
Other edible	D	D	D	D	D	D	D
Total edible	21,823	21,525	15,425	14,780	17,673	15,340	198,054
Palm oil:							
Baking or frying fats	D	D	D	D	D	D	D
Total edible	D	D	D	D	D	D	D
Peanut oil:							
Salad or cooking oil	D	D	D	D	D	D	D
Total edible	D	D	D	D	D	D	D
Edible rapeseed oil:							
Baking or frying fats	D	D	D	D	D	D	D
Salad or cooking oil	78,287	66,600	50,015	53,575	61,207	65,490	666,293
Total edible	95,117	81,400	62,594	63,322	72,803	77,530	809,413
Safflower oil:							
Consumption, total	D	D	D	D	D	D	D
Soybean oil:							
Baking or frying fats	818,750	678,675	622,495	672,673	646,872	696,112	7,640,653
Margarine	126,923	116,526	91,382	91,246	87,372	90,723	1,079,206
Salad or cooking oil	707,009	624,782	585,096	677,748	697,105	638,744	7,248,926
Other edible	10,913	8,813	9,383	10,550	10,439	10,143	109,958
Total edible	1,663,595	1,428,796	1,308,356	1,452,217	1,441,788	1,435,722	16,078,743
Sunflower oil:							
Total edible	13,586	15,607	11,861	14,928	12,481	15,566	
Tallow, edible:							
Baking or frying fats	D	D	D	D	D	D	D
Total edible	23,288	22,372	18,744	19,852	15,226	18,581	214,217
Total fats and oils used in edible product:							
Baking or frying fats	923,585	770,422	702,737	750,445	728,045	780,565	8,605,542
Margarine	135,511	126,956	97,466	96,655	95,167	96,561	1,156,075
Salad or cooking oil	991,575	881,607	801,231	916,860	941,157	890,675	9,978,189
Other edible	40,128	25,632	30,096	32,569	30,748	33,492	362,630
Total edible	2,090,779	1,804,617	1,631,530	1,796,529	1,795,117	1,801,293	20,102,436

<sup>a</sup>Selective months shown. Total for Oct. 2002–Sept. 2003 includes entire 12-month period. D = Withheld to avoid disclosing figures for individual.

Source: Bureau of the Census.

in edible products for 2002–2003 (9). Table 2 gives production and disappearance of edible oils in the U.S. for the period 1988–2002 (10). Note that disappearance as defined by USDA-ERS means beginning food stocks, production, and imports, minus exports, shipment to U.S. territories, and ending stocks.

Lard usage in shortening decreased to a low point in 1940 while cottonseed oil became the major source oil used in shortenings. Interestingly, soybean oil accounted for almost 18% of the oil used for shortenings at this early date. The next decade was a time of change as illustrated by the shortening source oils utilization: (1) soybean oil replaced cottonseed oil as the highest volume oil to attain 49% of the total oil used and (2) the introduction of crystal-modified lard-based shortenings in 1950 returned this raw material to shortening production. Interestified lard shortenings were comparable to good-quality pure vegetable plasticized shortenings in appearance and creaming properties.

Soybean oil began its rise from a minor, little known, problem oil before 1940 due to the scarcity of other oils during World War II. After the hostilities ended, these gains were in jeopardy unless the technology could be developed to improve the flavor stability of soybean oil products. The technologies developed included metals deactivators, hydrogenation techniques and more selective catalysts, antioxidants, surface active agents, other surfactants, etc. These and other considerations helped soybean oil reach and maintain a dominant shortening source oil position in 1960 with over 50% of the total requirement. The soybean oil share had risen to 75% for the 1992–1993 crop year.

Palm oil threatened to become a major source oil for U.S. shortenings in the mid-1960s. It grew to command better than 15% of the total shortening requirement in less than 10 years. Palm oil was found to be an excellent plasticizing agent to force a shortening's crystal habit to  $\beta'$ . Therefore, the growth of palm oil usage in shortenings was primarily at the expense of cottonseed oil and tallow. Palm oil's decline, which began in the late 1980s, was due to unfavorable publicity highlighting nutritional concerns with saturated fatty acids. Palm oil dropped to an estimated 2–3% of the total shortening source oil requirement after this time.

Coconut oil and the other lauric oils are not among the more desirable materials for shortening manufacture because of their short plastic range and tendency to foam in deep-fat frying when mixed with other source oils. Nevertheless, coconut oil was a popular frying media for Mexican foods, which probably accounts for the high usage in 1980–1981. This use also suffered from unfavorable publicity that convinced the foodservice industry to change to frying shortenings with a more healthy image. This change probably accounted for the rise in corn oil utilization in 1990.

Lard and tallow were both important shortening raw materials after the meat fat shortenings regained popularity during the 1950s: interestified lard in bakery shortenings and tallow in frying shortenings and as a hardening/plasticizing agent for shortenings. Later, technology was developed to replace the crystal-modified lard with tallow. The meat fat usage continued to grow until cholesterol concerns brought pressure on the major users to provide products that had a better nutritional image. Meat fats account for less than 11% of the shortening

**TABLE 2. Production and Disappearance of Edible Fats and Oils in the United States from 1990–2002, 10<sup>6</sup> lb.**

Item	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001 <sup>a</sup>	2002 <sup>a</sup>
<b>Imports</b>													
Coconut	946	838	1,162	999	1,100	873	1,188	1,440	791	926	1,100	1,150	1,150
Cottonseed	3	18	38	26	0	0	0	0	48	8	0	0	0
Olive oil (net)	211	216	253	262	260	227	304	333	355	397	455	455	455
Palm	284	220	267	368	218	236	322	282	284	345	399	490	460
Palm kernel	306	342	302	304	280	262	392	359	401	393	351	330	379
Peanut	10	1	0	11	4	5	14	10	73	12	79	39	45
Canola	583	815	861	902	938	1,086	1,075	1,088	1,060	1,139	1,193	1,108	1,027
Safflower	22	22	15	16	26	35	30	51	51	33	34	40	43
Soybean	17	1	10	68	17	95	53	60	58	82	73	46	65
Sunflower	33	9	0	7	1	2	22	8	5	4	8	16	5
<b>Production</b>													
Corn	1,656	1,821	1,878	1,906	2,227	2,139	2,230	2,335	2,374	2,501	2,403	2,459	2,575
Cottonseed	1,154	1,279	1,137	1,119	1,312	1,229	1,216	1,224	832	939	847	870	865
Lard	934	1,016	1,011	1,015	1,052	1,013	979	1,065	1,106	1,069	1,050	1,080	1,085
Peanut	213	356	286	212	314	321	221	176	145	229	172	230	206
Canola	18	32	49	406	299	355	342	451	548	617	656	713	560
Safflower	78	69	87	111	115	127	103	115	111	91	88	76	89
Soybean	13,408	14,345	13,778	13,951	15,613	15,240	15,752	18,143	18,081	17,825	18,434	18,898	18,930
Sunflower	536	911	730	580	1,165	860	840	959	1,177	1,046	873	713	570
Tallow, edible	1,202	1,515	1,414	1,499	1,542	1,505	1,390	1,517	1,677	1,810	1,814	1,920	1,800
<b>Exports</b>													
Coconut	51	22	15	20	18	11	11	7	11	14	8	11	10
Corn <sup>b</sup>	498	566	712	717	865	977	986	1,118	989	952	843	1,130	1,150
Cottonseed	249	281	177	248	338	221	240	208	111	141	140	140	125
Lard <sup>b</sup>	107	129	126	116	138	92	102	120	138	187	92	85	98
Palm kernel	2	2	9	4	2	2	2	2	2	2	2	2	2
Palm	4	7	7	7	13	20	9	11	11	11	11	10	11
Peanut	25	151	52	61	97	108	21	13	10	18	14	18	12

Canola	7	15	16	76	153	147	295	349	272	284	187	276	187
Safflower	56	73	65	75	93	122	83	83	92	51	35	37	40
Soybean	780	1,648	1,461	1,531	2,683	992	2,033	3,079	2,371	1,376	1,430	2,500	2,400
Sunflower	359	471	586	450	978	628	709	815	800	630	545	465	235
Tallow, edible <sup>b</sup>	239	327	296	301	259	233	176	234	319	214	305	465	290
Domestic disappearance													
Coconut	897	906	1,082	1,067	1,082	941	1,111	1,190	1,021	927	968	1,100	1,201
Corn	1,149	1,202	1,220	1,228	1,250	1,298	1,244	1,268	1,397	1,417	1,711	1,342	1,400
Cottonseed	851	1,075	995	873	1,006	996	1,004	1,004	772	833	674	767	725
Lard	825	885	886	890	924	922	880	925	987	886	962	989	982
Olive	211	216	253	262	260	227	304	333	355	397	455	455	455
Palm	256	223	271	359	225	201	298	282	260	335	375	471	474
Palm kernel	362	344	254	315	295	293	362	344	390	414	243	355	440
Peanut	197	179	236	187	206	193	194	217	208	233	244	250	245
Canola	577	801	898	1,162	1,165	1,271	1,134	1,143	1,287	1,435	1,744	1,493	1,389
Safflower	58	15	47	40	57	17	67	73	59	86	102	89	93
Soybean	12,164	12,245	13,012	12,939	12,913	13,465	14,267	15,262	15,655	16,056	16,210	16,958	17,350
Sunflower	200	396	188	129	171	168	207	186	320	385	357	375	315
Tallow, edible	955	1,197	1,109	1,239	1,275	1,345	1,218	1,286	1,360	1,599	1,498	1,474	1,500

1/ Preliminary and estimated.

<sup>a</sup> ERS and WAOB forecast.

<sup>b</sup> Lard, corn oil, and tallow exports are net of imports.

Source: Bureau of the Census.

**TABLE 3. Genetically Modified Vegetable Oils (11).**

Oil Seed	Type	Fatty Acid Composition (%)					
		C-16:O	C-18:O	C-18:1	C-18:2	C-18:3	C-22:1
Soybean	Normal	11.0	3.0	22.0	56.0	8.0	—
	N-87-2122-4	5.3	3.2	48.0	38.9	4.6	—
	N85-2176	9.5	3.3	44.4	39.5	3.3	—
	A-6	8.4	28.1	19.8	35.5	6.6	—
	C-1727	17.3	2.9	16.8	54.5	8.3	—
Sunflower	Normal	7.0	5.0	20.0	68.0	—	—
	G-8	3.3	8.2	84.2	3.5	0.8	—
Rapeseed	Normal	4.0	2.0	18.0	14.0	9.0	53.0
	M-30	2.4	1.0	91.6	1.5	3.3	0.2

source oil requirements in 1992, after controlling slightly more than 25% for many years.

The U.S. shortening industry has been able to develop processing technologies for the utilization of many different source oils. The nutritional challenges may require that the technology effort be directed toward a change in the source oil's composition. Plant biotechnology research has developed the potential for genetic variation in the fatty acid composition of oils. This means that performance may be grown into the oil rather than relying on processing to provide these characteristics. Table 3 illustrates some of the potential changes in the fatty acid composition of vegetable oils with biotechnology.

## 2. SHORTENING ATTRIBUTES

The development of a shortening for a food application is dependent on many inter-laced factors. These requirements may differ from customer to customer depending on the equipment, processing limitations, product preference, customer base, and many other contributors. Fat and oil products are now being designed to satisfy individual specific requirements as well as the general-purpose products with a broader application potential. The design criteria for the general-purpose products must be of a less exacting nature than those developed for a specific product or process.

The important attributes of a shortening in different food products vary considerably. In some food items, the flavor contributed by the shortening is of minor importance; however, it does contribute a beneficial effect to the eating quality of the finished product. This fact has been relearned by the developers and experienced by the initial consumers, to their dissatisfaction, with many of the fat-free products recently introduced. A characteristic failing of the fat-free products was a lack of the eating characteristics normally contributed by shortening. In many products, such as cakes, pie crusts, icings, cookies, and other pastries, shortening

is the major contributor to the product structure and eating character as well as contributing other significant effects upon the finished product's quality. Satisfactory shortening performance is dependent on many factors. Five of the most important considerations, which affect most applications are: (1) flavor, (2) physical characteristics, (3) crystal structure, (4) emulsification, and (5) additives.

## 2.1. Flavor

Generally the flavor of shortening should be as completely bland as possible so it can enhance a food product's flavor rather than contribute a flavor. In some specific cases the desired shortening flavor is typical of the original flavor of the source oil, for example, a lardy flavor is somewhat desirable in some products. Also, artificial flavors are added to some shortening products to enhance the functionality. Both the bland or typical flavor must be stable throughout the life of the food product. Therefore, the oxidative stability requirements of the finished product must be established to determine the minimum requirements for the shortening. The oxidative reversion rate of a shortening is directly related to the type and amount of unsaturated fatty acids available. The expected oxidation rates for the three most common unsaturated fatty acids are as follows (12).

Fatty Acid	Oxidation Rate
Oleic C-18:1	1
Linoleic C-18:2	10
Linolenic C-18:3	25

Reversion of deodorized shortenings brings back the flavor characteristics of the original crude oil. Shortening must be designed with a flavor stability suitable for the finished product requirements. Reduction of unsaturated fatty acids can be accomplished with hydrogenation or fractionation to increase flavor stability.

## 2.2. Physical Characteristics

The characteristics of the fats and oils used for a shortening are of primary importance in the design of a shortening for a specific use. Oils can be modified through various processes to produce the desired properties. Hydrogenation has been the primary process used to change the physical characteristics of oils. Melting points or hardness of an oil can be completely altered with this process and the changes controlled by the conditions used to hydrogenate the oil. In the hardening process, hydrogen gas is reacted with oil at a suitable temperature and pressure in the presence of a catalyst with agitation. Control of these conditions and the end point enables the operator to better meet the desired physical characteristics of the shortening products. A range of typical shortening solids fat index curves are shown in Figure 1. This chart illustrates how the hydrogenation process can be used to produce physical characteristics suitable for the performance desired.

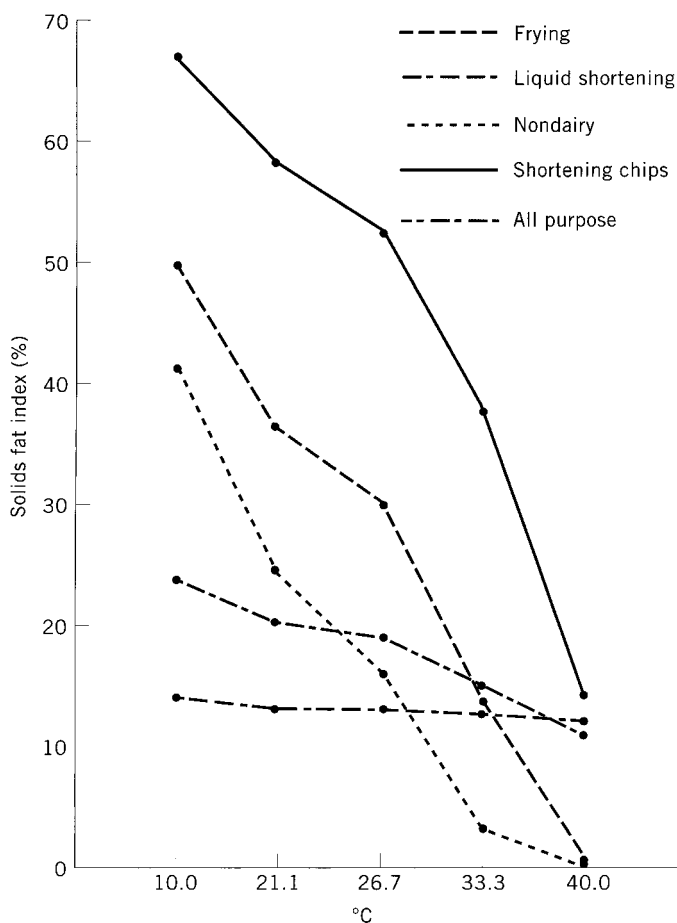


Figure 1. Typical shortening products.

### 2.3. Crystal Structure

Each source oil has an inherent crystallization tendency, either  $\beta$  or  $\beta'$ . The small, uniform, tightly knit  $\beta'$  crystals produce smooth textured shortenings with good plasticity, heat resistance, and creaming properties. The large  $\beta$  crystals can produce sandy, brittle consistency shortenings that result in poor baking performance where creaming properties are important. However, the large  $\beta$  crystals are desirable for some applications such as pie crusts or frying. Crystal habit is controlled by the oil source selection and complemented by the plastification conditions employed and tempering for slow crystallization after packaging.

## 2.4. Emulsification

Shortening emulsifying properties are accomplished with adjustment of the fat structure and with the addition of surface-active agents. The typical food emulsifiers supplement and improve the functionality of a properly developed shortening, i.e., act as lubricants, emulsify fat in batters, build structure, aerate, improve eating qualities, extend shelf life, crystal modifier, antisticking, dispersant, moisture retention, etc. Obviously, no single emulsifier or emulsifier system can perform all of these different functions. In selection of the proper emulsifier or system, the developer must consider the usage application, the preparation method, emulsion type, effects of the other ingredients, economics, and any other applicable criteria for the finished product. Most of the emulsifiers used in shortenings are listed in Table 4 with Code of Federal Regulations (CFR) Title 21 Part numbers and a suggested application guide.

## 2.5. Chemical Adjuncts

In addition to emulsifiers a number of other chemical compounds provide a specific function for certain types of shortenings. These additives can be classified into the following categories:

*Antifoamers.* Dimethylpolysiloxane serves as an antifoaming agent that forms a monomolecular layer on the surface of a heated frying shortening to retard oxidation and foaming. The silicone compounds are added to frying shortenings at levels of 0.5–3.0 ppm. Higher concentrations do not inhibit foaming any more effectively and can cause immediate foaming at levels in excess of 10 ppm, the maximum allowed by U.S. federal regulations. Other potential problem areas with antifoamer

**TABLE 4. Emulsifiers Used in Shortening.**

Emulsifier	CFR 21	Applications <sup>a</sup>
Mono- and diglyceride	182.4505	All
Lecithin	182.1400	All
Lactylated monoglyceride	172.852	C and M
Calcium stearyl lactylate	172.844	B and S
Sodium stearyl lactylate	172.846	B and S
Propylene glycol mono esters	172.856	C,M,B, and S
Diacetyl tartaric monoglycerides	182.4101	B
Ethoxylated monoglycerides	178.834	B
Sorbitan monostearate	172.842	C and M
Polysorbate 60	172.836	All
Polyglycerol esters	172.854	All
Succinylated monoglycerides	172.830	B
Sodium stearyl fumarate	172.826	B and S
Sucrose esters	172.859	C,B, and S
Stearyl lactylate	172.848	C,B, and M

<sup>a</sup>Applications code: B, bread; C, cake; F, fillings; I, icings; M, cake mix; S, sweet doughs; All, all applications.



TABLE 5. Antioxidants Effect Upon AOM Stability.

Antioxidant <sup>a</sup>	AOM Stability (h)			
	70 PV End Point		20 PV End Point	
	Soybean	Cottonseed	Lard	Tallow
None	11	9	4	16
TBHQ	41	34	55	133
BHA	10	9	42	95
BHT	13	11	33	138 <sup>b</sup>
Propyl gallate	26	30	42	<sup>c</sup>

<sup>a</sup>200 ppm added.

<sup>b</sup>100 each of BHA and BHT.

<sup>c</sup>Not analyzed.

use are (1) unintentional addition to bakery shortenings can cause cake failures, (2) glazes may not adhere to donuts fried with an antifoamer present, and (3) potato chips may lack crispness (13).

*Antioxidants.* Antioxidants are materials that can retard the development of off-flavors and odors by inhibiting oxidation. Vegetable oils contain natural antioxidants, tocopherols, that can survive most processing. Several phenolic compounds have been identified that can also provide oxidative stability. Table 5 presents active oxygen method (AOM) stability data for select source oils treated with four different phenolic compounds (13). This data should only be used as a guideline to distinguish effectiveness of the antioxidants. In general, tertiary butylhydroquinone (TBHQ) is the most effective synthetic antioxidant in unhardened vegetable oils, followed by propyl gallate. TBHQ also appears to be effective for the meat fats, but protection is also afforded by butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA).

*Metal Inactivators.* Fats and oils obtain metal contents from the soils where the plants are grown and later from contact during crushing, processing, and storage. Many of the metals promote autoxidation that results in off-flavors and odors accompanied by color development in the finished shortening. Studies have identified copper as the most harmful metal with iron, manganese, chromium, and nickel following. The results of a lard study is presented in Table 6. Metal scavengers, added at low levels during or immediately following deodorization, facilitate removal of the harmful metals. The most widely used chelating agent is citric acid at 50–100 ppm. Phosphoric acid at 10 ppm and lecithin at 5 ppm have also been used to inactivate metals.

*Colorants.* Pigments used in shortenings are usually the oil-soluble carotenoids in the yellow to reddish orange range. The carotenoids with U.S. Food and Drug Administration (FDA) approval include the carotenes, bixin, and apo-6-carotenol. Carotenoids are heat sensitive but can be stabilized with BHA and BHT for greater heat stability. Lakes are FDA approved and heat stable but violent agitation is required to keep these oil-insoluble colorants in suspension. Apocarotenol, a synthetic, FDA-approved pigment is used primarily as a color intensifier for  $\beta$ -carotene (15).

**TABLE 6. Trace Metals Concentration Required to Reduce Lard AOM by 50% (14).**

Trace Metal	ppm
Copper	0.1
Manganese	0.6
Iron	0.6
Chromium	1.2
Nickel	2.2
Vanadium	3.0
Zinc	19.6
Aluminum	50.0

*Flavors.* Most of the flavors used in shortenings are butterlike. Diacetyl was the major butter flavor used in fat and oil products until improved analytical techniques identified other flavor components in butter. Today, the U.S. FDA regulations allow safe compounds that impart a suitable flavor to the finished product (16). The choice of a particular flavor or blend of flavors depends on the expertise and the taste preference of the product developer.

### 3. BASE STOCK SYSTEM

In the past, prepared food products were formulated with the ingredients available. Today, most prepared foods are formulated with ingredients designed for their applications or, in many cases, specifically for the particular product and/or processing technique employed by the producer. These customer specialty products have expanded the product base for fat and oil processors from a few basic products to literally hundreds.

A shortening manufacturer could formulate products so that a special base fat or series of base fats is required for each different product. This practice, with the ever-increasing number of finished products, would result in a scheduling nightmare with a large number of product heels tying up tank space and inventory. A base stock system using a limited number of hydrogenated stock products for blending to meet the finished product requirements is practiced by many shortening producers.

#### 3.1. Base Stock System Advantages

The advantages provided by a base stock system are twofold (17).

*Control.*

1. Blending of hydrogenated oil batches to average minor variations.
2. Improved uniformity by producing the same hydrogenated product more often.

3. Cross-product contamination reduction by scheduling more of the same product consecutively.
4. Reduction of product deviations from attempts to use finished product heels.
5. Eliminate rework created by heel deterioration before use.

### *Efficiency.*

1. The hydrogenation process scheduled to maintain base stock inventories rather than reacting to customer orders.
2. Hydrogenation of full batches instead of some smaller batches to meet demands without creating excessive heels.
3. Better order reaction time to improve customer service.

## **3.2. Base Stock Uniformity Control**

Base stock consistency control is the secret to first pass yield, i.e., meeting finished product specification limits with the specified blend of base stocks. Most finished shortenings' physical characteristics are controlled with solids fat index (SFI) iodine value (IV), and/or a melting point analysis. However, time restraints during hydrogenation do not allow the long elapsed times required for these results. More rapid techniques to control base stock end points are:

1. Soft base stocks with IV of 90+ are adequately controlled by refractometer (RI or RN) determinations performed within the hydrogenation department. Mettler dropping points and most other melting point analysis have poor reproducibility at this level or are too time consuming.
2. Intermediate base stocks (55–89 IV) preferred controls are a combination of RI or RN readings and Mettler dropping point (AOCS Method Cc 18–80) (18). The oil is hydrogenated to the predetermined refractometer end point and then the reaction is stopped until a Mettler dropping point result can be determined. The melting point is the controlling analysis. If the analysis indicates that the base stock is softer than desired, hydrogenation is continued and the process repeated until the internally specified end point has been attained.
3. Low iodine value hard stocks can be controlled with “quick titer” determinations. There are many versions of this evaluation, but basically, the test involves dipping the bulb of a glass thermometer into liquid fat heated above the melting point, then rotating the thermometer stem to cool the fat. The end point is the temperature when the fat on the bulb clouds. Constants can be determined to add to the quick titer result to approximate “real tier” determined by AOCS Method Cc 12–59 (19).

The time-consuming but more accurate analytical methods should be performed as an audit function to ensure that the correlation with the quicker controls are still valid.

### 3.3. Soybean Oil Base Stocks

Base stock requirements will vary dependent on the customer base served, which obviously dictates the finished product mix. A wide variety of source oils are available that could be used for shortening base stocks, as illustrated by Table 1. The choices are narrowed by factors such as customer specifications, costs, religious prohibitions, traditional preferences, crop economics, legislation, availability, transportation, and others. These factors have favored soybean oil in the United States for several decades. Therefore, most U.S. shortening processors have base stock systems dominated by soybean oil with only minor representation by the other source oils. The minor oils in most base stock systems serve as  $\beta'$  promoters for plasticity, like cottonseed and palm oils, or those source oils required for specialty product preparation.

Table 7 outlines a soybean-oil-based system with seven hydrogenated base stocks; ranging from a partially hydrogenated 108 IV base stock to a saturated hard stock with a 5 max IV. An eighth base stock is refined and bleached liquid soybean oil, the same oil that serves as the feedstock for the hydrogenated bases. Utilization of a similar base stock system should enable the processor to meet most shortening specifications by blending two or more base stocks, except for some specialty products that can only be made with special hydrogenation conditions.

Hydrogenation has two principal aims: to produce solid or semisolid products with certain plastic properties and to increase the stability of the oil. A huge variety of products can be produced with the hydrogenation process depending on the conditions used and the degree of saturation. The fatty acid composition and resultant characteristics of the hydrogenated products depend on the controllable factors: temperature, pressure, agitation, catalyst type and activity, and catalyst concentration. Agitation is fixed on most converters by design and, therefore, cannot be

**TABLE 7. Soybean Oil Base Stock.**

Iodine Value	Solid Fat Index					Mettler Dropping Point (°C)	Quick Titer (°C)
	10.0°C	21.2°C	26.7°C	33.3°C	40.0°C		
108	4.0 max.	2.0 max.	—	—	—	—	—
85	15–21	6–10	2–4	—	—	28–32	—
80	22–28	9–15	4–6	—	—	31–35	—
75	38–44	21–27	13–19	3.5 max.	—	34–37	—
65	59–65	47–53	42–48	23–29	3–9	41–45	—
60	65–71	56–62	51–57	37–43	14–18	45–48	—
>5	a	a	a	a	a	a	50–54
Hydrogenation Conditions	Temperature		Pressure	Catalyst Concentration		Agitation	
Nonselective	Low		High	Low		Fixed	
Selective	High		Low	High		Fixed	
Selectivity unimportant	High		High	High		Fixed	

<sup>a</sup>Too hard to analyze.

considered an operator controllable factor. Changes in the reaction conditions affect the selectivity of the hydrogenated base stock; e.g., saturation of linoleate over oleate and the rate of trans acids formation. Selectivity affects the slope of the SFI curve; steep SFI slopes are produced with selective hydrogenation conditions while flat SFI slopes are the result of nonselective hydrogenation conditions.

The soybean oil base stock system in Table 7 uses both selective and nonselective conditions for the partially hydrogenated bases. Selectivity is not important for low-IV hard stocks because these reactions are continued to almost complete saturation. The main objective for these products is to reach maximum saturation as quickly as possible. Actual values for the hydrogenation conditions were not identified because results vary from one converter to another due to design and other variables within each plant (20). It is necessary to develop conditions for each installation separately to meet the SFI, IV, and melting point relationships.

## 4. SHORTENING FORMULATION

Most shortenings are identified and formulated according to usage. Figure 1 illustrates the diverse SFI and melting point relationships among five different shortening products. This figure indicates the differences in plastic range necessary to perform the desired function in the finished products. Shortenings with the flattest SFI curves have the widest plastic range for workability at cool temperatures as well as elevated temperatures. All-purpose shortenings have the widest plastic range. Nondairy and solid frying shortenings have relatively steep SFI curves, which will provide a firm, brittle consistency at room temperature but will be almost fluid at only slightly elevated temperatures.

The product with the very flat SFI slope is a fluid opaque or pumpable liquid shortening that has become popular due to the convenience offered, handling cost savings in some situations, and lower saturated fatty acid levels. In these systems, the  $\beta$ -crystal form is necessary to produce and maintain fluidity.

Shortening chips are a somewhat recent development as a specialty ingredient for addition to doughs, biscuits, cookies, and other baked products. These specialty products are modifications of fat flakes, which formerly indicated only the saturated oil products or hard fats. The chips are formulated with selectively hydrogenated base stocks with melting points high enough to flake but low enough for good eating characteristics. This product type now includes the traditional hard fat flakes, shortening chips, and stabilizers for icings and glazes.

### 4.1. Wide Plastic Range Shortenings

The basic all-purpose shortening has been the building block for shortenings where creaming properties, a wide working range, and heat tolerance are important. The functionality of an all-purpose shortening at any temperature is largely a function of

the solids content at that temperature. The all-purpose shortenings are formulated to be not too firm at 10–16°C (50–60°F) and not too soft at 32–38°C (90–100°F). Initially, a liquid oil was blended with a hard fat to make a compound shortening that had a very flat SFI curve, which provided an excellent plastic range. However, the low oxidative stability of these shortenings preclude their use today for most products. Currently, most of these products are formulated with a partially hydrogenated soybean oil base stock and a low-IV cottonseed or palm oil hard stock. Hard fats are added to shortenings both to extend the plastic range, which improves the tolerance to high temperatures, and for crystal type and stability. The  $\beta'$ -crystal-forming cottonseed oil hard stock functions as a plasticizer for improved creaming properties.

Hydrogenation of a shortening base increases the oxidative stability. As a rule, the lower the base IV the longer the AOM stability. However, as base hardness is increased, the level of hard stock required to reach a desired consistency decreases. Hard stock reduction reduces the plastic range and heat tolerance. Therefore, oxidative stability improvements are achieved at the expense of plasticity. The extent that one attribute can be compromised to improve another must be determined by the requirements of the intended food product.

Shortening plastic range is important for bakery shortenings intended for roll-in and creaming applications alike because of the consistency changes with temperature. Shortenings become brittle above the plasticity range and soft below the range; both conditions adversely affect creaming and workability alike. Shortenings are normally plastic and workable at SFI values between 15% and 25%. Therefore, shortenings with flatter SFI slopes fall within the plasticity window for a much greater temperature range than products with steep SFI slopes. The all-purpose shortening in Figure 1 has a plastic range of 23°C (73.4°F) while the frying shortening has only a 4°C (39.2°F) plastic range. Theoretically, the frying shortening should perform equally as well as the all-purpose shortening for baking applications if it is used within the 4°C (39.2°F) range from 29°C to 33°C (85°F to 92°F). The frying shortening's use for the baking application would require very strict controlled temperatures probably not available in most bakeries. The 23°C (73.4°F) range from 10°C to 33°C (50°F to 92°F) for the all-purpose shortening is decidedly more practical.

Two of the basestocks outlined in Table 7 are designed for shortenings requiring a wide plastic range, e.g., the nonselectively hydrogenated 80 and 85 IV base stocks. Even these two base stocks with only a 5 IV difference provide measurable differences in plastic range and stability when cottonseed oil hard fat is added to produce equivalent consistencies at 26.7°C (80°F). The softer 85 IV base stock required 2.5% more hard fat to achieve the targeted 20% SFI at 26.7°C (80°F). The higher hard fat level indicates greater heat resistance and a wider plastic range but a lower AOM stability from the lower IV value. The firmer 80 IV base stock required 2.5% less hard fat to attain the 20% SFI at 26.7°C (80°F), which helped reduce the plastic range by 4.5°C (40.1°F) but increased the AOM stability to 100 h versus the 65 h for the shortening with the 85 IV base. Figure 2 graphically illustrates these effects.

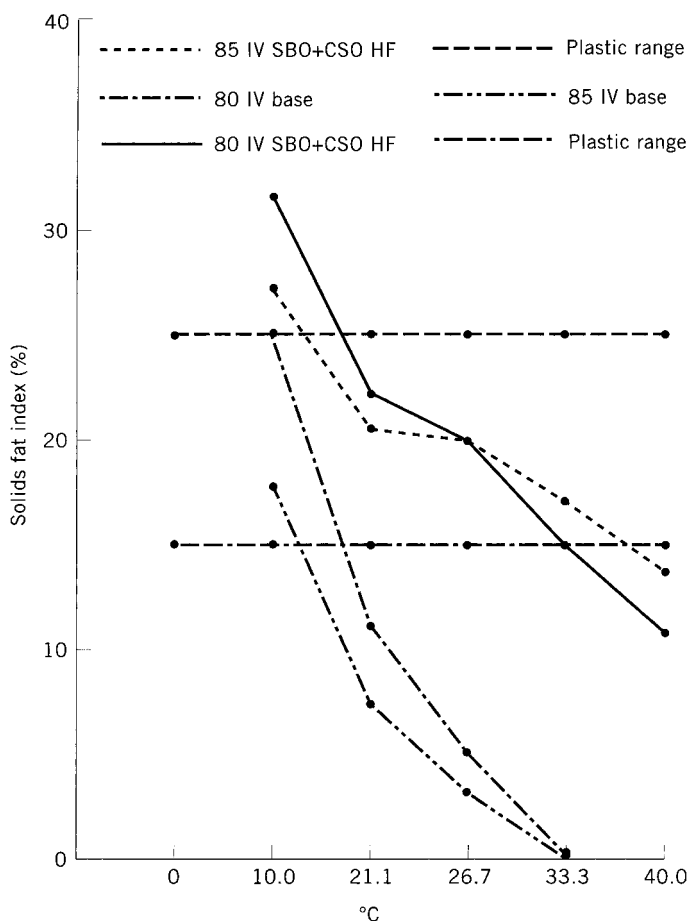


Figure 2. Base stock effect.

The effect of hard stock upon the SFI slope and plastic range is illustrated by Figure 3. Cottonseed oil hard fat was added to the 85 IV soybean oil base stock to demonstrate the plasticizers effect; as the hard fat level is increased the shortening becomes firmer with a flatter slope. The higher hard fat levels are used to formulate roll-in, puff pastry, and other shortenings where a plastic but firm consistency is desired.

The use of a partially hydrogenated base plus hard fat to produce a wide plastic range with good creaming properties has been expanded into a whole family of specialized shortenings. The development of these products has involved the selection of the proper hydrogenated base stock and hard fat to produce the desired plastic range and AOM stability. These developments have taken two directions: (1) the addition of an emulsifier or an emulsifier system to an all-purpose shortening base or (2) formulating nonemulsified products for a specific functionality. Table 8 outlines shortening types in these two categories.

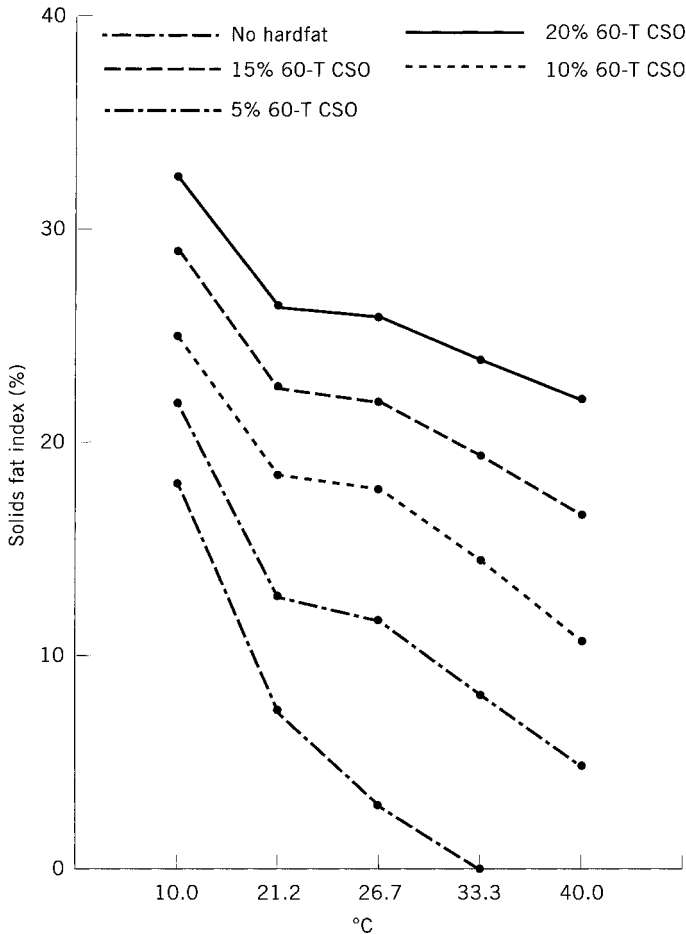


Figure 3. Hard fat effect.

TABLE 8. Wide Plastic Range.

Nonemulsified	Emulsified
All-purpose	Household
Danish roll-in	Cake and icing
Puff pastry	Icing and filling
Cookie	Cake mix
Pie crust	Specialty cakes
Donut frying	Yeast raised



## 4.2. Narrow Plastic Range Shortenings

Plasticity is of minor importance and can be a detriment for products requiring high oxidative stability and/or sharp melting characteristics. Shortenings specially designed for specific frying situations, nondairy systems, cookie fillers, and confectionery fats require flavor stability's and eating characteristics not possible with blends of moderately hydrogenated oils with hard fats. These products require lower IV shortenings for oxidative stability with steeper SFI slopes and a melting point lower than body temperature for good eating characteristics. The frying and nondairy shortenings plotted on Figure 1 illustrate the steep SFI slopes.

The frying shortening meets the restaurant industry requirements for a stable heat transfer media that becomes a part of the food to supply texture, mouth feel, and to enhance the food flavor. The nondairy shortening requirements are similar to those of the frying shortening. Shortening effects both texture and mouth feel of the finished products. A shortening with a flat SFI will mask the desirable flavors and coat the mouth rather than melt cleanly. Flavor stability is also a concern because reverted or oxidized flavors change the flavor profile of dairy-type products.

The steep SFI products are generally produced with selectively hydrogenated bases that have high solids at the lower temperature readings but fall off rapidly to a low melting point for the desired eating or mouth feel characteristics and excellent oxidative stability. These products are usually composed of a single straight hardened base or possibly two selectively hydrogenated bases for a slightly different slope than available with a single base. For example, the nondairy shortening in Figure 1 is composed of one straight hydrogenated base; it is the 75 IV selectively hydrogenated soybean oil base stock. The frying shortening can be produced with a blend of the 65 and 75 IV base stocks.

## 4.3. Liquid Shortenings

Liquid or pumpable shortenings are flowable suspensions of solid fat in liquid oil. The liquid oil phase may or may not be hydrogenated depending on the finished product's consistency and oxidative stability required. Low-IV soybean oil hard fat seeds crystallization. It can vary from as low as 1% to higher levels as required to produce the desired finished product viscosity. The ease in which soybean oil converts to the stable  $\beta$  crystal form makes it ideal for liquid shortenings (21).

Liquid opaque shortenings have been developed for food products where pourability or pumpability at room temperature and below is important. The major uses identified for these products are:

1. *Frying.* Liquid frying shortenings provide a convenience to the operator; it can be poured directly into the fryer and quickly heated to frying temperature.
2. *Bread and Cake.* Bakery liquid shortening systems usually contain high melting emulsifiers, which provide optimum performance, combined with the oil phase, which allows bulk handling without heating while still maintaining the desired specialty product performance.

**TABLE 9. Typical Liquid Opaque Shortening Formulations.**

Soybean Oil Basestock (%)	Frying	Bread	Cake	Nondairy
135 IV	—	90	—	—
108 IV	98	—	99	98
5 IV	2	10	1	2
Base total	100	100	100	100
Additives				
Dimethylpolysiloxane	1.0 ppm	None	None	None
Emulsifiers (%)	None	None	Yes	Yes
Analytical limits:				
% SFI at 10.0°C	4.0 max	13.0	7.0	5.5
% SFI at 26.7°C	2.0 max	12.0	5.5	2.5
% SFI at 40.0°C	0.5 max	10.0	1.0	1.0
$\alpha$ monoglycerides (%)	—	—	1.5	4.0
PGME (%)	—	—	2.8	—

3. *Nondairy*. Liquid shortening systems offer the room temperature pumpable convenience with a stable shortening high in polyunsaturates, which are attractive for some nondairy applications such as creamers, filled milks, toppings, etc.

Typical compositions and SFI results for four different types of liquid shortenings are compared in Table 9. These products must contain high levels of liquid or only lightly hydrogenated oils to maintain fluidity. Therefore, additives must contribute heavily to the product performance in all liquid shortenings. The major additives in liquid shortenings, as indicated with the compositions in Table 9, are antifoaming agents for frying applications and emulsifiers for the cake and nondairy products. Bread shortenings are the only product where an additive is not mandatory for liquid shortening performance. Lubrication is the primary function of a bread shortening. However, the emulsifiers that improve bread shelf life are usually produced with fully saturated fats that are difficult for the baker to add to doughs. Liquid shortening is an ideal carrier for these emulsifiers, providing another convenience for the user.

Other additives used with liquid shortenings are color and flavor. Butterlike flavors and yellow colors have been added to the nondairy liquid shortenings to make them more complete systems. In the foodservice area, a modification of the liquid frying shortening has become another product type—*pan and grill* liquid shortening. Additions of butterlike flavors, yellow colors, and lecithin to the liquid frying shortening are the usual procedure employed for making the pan and grill liquid products. Lecithin, a natural emulsifier, functions as an antisticking agent in this application.

#### 4.4. Shortening Flakes and Chips

Shortening flake describes the high melting edible oil products solidified into a thin flake form for ease in handling, quicker remelting, or for a specific function in a

food product. The traditional flaked products have been the saturated oil products known as low IV hard fats or stearines. The hard fat flakes have been joined by products formulated for special uses where the flake form is desirable. These specialty flaked products are shortening chips and stabilizers for icings and glazes.

Hydrogenation selectivity is not important for the hard fats or stearines because the reactions are carried to almost complete saturation. However, selectivity is very important for the proper functionality of both the shortening chips and icing stabilizers. The shortening chips are selectively hydrogenated to attain a steep SFI slope with a melting point as low as possible to still allow the product to maintain the chip form after packaging, during shipment to the food processor, while processing into the finished product, and until heated by the homemaker during baking to produce a flaky, tender product. The chips can be formulated from the base stock system with a blend of the 60 and 65 IV base stocks depending on the desired melting point and SFI requirements. Shortening chips made with nonlauric oils, melting points usually range from 43°C to 48°C (110°F to 118°F). Shortening chips can be made with hydrogenated lauric oils (palm kernel or coconut) as well. The lauric-oil-based chips offer a sharper melt with a lower melting point than the chips made with soybean or cottonseed oils. The melting points for the lauric oil chips normally range from 38°C to 42°C (101°F to 108°F).

Flavors, colors, and/or spices can be encapsulated in the shortening chips to leave pockets of color and flavor where the chips have melted during baking. Shortening chips have been commercially available in flavors such as plain, butter, cinnamon sugar, and blueberry.

Icing stabilizers are also selectively hydrogenated with melting points centered at 45°C, 47°C, and 52°C (113°F, 116.6°F, and 125.6°F) and formulated with and without lecithin dependent upon the desired degree of fluidity in the finished icing or glaze. Lecithin addition increases fluidity up to 0.5% but restricts this function at higher levels. The icing stabilizers must be hydrogenated specially to meet the SFI–melting point requirements, except for the 45°C (113°F) melting point product; it can be blended like the shortening chips. In either case, low-IV hard fats should not be used to adjust melting points, due to the effect upon mouth feel.

#### **4.5. Powdered Shortenings**

Two types of powdered shortenings are produced: (1) spray dried fat emulsions with a carrier and (2) spray chilled or beaded hard fat blends. The spray dried powdered shortenings are partially hydrogenated shortenings encapsulated in a water-soluble material. The fats can be homogenized in solution with a variety of carriers; i.e., skim milk, corn syrup solids, sodium caseinate soy protein isolate, and others. Emulsifiers may be included with the fat for finished product functionality. Fat contents range from 50% to 82% depending on the original emulsion composition (22). The spray dried powdered shortenings are used in some prepared mixes for their ease in blending with the other dry ingredients.

Hard fats can be powdered or beaded without the aid of a carrier. Spray chilling in a tower or solidification on a chill roll followed by grinding and screening for

particle sizing are two methods used for powdering or beading the hard fats (23). Some of the uses for these products are as peanut butter stabilizers, specialty prepared mix products, and in place of flaked hard fats where more rapid melting is desirable.

## 5. SHORTENING CRYSTALLIZATION

The functionality of solidified edible oil products is influenced by three basic processes: (1) formulation, which includes the choice of source oils and the hydrogenation techniques used for the base stocks; (2) chilling, which initiates the crystallization process; and (3) tempering, where the desirable crystal nuclei are developed and stabilized. Formulation, the first requirement for consistency control, has been reviewed in the preceding section. Chilling and tempering, which develop, mature, and stabilize the desired crystal structure introduced by the product composition, is discussed in this section.

Chilling and tempering processes are employed when shortenings are packaged. The physical form of edible oil products is important for the proper handling and performance in food products. The importance of shortening consistency cannot be overemphasized, many applications depend on the physical properties peculiar to each packaged product, such as softness, firmness, oiliness, creaming properties, melting behavior, surface activity, workability, solubility, aeration potential, pourability, and others. In the case of plastic shortenings, consistency is important from the standpoint of usage and performance in bakery and related products. And the importance of the consistency for a liquid shortening is obvious; it must be maintained as a uniform, homogeneous suspension to perform properly (24).

### 5.1. Fat Plasticity

Edible fat products appear to be soft homogenous solids; however, microscopic examination shows a mass of very small crystals in which a liquid oil is enmeshed. The crystals are separate discrete particles capable of moving independently of each other. Therefore, shortenings possess the three conditions essential for plasticity in a material:

1. A solid and a liquid phase.
2. The solid phase dispersed finely enough to hold the mass together by internal cohesive forces.
3. Proper proportions of the two phases.

Plasticity, or consistency, of an edible oil product depends on the amount of solid material, the size, shape, and distribution of the solid material, and the development of crystal nuclei capable of surviving high-temperature abuse and serving as starting points for new desirable crystal growth. The factor most directly and obviously influencing the consistency of a plastic shortening is the proportion of the solid

phase. As the solids contents increase, an edible oil product becomes firmer. The proportion of the solid phase is influenced by the extent of hydrogenation and attendant isomerization. Shortening products are also firmer with smaller crystal sizes due to an increased opportunity for the solids particles to touch and resist flow. Stiffening is also increased more with the interlacing of long needlelike crystals than with more compact crystals of the same size. The crystal nuclei (“memory”) are developed by the further treatment of a newly solidified product. Exposure immediately after chilling to 29°C (85°F) for 24+ h before cooling to 21°C (70°F) provides a softer consistency with the ability to withstand widely fluctuating temperatures and still revert back to the original consistency at room temperature (25).

## 5.2. Fat Crystal Habit

Fat crystals represent lower energy states of molecular configuration. At elevated temperatures, fats retain enough molecular motion to preclude organization into stable crystal structures. Edible oils go through a series of increasingly organized crystal phases with cooling until a final stable crystal form is achieved. This process can occur in fractions of a second or over a number of months. The crystal types formed define the textural and functional properties of most fat-based products.

The crystal structure of a shortening or other fat-based product is determined by: (1) source oil composition, (2) processing, and (3) tempering or maturing. Crystallization is induced when melted fat is cooled rapidly to initiate the formation of crystal nuclei or seed. The seeds form templates upon which crystals grow. Formulation, cooling rate, heat of crystallization, and agitation levels affect the number and type of crystals formed.

Each source oil exhibits inherent crystallization tendencies. A fat may pass through one or more unstable crystalline stages before assuming either  $\beta$  or  $\beta'$  crystal forms. The differences among the three crystal forms are (26).

1.  $\alpha$ -crystal forms are unstable and will convert into the more stable  $\beta$  or  $\beta'$  crystal forms.
2.  $\beta$  crystals are large, coarse, and self-occluding.
3.  $\beta'$  crystals are small and needlelike and can pack together into dense fine-grained structures.

Each common fat or oil has a definite crystal habit that is determined by four factors: (1) palmitic fatty acid content, (2) distribution and position of palmitic and stearic fatty acids on the triglyceride molecule, (3) degree of hydrogenation, and (4) the degree of randomization. Table 10 identifies the crystal habit of hydrogenated edible oils (27).

Many edible oil products contain various combinations of  $\beta$  and  $\beta'$  tending components. The ratio of  $\beta$ - $\beta'$  crystals helps to determine the dominant crystal habit, but the higher melting triglyceride portions of a solidified fat product usually force the fat to assume that crystal form. The crystal form of the solidified fat product has a major influence upon the textural properties. Fats exhibiting a stable  $\beta'$  form

**TABLE 10. Hydrogenated Oils Crystal Structure.**

$\beta$	$\beta'$
Canola oil	Cottonseed oil
Cocoa butter	Butter oil
Coconut oil	Herring oil
Corn oil	Menhaden oil
Olive oil	Modified lard
Lard	Palm oil
Palm kernel	Rapeseed oil
Peanut oil	Tallow
Safflower oil	Whale oil
Sesame oil	
Sesame oil	
Soybean oil	
Sunflower oil	

appear smooth, provide good aeration, and have excellent creaming properties for the production of cakes, icings, and other bakery-type products. Conversely, the  $\beta'$ -polymorphic form tends to produce large granular crystals for products that are waxy, grainy, and provide poor aeration. These  $\beta$  formers perform well in applications such as pie crusts where a grainy texture is desirable (28) and opaque liquid shortenings where the large granular crystals are preferred for stability and maintenance of fluidity.

## 6. PLASTICIZED SHORTENING CONSISTENCY

The consistency of a shortening is the culmination of all the factors influencing crystallization and plasticity: chilling, working, creaming gas, pressure, and tempering. Each process is individually and collectively important; a shortening's performance can be adversely affected if any of the consistency factors do not conform to the standards established for each individual shortening product during development. Table 11 summarizes the effects of the crystallization processes upon shortening consistency covered in greater detail in the following paragraphs.

**TABLE 11. Factors Influencing Shortening Consistency.**

Soft	Process	Firm
Cold	Chilling	Warm
More	Working	Less
High	Creaming gas	None
High	Pressure	Low
Warm	Tempering	Cold

## 6.1. Chilling

One of the earliest methods of solidifying shortenings involved the use of a chill roll that was internally refrigerated by circulating cold brine. The melted fat was fed into a trough from which a thin film of the liquid was picked up as the roll revolved and crystallized into a semisolid state during a revolution. A blade removed the solidified fat film, which fell into a trough equipped with a rotating shaft with metal fingers called a picker box, which worked the product to make it homogeneous and to incorporate air. Pumps then picked up the shortening for transfer to filling machines. This process has now become obsolete in the United States except for some very special products. The reason for obsolescence for shortening processing was the difficulty in controlling the variables to produce a uniform product (29), poor thermal efficiency, and moisture condensation on the chilled roll surface.

Most shortenings are now quick-chilled in closed thin-film scraped-surface heat exchangers. The principal of operation for most of these units is a combination of chilling the fat in a very thin film, which is continually removed by scraper blades with concise temperature control. The residence time within the heat exchanger tubes is very short, almost always less than 20 s. Since all triglycerides exhibit a high propensity for supercooling, the product exits from the heat exchanger cooled well below the equilibrium crystallization temperature, usually within a range of 15.5–26.7°C (60–80°F) depending on the shortening type.

The chilling unit temperature control limits are determined by fill tests to identify the condition necessary to produce the consistency and plasticity requirements for each shortening product. The initial guidelines are usually identified by trial and error to determine the temperature and pressure where the shortening forms a slight rounded or mounded surface upon filling plus evaluation of the product characteristics after tempering. The finished shortening's consistency becomes softer as the temperature is decreased and firmer more brittle shortenings develop with higher temperatures. After the operating limits are identified for a product, the processing control should be within 1°C (33.8°F) for uniformity. Thereafter, if the plasticization controls and the formulation specifications are met, the original product should be duplicated.

## 6.2. Working and Filler Pressure

If the product is allowed to solidify without agitation at this point, it will form an extremely strong crystal lattice and exhibit a narrow plastic range. This consistency may be desirable for stick margarine but is harmful for products requiring a plastic-like consistency. Therefore, processing after the initial quick chilling cycle has been adapted to the product consistency or form desired (30, 31). Enclosed worker units with speed controls have replaced the picker boxes that followed the chill roll in the early plasticization process. The heat of crystallization is dissipated rapidly in these units while working the product to provide fine crystals. Extrusion valves are employed in most systems to deliver a homogeneous smooth product to the filler at 17–27 atm (250–400 psig) (29).

### 6.3. Creaming Gas

Air incorporation, while agitating in the open picker box, has been replaced with nitrogen injection into the inlet side of the chiller, in precisely controlled quantities, normally 12–14% by volume for standard plasticized shortenings, which provides a white creamy appearance and increases the shortening's workability. The correct gas content is important for appearance and stability. Shortenings containing the proper levels are white and creamy with a bright surface sheen. Too low a gas content gives a yellowish greasy appearance and shortenings packed without creaming gas develop a Vaseline-like appearance. High gas levels cause dead-white chalky appearances with a lifeless surface appearance and often large air pockets within the product, which give a puffy feel or consistency. Nonuniform dispersion of the gas gives an unattractive streaked appearance.

Gas levels are varied from none to as high as 30% in the shortenings produced depending on the product requirements. The typical creaming gas additions for the different shortening types are identified in the following: regular plasticized, 12–14%; precreamed, 19–25%; puff pastry, none; liquid shortening, none; shortening chips, none; and speciality shortenings, as needed.

### 6.4. Tempering

Most technologists agree that a shortening is tempered when the crystal structure of the hard fraction reaches equilibrium by forming a stable crystal matrix. The crystal structure entraps the liquid portion of the shortening. The mixture of low- and high-melting components of the solids undergoes a transformation in which the low-melting fractions remelt and then recrystallize into a higher melting, more stable form. This process can take from 1 to 10 days depending on the shortening formulation and package size. After a shortening takes an initial set, some  $\alpha$  crystals are still present. These crystals remelt and slowly recrystallize in the  $\beta'$  form during tempering.  $\beta'$  crystals are preferred for most plastic shortenings, especially those designed for creaming or laminating (31). Therefore, soybean-oil-based shortenings requiring a plastic range are formulated with 5–20% of a  $\beta'$  tending hard fat. The  $\beta'$  hard fat must have a higher melting than the soybean oil base stock in order for the entire shortening to crystallize in the stable  $\beta'$  form.

The effect of tempering on a plastic shortening can be demonstrated best by performance testing. In some cases penetration values undergo some change during tempering, showing a softening of the conditioned shortening versus a nontempered one. The effect of tempering can be identified with the feel or workability of the shortening; a tempered product is more smooth with good plasticity while the nontempered shortening will be more brittle with less plasticity.  $\beta'$ -crystal-forming shortenings transferred to a cool temperature immediately after filling become permanently hard and brittle and attempts to recondition these products by subjecting them to tempering conditions have not been successful.

Immediately after packaging, a shortening requiring a plastic consistency should be stored 40 h or more in a quiescent state at a temperature slightly above the fill



temperature. In practice, holding at 29°C (85°F) for 24–72 h or until a stable crystal form is reached is an acceptable compromise (28). The primary purpose of tempering is to condition the solidified shortening so that it will withstand wide temperature variations in subsequent storage and still have a uniform consistency when brought back to 21–24°C (70–75°F), which is the use temperature for a majority of the plasticized shortenings (30).

### 6.5. Quick Tempering

The expense and logistical problems associated with constant temperature rooms for tempering have led several equipment manufacturers to investigate the development of mechanical systems to eliminate the need for tempering. The majority of the systems developed do not claim complete elimination but rather a 50% or more reduction in tempering time. Most of the so-called quick tempering systems add a postcooling and kneading, or working, unit to the conventional-type chilling and working systems used with tempering. The theory postulated for these systems is that liquid fat is forced to crystallize individually and rapidly, thus creating smaller more stable crystals, rather than crystallizing onto existing crystals and increasing their size as happens with normal tempering (30, 31).

Performance characteristics equivalent to well-tempered shortenings with only 24 h conditioning have been claimed for the quick temper products (32). These systems have been accepted by some shortening processors, but the standard tempering procedures are still practiced by many for shortenings requiring a wide plastic range.

### 6.6. Quality Assurance Evaluations

The consistency of a shortening is controlled by two dominating factors: (1) the SFI, which depends on the composition of the fat blend and (2) the processing conditions used to solidify, package, and temper the product. When the solidification conditions are constant, a strong correlation is expected between the analytical characteristics of the blend and the consistency of the shortening. Solidified shortening's appearance, texture, and consistency characteristics should be evaluated after tempering to identify potential plasticization defects and devise corrective actions for future production. A suggested evaluation method is to inspect a package of the shortening visually and physically. The physical evaluation involves picking up a small portion and kneading it in your hand. A good wide plastic range product will be smooth and will not become sticky or fall apart when worked. A review of shortening faults previously encountered with corrective actions are:

1. *Striking* can be caused by a number of plasticization deficiencies: (1) chilling too low for the operating backpressure; (2) erratic creaming gas dispersion; (3) channeling, which allows semiliquid oil to pass through the chilling unit insulated from chilling properly; (4) mixing different chiller unit streams operating at different temperatures; and (5) erratic chilling unit pressures (33).

2. *Sandy* or small lumps can be caused by too cold chilling unit temperatures or precrystallization prior to the chilling unit.
3. *Ribby* is a condition of alternating layers of hard and soft product caused by (1) too cold chilling unit with low pressure, (2) mixing streams from two chilling units operating at different temperatures, or (3) too cold chilling with excessive mounding when filling.
4. *Puffy* conditions are caused by too high creaming gas content or too low pressure to finely distribute the air cells.
5. *Brittle* feel is contributed by (1) high chilling unit temperature, (2) absence of low working after chilling, or (3) a formulated narrow plastic range.
6. *Oil separation* or Free oil, is caused by (1) high chilling unit temperature, (2) precrystallization (3) temperature abuse after packaging, or (4) formulation.
7. *Chalky* appearance is caused by too high creaming gas content.
8. *Vaseline-like* or a yellowish greasy appearance is typical of shortenings packaged without creaming gas.
9. *Whiteness* may be controlled by the amount and degree of dispersion of the creaming gas (34).

## 7. LIQUID OPAQUE SHORTENINGS

Liquid opaque shortenings are distinguished from liquid oils by composition and appearance. Both are pourable, but liquid oils are clear while liquid shortenings are opaque due to their suspended solids. The suspended solids may be hard fats, emulsifiers, or a combination of the two depending on the intended use, i.e., frying, bread, cake, or nondairy products. Simply described, liquid shortenings are flowable suspensions of solid fat and/or emulsifiers in liquid oil.

### 7.1. Crystallization Processes

1.  $\beta$  tending hard fats, such as the  $>5$ -IV soybean oil hard fat identified in the base stock system, serve as quick-forming nuclei that cause solids in the base oil to precipitate in small enough crystals to ensure pourability and prevent separation. Many different crystallization procedures for liquid opaque shortenings have been patented, some of which are briefly described below (35–38):
2. Gradual cooling of melted product to form large  $\beta$  crystals with gentle agitation. This procedure normally requires 3–4 days elapsed time.
3. Gradual cooling followed by comminution with a homogenizer or colloid mill. An estimated 3–4 days elapsed time required for processing.
4. Rapid cooling with a swept-surface heat exchanger followed by a holding period of at least 16 h with gentle agitation, to fluidize, just prior to packaging.

5. Suspension of finely ground hard fat or emulsifier in a cool liquid oil with subsequent comminution using a homogenizer, colloid mill, or shear pump.
6. Quick chilling a concentrated mixture of hard fat in liquid oil followed by a holding period in an agitated tank to allow  $\beta$ -crystal growth. The stabilized concentrate is then blended with a diluent oil at room temperature and seeded with an oil containing  $\beta$ -hard fat crystals.
7. Quick chilling a solution of  $\beta$ -crystal-forming hard fat in liquid oil to 38°C (100°F) and allowing the heat of crystallization to carry the temperature of the cooled oil to not over 54°C (130°F). Complete crystallization reportedly requires 20–60 min.
8. Recirculating a hot oil solution of hard fat from an agitated holding tank through a scraped-surface heat exchanger and back into the holding tank. The oil in the tank cools slowly and the crystals formed in the early stages melt in the hot oil. Eventually the mass cools to a point where the crystals do not melt but form  $\alpha$ , then  $\beta'$ , and finally stable  $\beta$  crystals. At this point, further chilling is stopped and the resulting opaque pourable shortening is packaged.
9. Double chilling/tempering system of first cooling from 65°C to 43°C (150°F to 110°F) followed by a 2-h crystallization period with gentle agitation and subsequent supercooling to 21–24°C (70–75°F) followed by a second crystallization period of approximately 1 h. The opaque liquid shortening is packaged after an expected heat of crystallization rise of approximately 9°C (15°F) has been experienced.

The summarized processing procedures for opaque liquid shortenings agree on several points: (1) all are dispersions of solid and liquid fractions, (2)  $\beta$ -forming hard fats is the preferred solid fraction to seed crystallization, and (3) heat of crystallization must be dissipated before a stable product is achieved. Another agreement is that aeration of the liquid shortenings must be avoided at all stages of processing before, during, and after crystallization. Air incorporation makes the product more viscous or less pourable and promotes product separation. Storage studies indicate an air content of less than 1.0% is required for a stable suspension stability.

## **7.2. Tempering and Storage**

Opaque liquid shortenings do not require any further tempering after packaging, but the storage temperatures are critical. Storage below 18°C (65°F) will cause the liquid shortenings to solidify with a loss of fluidity. Storage above 35°C (95°F) will result in partial or complete melting of the suspended solids. The solidification due to cool temperatures can be reversed by a controlled heating not to exceed the melting point. However, the damage caused by high temperature cannot be remedied except by complete melting and reprocessing.

## 8. SHORTENING CHIPS AND FLAKES

As stated previously, shortening flake describes the higher melting edible oil products solidified into a thin flake form for ease of handling, quicker remelting, or for a specific function in a food product. Flaking rolls, utilized for the chilling of shortening and margarine prior to the introduction of scraped-wall heat exchangers, are still used for the production of shortening flakes. Chill rolls have been adapted to produce several different flaked products used to provide distinctive performance characteristics in specialty formulated foods. Consumer demands have created the need for such specialty fat products. Specialty high-melting fat flakes have been developed for specific applications with varied melting points as shown on Table 11. Instead of becoming obsolete, chill rolls have fulfilled an equipment requirement to produce specialty-fat-based products.

### 8.1. Chill Rolls

Chill rolls are available in different sizes, configurations, surface treatments, feeding mechanisms, etc., but most are a hollow metal cylinder with a surface machined and ground smooth to true cylindrical form. Rolls, internally refrigerated with either flooded or spray systems, turn slowly on longitudinal and horizontal axes. Several options exist for feeding the melted oil product to the chill roll: (1) a trough arrangement positioned midway between the bottom and top of the roll, (2) a dip pan at the bottom of the roll, (3) overhead feeding between the chill roll and a smaller applicator roll, and (4) a double- or twin-drum arrangement operating together with a very narrow space between them where the fat product is sprayed for application to both rolls. The coating of fat is carried over the roll to solidify and is removed by a doctor blade, positioned ahead of the feed mechanism with all of the designs.

### 8.2. Flake Crystallization

In the crystallization of hydrogenated edible oil products, the sensible heat of the liquid is removed until the temperature of the product is equal to the melting point. At the melting point, heat must be removed to allow the crystallization of the product. The quantity of heat associated with this phenomenon is called heat of crystallization. Sensible heat (specific heat) of most common hard fat products is equal to about 0.27 cal/g (0.5 Btu/lb) and the heat of crystallization is equal to 27.8 cal/g (50 Btu/lb). The amount of heat that must be removed to crystallize hardened oil is 100 times the amount of heat that must be removed to lower the product temperature (39).

### 8.3. Flaking Conditions

The desired shortening flake product dictates the chill roll operating conditions and additional treatment necessary before and after packaging. However, some generalizations relative to chill roll operations and product quality can be made.

1. *Crystal structure*: each flaked product has crystallization requirements dependent on source oils, melting points, degree of saturation, and the physical characteristics desired.
2. *Flake thickness*: four controllable variables help determine flake thickness: (1) oil temperature to the roll, (2) chill roll temperature, (3) speed of the chill roll, and (4) the feed mechanism.
3. *In package temperature*: heat of crystallization will cause a product temperature rise after packaging if it is not dissipated prior to packaging. The product temperature can increase to the point where partial melting coupled with the pressure from stacking will cause the product to fuse together into a large lump.
4. *Flake condition*: glossy or wet flakes are caused by a film of liquid oil on the flake surface due to incomplete solidification. Either too warm or too low chill roll temperatures can cause this condition. High roll temperatures may not provide sufficient cooling to completely solidify the flake. Low roll temperatures may shock the oil film, causing the flake to pull away from the surface before completely solidified. Wet flakes from either cause will lump in the package.

## REFERENCES

1. K. F. Mattil, in D. Swern, *Bailey's Industrial Oil and Fat Products*, 3rd ed., Interscience, New York, 1964, p. 265.
2. W. H. Shearon, Jr., *Ind. Eng. Chem.*, **July**, 1278 (1950).
3. Ref. 1, p. 267.
4. J. E. Slaughter, Jr., *Proceedings: A Six Day Short Course in Vegetable Oils*, sponsored by The American Oil Chemists' Society, Aug. 16–21, 1948, p. 120.
5. K. S. Markley, *Fatty Acids*, Part I, 2nd ed., Interscience, New York, 1960, pp. 6–13.
6. K. S. Markley, *Chem. Soc.*, **54**, 557 (1977).
7. D. I. Hartnett, *J. Amer. Oil Chem. Soc.*, **54**, 557 (1977).
8. H. E. Robinson and K. F. Mattel, *J. Amer. Oil Chem. Soc.*, **36**, 434 (1959).
9. *USDA Oil Crops Situation and Outlook* Economic Research Service, USDA, Oct. 2000, Table 40 updated online 2004.
10. Ref. 9, Table 36.
11. R. F. Wilson, *INFORM*, **4**, 193 (1993).
12. F. D. Gunstone and T. P. Hiloitch, *J. Amer. Oil Chem. Soc.*, **22**, 836 (1945).
13. T. J. Weiss, *Food Oils and Their Uses*, AVI Publishing, Westport, Conn., 1983, p. 112.
14. F. J. Flider and F. T. Orthoefer, *J. Amer. Oil Chem. Soc.*, **58**, 270 (1981).
15. Ref. 13, p. 114.
16. Ref. 13, p. 116.
17. E. G. Latondress, *J. Amer. Oil Chem. Soc.*, **58**, 185 (1981).

18. *Official Methods and Recommended Practices of American Oil Chemists' Society*, 4th ed., American Oil Chemists' Society, Champaign, Illinois, 1994, Method Cc 18–80.
19. Method Cc 12–59, in Ref. 17.
20. E. J. Latondress, in D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soy Oil Processing and Utilization*, American Soybean Association and AOCS, Champaign, Illinois, 1980, pp. 145–152.
21. R. D. O'Brien, in R. Hastert, ed., *Hydrogenation: Proceedings of an AOCS Colloquium*, American Oil Chemists' Society, Champaign, Illinois, 1987, pp. 157–165.
22. Ref. 13, pp. 129–130.
23. M. M. Chrysam, in T. H. Applewhite, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 3, Wiley-Interscience, New York, 1985, pp. 97–98.
24. R. J. Bell, in P. J. Wan, ed., *Introduction to Fats and Oils Technology*, American Oil Chemists' Society, Champaign, Illinois, 1992, pp. 187–188.
25. Ref. 1, pp. 272–281.
26. D. Best, *Prepared Foods*, May, 168 (1988).
27. L. H. Wiedermann, *J. Amer. Oil Chem. Soc.*, **55**, 825 (1978).
28. A. E. Thomas, *J. Amer. Oil Chem. Soc.*, **55**, 830 (1978).
29. C. E. McMichael, *J. Amer. Oil Chem. Soc.*, **33**, 512 (1956).
30. N. T. Joyner, *J. Amer. Oil Chem. Soc.*, **30**, 526 (1953).
31. C. W. Hoerr and J. V. Ziemba, *Food Eng.*, 90 (May 1965).
32. Ref. 13, pp. 96–97.
33. Ref. 13, p. 125.
34. D. R. Erickson, *J. Amer. Oil Chem. Soc.*, **44**, 534A (1967).
35. Ref. 12, pp. 126–129.
36. T. Petricca, *Baker's Digest*, **Oct.**, 39 (1976).
37. Ref. 1, pp. 1064–1068.
38. Ref. 23, pp. 100–104.
39. P. J. Wan and A. H. Chen, *J. Amer. Oil Chem. Soc.*, **60**, 743 (1983).

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## *Confectionery Lipids*

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### **1. INTRODUCTION**

Chocolate not only has nutritional value, but it is an emotional confection—it can be used to comfort, apologize, celebrate, and as a token of gratitude. Chocolate is derived from the cocoa bean, which in turn comes from the cacao (ka-ka-o) tree, *Theobroma cacao*. Theobroma means “food of the gods,” and cacao is as rich in history as it is in flavor.

The tree is said to have been discovered in the Amazon or Orinoco basin at least 4000 years ago. Christopher Columbus was the first European to encounter the beans, during his fourth voyage to the New World in 1502, but he virtually ignored them. It was two decades later that the Spanish conquistador Hernando Cortés helped spread the valuable cocoa bean crop to the Caribbean and Africa, and then he introduced drinking chocolate into Spain in 1528. The cacao tree is now cultivated in West Africa, South America, Central America, and the Far East. At world level, the demand for cocoa is generally measured by reference to world grindings. The world grindings of cocoa beans in 2003/2004 approached three million metric tons.

### **2. CHEMISTRY OF CHOCOLATE**

Cocoa beans are converted into chocolate liquor by a combination of roasting, winnowing, and grinding. Until 1828 the only known product was “drinking

chocolate,” but high fat levels were a deterrent to an acceptable product. It was not until the 1828 invention of the cocoa press, by Van Houten in Holland, that a more acceptable product was forthcoming. The chocolate liquor could now be further processed by pressing into cocoa butter and cocoa powder. The availability of quantities of cocoa butter made it possible to produce “eating chocolate.” Cocoa liquor, cocoa butter, and cocoa powder are now all important ingredients in the chocolate/confectionery industry. Out of these, cocoa butter is the most expensive one, followed by cocoa liquor and cocoa powder. Cocoa powder is mainly used in chocolate drinks and in confectionery coatings. Chocolate liquor, cocoa butter, lecithin, and sugar are the main ingredients in dark chocolate. Milk crumb or milk powder may be also added to produce milk chocolate. Various vegetable confectionery fats described in detail later are also used to implement new functionalities to the chocolate system. Chocolate is produced by a combination of techniques such as refining, conching, standardizing, and molding.

### 3. CHARACTERISTICS OF COCOA BUTTER

The total fat content of the whole bean on a dry basis is around 48–49%, and triacylglycerol is the major storage component. A mature cocoa bean can store up to 700 mg of cocoa butter. As a tree may produce as many as 2000 seeds a year, a single tree could yield up to 15 kg of cocoa butter annually.

Cocoa butter is the most expensive constituent of chocolate formulations as well as an extremely important component. It is composed predominantly (>75%) of symmetrical triacylglycerols with oleic acid in the 2-position (1). Approximately 20% of triacylglycerols are liquid at room temperature, and cocoa butter has a melting range of 32–35°C and softens around 30–32°C. This is essential to the functionality of cocoa butter in its applications. It contains only trace amounts of the unsymmetrical triacylglycerols (PPO, PSO, and SSO). (P = palmitic acid, O = oleic acid, and S = stearic acid; the order of letters indicates the position of the acids in the triacylglycerols molecule.)

The unique triacylglycerol composition together with the extremely low level of diacylglycerols gives cocoa butter its desirable physical properties and its ability to recrystallize during processing in a stable crystal modification. The complexity of the crystallization of cocoa butter is because triacylglycerols can crystallize in a number of different crystal modifications, dependent on triacylglycerol composition and on crystallizing and tempering conditions during manufacturing and storage.

The fatty acid composition, various analytical constants, and triacylglycerol composition of different cocoa butters are presented in Tables 1–3, respectively. These results show that Malaysian cocoa butter contains the highest levels of mono-unsaturated triacylglycerols. The Brazilian cocoa butter contains the lowest levels of monounsaturated triacylglycerols and the highest levels of other unsaturated triacylglycerols. The cocoa butters from India and Sri Lanka are close to Malaysian cocoa butter in terms of hardness and triacylglycerol composition.



**TABLE 1. Fatty Acid Composition of various Cocoa Butters by GLC (wt%).**

Sample Cocoa Butter	C14	C16	C16:1	C17	C18	C18:1	C18:2	C18:3	C20	C20:1	C22	C24
Ghana	0.1	24.8	0.3	0.3	37.1	33.2	2.6	0.2	1.1	Trace	0.2	0.1
India	0.1	25.3	0.3	0.2	36.2	33.5	2.8	0.2	1.1	0.1	0.2	Trace
Brazil	0.1	23.7	0.3	0.2	32.9	37.4	4.0	0.2	1.0	0.1	0.2	Trace
Nigeria	0.1	25.5	0.3	0.3	35.8	33.2	3.1	0.2	1.1	0.1	0.2	0.1
Ivory Coast	0.1	25.4	0.3	0.2	35.0	34.1	3.3	0.2	1.0	0.1	0.2	0.1
Malaysia	0.1	24.8	0.3	0.3	37.1	33.2	2.6	0.2	1.1	Trace	0.2	0.1

**TABLE 2. Analytical Constants of Various Cocoa Butters.**

Sample	IV	C3 <sup>a</sup>	% DAG	% FFA	Pulsed NMR Extended <sup>b</sup>				Pulsed NMR BS684 method <sup>c</sup>			
					20°C	25°C	30°C	35°C	20°C	25°C	30°C	35°C
Ghana	35.8	32.2	1.9	1.53	84.0	78.0	36.0	0.1	76.0	69.6	45.0	1.1
India	34.9	32.4	1.5	1.06	88.1	83.3	44.7	1.8	81.5	76.8	54.9	2.3
Brazil	40.7	32.0	2.0	1.24	67.7	56.6	18.5	0.6	62.6	53.3	23.3	1.0
Nigeria	35.3	33.1	2.8	1.95	83.7	77.3	35.4	0.1	76.1	69.1	43.3	0.0
Ivory Coast	36.3	32.0	2.1	2.28	82.3	74.8	32.7	0.9	75.1	66.7	42.8	0.0
Malaysia	34.2	34.3	1.8	1.21	89.3	83.7	49.6	1.8	82.6	77.1	57.7	2.6
Sri Lanka	35.2	33.2	1.1	1.58	—	—	—	—	79.7	74.2	50.4	0.1

IV: iodine value; DAG: diacylglycerols; FFA: free fatty acids.

<sup>a</sup>Melting Point Stabilization 64 hours at 25 °C.

<sup>b</sup>Tempering 64 hours at 20 °C.

<sup>c</sup>BS 684: British Standard method 684. Tempering 40 hours at 26 °C.

**TABLE 3. Triglyceride Composition (Mole %) of Various Cocoa Butters by High-Speed Liquid Chromatography.**

	TAG	Ghana	India	Brazil	Ivory Coast	Malaysia	Sri Lanka	Nigeria
Tri.	PPS	0.3	0.6	tr.	0.3	0.8		0.3
Saturated	PSS	0.4	0.5	tr.	0.3	0.5	1.9	0.5
Total		0.7	1.1	tr.	0.6	1.3	1.9	0.8
Mono-	POS	40.1	39.4	33.7	39.0	40.4	40.2	40.5
Unsat.	SOS	27.5	29.3	23.8	27.1	31.0	31.2	28.8
	POP	15.3	15.2	13.6	15.2	15.1	14.8	15.5
	SOA	1.1	1.3	0.8	1.3	1.0	1.0	1.0
Total		84.0	85.2	71.9	82.6	87.5	87.2	85.8
Di-	PLiP	2.5	2.0	2.8	2.7	1.8	2.5	2.2
Unsat.	POO	2.1	1.9	6.2	2.7	1.5	2.3	1.7
	PLiS	3.6	3.1	3.8	3.6	3.0	1.4	3.5
	SOO	3.8	3.3	9.5	4.1	2.7	3.9	3.0
	SLiS	2.0	1.7	1.8	1.9	1.4		1.8
	AOO		0.8		0.5	0.5		0.5
Total		14.0	12.8	24.1	15.5	10.9	10.1	12.7
Poly-	PLiO	0.6	0.5	1.5	0.8	0.3	0.8	0.4
Unsat.	OOO	0.4	tr.	1.0	tr.			tr.
	SLiO	0.3		1.2	0.5	tr.		0.3
	ALiO		0.4					
	LiOO			0.3				
Total		1.3	0.9	4.0	1.3	0.3	0.8	0.7

TAG: triacylglycerol.

There is a good correlation between the triacylglycerol composition and solid fat content of these cocoa butters. Malaysian, Sri Lankan, and Indian cocoa butters are the hardest, and Brazilian is the softest, whereas others lie in between. The quality of the Brazilian cocoa butter can be improved by mixing it with Malaysian cocoa butter, which will result in higher solid fat content at various temperatures.

At International Food Science Center, we have measured diacylglycerols levels varying between 1.5% and 2.8%. Higher diacylglycerol levels affect the crystallization of cocoa butters remarkably, and thus all efforts should be made to reduce these levels in good quality cocoa butters.

The main drawbacks of Malaysian cocoa beans are their excessive acidic flavor, weak chocolate flavor, and certain other off flavors. Several attempts have been made to improve these characteristics (2).

The deodorization of cocoa butter is necessary to reduce free fatty acid content and to give a product that satisfies the present day requirement of a neutral bland flavor. Deodorization is a suitable method for partially eliminating chlorinated insecticides from cocoa butter. The normal deodorization temperatures are in the range 160–180°C. The oxidative stability of various cocoa butters listed in Table 4 shows extremely high values, and these are unaffected during the deodorization process. Stability against oxidation depends on natural antioxidants present in cocoa butters. The tocopherol composition in Table 5 shows a predominance of

**TABLE 4. Oxidative Stability of Cocoa Butters.**

Samples	Induction time at 120°C in hours
CB Trinidad	42.3
CB Brazil	35.3
CB Colombia	38.4
CB Venezuela	41.3
CB Ecuador	19.1
CB Ivory Coast	42.9
CB Ghana	42.2

**TABLE 5. Tocopherol Composition of Various Cocoa Butters by High-Speed Liquid Chromatography.**

Sample	Total	Tocopherols (mg/kg)						
		$\alpha$ -T	$\beta$ -T	$\tau$ -T	$\delta$ -T	$\alpha$ -TT	$\tau$ -TT	$\delta$ -TT
Brazil	176	0.7	1.2	164	6.9	—	2.0	0.7
Ghana	198	2.7	1.5	183	6.8	0.6	2.3	0.7
India	265	6.5	2.2	245	9.1	—	2.3	—
Ivory Coast	126	0.4	0.4	117	6.2	—	2.3	—
Malaysia	149	0.5	—	140	7.4	—	0.6	—

$\alpha$ -tocopherol and total tocopherol levels ranging between 100 mg/kg and 300 mg/kg. Dimick has found phospholipids levels ranging from 3.62  $\mu$ g to 4.72  $\mu$ g per 500  $\mu$ g in cocoa butters of differing origins (Table 6). Dimick is extending the scope of this research to determining the influence of phospholipids during the crystallization of hard and soft cocoa butters (3).

The results of thermorheographic experiments presented in Table 7 show very high correlation with the pulsed NMR data in Table 2. Thus, Malaysian cocoa butter crystallizes quickly, and Brazilian is the slowest crystallizing, which correlates with the hardness of these cocoa butters as measured by pulsed nuclear magnetic resonance technology.

**TABLE 6. Phospholipid Quantities of Origin Cocoa Butter Samples (% by wt).**

Cocoa Butter	Phospholipid ( $\mu$ g)	% by weight*
Malaysia	3.62	0.72
Ivory Coast	4.35	0.87
Ghana	4.72	0.94
Ecuador	3.80	0.76
Dominican Republic	4.72	0.94
Brazil	4.54	0.91

\*Ratio of phospholipid mass divided by sample mass (500  $\mu$ g) times 100.

**TABLE 7. TRG Values of Different Types of Cocoa Butters.**

Cocoa Butter	Measuring temperature: 22 °C Times of different MPS (minutes)		
	30 mp	50 mp	80 mg
Malaysia	13	16	20
Sri Lanka	12	14	15
Ghana	31	36	42
Brazil	178	187	199

TRG: thermorheogram.

The addition of milkfat to cocoa butter (4) results in marked lowering of the melting point, adversely affecting the crystallization behavior and the hardness as shown in Table 8. An obvious decrease is clearly evident in the solid fat content and a deterioration in solidification properties as shown in the values of the Jensen curve. These results are further confirmed by comparing the curves for milkfat and cocoa butter in various proportions. There are two reasons for this strong decrease in hardness (5, 6);

- Liquid oil components of the milkfat soften the cocoa butter because of their fluidity.
- The solid fat components form eutectics with the triacylglycerols of cocoa butter.

The analytical results of the fractionation of Malaysian cocoa butter are given in Table 9. The stearin thus produced is primarily a mixture of POP, POS, and SOS and has virtually no components that are liquid at or near room temperature. With the removal of the moderating influence of the more liquid components of cocoa butter, the cocoa butter fraction (CBF) becomes more crystalline and complex, the melting range becomes shorter, and the heat of fusion increases.

This stearin is extremely hard and can be used effectively to improve the quality of soft cocoa. Attempts have been made to improve the quality of Brazilian cocoa butter by fractionation.

#### 4. CONFECTIONERY FATS

The historical uncertainty in the cocoa butter supply and the volatility in cocoa butter prices depending on fluctuating cocoa bean prices forced confectioners to seek other alternatives, which may have a stabilizing influence on the prices of cocoa butter. Ever increasing demand for chocolate and chocolate-type products increases the demand for cocoa beans from year to year. However, it is difficult to predict the supply of cocoa beans. This ensures a continuing need for economical

**TABLE 8. Analytical Constants of the blends of Cocoa Butters (CB) and Milkfats (MF).**

Sample	IV (Wijs)	Pulsed NMR BS684 Method 2				Solidification Curve (Jensen)		
		20 °C	25 °C	30 °C	35 °C	Max. Temp. (°C)	Time min/max (min)	Temperature Rise (°C)
CB Malaysia	35.6	82.1	78.3	57.9	2.1	31.0	40.0	7.3
CB Malaysia (90%) MF (10%)	35.4	69.1	63.9	43.0	1.2	29.5	39.0	6.0
CB Malaysia (85%) MF (15%)	35.3	61.4	56.7	37.1	1.0	29.0	37.0	6.1
CB Malaysia (80%) MF (20%)	35.2	53.9	49.3	31.0	1.0	28.5	35.0	5.7
CB Malaysia (75%) MF (25%)	35.1	46.0	42.1	25.5	1.3	27.5	32.5	5.0
CB Brazilian	39.7	62.8	53.5	29.9	0.4	29.0	30.5	4.6
CB Brazilian (90%) MF (10%)	39.1	53.5	42.9	19.8	0.0	27.5	37.0	5.9
CB Brazilian (85%) MF (15%)	38.8	46.1	36.1	13.0	0.5	26.5	34.5	4.9
CB Brazilian (80%) MF (20%)	38.4	36.3	29.9	11.5	0.0	26.0	37.5	4.7
CB Brazilian (75%) MF (25%)	38.1	27.2	17.9	6.3	0.0	25.5	45.0	4.6
CB Ghana	35.6	77.8	72.9	49.2	0.6	30.5	36.5	5.6
CB Ghana (90%) MF (10%)	35.4	64.2	58.6	35.2	0.0	29.0	35.0	5.0
CB Ghana (85%) MF (15%)	35.3	56.8	51.7	29.7	0.3	28.5	37.0	5.2
CB Ghana (80%) Brazilian (20%)	35.2	48.3	43.3	24.5	0.0	27.5	42.0	5.2
CB Ghana (75%) MF (25%)	35.1	39.6	34.9	18.4	0.1	27.0	47.5	4.4

**TABLE 9. Fractionation of Malaysian Cocoa Butter.**

Sample	Pulsed NMR BS684 method 2						Triglyceride composition (Mol%)( HPLC)										
	% Yield	IV (Wijs)					Monounsaturated				Diunsaturated						
			20°C	25°C	30°C	35°C	POP	POS	SOS	Total	PLiO	PLiP	POO	PLiS	SOO	SLiS	Total
Malaysian Cocoa Butter			82.1	78.7	58.3	2.4	12.5	45.3	37.2	95.0	tr.	2.2	0.7	0.6	1.6		5.1
Cocoa butter																	
Stearine	79.4	29.7	96.6	95.7	89.1	13.7	11.4	51.3	37.3	100.0			tr.	tr.			
Cocoa Butter																	
Oleine	20.6	52.2	1.2	0.0	0.0	0.0	11.3	19.1	17.5	47.9	3.6	16.3	6.6	9.0	15.6	1.0	52.1

BS 684: British Standard, method 684.

vegetable fats to replace cocoa butter in chocolate and confectionery products. Attempts were made as early as 1930 by confectioners to use fats other than cocoa butters in their formulations. These experiments did not succeed because of the incompatibility of the fat blends used, which resulted in discoloration and fat bloom. However, these experiments demonstrated the need for cocoa butter-type fats in the chocolate and confectionery industry.

The continued research in the field of confection science resulted in the development of fats resembling the characteristics of cocoa butter. These fats have become known by the term *hard butters*. These fats were developed using palm-kernel, coconut, palm, and other exotic oils such as sal, shea, and illipe as raw materials. The processes involved in producing such fats included hydrogenation, interesterification, solvent or dry fractionation, and blending. The most elementary hard butters are manufactured by combining the processes of hydrogenation and fractionation.

## 5. HARD BUTTERS

The hard butters can be divided into the following three main groups based on their characteristics and raw materials used to produce them:

1. Lauric cocoa butter substitutes (lauric CBS). These are fats that are incompatible with cocoa butter but that have physical properties resembling those of cocoa butter.
2. Non-lauric cocoa butter substitutes (non lauric CBS). These are fats that are partly compatible with cocoa butter.
3. Cocoa butter equivalents or extenders (CBE). These are fats that are fully compatible with cocoa butter (chemical and physical properties similar to those of cocoa butter).

Other terms used to describe hard butters include *cocoa butter partial replacers*, *total replacers*, *modifiers*, and *extenders*. All of these categories can be further subdivided into a range of speciality fats, tailored to suit particular purposes.

## 6. LAURIC CBS

This category offers a range of confectionery fats with different levels of physical properties, but all having triacylglycerol compositions that make them incompatible with cocoa butter; i.e. they are all used in formulations with cocoa powder, mainly for compound coating.

Cocoa butter substitutes are produced from lauric fats that are obtained from various species of palm tree, the main varieties being palm, which yields palm-kernel oil and coconut. These fats differ from non-laurics in that they contain 47–48%



lauric acid, together with smaller amounts of other medium- and short-chain fatty acids. This gives the fats a solid consistency at cool ambient temperatures, but they nevertheless melt below 30°C. From a practical point of view, cost economy probably has been the main incentive behind the search for suitable and reliable substitutes for cocoa butter. The introduction of hydrogenation added another dimension to the alteration of lauric fats, but it must be emphasized that the palm-kernel stearins exhibit characteristics considerably better than those of hydrogenated palm-kernel oil (HPKO), all dependent on the sharpness of the fractionation.

Palm-kernel stearins today have functional properties similar to those of cocoa butter: a steep NMR curve, a very brittle texture, and a narrow melting range that ensures a quick meltdown and a pleasant mouthfeel. The interval between setting and melting points is short. This offers a technological advantage over cocoa butter. The tempering is simplified or may be omitted for normal coating purposes. Vegetable fats can crystallize in several polymorphic forms, the most common being alpha, betaprime, and beta, which in the same order display an increasing stability, melting point, heat of fusion, and density. In general, lauric fats are stable in betaprime form. The rate of crystallization of the alpha form is higher than that of the betaprime form, which in turn crystallizes faster than the beta form. The other sources for the manufacture of lauric CBS includes coconut, South American palm-kernel oils, tucum, cohune, babassu, and ouri-curi. Most of these minor varieties are seldom encountered in Europe except in the country of origin and they do possess specific properties.

The principal advantages and disadvantages of lauric CBS are as follows.

### 6.1. Advantages

1. Good oxidative stability, long shelf life.
2. Excellent eating quality and flavor release, no waxy aftertaste.
3. Texture very similar to that of cocoa butter, i.e., excellent hardness and snap and not greasy to the touch.
4. Solidify quickly tempered or untempered.
5. Excellent gloss and gloss retention.
6. Available at a cost far less than cocoa butter.

### 6.2. Disadvantages

The principal disadvantages of lauric CBS include the following:

1. Mixing with cocoa butter results in a eutectic state. If the manufacturer is to change from chocolate to confectionery coatings, an absolute cleanout of all tanks and enrobing systems is required. Separate production lines are preferred. These fats do not tolerate more than 6% cocoa butter.
2. When lauric CBS are exposed to moisture and fatsplitting enzymes (lipase), there is a danger of fat hydrolysis and the liberated lauric acid has a distinct

soapy flavor that can be detected even at low concentrations. These liberated fatty acids also have a lower flavor threshold as compared with the longer chains. This is shown below:

Butyric acid	(C4)	0.6 ppm
Caproic acid	(C6)	2.5 ppm
Caprylic acid	(C8)	350 ppm
Capric acid	(C10)	200 ppm
Lauric acid	(C12)	700 ppm
Stearic acid	(C18)	15000 ppm

3. Relatively low milkfat tolerance.

## 7. NON-LAURIC CBS

Non-lauric CBS consists of fractions of hydrogenated oils: soybean, cotton, corn, peanut, safflower, and sunflower oils. These oils are hydrogenated under selective conditions to promote the formation of *trans*-fatty acids, thereby increasing the solid contents considerably. The melting point of oleic acid—the *cis*-configuration—is 14°C, whereas the isomer elaidic acid melts at 51.5°C.

As a result of the similarity in the chain length and the molecular weight, products of this type can tolerate up to 25% cocoa butter on a fat basis when used in a confectionery coating.

Non-lauric CBS possesses good flavor, odor, and color properties and does not need tempering.

## 8. COCOA BUTTER EQUIVALENTS (CBEs)

CBEs are nonhydrogenated specialty fats containing the same fatty acids and symmetrical mono-unsaturated triacylglycerols as cocoa butter. They are fully compatible with cocoa butter and can be mixed with cocoa butter in any ratio in the chocolate formulations.

From the data presented earlier in Table 3, it is clearly evident that cocoa butter is a simple three-component system consisting of POP, POS, and SOS triacylglycerols, and if these three triacylglycerols are mixed in appropriate proportions, then the resultant vegetable fat will behave as 100% cocoa butter equivalents. Although CBEs are not produced by mixing individual triacylglycerols as they are very expensive to produce, this is the logic behind the whole game of producing CBE. Palm oil is fractionated to produce a middle-melting fraction rich in POP, and exotic fats such as shea, sal, illipe (Borneo Tallow), and so on are fractionated to get triacylglycerol cuts rich in POS and SOS. A careful preparation and blending of these results in a tailor-made fat equivalent to cocoa butter in physical properties. Therefore, these fats are called cocoa butter equivalents. The formulation of a suitable CBE is the greatest art in fat technology.

## 8.1. Disadvantages

The main drawbacks of cocoa butter are as follows:

1. Low milkfat tolerance.
2. Lack of stability at elevated temperatures.
3. Tendency to bloom.

## 8.2. Advantages

The principal advantages of incorporating CBEs are as follows:

1. Reduction in the production cost of chocolates as CBEs are cheaper than cocoa butter.
2. Stabilizing influence on fluctuating prices of cocoa butter.
3. Improvement of the milkfat tolerance.
4. An increasing resistance to storage at high temperatures.
5. Bloom control.

The greatest technological advantages lies in the compatibility of these fats with cocoa butter.

The most recent monograph on confectionery fats was developed by Society of Chemical Industry UK out of a symposium held in Oct. 1996 and chaired by the Author. (7).

## 9. ORGANIC CHOCOLATE AND CONFECTIONERY

The market for organic chocolates and confectionery has not been very successful earlier because of nonavailability of various ingredients in designing the final products. This market is booming as several new chocolate, ice cream, and other confectionery products are made available in the market. We have recently designed a number of organic products for organic ice cream, compound chocolate, and coffee creamer production. These products open new avenues for the future development in this field (see Table 10).

### 9.1. Ten Good Reasons to Go Organic

1. Safe, nutritious, unadulterated food.
2. No artificial chemicals, pesticides, and fertilizers.
3. Absence of antibiotics and growth-promoting drugs.
4. Environmentally friendly.
5. Produced without GMOs.

**TABLE 10. Designer Organic Fats developed by the industry.<sup>a</sup>**

Product Name	Iodine Value	Solid Fat Content (SFC) by Pulsed NMR			
		10°C	20°C	25°C	30°C
Coffee Creamer Fat	43–49	45–55	15–23	6–13	Max 5
Ice Cream Coating Fat	22–30	54–64	14–22	Max 2	Nil
Ice Cream Coating Fat	16–22	67–74	24–34	2–9	Max 1
Ice Cream Filling Fat	43–50	44–54	14–22	6–12	Max 6
Palm Shortening	50–55	52–62	23–31	13–21	6–12

<sup>a</sup>A courtesy of IFSC.

6. Places great emphasis on animal welfare.
7. Reduces dependence on nonrenewable resources.
8. Based on modern and scientific understanding of ecology.
9. Based on soil science and ensures soil fertility by crop rotation.
10. Better taste.

## 9.2. Advantages

1. There is a continuously growing demand for organic foods driven primarily by consumer perceptions of quality and safety (25–50% increase each year).
2. The establishment of regional (EU) and international (Codex) guidelines for production, processing, labeling, and marketing of organic foods has been the key step in the international harmonization of requirements for organic foods.
3. The organic label is not a health claim; it is a process claim. No clear trends have been established in terms of organoleptic quality differences between organic and conventional food.
4. Because of lower chemical usage in organic food production, we have to apply better principles of storage and transport to guarantee freshness of the product.
5. Future research must focus on critically designed experiments to reveal the nutritional quality of organic food as compared with conventional food.

## 10. RECIPÉ ENGINEERING AND OIL PROCESSING

In the management of speciality fats, recipé engineering plays a major role. The major analytical techniques such as gas liquid chromatography, high-performance liquid chromatography, low-resolution pulsed nuclear magnetic resonance spectroscopy, differential scanning calorimetry, various static and dynamic crystallization measurement techniques, and rheology are the tools employed to define the final product and its application in the confectionery industry.

Large food manufacturers demand and receive ever improving standards and consistency. Equipment for monitoring trace impurities and physical and chemical properties has led to new sophisticated standards, which are directing the way in which we transport our raw materials and refining equipment construction. In this context, the type of heating systems in use can also be important. Refiners everywhere are being asked by their customers to change from traditional oil heating systems to systems using high-pressure steam, because of the real risk of product contamination using thermal fluid heating systems. There are ways of converting older plants without scrapping their boilers, but generally the best option is to simply build new and take advantage of the sophisticated equipment available today.

Finally, fats not only provide functionality and structure to products but can be dominant in their contribution to taste and flavor, including off-flavors. Thus, it is of utmost importance to process speciality products, taking into account minute details to produce a product that not only tastes fresh but also lasts longer (8).

## 11. CONCLUSIONS AND FUTURE PROSPECTIVES

Confection science is extremely complicated because of the great diversity of triacylglycerol molecules and the presence of several surface-active components that play a major role in defining the quality of the final products.

Sophisticated analytical methodologies play a major role in unfolding the mysteries of speciality fats in chocolate. Recent harmonization of European Union law for speciality fats in chocolates will demand newer sources of exotics to be evaluated to fulfill future requirements.

## REFERENCES

1. V. K. S. Shukla, W. Schiøtz-Nielsen, and W. Batsberg, *Fette Seifen Anstrichmittel*, **85**, 274–278 (1983).
2. R. J. E. Duncan, et al., *The Planter, Kuala Lumpur*, **65**, 157–173 (1989).
3. P. S. Dimick, personal communication.
4. V. K. S. Shukla, in R. J. Hamilton, ed., *Developments in Oil and Fats*, Blackie Academic & Professional, Glasgow, United Kingdom, 1995, pp. 66–94.
5. V. K. S. Shukla, D. P. J. Moran and K. K. Rajah, eds., *Fats in Food Products*, Blackie Academic & Professional, Glasgow, United Kingdom, 1994, pp. 255–276.
6. V. K. S. Shukla, *World Ingredients*, **January-February**, 30–33 (1995).
7. W. Hamm and R. E. Timms, eds., *Production and Application of Confectionery Fats*, Society of Chemical Industry and Lipid Technology, 1997.
8. V. K. S. Shukla, *European Food and Drink Directory*, 11–16 (1994/1995) (Contract Communications Ltd., London, United Kingdom).

# 6

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## *Cooking Oils, Salad Oils, and Dressings*

Steven E. Hill and R. G. Krishnamurthy

### 1. INTRODUCTION

Lipids used in food products have been conventionally divided into two classes based on their consistency at about 25°C (72°F): (1) liquid oils, such as soybean, cottonseed, sunflower, safflower, peanut, olive, and rapeseed oils, and (2) solids and semisolids, such as lard, tallow, palm oil, coconut oil, palm-kernel oil, and cocoa butter. In the preparation of some foods, it is of no particular consequence whether the lipid material is liquid or solid, but in others the consistency is important, depending on the amount of the lipid used. For example, in the preparation of dressing for green salads, the object is to provide an oily mouthfeel; hence, a liquid oil must be used. When the oil content of such a dressing is low, a low melting fat may be desired for texture benefits. On the other hand, baked food products generally require plastic fats for the incorporation of air needed for leavening. But with the development of emulsifiers, in many of these areas, it is possible to use liquid oils with a suitable emulsifier (1, 2) to achieve this goal.

In the past, for reasons related to both history and climate, there were distinct geographical divisions of people by fat- and oil-consuming groups. Inhabitants of central and northern Europe derived most of their lipids from domestic animals. Consequently, the food habits and cuisine of these people developed around the use of plastic fats. On the

other hand, the main form of lipids used by people from the warmer climates of southern Europe, Asia, and Africa were liquids. In the Western Hemisphere, plastic fats are the most widely used lipids because of the predominantly northern European extraction of the early settlers. However, a significant shift in this distinction has taken place because of the global movement of peoples, the implication of plastic fats in cardiovascular diseases, and the development of new oil compositions through biotechnology. In general, there is greater use of liquid oils except where functionality, such as highly developed dough structure, cream icings, or fillings, dictates use of a plastic fat.

In addition to their household uses, cooking oils are in considerable demand for deep-fat frying of many food products that are consumed immediately after frying. In the past, for reasons of stability during storage, fats were the preferred frying medium for packaged foods, such as potato, corn chips, and other snack products that remain in storage for considerable periods. In recent times, the use of oils, which are less unsaturated and more stable in such operations, has significantly increased. Products like doughnuts still require plastic fats for frying, because liquid oils tend to give them a greasy appearance.

Most cooking oils are of vegetable origin, although such oils can be produced by fractionation (3) of animal fats, like butter fat and tallow, and vegetable oils like palm oil. The only naturally occurring liquid oil from animal sources is of marine origin. But marine oils in their natural form are highly susceptible to oxidation, and therefore, special handling is desired for their use. In various parts of the world, considerable quantities of marine oils are used in food after hydrogenation. However, stabilization technologies (4) are being developed for use of liquid marine oils because of the health benefits (5) of highly unsaturated fatty acids, such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA).

## 2. NATURAL AND PROCESSED COOKING AND SALAD OILS

Cooking oils are used either in their natural state or after processing, depending on the method of production, local taste, custom, and nutritional beliefs. In the Old World, oils were used in their natural state. In Occidental countries, the oil is processed to a bland state. This geographical difference in the use of oils is probably caused by the different methods of obtaining the oil from its source. However, use of processed oils has become more common in all parts of the world because of the global expansion of the more efficient technologies, such as high-pressure expression and solvent extraction to obtain higher yields of oil, which also produce an oil with considerable amounts of nontriglyceride components, such as coloring bodies and gums. These oils, because of the presence of nontriglyceride substances and their strong flavor and pronounced color, must be suitably processed to make them acceptable and edible. In general, bland neutral oil is produced from crude oils by refining, bleaching, and deodorizing. An important distinction between salad and cooking oils is the difference in their oxidative and thermal stability. Cooking oils have to be more stable than salad oils at higher temperatures, such as deep-fat frying. The term *salad oil* is applied to oils that remain substantially liquid in a refrigerator, i.e., about 4.4°C (40°F). The standard method of evaluating salad

oils (AOCS Method Cc 11-53) is the cold test. The oil sample is put in a sealed 4-oz bottle and placed in an ice bath at 0°C (32°F). If the oil remains clear after 5.5 h, it meets the criterion of a salad oil or suitably winterized oil. Well-winterized oils remain clear for periods much longer than 5.5 h. From a practical standpoint, the number of hours required for the oil to cloud is of less significance than the character and the amount of the crystals deposited after a prolonged storage at 0°C (32°F), or refrigeration temperature. Oils with good cold test hours are necessary for manufacture of stable emulsions. Crystallization inhibitors, such as lecithin and polyglycerol esters, have been used for extending the cold test. Other crystal inhibitors, such as disaccharide esters (6), monosaccharide esters (7), and polysaccharide esters of hydroxy fatty acids (8), have been patented. The extent of commercial use of these additives is not known. Oxystearin, which is produced by controlled oxidation of partially hydrogenated cottonseed oil, was once extensively used, however, is no longer used as a crystal inhibitor. Sunflower, safflower, and corn oils need dewaxing before they can meet the criteria of a salad oil. Soybean, high erucic, and low erucic rapeseed oils conform to the definition of a salad oil. However, because they contain significant amounts of relatively unstable linolenic acid, they are partially hydrogenated to improve stability for many food applications. However, both soybean and low erucic acid rapeseed (LEAR or Canola) oils are used without hydrogenation in the preparation of salad dressings. Cottonseed oil, because of its high content of higher melting triglycerides, must be winterized to obtain a salad oil. Oils meeting the criteria of a salad oil cannot be prepared from peanut oil (because of a noncrystalline gel structure of its higher melting fraction) or palm oil (because of its high content of palmitic acid and resulting crystallization). However, liquid cooking oil is manufactured from palm oil by winterization.

High oleic (up to 80%) safflower and sunflower oils (9, 10), created through crop biotechnology, can serve as stable cooking and salad oils. More recently, a sunflower oil (termed NuSun, with oleic levels of approximately 50-60%) has been developed through conventional breeding (11) and has been found to be a suitable cooking oil. Dewaxed rice bran oil, because of its stability, is gaining significant commercial status, particularly in Asian countries. Linola (a low linolenic flax seed oil created through crop biotechnology) was approved in Canada for food use. Lines of peanuts that contain high levels of oleic acid have been described (12). Benefits of a high oleic peanut oil are obtained by the food manufacturer in the form of increased stability and by the consumer for the improved nutritional aspect. Olive oil, a high oleic oil, is used as both a cooking oil and a salad oil in many parts of the world. Olive oil, because of its unique natural flavor, is relished by users; however, its use is limited by its high cost. Manufacture of naturally flavored salad oils such as olive oil require nothing more than expression of the oil from oil-bearing materials and clarification of the oil by filtration. Commercial olive oil is typically a blend of oils from different sources. The quality and the flavor of olive oil vary considerably from one season and one locality to another. To make a product of uniform quality, the packer must have access to a variety of oil sources to blend.



**TABLE 1. Typical Composition of Regular and Modified Salad Oils of Commerce.**

Oil Source	Fatty Acid Composition (% total)					
	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Others
Soybean <sup>a</sup>	11.0	4.0	23.0	55.0	7.0	1.0
Canola <sup>a</sup> (LEAR)	4.0	2.0	61.0	20.0	9.0	4.0
Sunflower <sup>a</sup>	7.0	2.0	20.0	70.0	0.0	1.0
Sunflower <sup>a</sup> (high oleic)	4.0	4.0	86.0	3.0	0.0	1.0
Sunflower <sup>b</sup> (NuSun)	4.5	3.7	58.9	30.9	0.0	1.5
Safflower <sup>a</sup>	6.5	2.5	20.0	75.0	0.0	1.5
Flax <sup>a</sup>	6.4	3.5	20.2	17.1	53.0	
Cottonseed <sup>a</sup>	25.0	2.0	18.0	53.0	0.0	2.0
Corn <sup>a</sup>	11.0	2.0	26.0	59.0	1.0	2.0
Palm <sup>a</sup>	45.0	5.0	39.0	9.0	0.0	2.0
Peanut <sup>a</sup>	10.0	3.0	56.0	24.0	0.0	7.0 <sup>c</sup>
Rapeseed <sup>a</sup>	4.0	1.0	15.0	14.0	9.0	55.0 <sup>d</sup>

<sup>a</sup>Values from (16).<sup>b</sup>Values from (17).<sup>c</sup>Mostly C20–24 acids.<sup>d</sup>Primarily C22:1 (45%) and C20:1 (10%).

There is a substantial market for cold-pressed peanut, sesame, safflower, and sunflower oils in many countries. In the United States, the use of such unprocessed oils has become more popular in recent years because of nutritional considerations and an interest in less processed foods. However, there are no reliable data available for the quantities of such oils consumed. Consumption of all types of food fats and oils has been gradually increasing all over the world. Fats and oils consumed as part of foods can be conveniently divided into two broad categories: visible fats that are used in the preparation of foods and invisible fats that are present in the food naturally. Per capita consumption of visible fats in the United States grew from 104.9 kg (47.7 lb) in 1965 to 144.5 kg (65.7 lb) in 1992, and to 149.6 kg (68.0 lb) in 2002 (13).

Predominate use of a particular oil in a given region of the world is dependant on world trade movement. For many years, cottonseed oil was the main source of cooking and salad oils in the United States. However, since World War II, soybean oil has become the most prominent oil. Other oils, particularly those with high oleic content or low linolenic content, are becoming more important, as they do not require hydrogenation for stability (14, 15). Typical compositions of some commercially important cooking and salad oils are shown in Table 1.

### 3. STABILITY OF SALAD AND COOKING OILS

By proper handling and processing, most oils can be made bland and stable. Complete removal of the “corny” flavor from corn oil is rather difficult. However,

this flavor of corn oil is preferred by many consumers in the preparation of some foods, such as corn chips. During storage and cooking, even bland oils undergo physical and chemical changes, because they are sensitive to heat and light. In addition, trace metals (e.g., copper and iron), chlorophyll, and other pro-oxidants of unknown nature catalyze such chemical changes, particularly in the presence of oxygen.

At low levels of oxidation, the degree and type of change known as flavor reversion is characteristic for each oil. For example, soybean oil develops a flavor that is described as beany or grassy. This flavor has been attributed to the formation of 2-pentylfuran (18) and 3-*cis*-hexenal (19). Canola oil (LEAR) develops flavors similar to those of soybean oil. The flavor reversion of sunflower seed and safflower seed oils are described as “seedy.” Similarly, corn and palm oils develop flavors of distinct type. These reversion flavors are observed long before other objectionable oxidative off-flavors are formed. With many of these oils, the cause of the reversion flavors is not well understood. In the case of most well-processed oils, the reversion flavor is not a frequent problem.

The initial product of reaction of oils with oxygen is the hydroperoxide, which has no odor or flavor. When hydroperoxide decomposes, it forms diverse compounds, including aldehydes, ketones, acids, alcohols, hydroxy compounds, lactones, hydrocarbons, dienals, epoxides, and polymers. The breakdown products are often the cause of off-flavors observed in oils and their products. Many of these compounds are present only in parts per billion, and not all of these compounds are objectionable. Many of the related esters, alcohols, acids, ketones, and lactones are responsible for the desirable flavors of tomato juice, ripened banana, butter, olive oil, and other foods. Similarly, the flavor of fried foods has been attributed to 2,4-decadienal, which is also a component of off-flavor soybean oil. The amounts and proportions of these compounds in the oil are more important in determining whether a given flavor is objectionable or favorable, rather than the types of compounds (20). Shelf stability of oils and fats depends on many factors, such as composition and structure, initial quality of the crude oil, conditions of processing and subsequent handling, presence and amount of natural antioxidants, type of container, conditions of light, and temperature of storage (21). However, it can be generally stated that the oils containing linolenic acid—whether hydrogenated or unhydrogenated—are less stable than oils with corresponding iodine value but without linolenic acid. It has been shown that the oxidative stability of polyunsaturated oils can be improved by blending with a less polyunsaturated oil, probably because of a decrease in total polyunsaturation (22).

In the case of cooking oils, both thermal and oxidative degradation are important. Thermally caused changes result in polymer formation and other types of alteration of the oil. These changes have important nutritional and aesthetic aspects. Therefore, they have been extensively studied and discussed (23–31). Unfortunately, many of these studies have been conducted on fats and oils degraded under conditions far different from practical cooking operations. This has caused a considerable amount of uncertainty about the nutritional qualities of cooking oils that are consumed through fried foods. It has been shown that in fast oil turnover frying

operations, the changes slow down after initial rapid changes (32). Both volatile and nonvolatile decomposition products of different cooking oils have been reported (33–37).

## 4. QUALITY EVALUATION OF SALAD AND COOKING OILS

Handling and storage of finished oils have been reviewed (38). Minimizing the oxidation of oils and fats from harvest to consumption is important to the quality and stability of oils and the products in which they are used. Therefore, many quality control methods have been used to measure the extent of such oxidation. The most important quality criterion in the case of salad and cooking oils is their blandness, which is changed by any degree of oxidation. A common method of determining blandness is organoleptic evaluation, which is subjective and is influenced by many variables. To minimize variability, a certain amount of expertise is developed by the tasters through training. In addition, several objective methods to evaluate the quality of oils and fats are in use. These methods are generally based on the fact that oxidative degradation produces both volatile and nonvolatile chemical compounds with functional groups that can be measured by chemical and physical methods. However, for any method to be useful, the results must correlate with the organoleptic properties of the oils and fats in question. A second, but equally important requirement, is that the method should be capable of predicting the storage stability of the oils as well as any products made with them. Older methods, like the Kreis test and carbonyl value, are rarely used (39–41).

### 4.1. Peroxide Value

Peroxide value is the most widely used method (AOCS methods Cd S-53 and Cd 8b-90) to determine the quality of the oil. The primary oxidation products of oils and fats are the hydroperoxides. They can be quantitatively measured by determining the amount of iodine liberated by their reaction with potassium iodide. The peroxide content is expressed in terms of milliequivalents of iodine per kilogram of fat. However, when these hydroperoxides start breaking down to produce off-flavor compounds, correlation to the quality and stability of the oil will no longer be valid. Freshly deodorized oil should have zero peroxide value. In most cases, for the product to have acceptable storage stability, the peroxide value of oils used should be less than 1.0 meq/kg fat at the point of use.

### 4.2. Benzidine or Anisidine Value

The benzidine or anisidine value method uses the reaction of nonvolatile  $\alpha$ - and  $\beta$ -unsaturated aldehydes with these reagents (42, 43). Absorption readings are made at 350 nm in 1-cm cells. Originally, benzidine was used as a reagent. Later, because of the carcinogenicity of benzidine, anisidine was recommended. This method of determining the degree of oxidation is extensively used in Europe and

other parts of the world. Results, thus far, indicate that this method is more useful in determining the quality of crude oils and the efficiency of processing procedures than the quality of the finished oil during shipment and storage. An extension of this method is the total oxidation (TOTOX) value (44), which is the sum of anisidine value and two times the peroxide value.

### 4.3. Pentane and Hexanal Values

One of the compounds produced by the oxidation of salad and cooking oils is pentane. The quantity of pentane is measured by gas liquid chromatography (GLC) procedure. There is good correlation of this value with the organoleptic qualities of the oil for the first few weeks of storage life. However, correlation at later periods of storage has not been unequivocally established. Similarly, level of hexanal has been used as a quantitative indicator of the quality of oils and products made with them. As the oil or the product degrades during storage, other degradation products seem to be more important than hexanal in determining the organoleptic quality (45, 46).

### 4.4. Thiobarbituric Acid Test

The thiobarbituric acid test (AOCS method Cd 19-90), developed initially for measurement of malondialdehyde, is used to measure the oxidative degradation product of polyunsaturated fatty acids (47, 48). However, it lacks sensitivity (49).

All the preceding methods serve as useful tools under a given set of conditions, but seem to fail in their universal applicability to measure the quality or predict the stability of oils, fats, and their products.

### 4.5. Volatile Profile Method

The volatile profile method is a direct gas chromatographic method to examine the volatiles in oils, fats, and their products (50–54). Under control conditions, the method has good reproducibility. When the results are statistically treated, good correlation with organoleptic results is obtained. Two methods have been developed and standardized for this approach (AOCS methods Cg 4-94 and Cg 1-83)

### 4.6. Accelerated Tests

Storage stability studies of salad oils are time-consuming and require several months to complete. From a commercial standpoint, it is necessary to complete this determination in a shorter period. Therefore, partially successful attempts have been made to develop accelerated tests using light- and temperature-susceptible properties of oils and fats. Moser et al. (55) have designed an apparatus using fluorescent light to accelerate the aging of oils and fats. Radtke et al. (56) have studied the influence of the intensity and wavelength of light on the oxidative deterioration of salad oils. They observed that photochemical action depended on the wavelength

and increased with decreasing wavelength to a much greater extent than could be predicted from energy considerations. Similar studies have been done by others (57, 58). From these light studies, it may be possible to reduce the time of the storage stability test by exposing the oils to short wavelengths, followed by an analysis of the volatiles, using volatile profile methods. These methods can indicate comparative stability, but cannot predict absolute stability.

Several temperature-catalyzed stability tests are used in evaluating the oxidative stability of oils and fats. The oldest method is the Schaal oven test (39). It is inexpensive but subjective, because it uses organoleptic and odor intensities in the procedure and still requires days to obtain the result. This approach has been standardized into a recommended practice (AOCS method Cg 5-97). In the active oxygen method (AOM) (39), the development of peroxide is measured with time. As the formation and decomposition of peroxides are dynamic processes, the results obtained by this method do not correlate well to the actual stability of the oils and fats observed under practical application conditions. Other methods that have been based on oxygen absorption are the gravimetric (59) and the headspace oxygen concentration measurement (60, 61).

Recently, with the advent of more sophisticated technology, the older thermal methods are being replaced by procedures using automated instruments (Rancimat, oil stability index or OSI, and oxidograph) for measurement of oil stability. The Rancimat and OSI plot the change in electrical conductivity of distilled water with time by the dissolution of gaseous products from the oxidizing oil (62-65). In both of these instrumental procedures, just as in AOM, air is passed through heated oil under defined conditions of temperature and flow rate. The decomposition products are absorbed in distilled water. The end of the induction period is indicated by the rapid increase in the conductance of the distilled water. The OSI method has recently been adopted as a recommended method (AOCS method Cd 12b-92). The oxidograph (66) is a modified version of FIRA Astell apparatus (67) and is based on a plot of the change in oxygen pressure of the system containing the oil with time. The plots from this method resemble the conductivity versus time graphs from Rancimat.

In the case of cooking oils, degradation similar to that observed in salad oils occurs, but to a lesser degree. However, during cooking, more severe types of breakdown are caused by high temperatures and the presence of moisture, oxygen, and food materials. These changes are reflected by increases in color, free fatty acids, polymers, polar materials, foaming, and so on. Even today there are no reliable methods to predict the performance of a cooking oil and establish a discard point for the used oil. However, several empirical methods have been suggested to determine the extent of damage to the oil caused by the frying process (68).

#### 4.7. Free Fatty Acid

Free fatty acids are probably the most widely used characteristic of oil quality control (AOCS Ca 5a-40). Deodorized oils, generally, have a free fatty acid level of less than 0.05%. During use for frying, there is a buildup of free fatty acids. In the initial

stages of cooking, free fatty acids are produced by oxidative breakdown, but in later stages, hydrolysis of the fat caused by the presence of moisture in the food causes free fatty acids to increase. This is a dynamic process, as some of the free fatty acids are lost through oxidation and steam distillation from the food. Furthermore, free fatty acids seem to catalyze hydrolysis of the cooking oils. An increase in free fatty acid decreases the smoke point of the oil. When significant amounts of free fatty acids have accumulated in the oil, smoking becomes excessive and the quality of the food decreases. The oil then must be discarded.

#### 4.8. Color

Oils used for cooking darken because of the formation of oxidative materials, including polymers and the presence of oil-soluble products from the fried food. Generally speaking, mere darkening of the oil without corresponding increase in the other degradative products will simply make the food *look slightly darker*, but does not significantly affect the taste of the food. Color (AOCS Cc 13b-45) alone as a measure of the extent of degradation of the cooking oil is highly unreliable.

#### 4.9. Oxidized Fatty Acids

Many methods have been proposed to measure the amount of oxidized lipids in a cooking oil (69, 70). Oxidative degradation will affect the appearance, the taste, and the nutritional quality of the food.

#### 4.10. Polymers

When a cooking oil oxidizes, polymers are formed that cause the oil to foam. In addition, the viscosity of the oxidized oil increases, making the cooked food look oily as a result of retention of a higher amount of oil on the surface of the food. Many methods have been proposed for the determination of polymers in the oil (71–74).

### 5. ADDITIVES FOR SALAD AND COOKING OILS

Salad and cooking oils have varying amounts of natural antioxidants, mostly tocopherols. With the introduction of synthetic antioxidants, it has been customary to supplement the natural antioxidants with the synthetic ones. Most commonly used synthetic antioxidants are butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate (PG), ascorbyl palmitate, and tertiary butyl hydroquinone (TBHQ). As a result of the questionable health implications of BHA and BHT, use of BHA and BHT is becoming rare. In recent times, TBHQ, because of its superior performance, has been used more extensively than the other synthetic antioxidants. Application and status of antioxidants have been reviewed (75).

Antioxidants derived from sage, rosemary, and green tea are becoming more popular because they are from natural sources (76–78). In addition, the antioxidants from these sources show a greater thermal stability than the synthetic antioxidants, particularly in meat products. However, they do impart flavors and colors to the product in which they are used.

Citric acid is added primarily to chelate trace metals, which otherwise accelerate the oxidative process. Methyl silicone (dimethyl polysiloxane), which act at the interface of oil, air, and cooking utensil surface, is added in 1–10 ppm quantities to cooking oils as an anti-foaming agent that also reduces the rate of oxidation at high temperature usage. Many other additives have been suggested or patented to improve either stability or functionality, but none are being used commercially (79–82).

## 6. NUTRITION-ORIENTED SALAD AND COOKING OILS

The U.S. diet, as well as diets of many industrialized nations, is rich in calories contributed by oils and fats. Technological innovations are, therefore, directed toward finding low- or no-calorie substitutes that will perform like normal oils and fats and that do not affect the aesthetic or health values of food products (83–85). The manufacturers of many of these synthetic fats must still establish that the substitutes are fit for human consumption. Even sucrose polyesters, which have been shown to be safe for food use and have been granted FDA approval for certain foods, still have not gained significant use in food products because of certain side effects. Other attempts to improve on the nutritional significance of salad and cooking oils have been made (86, 87).

Recently, several reports and patents have been published that document the process, composition, and effect of a diacylglyceride (DAG) oil. The DAG oil literature indicates unique metabolic effects associated with reduced-fat deposition and weight loss (88–90). The DAG oil is unique in structure, as it contains fatty acids in the 1 and 3 position of the glycerol molecule, but not the 1 and 2 or 2 and 3 positions. In this molecule, the hydroxy group in the 2 position is free. The biochemical mechanism by which the DAG oil works to affect body weight and body fat mass is poorly understood. However, the DAG oil is being used as both a salad and cooking oil in Japan and, recently, was introduced into the U.S. market. With the increased polarity of the 1,3 DAG structure, creation of a high-oil-containing food, like mayonnaise, can only be accomplished if formulation modifications are taken to emulsify the oil. Reports indicate that flavor of the DAG oil is bland similar to that of a salad or cooking oil (91).

## 7. NEW SALAD AND COOKING OILS

As a result of the health concerns of certain fatty acids, such as saturated fatty acids and *trans*-fatty acids produced by hydrogenation and erucic acid in rapeseed, there

**TABLE 2. Composition and Functionality of Some Cooking and Salad Oils Commercialized and in Development (16).**

Crop	Functionality	Approximate Fatty Acid Composition (% total)					
		Palmitic	Stearic	Oleic	Linoleic	Linolenic	Others
Soybean	Multifunctional	11.0	4.0	23.0	55.0	7.0	1.0
Low saturate	Nutritional	3.5	2.8	22.7	60.3	9.8	1.0
High oleic	High Temperature Stability	11.0	4.9	26.2	53.1	3.5	1.0
High Stearic	Stability	8.0	24.7	17.2	39.2	8.3	2.4 <sup>a</sup>
Canola (LEAR)	Multifunctional	4.0	2.0	61.0	20.0	9.0	4.0
High oleic	Stability	4.0	2.0	65.0	26.0	2.5	—
Peanut	Stability	10.0	3.0	37.0	41.0	0.0	7.0 <sup>a</sup>
Peanut (high oleic)	Stability	7.0	3.0	76.0	4.0	0.0	9.0 <sup>a</sup>

<sup>a</sup>Mostly C20-24 acids.

has been significant biotechnological work to develop crop oils with low saturates and oils that will be stable at high frying temperatures. Some of the oils (high and mid oleic sunflower, low erucic acid rapeseed, high oleic soybean, and high oleic canola) are already in commerce. Approximate compositions of some other oils in early commercialization stages or in development are shown in Table 2

## 8. OIL-BASED DRESSINGS

Oil-based dressings are divided into two broad categories by their texture: spoonable dressings (salad dressings, mayonnaise, and their reduced-calorie counterparts) and pourable dressings (various varieties, including reduced calorie).

## 9. VISCIOUS OR SPOONABLE DRESSINGS

### 9.1. Usage

Beginning about 1988, there was an increased consumer awareness of the contribution of dietary fats to various debilitating diseases. The consumption of many oil-containing products, including salad products, slowed as consumers began seeking healthier alternatives in their diet. Data from AC Nielson, a company that measures sales of consumer products, has documented the general decline of the purchases in the salad dressing and mayonnaise categories from 1988 through 2003. This measured decline in purchases can be linked to a decreased consumption. Reduced-calorie salad dressings and mayonnaise peaked in volume in 1989, then declined as fat-free versions were introduced in 1989. However, fat-free salad dressing and mayonnaise-type products peaked in volume in 1994, and have been



decreasing in consumption from 1994 through 2001. According to AC Nielson data, since 2001, consumption of fat free has plateaued.

## 9.2. Composition

**9.2.1. Mayonnaise** The official definition adopted in 1950 by the U.S. Food and Drug Administration (FDA) describes mayonnaise as follows: “Mayonnaise is the semisolid food prepared from edible vegetable oil, egg yolk, or egg whites (fresh or frozen or dried); any vinegar diluted with water to an acidity, calculated as acetic acid, of not less than 2.5% by weight; lemon and/or lime juice; with one or more of the following: salt, sweetener, paprika, monosodium glutamate, and other suitable food seasonings. The finished product contains not less than 65% of edible vegetable oil.” This definition is not universal, and other types of dressings, with different percentages of oil, are marketed as mayonnaise in other countries around the world. The Standard of identity is indicated in Table 3. The sweetener used may be sucrose, dextrose, corn syrup, invert sugar syrup, nondiastatic maltose syrup,

**TABLE 3. Standard of Identity of Mayonnaise (section 169.140 Code of Federal Regulations). Description: Mayonnaise is the emulsified semisolid food prepared from vegetable oil(s), one or both of the acidifying agents specified (below), and one or more of the egg containing ingredients specified (below).**

Ingredient	Percent by Weight
Oil Ingredient	Vegetable oil, not less than 65%
Acidifying Ingredients	<ol style="list-style-type: none"> <li>1. Vinegar or vinegar plus an optional acidifying agent contributing an acidity, calculated as acetic acid, of not less than 2.5%</li> <li>2. Lemon Juice and/or lime juice contributing an acidity, calculated as citric acid, of not less than 2.5%</li> </ol>
Egg Ingredient	Egg yolk containing ingredients including forms of egg yolks and whole eggs, to which can be added egg whites
Optional Ingredients	<ol style="list-style-type: none"> <li>1. Salt</li> <li>2. Nutritive carbohydrate sweeteners</li> <li>3. Any spice (except saffron or turmeric) or natural flavorings</li> <li>4. Monosodium Glutamate</li> <li>5. Sequesterant(s), including but not limited to EDTA</li> <li>6. Citric and/or malic acid in an amount not greater than 25% or the weight of the acids of the vinegar, calculated as acetic acid</li> <li>7. Crystallization inhibitors</li> </ol>

glucose, honey, or high-fructose corn syrup. Mustard, paprika, other spices, or any spice oil or spice extract can be used, but not turmeric, saffron, spice oil, or spice extract, which imparts to the mayonnaise a color simulating the color imparted by egg yolk. Both mustard and oleoresin paprika impart a yellow color, but, because of the small amounts used, do not cause any variance from the standard of identity. Monosodium glutamate, because of its implications as an allergen, has come under the scrutiny of the FDA and various consumer watchdog groups. Although not delisted, monosodium glutamate is rarely used in mayonnaise. Any other harmless food seasoning or flavoring can be used, provided it is not an imitation and does not impart to the mayonnaise a color simulating the color imparted by egg yolk.

Acidity, calculated as acetic acid and contributed by vinegar, lemon, or lime juice, should not be less than 2.5% by weight of the product. When an optional acidifying ingredient such as citric acid is used, the label on the package must bear the statement "citric acid added." As of January 1, 1978, malic acid has been permitted as an acidifying agent by the FDA.

Calcium disodium ethylenediaminetetraacetic acid (EDTA), or disodium EDTA, is permitted to protect the flavor. Physically, mayonnaise consists of an internal or discontinuous phase of oil droplets dispersed in an external or continuous aqueous phase of vinegar, egg yolk, and other ingredients. Therefore, it is an oil-in-water emulsion. Consistency of the emulsion depends, to a large extent, on the ratio of its aqueous and oil phases and the amount and type of egg solids. Stability of the emulsion not only depends on the ingredients, but is also greatly affected by the type of equipment and how it is operated during manufacture. Table 4 gives a general composition for mayonnaise. The color of mayonnaise is pale yellow and is obtained mainly from the egg rather than from the oil.

Edible vegetable oil used in mayonnaise may contain not more than 0.125% by weight of crystal inhibitors to prevent crystallization of high melting triglycerides. Mayonnaise may legally contain as little as 65% oil. However, in practice, most mayonnaise manufactured in the United States has 75–82% oil, usually nonhydrogenated soybean oil. Other salad oils, including winterized cottonseed oil, safflower oil, corn oil, and partially hydrogenated winterized or nonwinterized soybean oil, can be used in mayonnaise (92, 93). Oils with large quantities of saturated acids,

**TABLE 4. Typical Composition of Mayonnaise.**

Ingredient	Percent by Weight
Vegetable Oil	75.0–80.0
Vinegar (4.5% acetic acid)	9.4–10.8
Egg yolk	7.0–9.0
Sugar	1.5–2.5
Salt	1.5
Mustard	0.5–1.0
White pepper	0.1–0.2

such as palm oil, or oils that solidify at refrigeration temperatures, such as peanut and olive oils, are seldom used because they tend to break the emulsion at refrigerator temperatures. Oil levels 1–2% higher than the range given in Table 4 are used in mayonnaise when a stiffer body is needed. If the oil content exceeds 85%, the emulsion tends to be unstable.

Vinegar has a dual purpose in mayonnaise and in most other dressings. The acetic acid acts as a preservative against microbial spoilage, including some types of yeasts (94). The theory of action of acetic acid on microbes has been reviewed (95). Acetic acid also serves as a flavor ingredient when used at the proper level. However, excessive quantities of the acid will impair the flavor. Added water and the water in vinegar forms the continuous phase in the emulsion. Generally, the concentration of acetic acid is about 3.5% of the volatiles present in vinegar. Vinegar may contain high quantities of trace metals that can be detrimental to the stability of the mayonnaise. Therefore, the quality of the vinegar used is important.

The main function of the egg solids in mayonnaise is to serve as an emulsifying agent. The mayonnaise emulsion is built around egg yolk, which is one of nature's perfect emulsifiers. One of the factors influencing the stiffness and the stability of the emulsion is the amount and kind of egg yolk. Egg yolk is a complicated chemical entity with a high lipid content. The lipids of the egg yolk form about 65% of the total solids. The composition (96) of the lipids in egg yolk is as follows: glycerides 40.3%, phospholipids 21.3%, and cholesterol 3.6%, based on the total solids. Of the fatty acids, 30% are saturated and the rest are unsaturated. Lipid components of egg yolk have a profound effect on flavor, color, and stability of mayonnaise and other dressings. Egg white is a complex system of proteins that aids in emulsification by forming a gel structure on coagulation by the acid component (97). More detailed information on egg and egg protein composition is available (98).

Mustard is generally used in mayonnaise in the form of a flour. There are two varieties of mustard: white and brown. White mustard is hot to taste but practically odorless. The brown variety, on the other hand, has a sharp odor. Therefore, the two varieties are blended in various proportions to achieve a desired level of flavor and pungency (99). Mustard contains a glucoside that, when hydrolyzed, releases the pungent oil of mustard, allyl isothiocyanate. This compound imparts the bite to mayonnaise. According to Corran (100), in addition to its flavor contribution, the nature, the origin, and the method of addition of mustard influence the emulsion. He stresses that mustard is an efficient emulsifier and is effective if incorporated with egg yolk.

As a result of the variation in the essential oil content and immiscibility of the mustard flour with the vegetable oil, Cummings (101) recommends the use of oil of mustard in place of mustard flour. If oil of mustard is used, the advantage of mustard flour as an emulsifying adjunct and as a possible contributor of color in mayonnaise may be lost.

Of the other optional ingredients permitted, paprika adds to the flavor and salt and sugar contribute taste and flavor and impart some microbial and physical stability to the mayonnaise.

**9.2.2. Other Spoonable Salad Dressings** About 1935, salad dressings were first developed as an alternative to mayonnaise during the Depression years. These products are commonly called salad dressings and, like mayonnaise, have a standard of identity as defined by the FDA. The composition of salad dressings as defined in the standard of identity is as follows. Salad dressing is the emulsified semisolid food prepared from not less than 30% vegetable oil; acidifying ingredients (usually vinegar); not less than 4% egg yolks; and a starchy paste component prepared from one or more of a combination of tapioca starch, wheat starch, rye starch, or corn starch (dent or amioca). The starches can be native or chemically modified to impart physical stability against syneresis, or acid breakdown. The starch paste is prepared, usually with vinegar and spices, and then blended with a modified mayonnaise base to yield the finished spoonable salad dressing. The following optional ingredients may also be included: salt, nonnutritive sweeteners, spices, monosodium glutamate, thickeners and stabilizers, sequestrants, and crystallization inhibitors. The oils selected use the same criteria as for mayonnaise. However, a process for making stable salad dressings containing mixtures of liquid triglycerides that have an iodine value greater than 75 and solid triglycerides that have an iodine value not exceeding 12 has been patented (102).

### 9.3. Manufacture: Mayonnaise

As mayonnaise contains seven times more oil than water and the emulsion is an oil-in-water type, preparation of a stable mayonnaise emulsion is difficult. Thus, major manufacturers of these products have developed proprietary techniques to achieve this goal. Interactive effects of various factors on the stability of mayonnaise emulsions are not well understood. Therefore, production of stable mayonnaise has remained an art to some degree. Although egg solids are the backbone of a stable emulsion, processing conditions whose interactions are only partially understood play an important role.

A batch process for making mayonnaise in a batch mixer is to mix egg yolks, sugar, salt, spices, and a portion of the vinegar, followed by gradually beating in the oil and then thinning out by mixing the remainder of the vinegar. This method of mixing is believed to give a product of better consistency than is obtained by adding all the vinegar at the beginning of the operation.

Gray et al. (103) studied the effect of the method of mixing on the consistency of the emulsions and recommended that one-third of the vinegar be added initially and the balance be added at the end. On the other hand, Brown (104) recommended that the mixture of eggs and other ingredients be as stiff as possible when the oil is incorporated. The stiffness can be controlled during the addition of the oil by adding small portions of vinegar. This method of preparation has been claimed to produce small oil droplets, thus making the emulsion highly stable.

The temperature of the oil and other materials during mixing also influences the body characteristics of mayonnaise. A thin product results if the operation is carried out with materials that are warm. Gray and Maier (105) suggest a temperature of 15.5–21.1°C (60–70°F). The inconvenience of attempting to operate at temperatures

below 15.5°C (60°F) was not worthwhile, as the initial superiority of the low temperature processed product is lost with slight aging of the product. Brown (104), however, recommends a temperature of 4.4°C (40°F).

The equipment most popular in the manufacture of mayonnaise and salad dressings are the Dixie mixer and the Charlotte colloid mill connected suitably with valves and pumps. A colloid mill is a mechanical device in which the product passes between a high-speed rotor (3600 rpm) and a fixed stator. Product enters the area at a low velocity and is subjected to a high shear with reduction of the particle size. Clearance between these two parts determines (1) the amount of shear imposed and the viscosity of the final product and (2) throughput of the mill. Weiss (99) recommended an opening within a range of 25–40 mils. The mixer is used to prepare a coarse emulsion that is then passed through the colloid mill to give the creamy texture. A second system is the AMF system (106), which consists of a premix feed tank and two mixing stages. In the premix tank, slow agitation is maintained to keep the ingredients well dispersed, but not to thicken the emulsion. The mix containing all the ingredients except a portion of the vinegar from the premix tank is converted to a coarse emulsion in the first stage of mixing and is then pumped to the second stage. At this stage, the balance of the vinegar solution is added. This mixer rotates at about 475 rpm, which is much slower than a colloid mill, but, because of its teeth design, the mixing is very intense. It is claimed that this system produces much smaller and more uniform droplets of oil than the colloid mill does and, thus, makes a highly stable emulsion.

Other equipment suggested are the Girdler CR mixer (107) and the Sonalator (108), which is an ultrasonic homogenizer. A comparative study of homogenizers and colloid mills for producing dressings showed that homogenizers can be used for certain types of dressing, but not for mayonnaise (109). Automation techniques for the salad dressing industry have been discussed (106). Advanced techniques for making mayonnaise and other salad dressings have been reviewed (110). Continuous systems that can improve productivity and have built-in versatility to permit production of different dressings have been described (111–114). A streamlined production line intended to assure quality of dressings has been outlined (113).

#### **9.4. Quality Control and Stability Measurements**

Mayonnaise may be classified as a semiperishable product. It is sufficiently stable to keep for reasonable lengths of time without refrigeration. Mayonnaise gradually becomes thinner with age. Thinning and separation of the phases of mayonnaise are greatly accelerated by mechanical shock. Separation of phases can also be caused by exposure to low temperatures. Gray (115, 116) studied the breakdown of mayonnaise and concluded that the higher the water content, the greater the amount of egg solids needed to stabilize the emulsion. Other causes of breakdown are rapid addition of the oil and unregulated agitation during emulsifying, high-temperature storage, and vibration during transit.

Other common forms of spoilage in mayonnaise are a result of oxidative degradation of various components, especially vegetable oil and egg lipids. Microbial

spoilage is a rare occurrence, as the product is well protected by the high acid content. However, molds and yeast and, to a lesser extent, Lactobacilli may sometimes find conditions favorable for growth (117).

As the quality of the oil plays a major role in the flavor stability of mayonnaise and other dressings, only the best grade of oil should be used. The quality of flavor is generally judged by the subjective method of tasting. However, Dupuy et al. (50) have developed a gas chromatographic method to evaluate the flavor quality objectively.

Poor oils will produce inferior quality products with shortened shelf life. Similarly, the quality of the egg, both microbiologically and chemically, should be high; otherwise, both flavor and emulsion stability problems can be encountered. Mustard flour must be free from impurities. Strict microbiological control of raw materials is important. During processing, control must be exercised in maintaining correct temperature and emulsification procedures. Proper sequential addition of ingredients and mixing times are important for emulsion stability (119). For good emulsification, the oil globules should be even and should be 1–4  $\mu$  in size (120). It is reported that freshness of mayonnaise can be retained substantially longer by processing in the absence of oxygen (121). Processing under nitrogen increased the shelf life of mayonnaise from 180 days to more than 240 days (117). Specific gravity of the product with proper amount of inert gas is about 0.90–0.92, and that of product without gas injection is about 0.94. Amount of entrapped gas must be controlled below 6%; otherwise, shrinkage during transit will cause deterioration of the product (117). Tryptophan or tryptophan derivatives (122), as well as L-cystine (123), have been found to improve the storage stability of mayonnaise.

Various instruments have been proposed for testing the important properties of consistency, stability, and flavor quality of mayonnaise and other dressings. Consistency is measured by a device that is a modified form of the Gardner mobilometer (124), which was originally designed for testing paints, varnishes, and enamels. The mobilometer is, in reality, a special form of viscometer in which a weighted, perforated plunger is allowed to fall through the sample. Consistency of the product is reported in seconds. Kilgore (125) devised a simple method that is particularly suitable for control work, as it involves nothing more than dropping a pointed rod, or a “plummet,” into a sample from a definite height and noting the depth of its penetration. Penetration correlates inversely with viscosity. The Brookfield Helipath viscometer is commonly used to measure viscosities. These consistency measurements do not totally describe the attributes of body and texture of mayonnaise or salad dressing. The ability of mayonnaise emulsion to resist mechanical shock is commonly evaluated by testing samples under simulated shipping conditions.

## 10. POURABLE DRESSINGS

Pourable dressings are relative newcomers as a grocery store food category compared with mayonnaise and spoonable dressings. They first appeared on the market in limited flavors, such as French and Thousand Island. Over the last several decades, many more varieties have become available. The only legal definition in

the United States for pourable dressings pertains to French dressing. To be labeled “French dressing,” the product requires a minimum of 35% oil and one or more of the following ingredients: salt, nutritive sweeteners, mustard, paprika or other spice extractives, monosodium glutamate (MSG), any suitable flavoring other than imitations, tomato in any suitable form, sherry wine, color additives, vegetable gums or thickeners, sequestrants, such as EDTA, or crystallization inhibitors. The lack of restrictions in legally defining a “dressing” gives the dressing manufacturer flexibility to make any flavored product using almost any formula and call it a “dressing,” with the exception of French dressing.

Unlike salad dressings and mayonnaise, pourable dressings have shown a slow, steady increase in production from 1988 through 2003. Reduced-calorie pourable dressings peaked in volume in 1989, which is also the year fat-free pourable dressings were introduced. The fat-free pourables category has continued to grow through 1993, at the expense of the reduced-calorie category. However, since 1994, this trend has reversed. The fat-free market has slowly declined, while reduced-fat products began to increase again. Partially contributing to these trends are the healthier lifestyles and dietary awareness of the buying public during these years. Salads have become a staple food in the American meal, and production of total pourable dressings reflects this trend.

Most pourable dressings are distributed and sold at ambient temperature. As many of the ingredients used support microbiological growth, spoilage can occur. Most pourable dressings are formulated within acid-salt-preservative tolerances to avoid spoilage on the grocery store shelves. Refrigerated dressings (discussed below) are marketed in the grocery store produce section.

Dressings may be formulated in two different finished forms. These are broadly described as two phase and single phase. This distinction generally represents the presence or absence of homogenization. Two-phase dressings are characterized by the presence of a layer of free oil above the aqueous layer. Two-phase dressings usually contain seasonings and flavors in the aqueous portion and the oil is added at the time of filling. Examples of two-phase dressings include oil and vinegar, Italian dressing, and some French-style dressings. These products are shaken before use, and they return to separated phases after sitting.

Single-phase dressings can be homogenized or blended. If homogenized, they are passed through a homogenizing device that reduces oil droplet size to produce a smooth, creamy dressing. Examples of homogenized dressings are Ranch, French, and Creamy Cucumber varieties. Blended dressings are characterized by the presence of both oil and aqueous portions in a loose blended matrix that is stabilized and thickened with gums. Typical blended examples are certain sweet French-style dressings and Italian varieties.

### 10.1. Composition

As a result of the variety of pourable dressings, the number of ingredients used in their manufacture is large. The following is a discussion of some of the major functional and flavor ingredients used in pourable dressing manufacture.

**10.1.1. Vegetable Oils** Vegetable oils have traditionally been a major ingredient in many dressing varieties. The first vegetable oil used in pourable dressings was cottonseed oil. As supplies of high-quality soybean oil became plentiful in the United States during the 1960s, its use became predominant. More recently, health concerns have opened the way for use of canola and other low-saturated vegetable oils. Canola oil is predominately used in Canada; other oils, such as sunflower, rapeseed, and soybean, are used in European markets. Vegetable oils impart lubricity and a creamy, pleasant mouthfeel to dressings. The action of homogenization on an oil-water dressing mixture causes an increase in viscosity. Other functions of vegetable oil include the solubilization of certain oil-soluble flavors and the contribution of an overall balanced flavor profile.

**10.1.2. Water** Water is present in all dressings. Its presence contributes liquidity to the dressing system and serves as a vehicle for delivering water-soluble flavors. Many ingredients must be dissolved to be functional, and water is the major solute for these ingredients. Water content is a critical consideration for developing a microbiologically stable dressing system.

**10.1.3. Vinegar** Vinegar, as in the case of spoonable dressings, has a dual role: flavor and microbiological preservation. Its role in flavor is emphasized in dressing varieties such as zesty-style Italian, but in buttermilk-based dressings, vinegar flavor is not desired. Shelf-stable dressings are formulated to a pH of 4.2 or less to control pathogenic organisms. Other acidifying agents have been used, and several patents are in effect that claim the use of lactic acid, phosphoric acid, malic acid, citric acid, etc. (126–129). Most of these acids impart tart flavors and control microbial growth. However, certain acids (such as phosphoric and lactic) are more efficient in pH reduction as a result of their relatively low pKa. They are valuable in controlling pH in dressings with high levels of buffering ingredients, such as buttermilk and blue cheese.

**10.1.4. Dairy Ingredients** Dairy products are used in pourable dressings to give characteristic flavors and textures. Buttermilk is a major ingredient in Ranch and, of course, buttermilk dressings. Sour cream and yogurt have been used in some creamy-style dressings. Blue cheese is a popular dressing variety; if its process of manufacture includes cooking, it yields a smooth, creamy dressing. Blue cheese can also be added after emulsion formation, which yields a chunky Blue Cheese dressing. Buttermilk and cheese also supply protein, which aids in emulsification of the oil. This protein also contributes to development of a pleasing mouthfeel. Protein from milk and cheese buffers pH upward, and formulation adjustment may be needed to meet pH criteria.

**10.1.5. Salt** Salt has been a known preservation agent for centuries. Its use in dressings complements vinegar and other preservatives in producing shelf-stable dressing products. Salt also serves as an important source of zesty flavor as well as a flavor enhancer for a number of other ingredients.



**10.1.6. Sugar** Sugar is included to add flavor to dressings as well as to counteract the harsher flavor effects of vinegar and salt. Sugar may be used in any of several different forms, which include sucrose, fructose, corn syrup, and maltodextrins. Sugar also increases the solids level in dressings, which contributes to smoothness and a creamy mouthfeel. In addition to its own flavor contribution, which may differ with different sugars, sugar is also a flavor enhancer for other ingredients.

**10.1.7. Spices** The essence of pouring a dressing on a salad is to add flavor to otherwise relatively bland vegetables. Spices deliver characteristic flavors to variety dressings. Thus, clove is added to Thousand Island dressing, mustard is used in French dressings, and thyme and oregano are associated with Italian dressings. Certain spices also contribute antimicrobial and antioxidant properties.

**10.1.8. Emulsifiers** Emulsifiers help form one continuous emulsion phase of water and oil. Two-phase dressings, such as oil and vinegar, have no emulsifier or a very low level of emulsifier. A small amount of emulsifier may be added to stabilize the loose emulsion of two-phase dressings momentarily, after shaking but before pouring on the salad. Several different ingredients function as emulsifiers and are used in pourable dressings. Eggs (yolks or whole eggs) are used as emulsifiers in Thousand Island dressing and also contribute some flavor. Polysorbate 60 is a synthetic emulsifier that is often used in creamy-style dressings. It produces small oil droplets and yields a very white emulsion. Emulsifiers also help solubilize flavors and improve overall flavor perception.

**10.1.9. Gums** Gums are added to dressings to produce creaminess, increase or modify viscosity, and control pourability. Commonly used gums include xanthan gum, propylene glycol alginate (PGA), carrageenan, and cellulose gums. Xanthan gum is the most commonly used gum in pourable dressings. It resists hydrolysis by the acidic dressing medium and maintains the product viscosity over shelf life. Each of these materials contributes characteristic rheological properties to the dressing; they are often used in combination. In addition to viscosity modification, certain gums, such as PGA, also aid in emulsion formation and stabilization.

**10.1.10 Stabilizers** In dressing manufacture, two different types of ingredients are often referred to as stabilizers: chelating agents (EDTA) and hydrocolloids (gums). Gums have already been discussed.

Chelating agents are used in dressings to stabilize the oil component against oxidation. EDTA is the most commonly used chelating agent, and the usual form of this material in dressings is the calcium disodium salt. Vegetable oils are prone to oxidation, which causes off-flavors in dressings. Oil oxidation is accelerated by the presence of iron and copper ions, but chelating agents inactivate, or chelate, these metal ions so they are not available to promote oil oxidation. EDTA also helps stabilize flavor and color in finished dressings.

**10.1.11. Colors** Several different colors used in dressings include  $\beta$ -carotene, apo-carotenal, FD&C colors, tumeric, and titanium dioxide. They are added to make pourable dressings eye appealing and to augment naturally occurring colors.

**10.1.12. Flavors** Flavors are used to extend the range of dressings available to the consumer. They can be either natural or artificial. Flavors are added to supplement the use of more expensive ingredients or when certain flavors cannot be obtained from the ingredients.

**10.1.13. Preservatives** Although vinegar is often the preservative of choice, the level of vinegar needed to ensure microbiological stability in mild-tasting varieties, such as Ranch, produces a dressing product that has too tart and too harsh a flavor. In these products, preservatives can be added to give the additional microbiological stability needed while still maintaining a desirable mildly acidic-tasting dressing. The most commonly used preservative is sorbic acid (sometimes added as potassium sorbate). Sodium benzoate is another preservative; it is not commonly used.

## 10.2. Manufacture

Pourable dressing manufacture is a relatively simple unit process. There are some order of addition considerations that should be incorporated when designing manufacturing procedures. Most gums tend to dissolve rather slowly with simple mixing into water. Their hygroscopic nature causes the outer layer of a gum particle to hydrate rapidly, but does not allow hydration of the inner layers of the gum particles. This results in partially hydrated gum particles, commonly called "fish eyes." There are three methods used to incorporate gums into water. First, high shear mixing can be used, but this often incorporates a large amount of air. Second, preblending gums with other dry substances (sugar, salt) will keep the individual dry gum particles separated. When this mixture is added to water, the gum will disperse effectively. This method is used in dry mix dressings, sauces, and gravies. Another, more common, method is to disperse the gums in vegetable oil before addition to the water. Vegetable oil effectively separates the individual gum particles, and hydration is effected on addition to water.

Mustard flour should be added to water without vinegar or salt. The hydration of mustard flour initiates an enzymatic process that develops the proper flavor. However, in the presence of acid, this enzyme process results in less desirable flavor components.

Most pourable dressing manufacturing process are batch designed. As a result of the large number of varieties usually produced, variety changeover is more efficient in a batch process than in a continuous or semicontinuous process.

**10.2.1. Gum Slurry** The gum slurry tank is usually adjacent to the main mix tank and contains about 0.1 of the volume of the main mix tank. Gums are dispersed in a portion of the formula vegetable oil with a lightening mixer or similar type agitator.

**10.2.2. Primary Mix Tank** Water is added to the mix tank, and the gum slurry mixture is added. Between two and three minutes of mix time is required for the gum to dissolve and hydrate. Additional dry ingredients and water-soluble liquid ingredients are then added. Certain ingredients, such as mustard flour, PGA, garlic powder, and microcrystalline cellulose, should be added before salt and acids. These ingredients are sensitive to salt and acid and may lose functionality if added in their presence. Addition of the acid follows most other ingredients, and vegetable oil is usually the last ingredient to be added.

**10.2.3. Homogenization** Homogenization produces a uniform, stable emulsion. There are several pieces of equipment that can be used for homogenization, including piston type, static shear, and colloid mills.

**10.2.4. Particle Addition** Following homogenization, additional ingredients that should not be homogenized are added in the secondary mix tank. These items are often large spice particles (typical of Italian dressings) and pickle relish (a common ingredient in Thousand Island dressing).

### 10.3. Quality Control and Stability Measurements

Quality control considerations for pourable dressings are similar to those discussed for mayonnaise. The use of high-quality ingredients is an important consideration for producing a high-quality dressing that will maintain its flavor for 8–12 months of shelf life. Certain pourable dressings contain a relatively high content of spices and seasonings. Spices, in particular, can contribute a significant load of microorganisms. Appropriate microbiological specifications must be adhered to, especially ingredients such as root crops. Spices and other ingredients used in pourable dressing manufacture can also contribute a high level of iron and copper ions, which catalyze the oxidation of oils and contribute to poor flavor.

Ranch dressings containing fermented dairy products, such as buttermilk, are often a source of wild cultures. These cultures, often heterofermentative *Lactobacillus*, can cause gassiness and product spoilage. Processing often includes pasteurization of the dairy component.

Quality measurements most often employed in pourable dressing manufacture include percent fat, moisture, and salt. Acidity and pH are critical control points to ensure a microbiologically stable dressing. Viscosity depends on gum hydration and mixing conditions. Viscosity is most often determined by a Brookfield viscometer, using standard spindles.

## 11. REDUCED-CALORIE DRESSINGS

Before 1993, dressings (both spoonable and pourable) could be manufactured and claimed to be reduced calorie if the caloric contribution was at least 30% reduced from a similar full-calorie product. Reduction in calories is largely obtained by

reducing the oil content. The Nutrition Labeling Education Act (NLEA) of 1993 (130) changed the requirements for reduced-calorie claims. This causes quality problems in terms of lower viscosity, shelf-life stability, and flavor-mouthfeel perceptions.

Use of proper stabilizers to improve the quality of reduced-calorie dressings has been discussed (131). The advantages of using apo-carotenal, a naturally occurring carotenoid that can be synthesized, as a coloring agent in nonstandardized dressings and spreads has been reviewed (132). Freeze-thaw stable salad dressings have been prepared using cooked starch mixtures made from freeze-resistant starch and non-winterized oil (133). Use of highly esterified sugar esters to reduce the caloric content of dressings has been described (134). A stable, low-calorie, cream-type salad dressing containing only 10–12% of fat and 5–20 Kcal per tablespoon has been claimed (135). A patent has claimed the use of agar and methocellulose mixture to produce a low-calorie dressing (136). An emulsion base prepared by homogenizing fats and oils like sunflower, coconut, lard, and cottonseed, with a solution containing proteins from whey, whole milk, sunflower seed globulins, or rapeseed, has been used for preparing low-calorie dressings (137). Combination use of carrageenan and carob bean or guar gum to prepare low-calorie dressings has been patented (138). Weidemann and Reinicke (139) prepared a dietetic salad dressing with only 15–35% oil. In addition to protection provided by the low pH, pasteurization of the product at 95°C for 40 min gives the product longer life.

### 11.1. Composition

In 1993, the NLEA (130) was implemented. This law set a new standard for reduced-calorie dressings. Briefly, with reference to pourable dressings, the act states that to be declared “reduced calorie,” a dressing must contain 25% fewer calories than a reference dressing. The reader is referred to the text of the act for more information (130). In addition, the act declared the serving size for pourable dressings at two tablespoons. (Previous regulations were based on a 1-tablespoon serving size.)

This law set a new direction for dressings with lower calories. Many products on the market had to be reformulated to retain the claim; some were discontinued. Oil-free dressings were entering the market at about the same time, and these new products offered another option for the consumer.

Reduced-calorie pourable dressings generally follow the flavor trends of full-calorie dressings. Similar ingredients are used. However, adjustments for microbiological stability must be made. Reduced calorie is usually synonymous with reduced oil, because carbohydrates are present in low amounts and contribute only a small portion of calories in most products. To produce a reduced-calorie dressing, the fat is often reduced and replaced with water and additional gums. The higher level of water increases the susceptibility to microbial spoilage. Acetic acid (vinegar) and salt are concentrated in the aqueous phase and control microbial growth. As fat is reduced, the percentage of water must be increased. More acid and salt are needed to maintain the concentrations necessary to control spoilage. In

practice, salt is usually held constant, and additional vinegar is added to maintain microbiological stability. The additional acidity contributes to a harsher acidic flavor in the reformulated dressing. The challenge the product developer faces is to use the correct amount of additional acid needed to ensure microbiological stability while choosing other ingredients that will offset the higher acidic taste. Reduced-calorie dressings, both spoonable and pourable, are processed similar to regular (full-fat) dressings.

## 12. FAT-FREE DRESSINGS

Health trends in the 1980s initiated a relentless spiral of food product development activity centered around products with very low fat or no fat. Oil-free (fat-free) dressings had appeared on the market before 1980; but these products gained little consumer attention and effectively were niche products. Most, if not all, could be described as poor quality; they exhibited watery textures and harsh acidic flavors as a result of the relatively high amount of acid needed to stabilize them against microbial spoilage. The definitions surrounding what constituted fat free abounded, but with little uniformity either in composition or product labeling. In the late 1980s, there was some agreement in the pourable dressing category that less than 0.5 g of fat per serving (1 tablespoon = 16 g) was fat free.

### 12.1. Composition

In 1993, the FDA unveiled the NLEA (130). This act set the maximum amount of fat permitted for a dressing to be called fat free. It also defined a format for labeling the nutritional composition of most food products and redefined the serving size for pourable dressings. Previously, the serving size was one tablespoon (16 g); NLEA defined a serving of pourable dressing as two tablespoons. To be labeled fat free, pourable dressings must contain less than 0.5 g of fat per serving. This is equal to 1.56% fat in the product formulation.

The serving size for viscous (spoonable) dressings remains one tablespoon. Here, too, fat free requires less than 0.5 g of fat per serving. Therefore, a fat-free viscous dressing product may contain about 3% oil or fat in the formulation.

### 12.2. Fat Replacement

Fat or vegetable oil plays a key role in the mouthfeel and flavor release of dressings. Addition of enough oil to produce a reduced-calorie dressing yields a product with considerable mouthfeel and acceptable flavor quality. In essence, small quantities of oil, about 5–15% contribute generously to the eating quality of dressings. When no oil is present, the flavors are perceived as harsh, the mouthfeel is absent, and the dressing just does not taste good. Consumers generally are unwilling to trade flavorful eating satisfaction for the benefits associated with a fat-free dressing.

Many types of fat replacers have been developed with the goal of replacing fat in food products. These materials have generally been classified as fat substitutes and fat mimetics.

**12.2.1. Fat Substitutes** A fat substitute is a material that has fat properties, but reduced calories or no calories. Several of the latter materials have been developed. These materials have been reviewed, but only olestra has received FDA approval for food use (140, 141).

**12.2.2. Fat Mimetics** A fat mimetic is a material that mimics the role of fat when properly processed. Many different food ingredients can be produced to give texture properties that mimic some of the properties of oil. The challenge for the product developer designing fat-free dressings is to choose existing food ingredients and process options that mimic the eating qualities of fat-containing dressings.

### 12.3. Flavor Quality

From an empirical view, fat in a dressing causes a slow buildup of flavor in the mouth. The desirable flavor plateaus for a distinct time and then slowly dissipates, with a lingering desirable aftertaste. The same dressing formulation without fat will immediately give a very sharp flavor peak, sometimes two to three times higher in intensity than the fat-containing product, then rapidly decline with minimal or no desirable lingering aftertaste. The flavor developer is challenged to shape the flavor perception of fat-free products to match more closely the fat-containing dressing through choice of ingredients.

**12.3.1. Bulking Agents** A common approach to improving low-fat dressings is the addition of bulking agents. A number of different bulking agents are used in dressings; among them are microcrystalline cellulose, polydextrose, starches, and dextrans. Patents have been granted for specially processed microcrystalline cellulose (142) and a combination of microcrystalline and other bulking agents (143). As these materials are soluble or dispersible in water, they help build body and mouth-feel in fat-free dressings. Bulking agents also increase solids, which lowers water activity and aids in preservation. The technology of fat replacement through the use of various bulking agents has its roots in the fat mimetic theory, and many different types of bulking agents have been developed for dressings.

**12.3.2. Flavor Technology** Flavor compounds are complex chemicals, some of which are fat soluble and some of which are water soluble. In conventional fat-containing products, the partition of flavors seeks a natural equilibrium between the fat and aqueous phases. This natural partitioning provides a desirable release of flavor throughout the eating experience. If fat or oil is removed, the natural partitioning is removed. If the same flavor system used in fat-containing products is attempted in fat-free versions, the flavor will seem unbalanced.

Fat also functions as a coating for the flavor receptors in the mouth, effectively slowing the perception of the flavor. Again, the absence of fat results in a quick perception of flavor that often seems unnatural.

Many attempts have been made to overcome these flavor problems. Flavor suppliers have developed many prototype products that address the flavor problem. Suffice to say the technology of flavor development for oil-free dressings and other foods is in an evolutionary state.

#### **12.4. Microbiological Stability**

The issue of microbiological stability, discussed earlier, is more significant with fat-free dressings than with reduced-calorie dressing because the entire formulation is aqueous based. Microbiological stability is commonly addressed through use of various preservatives, such as sorbic acid-potassium sorbate and sodium benzoate, in addition to adjusting the level of vinegar. Acid harshness can be a problem and can be addressed through the selection of combinations of fillers and bulking agents that mask the harsher acidic sensation. Commonly, microbiological stability is evaluated by inoculation of product with organisms isolated from spoiled dressings.

#### **12.5. Processing**

Conventional dressing manufacture involves the mixing of liquid and dry ingredients together with the development of viscosity during the process. Fat-free dressing manufacture involves the hydration of various bulking agents. This unit operation often requires high shear mixing to disperse and hydrate the fat mimetics adequately. As there is no oil in these formulas, gums are dry blended with sugar before dressing manufacture. This dry blending helps prevent the formation of “fish eyes.” No homogenization step is necessary, but, through intimate mixing, it is necessary to effect full functionality from the fat replacer system.

### **13. REFRIGERATED DRESSINGS**

Refrigerated dressings comprise a small portion of the pourable dressing category. They are marketed in the produce section of the supermarket and appeal to the consumer interested in freshness. Although generally thought of as refrigerated and distributed under refrigeration, they are often displayed at room temperature. As a result, they are generally formulated in a similar manner as shelf-stable products to resist spoilage during periods of nonrefrigeration.

### **14. HEAT-STABLE DRESSINGS**

Improvement in the shelf stability of dressings can be expected if the product can be heated to high temperatures without losing body or color. Furthermore, such

heat-stable dressings can be incorporated into canned meat, fish, vegetable salads, and other foods. A certain microcrystalline cellulose imparts this stability (144), even when the product is retorted at 115.5°C (240°F) in the presence of food acids. Similarly, xanthan gum has been shown to produce a product with no visible signs of oil separation and with a viscosity that remains practically unchanged over a wide range of temperatures.

A dry preparation of the ingredients that may be reconstituted to form a salad dressing simply by the addition of water has been patented (145). Several such preparations are being marketed by different companies in the United States.

## REFERENCES

1. G. P. Lensack, *Food Eng.*, **12**, 98–100 (1969).
2. ICI United States, Inc., Bulletin, 222–228.
3. E. Deffense, *J. Amer. Oil Chem. Soc.*, **70**, 1193–1201 (1993).
4. C. Jacobsen, K. Hartvigsen, M. K. Tomsen, L. F. Hansen, P. Lund, L. H. Skbsted, G. Holmer, J. Adler-Nissen, and A. S. Meyer, *J. Agric. Food Chem.*, **49**, 1009–1019 (2001).
5. E. B. Schmidt and J. Dyerberg, *Drugs*, **47**, 405–424 (1994).
6. F. J. Baur and E. S. Lutton (to Procter & Gamble Co.), U.S. Patent 3,158,900, November 24, 1964.
7. F. J. Baur (to Procter & Gamble Co.), U.S. Patent 3,211,558, October 12, 1965.
8. F. R. Hugenberg and E. S. Lutton (to Procter & Gamble Co.), U.S. Patent 3,353,966, November 21, 1967.
9. G. Fuller, G. O. Kohler, and T. H. Applewhite, *J. Amer. Oil Chem. Soc.*, **43**, 477–478 (1966).
10. G. N. Fick (to Sigco Research Inc.), U.S. Patent 4,627,192, December 9, 1986.
11. L. W. Kleingartner, *NuSun Sunflower Oil: Redirection of an Industry*, ASHS Press, Alexandria, Virginia, 2002, pp 135–138.
12. D. A. Knauff, D. W. Gorbet, A. J. Norden, and C. K. Norden, (to University of Florida), U.S. Patent 5,922,390, July 13, 1999.
13. USDA, Oil Crops Situation and Outlook (2002).
14. R. Purdy, *J. Amer. Oil Chem. Soc.*, **62**, 523–525 (1985).
15. T. L. Mounts, K. Warner, G. R. List, W. E. Neff, and R. F. Wilson, *J. Amer. Oil Chem. Soc.*, **71**, 495–499 (1994).
16. D. Firestone, *Physical and Chemical Characteristics of Oils, Fats and Waxes*. AOCS Press, Champaign, Illinois, 1999.
17. K. Warner, B. Vick, L. Kleingartner, R. Issak, and K. Doroff, Compositions of Sunflower, NuSun (Mid-Oleic Sunflower) and High-Oleic Sunflower Oils, presented to the 18th session of the Codex Committee Fats and Oils, February, 2003.
18. S. S. Chang, T. H. Smouse, R. G. Krishnamurthy, B. D. Mookherjee, and B. R. Reddy, *Chem. Ind.*, **46**, 1926–1927 (1966).
19. G. Hoffmann, *J. Amer. Oil Chem. Soc.*, **38**, 1–3 (1961).



20. S. S. Lin, T. H. Smouse, and R. R. Allen, paper presented at the American Oil Chemists' Society Spring Meeting, Mexico City, 1974.
21. W. E. Neff, T. L. Mounts, W. M. Rinsch, and H. Konishi, *J. Amer. Oil Chem. Soc.*, **70**, 163–168 (1993).
22. E. N. Frankel and S. W. Haung, *J. Amer. Oil Chem. Soc.*, **71**, 255–259 (1993).
23. N. R. Artman, *Adv. Lipid Res.*, **7**, 245–330 (1969).
24. H. W. Schultz, E. A. Day, and R. O. Sinnhuber, *Symposium on Foods: Lipids and Their Oxidation*, The AVI Publishing Co., Westport, Connecticut, 1962.
25. Anonymous, *BIBRA Bull.*, **10**, 4–8 (1971).
26. R. Guinaman, *Rev. Fr. Corps. Gras.*, **18**, 445–456 (1971).
27. J. P. Freeman, *Food Process Mark. London*, **38**, 303–306 (1969).
28. R. J. Sims and H. D. Stahl, *Baker's Dig.*, **44**, 50–52 (1970).
29. U. Shimura, *J. Jpn. Oil Chem. Soc.*, **19**, 748–756 (1970).
30. E. Yuki, *J. Jpn. Oil Chem. Soc.*, **19**, 644–654 (1970).
31. W. W. Nawar, *J. Agr. Food Chem.*, **17**, 18–21 (1969).
32. C. Cuesta, F. J. Sanchez-Muniz, C. Gorrido-Polonio, S. Lopez-Varela, and R. Arroyo, *J. Amer. Oil Chem. Soc.*, **70**, 1069–1073 (1993).
33. B. R. Reddy, K. Yasuda, R. G. Krishnamurthy, and S. S. Chang, *J. Amer. Oil Chem. Soc.*, **45**, 629–631 (1968).
34. K. Yasuda, B. R. Reddy, and S. S. Chang, *J. Amer. Oil Chem. Soc.*, **45**, 635–628 (1968).
35. R. G. Krishnamurthy and S. S. Chang, *J. Amer. Oil Chem. Soc.*, **44**, 136–140 (1967).
36. M. C. Dobarganes, M. C. Perez-Camino, and G. Marqucz-Ruiz, *Fat Sci. Technol.*, **8**, 308–311 (1988).
37. D. M. Lee, *Bull. Brit. Food Manuf. Ind. Res. Assoc.*, **80**, (1973).
38. L. M. Wright, *J. Amer. Oil Chem. Soc.*, **53**, 408–409 (1976).
39. V. C. Mehlenbacher, *Analysis of Fats and Oils*, Gerrard Press, New York, 1960.
40. S. S. Chang and F. A. Kummerow, *J. Amer. Oil Chem. Soc.*, **32**, 341–344 (1955).
41. M. Loury and L. Garber, *Rev. FR Corps. Gras.*, **15**, 301 (1968).
42. U. Holm, K. Ekbohm, and G. Wode, *J. Amer. Oil Chem. Soc.*, **34**, 606–609 (1957).
43. G. R. List et al., *J. Amer. Oil Chem. Soc.*, **51**, 17–21 (1974).
44. G. Johansson and V. Persmark, *Oil Palm News*, **3**, 10–11 (1971).
45. R. G. Scholz and L. R. Ptak, *J. Amer. Oil Chem. Soc.*, **43**, 596–599 (1966).
46. C. D. Evans, G. R. List, R. L. Hoffmann, and H. A. Moser, *J. Amer. Oil Chem. Soc.*, **46**, 501–504 (1969).
47. G. A. Jacobson, J. A. Kirkpatrick, and H. E. Goff, *J. Amer. Oil Chem. Soc.*, **41**, 124–128 (1964).
48. J. A. Fioriti, M. J. Kanuk, and R. J. Sims, *J. Amer. Oil Chem. Soc.*, **51**, 219–223 (1976).
49. B. Tsoukalas and W. Grosch, *J. Amer. Oil Chem. Soc.*, **54**, 490–493 (1977).
50. H. P. Dupuy, S. P. Fore, and L. A. Goldblatt, *J. Amer. Oil Chem. Soc.*, **50**, 340–342 (1973).
51. A. D. Waltking and H. Zaminski, *J. Amer. Oil Chem. Soc.*, **54**, 454–457 (1977).
52. H. W. Jackson and D. J. Giacherio, *J. Amer. Oil Chem. Soc.*, **54**, 458–460 (1977).
53. J. L. Williams and T. H. Applewhite, *J. Amer. Oil Chem. Soc.*, **54**, 461–463 (1977).

54. D. B. Min and T. H. Smouse, *Flavor Chemistry of Lipid Foods*, American Oil Chemists' Society, Champaign, Illinois, 1989.
55. H. A. Moser, C. D. Evans, J. C. Cowan, and W. F. Kwolek, *J. Amer. Oil Chem. Soc.*, **42**, 30–33 (1965).
56. R. Radtke, P. Smits, and R. Heiss, *Fett Seifen Anstrichmit*, **72**, 497–504 (1970).
57. A. Sattar, J. M. deMan, and J. C. Alexander, *Lebensm. Wiss. Univ. Technol.*, **9**, 149–152 (1976).
58. A. Sattar, J. M. deMan, and J. C. Alexander, *J. Can. Inst Food Sci. Technol.*, **9**, 108–113 (1976).
59. H. S. Olcott and E. Einst, *J. Amer. Oil Chem. Soc.*, **35**, 161 (1958).
60. W. M. Gearhart, B. N. Stuckey, and J. J. Austin, *J. Amer. Oil Chem. Soc.*, **34**, 427 (1957).
61. B. S. Mistry and D. B. Min, *J. Food Sci.*, **52**, 831 (1987).
62. N. W. Labuli and P. A. Bruttel, *J. Amer. Oil Chem. Soc.*, **63**, 79 (1986).
63. J. M. deMan, F. Tie, and L. deMan, *J. Amer. Oil Chem. Soc.*, **64**, 993 (1987).
64. T. A. Jebe, M. O. Matlock, and R. T. Sleeter, *J. Amer. Oil Chem. Soc.*, **78**, 1055–1061 (1993).
65. S. E. Hill, *INFORM*, **5**, 104–109 (1994).
66. J. C. Allen and R. J. Hamilton, *Rancidity in Foods*, Elsevier Applied Science, Publishers, Ltd., Barking, United Kingdom, 1989.
67. British Food Manufacturing Industries Research Assoc., Technical Circular No. 605, Leatherhead, United Kingdom, 1989.
68. D. Firestone, R. F. Stier, and M. Blumenthal, *Food Technol.*, **45**, 90–94 (1991).
69. M. Ahrens, G. Guhr, J. Waibel, and S. Kroll, *Fette Seifen Anstrichmit*, **79**, 310–314 (1977).
70. U. J. SaIzar and J. Wurziger, *Fette Seifen Anstrichmit*, **73**, 705–710 (1974).
71. M. R. Sahasrabudhe and V. R. Bhalerao, *J. Amer. Oil Chem. Soc.*, **40**, 711–712 (1963).
72. E. G. Perkins, R. Tanbold, and A. Hsieh, *J. Amer. Oil Chem. Soc.*, **50**, 223–225 (1973).
73. G. Billek and G. Guhr, paper presented at the American Oil Chemists' Society Meeting, New York, 1977.
74. A. E. Waltking and H. Zaminski, *J. Amer. Oil Chem. Soc.*, **47**, 530–534 (1970).
75. E. R. Sherwin, *J. Amer. Oil Chem. Soc.*, **53**, 430–436 (1976).
76. Q. Chen, H. Shi, and C. Ho, *J. Amer. Oil Chem. Soc.*, **69**, 999–1002 (1992).
77. Z. Djarmati, R. M. Jankov, E. Schwirlich, B. Djulinac, and A. Djordjevic, *J. Amer. Oil Chem. Soc.*, **68**, 731–734 (1991).
78. Y. Hara, American Biotechnology Laboratory, 1994, p. 48.
79. R. G. Cunningham, R. D. Dobson, and L. H. Going (to Procter & Gamble Co.), U.S. Patent 3,415,658, December 10, 1968.
80. H. Enci, S. Okumura, and S. Ota (to Ajinomoto Co., Inc.), U.S. Patent 3,585,223, June 15, 1971.
81. S. S. Chang and P. E. Morne (to Swift and Co.), U.S. Patent 2,966,413, December 27, 1960.
82. E. R. Purves, L. H. Going, and R. D. Dobson (to Procter & Gamble Co.), U.S. Patent 3,415,660, December 10, 1968.

83. R. W. Fallat, C. J. Glueck, R. Lutmer, and F. H. Mattson, *Amer. J. Clin. Nutr.*, **29**, 1204 (1976).
84. S. M. Lee, *Fat Substitutes: A Literature Survey*, British Food Manufacturing Industries Research Association, Leatherhead, United Kingdom, 1989.
85. P. Seiden (to Proctor & Gamble), Eur. Patent 322,027 A2, 1989.
86. A. E. Blaurock, R. G. Krishnamurthy, and P. J. Huth (to Kraft Foods, Inc.), U.S. Patent 5,959,131, 1999.
87. J. Spinner, T. B. Guffey, P. Y. Lin, and J. Jandacek (to Proctor & Gamble Company), U.S. Patent 4,948,811, 1990.
88. M. Kenji, K. Yoshihisa, T. Tomoko., and T. Yasukawa (to Kao Corporation), U.S. Patent 6,495,536, 2002.
89. K. C. Maki et al., *Amer. J. Clin. Nutr.*, **76**, 1230–1236 (2002).
90. A. Takei, *J. Nutritional Food*, **4**, 1–13 (2001).
91. Anonymous, *INFORM*, **13**, 550 (2002).
92. CPC International, Brit. Patent 1,473,208, 1977.
93. F. J. Baur (to Proctor & Gamble Co.), U.S. Patent 3,027,260, March 27, 1962.
94. M. H. Joffe, *Mayonnaise and Salad Dressing Products*, Emulsol Corp., Chicago, Illinois, 1942.
95. S. Doores, in A. L. Branen and P. M. Davidson, eds., *Antimicrobials in Foods*. Marcel Decker, Inc., New York, 1983.
96. R. H. Forsythe, *Cereal Sci. Today*, **2**, 211 (1957).
97. W. Fluckinger, *Fette Seifen Anstrichmit.*, **68**, 139–145 (1966).
98. W. J. Stadelman and O. J. Cotterill, *Egg Science and Technology*, 3rd ed, Food Product Press, Binghamton, New York, 1990.
99. T. J. Weiss, *Food Oils and Their Uses*, AVI Publishing Co., Westport, Connecticut, 1970.
100. J. W. Corran, *Food Manuf.*, **9**, 17 (1937).
101. D. Cummings, *Food Technol.*, **11**, 1901–1902 (1964).
102. C. H. Japikse (to Proctor & Gamble Co.), U.S. Patent 3,425,843, February 4, 1969.
103. D. M. Gray, C. E. Meier, and C. A. Southwick, *Glass Packer*, **2**, 397–400 (1929).
104. L. C. Brown, *J. Amer. Oil Chem. Soc.*, **26**, 632–636 (1949).
105. D. M. Gray and C. E. Maier, *Glass Packer*, **4**, 23–25, 40 (1931).
106. M. H. Joffe, *Food Eng.*, **28**(5), 62–65, 100 (1956).
107. J. P. Bolanowski, *Food Eng.*, **39**(10), 90–93 (1967).
108. O. C. Samuel, *Food Process. Market*, 81–84 (1966).
109. L. H. Rees, *Food Prod. Develop.*, **9**, 48–50 (1975).
110. A. J. Finberg, *Food Eng.*, **27**(2), 83–91 (1955).
111. S. E. Potter, *Food Process.*, **31**, 43–44 (1970).
112. V. R. Carlson, *Food Eng.*, **42**(12), 54–55 (1970).
113. M. Lipschultz and R. E. Holtgrieve, *Food Eng.*, **40**(11), 86–87 (1969).
114. F. Taubrich, *Fette Seifen Anstrichmit.*, **65**, 475–478 (1963).
115. D. M. Gray, *Oil Fat Ind.*, **4**, 410 (1927).
116. D. M. Gray, *Glass Packer*, **2**, 311 (1929).

117. R. D. McCormick, *Food Prod. Dev.*, **1**, 15–18 (1967).
118. R. B. Smittle, *J. Food Protect.*, **40**, 415 (1977).
119. A. McKenzie and J. V. Ziemba, *Food Eng.*, **36**, 96–98 (1964).
120. D. R. Beswick, *Food Technol. N. Z.*, **4**, 332–339 (1969).
121. G. T. Muys and J. A. Schaap (to Unilever Ltd.), Brit. Patent 1,130,634, 1968.
122. Anonymous (to Kyowa Hakko Kogyo Co., Ltd.), Brit. Patent 1,155,490, 1969.
123. H. Enei, A. Mega, O. Ayato, S. Olumura, and S. Ota (to Ajinomoto Co.), Brit. Patent 1,152,966, 1969.
124. H. A. Gardner and A. W. VanHeuckeroth, *Ind. Eng. Chem.*, **19**, 724–726 (1927).
125. L. B. Kilgore, *Glass Packer*, **4**, 65–67, 90 (1930).
126. J. M. Antaki and D. T. Layne (to the Clorox Co.), U.S. Patent 4,927,657, May 22, 1990.
127. R. W. Wood, J. V. Parnell, and A. C. Hoefler (to General Foods Corp.), U.S. Patent 4,352,832, October 5, 1982.
128. J. E. Tiberio and M. C. Cirigliano (to Thomas J. Upton, Inc.), U.S. Patent 4,477,478, October 16, 1984.
129. J. G. Oles (to Kraft, Inc.), U.S. Patent 4,145,451, March 20, 1919.
130. *Federal Register*, Jan. 6, 1993, Vol. 58, pp. 632 and 2066.
131. G. Meer and T. Gerard, *Food Process.*, **5**, 170–171 (1963).
132. A. J. Froberg, *Food Prod. Develop.*, **4**, 46–47 (1971).
133. A. Partyka (to National Dairy Products Corp.), U.S. Patent 3,093,485, June 11, 1963.
134. F. H. Mattson and R. A. Volpenhein (to Proctor & Gamble Co.), U.S. Patent 3,600,186, August 17, 1971.
135. A. S. Szczesnia and E. Engel (to General Foods Corp.), U.S. Patent 3,300,318, January 27, 1967.
136. J. C. Spitzer, L. S. Nasareisch, J. L. Lange, and H. S. Bondi (to Carter Products, Inc.), U.S. Patent 2,944, July 12, 1960.
137. J. Kroll, G. Mieth, M. Roloff, J. PohI, and J. Broecker, Ger. Patent 106,777, 1974.
138. U. Steckowski (to Carl Kuhne KG), Ger. Patent 2,311,403, 1974.
139. H. Weidemann and H. P. Reinicke, Ger. Patent 1,924,465, 1970.
140. B. F. Hausmann, *J. Amer. Oil Chem. Soc.*, **63**, 278 (1986).
141. R. G. LaBarge, *Food Technol.*, **42**(1), 84 (1988).
142. C. C. Baer et al. (to Kraft General Foods), U.S. Patent 5,011,701, April 30, 1991.
143. R. Bauer et al. (to Thomas J. Lipton Co.), U.S. Patent 5,286,510, February 15, 1994.
144. C. T. Herald, G. E. Raynor, and J. B. Klis, *Food Process. Mark.*, **11**, 54–55 (1966).
145. M. H. Kimball, C. G. Harrell, and R. O. Brown (to Pillsbury Mills, Inc.), U.S. Patent 2,471,1435, May 31, 1949.

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## *Fats and Oils in Bakery Products*

Clyde E. Stauffer

### 1. INTRODUCTION

#### 1.1. Shortening in Bakery Usage

Fats and oils have been important bakery ingredients for centuries. Indeed, “shortening” is a baker’s term; fat in a bakery item “shortens” (tenderizes) the texture of the finished product. In bakery foods, shortenings impart tenderness, give a moister mouthfeel, contribute structure, lubricate, incorporate air, and transfer heat.

Properties of a fat or oil that determine its ability to carry out these functions are:

The ratio of solid to liquid phase.

The plasticity of a solid shortening.

The oxidative stability of the fat or oil.

Oil (or the oil fraction of a plastic shortening) in a baked food gives a tender bite, moist mouthfeel, and lubricity (the product clears more readily from the surfaces in the mouth). The solid portion of a shortening contributes to the structure of the dough and the final product, and entraps air bubbles during mixing.

These two functions are the key to selecting the proper shortening for a given application. Also, the fatty acids in the oil fraction are generally more unsaturated than those in the solid part, and polyunsaturated fatty acids are subject to the development of oxidative rancidity. Oxidative stability is especially important in shortenings that are exposed to air (cracker spray oils) and high temperatures (frying fats).

## 1.2. Other Uses: Icing, Filler, Coating Fats

In addition to inclusion in the dough, fats are also applied to the baked piece, providing additional enhancement of consumer appeal. Examples are: creme icings on cakes, aerated fillings in cornettos, fillings in sandwich cookies and sugar wafers, and chocolate and compound coatings on snack cakes.

These enhancements are primarily carriers of flavors and sweetness (they are typically high in sugar content). The solid portion of the fat is a major structural element in the material, while the oil part also enhances mouthfeel, particularly lubricity (1).

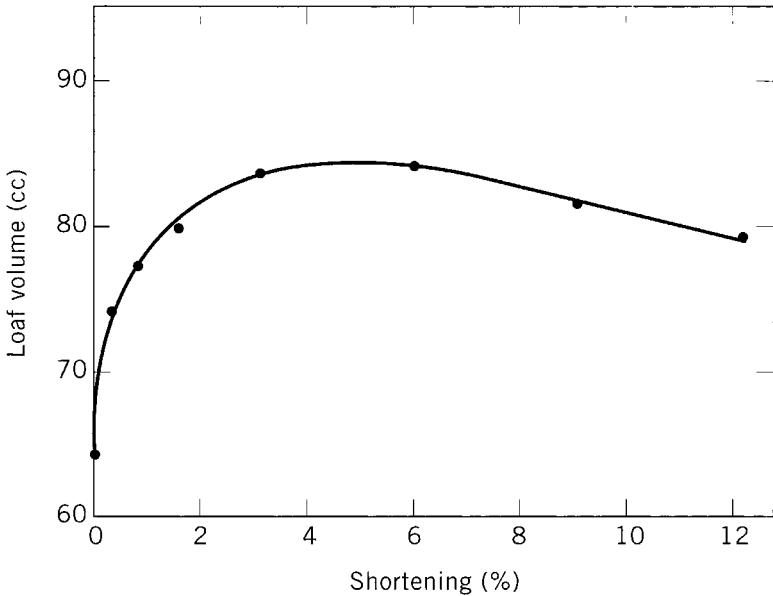
## 2. BREAD AND ROLLS

In yeast-leavened baked foods the volume and texture (fine-grained crumb with small, evenly distributed air cells) depends upon the strength of the gluten matrix. This is a mixture of hydrated storage proteins (glutenin and gliadin, found in wheat flour), surface active lipids (both those native to flour and added surfactants), and nonpolar fats. The structure of this matrix is still not clearly defined, but it is known that shortening is an integral part of it. During baking the protein is denatured, forming a glass that is in part responsible for the elasticity of the cooled bread. Shortening modifies both the baking process and the characteristics of the final product.

### 2.1. Effect on Texture

Up to 5% fat (flour weight basis) may be used in ordinary white pan bread although the usual level is 3 to 4% of a plastic fat such as all purpose shortening or lard. These amounts produce the optimum effect in the bread. In soft rolls such as hamburger buns 6 to 8% may be used to give a softer bun. This tenderizing effect also slows down the staling process so bread made with shortening remains softer after storage for several days as compared to bread made with the same formula but without fat in the dough.

The shortening is simply added to the mixer at the beginning of dough mixing. The statement has been made that the presence of fat slows down development of gluten (lengthens the required mixing time), but in experiments both in the laboratory and in the plant this thesis has not been confirmed.



**Figure 1.** Final loaf volume of white bread, as a function of added plastic shortening.

## 2.2. Effect on Volume

Loaf volume of bread increases as the amount of plastic shortening increases, up to about 5% (flour basis), then remains roughly constant (Figure 1). This is because the dough expands in the oven for a longer time when shortening is present, as compared to a dough made without added fat (2). Fat (or oil) seems to interact with dough components (starch and gluten) and delay the reactions that end loaf expansion during baking. In other words, in bakery terminology, the addition of shortening increases oven-spring of the bread.

## 2.3. Use of Oil Versus Plastic Fat

The tenderizing effect of shortening is due to the liquid phase. Since all-purpose shortening contains about 25% solid fat at room temperature, 3 kg of vegetable oil is equivalent to 4 kg of plastic fat in softening bread crumb. The solid fat portion of traditional bread shortening does play a structural role. It provides dough strength during proofing, contributing resistance to shocks during transfer from the proofer to the oven, and also appears to strengthen sidewalls of baked bread, minimizing “keyholing” of the finished loaf. In the continuous bread process hard flake [fat hydrogenated to an iodine value of about 5, and a melting point of about 60°C (140°F)] is often melted into the warm oil being pumped to the developer. When a switch is made from plastic fat to oil, it is necessary to use a dough strengthener such as sodium stearoyl lactylate (SSL) or diacetyl tartrate esters of monoglyceride (DATEM) to get good volume.

## 2.4. Frozen Doughs

Extremely lean bread doughs deteriorate more rapidly in frozen storage than doughs that contain sugar and shortening. For a lean French-type bread the inclusion of 0.5 to 1% vegetable oil in the formula will extend the shelf life by several weeks. In regular doughs (i.e., for baking pan bread or hamburger buns) the usual amount of shortening is used: 3 to 4% in bread, 6 to 8% in buns. The function in these cases is the same as in standard baking, that is, the shortening improves oven spring and tenderizes the finished product.

## 3. LAYERED DOUGHS

In many bakery products, fat is layered between sheets and dough, and this is manipulated to make a dough sheet consisting of up to 100 alternating layers of dough and fat. Such roll-in doughs include Danish pastry, puff pastry, and croissants. The dough is mixed, divided into pieces of about ten kilograms each, then cooled to 5–10°C in a retarder. The cooled dough is rolled out, two to three kilograms of fat is spread over part of the sheet, and the dough is folded over to cover the fat. The “sandwich” is then rolled out and folded; this is repeated several times, often with a retarding step included to keep the dough/fat mass cool.

The primary goal during the roll-in process is to preserve the structure of alternate layers of dough and fat. There are several important factors to consider in selecting the correct shortening or margarine for such a process. Some of them are: solid fat index (SFI) and plasticity of the fat; complete melting point of the fat; consistency (softness) of the dough; retarder temperature; number of folds given the dough before returning it to the retarder; and proofing temperature. Many of these factors are unique to a given product in a particular bakery, and they influence the specification for the roll-in fat which gives the best final product in that bakery.

### 3.1. Danish Pastry

The dough for Danish pastry contains higher levels of sugar and water than a bread dough, making it quite soft at the usual mixing temperature of around 20°C. After it is mixed (the gluten matrix is fully developed) it is divided and cooled as described above. The cooling and rest time (during which some fermentation occurs) makes the dough cohesive enough to handle, but if it is over-worked or allowed to warm up too much during the roll-in step, it again becomes soft, sticky, and easily torn. The plastic range of the roll-in fat must be rather broad. The consistency of the fat should nearly match that of the dough across the temperature range, which usually includes the temperature of the retarder (on the cool end) to room temperature or above. If the fat is significantly harder than the dough at the cool temperature, then when a retarded dough is rolled out, the fat does not spread into a uniform layer between the dough layers, but is likely to tear holes in the dough sheet. If the fat



is softer than the dough at room temperature, then as the dough mass warms up (during rolling and folding) the shortening soaks into the dough, the adjacent dough layers knit together, and the layering effect is lost. The proper plasticity of roll-in fat requires a relatively shallow SFI profile, and stabilization in the  $\beta'$  crystal phase. The latter is particularly important. If the shortening has begun to transform to the more solid  $\beta$  phase, the additional hardness due to  $\beta$  crystals will cause excessive tearing during roll-in.

The melting point of the fat must be higher than the proofing temperature for the Danish. If the proof box temperature is above the fat melting point, the fat layers turn to oil. This allows the dough layers to knit together to some extent during proofing, and the final product is less flaky than desired. As a general rule, the complete melting point should be at least 5°C higher than proof box temperatures.

A standard all-purpose shortening having a complete melting point of about 45°C usually works well in most Danish pastry production lines. This gives a flaky finished product. Some manufacturers use an emulsified shortening containing 3% monoglyceride, but with similar SFI and melting point specifications. This produces a Danish having a somewhat gummier mouthfeel, which is preferred in some parts of the country.

### 3.2. Croissants

A croissant dough is similar to Danish dough, although it generally contains somewhat less sugar and water, so it is somewhat firmer at the optimum mixed dough temperature of 20°C. As with Danish, the dough pieces are usually retarded before roll-in is begun. The best quality croissants are produced using unsalted butter for roll-in. The amount used varies from 20 to 35% of dough weight; higher levels give a product that has less volume and flakiness, and is often perceived as greasy in the mouth. The optimum is usually about 25% roll-in fat.

The factors involved in successful processing are similar to those discussed above for Danish pastry. Because butter has a steeper SFI curve than all-purpose shortening (it is much harder at retarder temperatures) more care is required to prevent tearing the dough as it is being rolled. The melting point is lower than that of shortening, so proofing temperatures are lower than for Danish. Puff pastry margarine is an acceptable substitute, although it does not contribute as much flavor as butter. Since puff pastry margarine has a higher melting point, the proofing may be done at a higher temperature (and for shorter times) if time is a factor.

### 3.3. Puff Pastry

Rolled-in doughs that contain no yeast (puff pastry) depend upon steam generation in the oven for their leavening. Usually margarine (which contains about 17–20% water) is used for the roll-in fat for these doughs. The water is trapped and held in

the fat layers in the dough. It evaporates and expands in the oven, giving an expanded structure to the final product. If the fat portion of the margarine is too soft, the water migrates into the dough during the roll-in step, and the leavening action in the oven is decreased. Puff pastry margarine has a higher SFI curve than all-purpose shortening, and it is somewhat more brittle during roll-in. In this case a smooth, continuous layer of fat between dough layers is not particularly desirable. The roll-in process is adjusted somewhat to achieve numerous discrete particles of margarine between the dough layers. These produce somewhat larger voids in the finished product, desirable in a puff pastry.

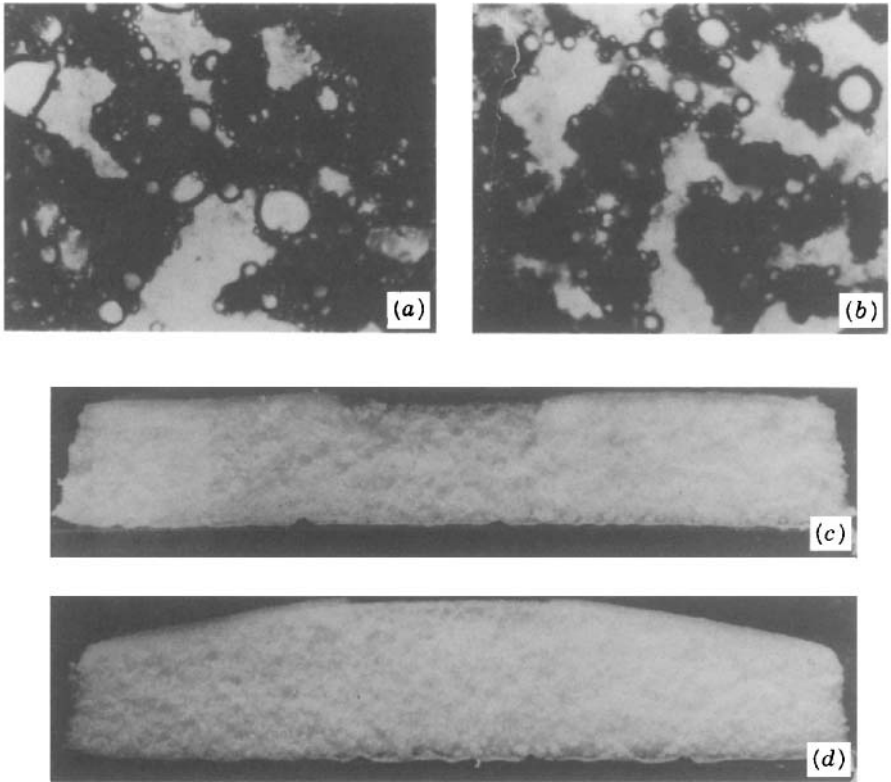
## 4. CAKES

### 4.1. Emulsified Plastic Fats

In layer cakes and related items the closeness of the internal grain, and to some extent the final volume, are strongly influenced by the characteristics of the shortening used. In the finished cake a high percentage of the total volume is open space, present as finally divided cells. These spaces are created by carbon dioxide (from the leavening system) and steam, formed during baking. When these gases are generated, by heat, they migrate to the nearest air bubbles that have been incorporated into the cake batter during mixing. If there are many small air bubbles in the batter the leavening gases are distributed widely. Each of the bubbles is small, and does not rise rapidly to the surface of the cake. The leavening gases are retained in the cake and contribute to final volume. If the air incorporated during mixing is present as relatively few, larger, bubbles, then during baking the bubbles are expanded by the leavening gases, and many of them are large enough that they rise to the surface, escaping and yielding a lower final cake volume.

If the original air bubbles are fewer and larger, the final cake will have less volume and a coarse (open) grain. If the original batter contains many small air cells, the final cake will have larger volume and a fine (close) grain. These two situations are exemplified in Figure 2. The shortening used plays a large role in determining the degree of subdivision of the air.

In the usual two-stage process of cake production, the shortening and sugar are combined and mixed. During this step the air is dispersed into the solid phase. Then the eggs are incorporated, followed by the flour, liquids, and other ingredients. During the first creaming step, the plastic shortening entraps air bubbles. In the presence of an emulsifier such as 4% monoglyceride, these bubbles are divided into numerous small air cells by the action of the beater. The shortening must be solid (so the bubbles do not escape), but also plastic so it can fold around each air pocket. This is best accomplished by a plastic shortening, crystallized in the  $\beta'$  phase. If the shortening has transformed into the  $\beta$  phase, the large plates of solid fat are much less effective in entrapping the fat. A good shortening for this type of cake batter production has the SFI profile of all-purpose shortening, containing added monoglyceride. Plastic shortening containing 5 to 8% of an  $\alpha$ -tending emulsifier such

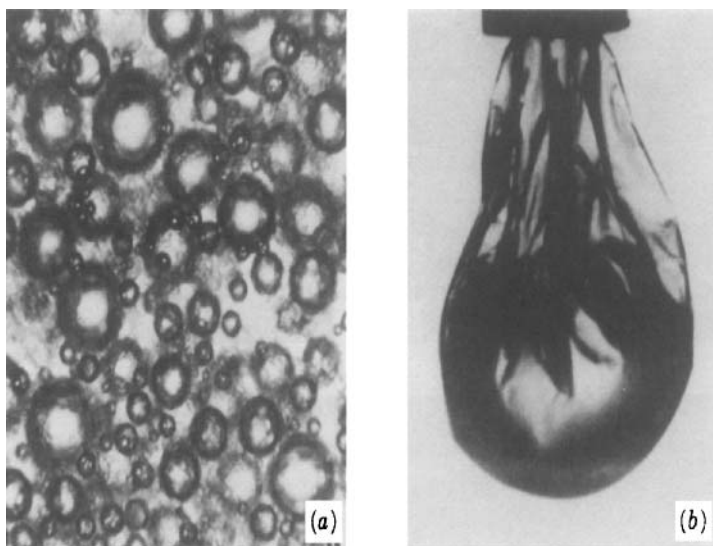


**Figure 2.** Emulsifiers help subdivide air particles in shortening. (a), Microphotograph of batter made with unemulsified shortening, showing large air bubbles; (c), cake baked from this batter. (b), Microphotograph of batter made with emulsified shortening, showing smaller air bubbles; (d), cake baked from this batter. (From Reference 3 by permission.)

as propylene glycol monoester plus 2 to 4% monoglyceride is also sold. These products are successfully used in one-stage cake production.

#### 4.2. Oil Shortening with $\alpha$ -Tending Emulsifiers

It is also possible to make cakes in a one-stage production process, in which all the ingredients are added at the beginning and the batter is mixed. In this case the air is entrapped in the water phase rather than in the shortening. In order to form the air-in-water foam, which is stabilized by proteins contributed from flour and eggs (3), it is necessary to prevent the defoaming action usually associated with fats and oils. This is accomplished by including an  $\alpha$ -tending emulsifier in the shortening; typical ones used are propylene glycol monoesters (PGME) or acetylated monoglyceride (AcMG). At high enough concentrations these emulsifiers form a solid film at the oil/water interface (Figure 3). This solid interfacial



**Figure 3.** (a) Microphotograph of batter of oil cake, with PGME in the oil, showing good air incorporation. (b) A water droplet was suspended in oil containing PGME, then some water was withdrawn through the syringe tip. The crumpled material is solidified emulsifier at the interface. (From Reference 4 by permission.)

film segregates the fat from the water phase, so it cannot destabilize the protein foam.

The film-forming tendencies of  $\alpha$ -emulsifiers enable the use of liquid oil as the shortening in a cake or muffin batter. Cakes made with oil shortening are more tender than when they are made with a plastic shortening. The cake gives an impression of moistness when eaten, even after storage for a week or longer. Packaged dry cake mixes, for use in the home, are usually made with oil containing 10 to 15%  $\alpha$ -tending emulsifier dissolved in the oil. Other emulsifiers such as Polysorbate 65 are also used in oil cakes. Because of the tenderness of the cake layer, this type of formulation is usually not suitable for commercial cake production.

#### 4.3. Muffins, Cupcakes

Muffins and cupcakes are quite similar to layer cakes in the batter characteristics and reactions during baking (timing of release of leavening gases, setting of the structure). They are less sweet than most cakes, and generally have a somewhat more open crumb structure (a close, fine grain is not desired). The shortening used is an all-purpose (nonemulsified) type. In some instances a vegetable oil shortening is used, when an extremely open texture accompanied by a moist mouthfeel is desired, for example, in a high fiber muffin. Shortening levels vary widely, from 18 to 35% based upon the amount of flour; tenderness of the finished product varies accordingly.

#### 4.4. Creme Icings

Layer cakes are generally covered with an icing, giving increased sweetness and flavor. Creme icings are made by creaming together the fat and sugar (in a ratio of about 3 to 4), then adding flavor and egg whites and perhaps a small amount of water, and mixing at high speed until the specific density is in the range of 0.4 to 0.6 g/cc. The fat used varies widely. Traditionally butter was used, giving a flavorful icing. Due to economics, some or all of the butter is frequently replaced with an emulsified shortening having an SFI curve similar to that of butter. It is important that the melting point of the shortening be about 37°C (body temperature), otherwise the residual hard fat leaves an undesirable greasy or waxy impression in the mouth.

Monoglyceride at 2 to 3% is commonly used as the emulsifier. At higher levels (e.g., the 4% level found in emulsified cake shortening) the aeration of the icing is less efficient. Polysorbate 65 at a concentration of about 0.5% is also included in some icing shortenings. It has a higher HLB, and allows the incorporation of somewhat more water in the icing (5).

### 5. CAKE DONUTS

Donuts are fried in hot (180–190°C) fat rather than being baked. Yeast-raised donuts are a sweet dough, cut into rings and proofed before being fried. Cake donuts are chemically leavened and similar to cake or cupcake batter in composition. In a cake donut batter the degree of subdivision of air entrapped in the shortening phase has much to do with the openness of grain in the final donut. Thus an emulsified cake shortening is generally used. The batter is extruded in a ring shape into the hot fat, where it is fried. Initially the ring sinks in the fat, but the leavening soon causes it to rise to the surface; it is flipped halfway through the frying cycle so both sides are cooked. A significant amount of the batter water is evaporated during the process, and an equal volume of frying fat is absorbed into the donut, accounting for about half the fat in the finished donut. The nature of the frying fat has much to do with the eating characteristics of the finished donut. If it has a rather high SFI curve and a high melting point, the cooled donut will have a solid texture and a dry mouthfeel. On the other hand, if a high stability frying *oil* is used (with a melting point at or below room temperature) the donut will be greasy and unappetizing. A good donut frying fat has an SFI somewhat higher than an all-purpose shortening, but with a lower melting point.

If the donut is to be coated with powdered sugar, the consistency of the absorbed fat is important to obtaining proper coverage. If the fat in the cooled donut is too hard, sugar pickup will be too small. On the other hand, if the fat is too soft the sugar will “melt” when the packaged donut is stored. For best results, in warm weather the SFI curve of the frying fat should be on the upper side of the specifications given in Table 1, while in cold weather it should be on the low side. The proper selection of frying fat is of major importance to the quality of the finished cake donut.

TABLE 1. Typical Physical Properties of Bakery Shortenings<sup>a</sup>.

Shortening Type	Solid Fat Index					AOM (min. h)
	10°C (50°F)	21°C (70°F)	27°C (80°F)	33°C (92°F)	40°C (104°F)	
RBD oil <sup>b</sup>	0	—	—	—	—	10
Lightly hydrogenated oil	<5	<1.5	—	—	—	25
Deep frying fat	47 ± 3	32 ± 3	25 ± 2	12 ± 1	<2	200
All-purpose	28 ± 3	20 ± 2	17 ± 1	13 ± 1	7 ± 1	75
Cake, icing <sup>c</sup>	32 ± 3	25 ± 2	22 ± 2	16 ± 1	11 ± 1	75
Pie crust	26 ± 3	17 ± 1	14 ± 1	10 ± 1	6 ± 1	75
Wafer filler fat	55 ± 4	39 ± 3	29 ± 3	4 ± 1	<1	100
Sandwich cookie filler	38 ± 3	24 ± 2	18 ± 1	9 ± 1	<2	100
Coating fat	64 ± 5	52 ± 4	44 ± 4	20 ± 2	0	200
Puff pastry margarine	34 ± 3	30 ± 3	27 ± 2	22 ± 2	16 ± 1	200
General use margarine	28 ± 2	21 ± 2	18 ± 1	15 ± 1	10 ± 1	200
Butter <sup>d</sup>	32	12	9	3	0	—
Cocoa butter <sup>d</sup>	62	48	8	0	0	—

<sup>a</sup>General chemical characteristics: peroxide value, 1 meq/kg max.; free fatty acid (as oleic acid), 0.05% max.; phosphorous content, 1 ppm max.

<sup>b</sup>RBD = Refined, bleached, and deodorized oil.

<sup>c</sup>Contains 3–5%  $\alpha$ -monoglyceride for cake, 2–3% for icing.

<sup>d</sup>Typical values. As natural products, these can vary somewhat.

## 6. COOKIES

### 6.1. Effect on Texture, Spread

Entrapped air bubbles in the shortening phase of a cookie dough serve as nuclei for leavening gas during baking, as in cakes. Thus if a fine-grained cookie, for example, a soft sugar cookie, is desired an emulsified shortening should be used. For a thin, crisp cookie a lightly hydrogenated oil may give the best product characteristics. For most wirecut cookies an all-purpose shortening works well. As the amount of shortening in the formulation increases, the tenderness of the baked cookie also increases, as would be expected.

The diameter of the baked cookie is important in commercial bakeries, where the finished product must fit a previously designed container. The height is also sometimes a factor, depending upon the type of packaging equipment in use. The amounts of both sugar and shortening (relative to flour) in the dough affect diameter and height, but in a rather complex fashion (6, 7). Briefly, in a 50% sugar (flour basis) dough, increasing shortening increases diameter and decreases height. In a 90% sugar formula, increasing shortening decreases diameter and has little effect on height.

### 6.2. Processing Considerations

*Dough.* For wirecut cookies, where the dough is extruded through a die and portions are cut and dropped onto the baking band, the main dough function

is incorporation of finely divided air bubbles. As the cookies bake, leavening gases (steam, carbon dioxide) collect in the air bubble nuclei. If the air cells are few and large, the grain of the resulting cookie will be open, while if the air nuclei are many and small, the cookie will have a finer grain. The shortening also lubricates the dough as it is extruded. If a low-fat cookie is being developed, difficulty is often seen at the wirecut depositor; the dough does not extrude readily. Using oil as the added allowable lipid may help this problem.

In rotary-molded cookies, air incorporation is important, but shortening also makes a structural contribution to cookie processing and quality. The solid phase of the shortening influences the consistency of the dough; if the fat is too soft (the SFI profile is too low) the dough will be soft, and not machine properly at the molder. Also, oil will tend to leak from the shaped pieces, and saturate the cloth takeaway belt, causing problems. If the SFI profile is too high, the dough will be stiff, it will not fill the mold properly, and it will not release cleanly from the die. In the former case the shortening is making an inadequate contribution to dough structure, while in the latter case lubrication by the shortening is lacking. It is necessary to maintain the proper balance between the solid and liquid phase in the shortening to have good machinability. To obtain the precise balance required it may be necessary to use a combination of lightly hydrogenated oil plus all-purpose shortening in the dough. The exact ratio for best results is determined by experiment: if bits of dough stick in the mold, use a little more oil, while if the pattern tends to smear out, use less oil (or a plastic shortening with a higher SFI profile).

For sheeted cookies, such as Marias biscuits, dough structure is primarily due to a modest amount of gluten development, and the grain of the final product depends upon proper extrusion and sheeting. The main contribution of shortening in these items is tender eating quality in the finished product. This is an attribute of the oil phase, and a liquid oil shortening should give good results, when used at about 75% of the amount of plastic fat in the formula. Cookies are a long shelf life item, and they must be acceptable to the consumer for up to six months or more. If oil is used in the dough, it should have good oxidative stability, so rancidity does not develop in the product during storage.

Wafer batter does not ordinarily contain fat or oil. However, on occasion a small amount of oil is included in the batter, to facilitate release of the baked wafer from the griddle. The amount used is just enough to effect release (perhaps 0.5% of total batter weight). An oil with high oxidative stability would give the best lubrication in this application.

*Filling Fat.* Fillings for sugar wafers or sandwich cookies consist of fat and sugar, with flavor and color added as desired. The consistency of the filling is determined to a large extent by the SFI profile of the fat used. This profile must meet three requirements:

1. The blend must have a soft consistency, so it can be extruded onto the basecake or wafer
2. The extruded filling must be firm at room temperature and below, so that it does not slide when the cookie is eaten

3. The fat must melt almost completely at mouth temperature, so it does not have a waxy mouthfeel

To achieve these goals, the SFI profile of a filler fat is rather steep, higher than that for all-purpose shortening at low temperatures, and lower at high temperatures. The plastic range is much narrower, and close control of temperature at the mixer and extruder is necessary.

The solid fraction of filler fat for wafers and sandwich cookies gives body to the filling. When the filled wafer or sandwich cookie is cooled to room temperature the cream sets up to the firm consistency needed. In the production of filled wafers it is crucial that the crystal structure be  $\beta'$ . If the shortening has started to go beta, the resulting oiliness causes the wafer sheets to slide during the transport and cutting operation. Also,  $\beta$  crystals set up slower than  $\beta'$  crystals, causing delays between the extruder and the cutting operations. In sandwich cookies this is not such a factor, because they are not cut after filling. Wafer filler fat has a higher SFI profile than sandwich cookie filler fat; because it must prevent slippage between the top and bottom wafer sheets during cutting. Filler fats with a wide range of SFIs are available and individual plants may want to develop their own specification to fit their equipment, processing conditions, and geographical locations.

*Coating Fat.* Cookies and other snack items are frequently coated with chocolate. The SFI profile of cocoa butter is unique among natural fats, being very high at room temperature and below, but melting rather sharply and completely at about 32–35°C. This characteristic is accepted as the norm for coating fats. A number of substitutes for cocoa butter have been sold. These are based upon shea butter, fractionated palm kernel oil, or soy and similar vegetable oils that have been hydrogenated in a special fashion. Such a fat is called a “hard butter.”

Hard butters do not have such a sharp SFI profile as cocoa butter and their melting point is generally slightly higher, around 38–42°C (the melting point is adjustable, by making slight changes in process parameters). When used to make a coating for a cookie or wafer, they are blended with cocoa powder, sugar, and milk solids. While the fat does not melt completely at mouth temperature, this is not a problem because it is chewed along with the other, nonmelting parts of the cookie and the slight residual solid fat is not noticed. The confectionery coating has an advantage over chocolate; it does not melt as readily when held in the fingers or when exposed to warm summer temperatures.

The crystallization behavior of cocoa butter is complex. Careful tempering of the chocolate is necessary, to obtain a covering that is smooth, glossy, and stable. If the cocoa butter in the covering undergoes crystal transformation, because of temperature fluctuations or a variety of other reasons, it takes on a dusty look, referred to as chocolate “bloom.” Hard butters are generally less complex in their crystal habit, tempering is easier, and bloom formation less likely to be a problem. The two kinds of fat generally are not compatible, and a coating made with hard butter should not contain any more cocoa butter than the few percent that is present in the cocoa powder, used for flavor and color. Likewise, trying to extend chocolate by adding hard butter enhances the tendency for bloom formation.



*Crackers.* Two main types of crackers are produced commercially: soda and snack. In both types about 10 to 12% of a plastic shortening is incorporated in the dough, providing tenderness and a desirable crisp “bite” in the final product. In addition, snack crackers are sprayed with oil as the hot product exits the oven to improve mouthfeel and help seasonings adhere to the piece. Traditionally lightly hydrogenated coconut oil with a complete melting point of 33°C has been used, but in recent years selectively hydrogenated soy oil has been successfully used. The polyunsaturated fatty acids are reduced to almost zero, but the m.p. is around 35°C. If a shiny appearance is desired an oil with an SFI around 10 at 20°C is used, while one with an SFI of about 20 at 20°C will give a drier appearance. Because the oil is on and near the surface of the cracker, and because the cracker should have a shelf life of up to six months, the spray oil must have good oxidative stability: AOM values should be at least 100 hours.

## 7. PIE CRUST, BISCUITS

### 7.1. Texture

Shortening functions in pie crust and American-style biscuits analogous to its action in layered doughs: layers of fat create regions of low tensile strength within the dough, giving it a flaky texture. The means of achieving this effect, however, is different, necessitating adjustment in the properties of the plastic shortening used. The shortening is mixed into the blended dry ingredients in such a way as to form small (pea-sized) pieces. Then liquid is added, the dough is gently mixed until it is just cohesive (the flour gluten is not developed), then it is sheeted out, the piece (crust or biscuit) is cut, and baked. The dispersion of shortening is more like that in puff pastry than in croissants. Intimate contact between all the shortening and the flour is not desired. The shortening should be slightly harder than the all-purpose shortening used, for example, for Danish roll-in. Alternatively, the crust or biscuit dough should be kept cool (even refrigerated) during processing to maintain the integrity of the fat pieces.

On the other hand, shortening used in biscuits should not have a melting point markedly higher than body temperature, or a waxy mouthfeel results. A shortening having an SFI profile similar to that suggested for sandwich cookie filler fat has been successfully used to accomplish all these aims.

## 8. SPECIFICATIONS FOR BAKERY SHORTENINGS

Certain properties of shortening are of particular importance to bakers. The solid fat index, plasticity, and oxidative stability of shortening are determined by the supplier’s production process. The source of the starting oils, the conditions and extent of hydrogenation, the blending and crystallization of various basestocks, the storage conditions after packaging, these production variables determine the factors which influence shortening functionality. Some understanding of the nature of the

three factors mentioned above clarifies their role in the bakery production process, and contributes to improved selection of shortenings for different bakery products.

### 8.1. Solid Fat Index/Content

The solid fat index (SFI) relates to the percent of shortening which is solid at various temperatures. This curve can have a variety of shapes, being rather humped like cocoa butter, or almost straight over most of the range, with a steeper or shallower slope. The whole curve cannot be predicted from a determination made at just one temperature. Curves for different fats may cross; the whole SFI curve is required in order to understand the properties of the shortening at different temperatures.

Solid fat index is measured by placing a sample of the fat in a dilatometer and measuring the volume at various temperatures. When a solid fat melts it expands. A solid triglyceride has a coefficient of expansion of about 0.00040 mL/g/°C, while a liquid triglyceride has a coefficient of expansion of about 0.00084 mL/g/°C. A shortening is a mixture of triglycerides that melt over a range of temperatures, and the actual volume change resembles the solid line shown in Figure 4. In this hypothetical example, the actual percent of solids in the fat at 20°C is  $S/T$ , or about 47%. While the specific volume line for oil is easy to determine, the corresponding line for fully solid fat is difficult to define. The standard SFI method (AOCS Method Cd 10-57) circumvents this difficulty by adopting a convention: the lower reference line is given the same slope as the line for the liquid, and is located 0.100 units

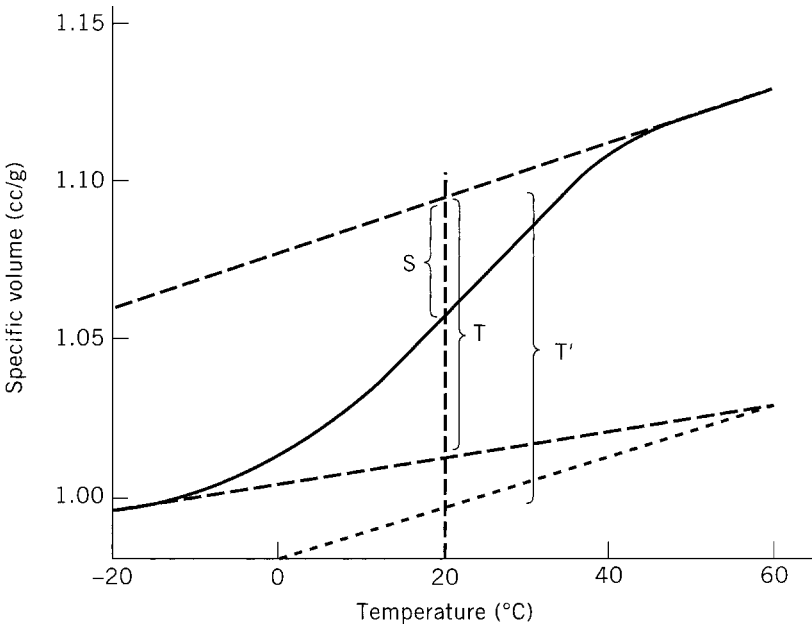


Figure 4. A curve exemplifying the determination of solid fat index by dilatometry.

below it (the dotted line in Figure 4). For the example the SFI value is  $S/T'$ , or about 40%.

The dilatometric method is time-consuming and subject to the bias introduced by the convention described. More recently pulsed Nuclear Magnetic Resonance (pNMR) has been used to measure the relative amounts of liquid and solid fat in a sample, based upon the difference in rates of relaxation of protons in the two phases after the sample has been pulsed (AOCS Method Cd 16-81). With proper calibration this gives a direct determination of the percentage of solid fat, and the results are termed solid fat content (SFC). The analysis takes less time than dilatometry, but the equipment is more expensive.

The relationship between SFI and SFC is a complex function of both temperature and the level of SFI. A comprehensive study of 46 plastic shortenings across the temperature range of 10–45°C provided data for deriving equations relating the two values (8). A study of 14 hard butters (9) showed that the relationship was not as complex as that for plastic shortenings.

Analytical measurements of SFI and SFC are relatively precise; duplicate determinations should agree within  $\pm 1$  unit. The suggested values given in Table 8.1 for SFI and SFC have rather large tolerances specified, because it is difficult to control the steps in shortening production to closer tolerances than those given.

Functionality of a plastic fat in the bakery depends not only on solids content, but also on the slope of the SFI curve. Meeting an SFI specification implies that the values for a particular batch lie within the specified ranges, and also that deviations from the target values are all on the same side, either higher or lower. This is particularly important in producing basestocks for blending for margarine and shortening production.

## 8.2. Plasticity

The plasticity of a fat is defined operationally; the shortening is smooth, not grainy, deforming readily when squeezed but holding its shape when set on a flat surface. No precise method of measuring these characteristics objectively has been developed to date. Various sorts of penetration tests give approximate results which are useful, although they must be used with some caution. One such test is the cone penetrometer method (AOCS Method Cc 16-60). A metal cone is set on the top surface of the fat, and the depth of penetration after a fixed time period is determined. The extent of penetration is larger for a soft fat than for a hard fat. A second method which is of value on the shortening production line employs a thick needle. A tube is held vertically on the surface of the fat, and the needle is dropped from the top of the tube. The depth of penetration is read from markings on the needle.

The plastic range refers to the range of temperatures over which a shortening will have the properties listed above. Plasticity is a function of two factors: SFI and crystal structure. Assuming the shortening has the proper  $\beta'$  crystal structure then it will be plastic over a range of about 10 to 25 SFI units (Figure 5). The choice of upper and lower limits of plasticity depend upon the experience of the individual choosing

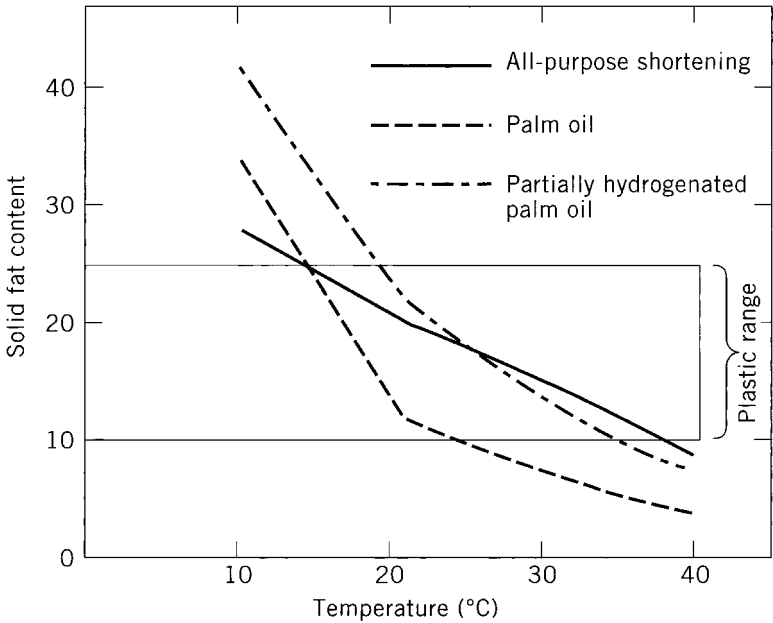


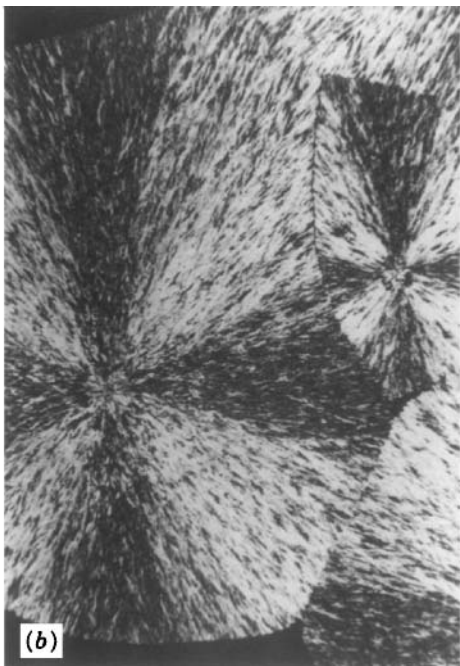
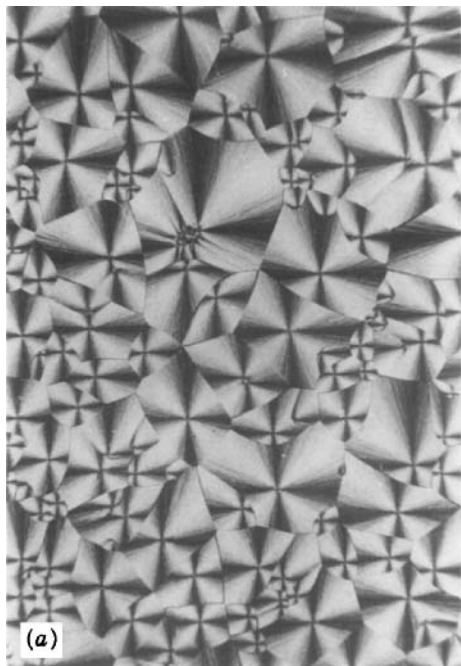
Figure 5. Plastic range of three shortenings having different SFI profiles.

them, and the application; they are broader for bakery shortening than for margarine for use in the home.

Triglyceride fats crystallize in three different crystal forms. Rapid cooling of melted fat forms a waxy solid called the alpha ( $\alpha$ ) form (Figure 6a). This is a rather unstable crystal which quickly changes into long needle-like elusters of beta prime ( $\beta'$ ) crystals (Figure 6b). This is the preferred crystal form for plastic shortenings. The long, thin crystals join together into a “brush heap” which immobilizes several times its own weight in liquid oil. The thin needles are readily broken when squeezed (and reform when deformation ceases) so the overall feeling is one of a very smooth, creamy solid.

If this crystal phase is not stabilized by proper tempering at the time of manufacture, or if the shortening is stored at too warm a temperature, the solid phase reorganizes into the most stable structure, beta ( $\beta$ ) crystals (Figure 6c). These are platelike, firm structures. Because there is less surface area per gram than in the case of  $\beta'$ , the  $\beta$  crystals immobilize less liquid. A fat that has converted to the  $\beta$  form feels grainy or sandy, and also oily. A shortening that has “gone beta” has lower plasticity than the same shortening stabilized in the  $\beta'$  phase.

Shortening made from 100% hydrogenated soy or sunflower oil converts to  $\beta$  crystals rather readily, but the addition of 5 to 7% hydrogenated palm or cottonseed oil stabilizes the  $\beta'$  phase. Most plastic shortening used in the United States today is made from partially hydrogenated soy oil plus a small amount of palm or cottonseed hard flakes (iodine value of 5). There are a few shortenings for which some  $\beta$  crystals are preferred, mainly in fluid shortenings and in puff pastry margarine.



**Figure 6.** Microphotographs of fat crystals, taken in polarized light. From left to right:  $\alpha$  crystals:  $\beta'$  crystals:  $\beta$  crystals.

The function of plasticity in various bakery products was discussed previously. In ordinary cookie production the plastic shortening is combined with sugar and then mixed, or creamed, to incorporate air bubbles. These air bubbles are the nuclei for gas expansion during baking. The air is physically trapped, and the brush heap structure of  $\beta'$  shortening is better able to do this than the plates of  $\beta$  crystals. The air is actually in the liquid oil, so if SFI is too high there is not enough oil volume for adequate aeration. On the other hand, if SFI is too low the air is not trapped, and it escapes before dough mixing is complete. There is a range of SFI values giving optimum aeration of the creamed shortening, corresponding to the plastic range.

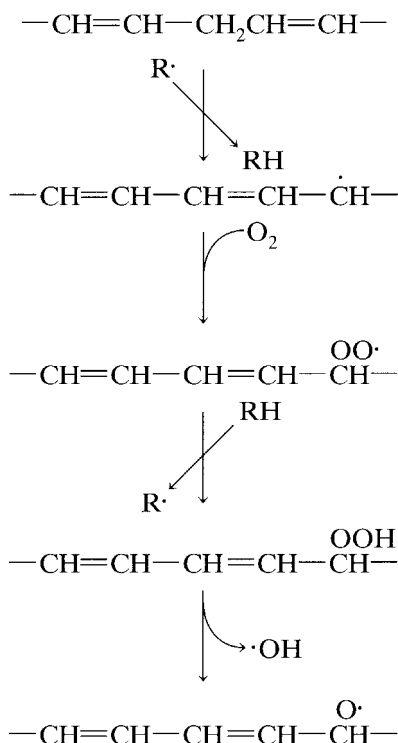
The machining of doughs, preparatory to baking, often causes some warming. If the SFI gets too low oiling out may be observed. The plasticity is suitable for the temperatures experienced in mixer operation, but because of warming during machining the shortening causes problems at the front of the oven. The plastic range of the shortening is too short to accommodate both the mixing and the forming operations. This is most often seen when a simple shortening (e.g., palm oil, as depicted in Figure 5) is used. Switching to a shortening with a broader plastic range will usually solve the problem.

Filling fat for wafers and sandwich cookies must meet a different set of requirements. If the solid fat index is too low at room temperature the wafers will tend to slide. The plastic range of filler fat is narrower, with less temperature tolerance at the mixer. When the wafer is cooled to room temperature the cream sets up to the firm consistency needed. In the production of filled wafers it is crucial that the crystal structure be  $\beta'$ . If the shortening has started to go beta, the resulting oiliness makes the wafer sheets slide during the transport and cutting operation. Also,  $\beta$  crystals set up slower than  $\beta'$  crystals, causing delays between the extruder and the cutting operations.

### 8.3. Oxidative Stability

The importance of oxidative stability of a fat or oil depends upon the intended use of the oil, in particular the use temperature and the finished product storage time. Two extremes are bread and a deep-fat fried snack. In bread the maximum temperature to which fat is exposed is 95°C (the final internal temperature during baking) and the storage time is at most 7 days; the least stable shortening, RBD oil, can be used for this application without danger of rancidity. The oil for deep-fat frying is heated to about 180°C, exposed to air during the frying operation, and has an expected shelf life up to 1 year. A stable fat is needed, so that rancidity does not make the snack prematurely unacceptable.

Autoxidation of fats occurs with unsaturated fatty acid chains. The relative rates of oxidation of oleic, linoleic, linolenic, and arachidonic acids (one, two, three, and four double bonds, respectively) are 1, 12, 25, and 50. Autoxidation is a free radical reaction, initiated and propagated by free radicals reacting with methylene ( $-\text{CH}_2-$ ) groups adjacent to double bonds (Figure 7). A hydrogen radical is extracted and one of the double bonds migrates into a conjugated position, moving



**Figure 7.** Reactions involved in autoxidation of a polyunsaturated fatty acid.

the radical site to the outer carbon. Dissolved oxygen adds to this site generating a peroxy radical; this abstracts a hydrogen from a donor, perhaps another methylene group, making a hydroperoxide. The hydroperoxide splits to generate two free radicals, a hydroxyl and an alkoxy radical. This cleavage is catalyzed by traces of metal ion such as copper or iron. The net result is three free radicals, each of which can initiate another chain of reactions. The rate of reaction is self-enhancing, i.e., it is an autocatalytic reaction.

The reactions detailed above can occur in the dark, as long as molecular oxygen and an initiating free radical species is present. If the oil is exposed to light, dissolved oxygen may be photoactivated to singlet oxygen, which can initiate the reaction chain at the second stage shown in Figure 7.

The extent of autoxidation of a fat is often measured as the Peroxide Value (PV), which is the amount of chemically reactive peroxide in the sample (AOCS Method Cd 8-53).

Four main factors contribute to oxidative rancidity:

1. Chain initiation by trace free radicals
2. Chain propagation by molecular oxygen

3. Hydroperoxide cleavage catalyzed by metal ions
4. Chain initiation by photoactivated oxygen

Proper refining and deodorization removes peroxides which are the source of trace free radicals. Oil should be processed, transported, and stored under a nitrogen atmosphere. Metal ions in the oil can be inactivated by chelation with citric acid. Finally, the exposure of oil to light should be minimal. With these precautions oxidative stability can be increased several-fold.

Antioxidants also increase oxidative stability. These bind active free radicals, thus preventing initiation of new reaction chains. If free radicals continue to form, due to the presence of oxygen and trace metals, eventually all the antioxidant will react, and the autocatalytic sequence will develop without hindrance.

Fat oxidative stability is measured by the active oxygen method (AOM, AOCS Method Cd 12-57). Oil or fat is held at 97.8°C while air is bubbled through it. The time required to develop a peroxide concentration of 100 meq/kg is the AOM stability of the sample. A closely related method, the oil stability index (OSI, AOCS Method Cd 12b-92), also bubbles air through hot oil. One of the breakdown products is formic acid, which is trapped in a water cell. The machine continuously monitors conductivity of the water, and records the time when it rises sharply. Rancimat times obtained at 110°C are 40–45% of the AOM times, so an OSI stability of 4 h is equal to an AOM stability of 10 h.

Oxidative stability is important for obvious reasons. The baker does not want the shortening or oil to develop rancidity in storage before use, and wants to avoid rancidity in the finished product as long as possible. The fat or oil as delivered to the bakery should have a peroxide value below 1 meq/kg. If the amounts of oil used are large enough, the baker may consider blanketing his storage tanks with nitrogen to extend storage stability.

## 8.4. Typical Specifications

Complete specifications for any bakery shortening include many factors beyond those discussed above and those listed in Table 8.1. These may include such items as the source material desired, packaging, storage, and numerous other factors related to Good Manufacturing Practices and HACCP guidelines. The items presented in Table 8.1 are related to the direct functionality of shortening in baked goods, as discussed in this Chapter. The conscientious Quality Assurance person writing the actual specification will want to include many more items.

The maximum peroxide value given relates to the potential resistance of the shortening against oxidative rancidity; a higher peroxide value means that the shortening will develop rancidity sooner. The value for AOM speaks directly to this characteristic. The free fatty acid and phosphorous limits given should be met by any supplier doing a good job of refining the initial raw materials (e.g., soybean oil) used to produce the shortening.

No melting points are given, because in general these have little relevance to functionality in baking. For reference, it is noted that different methods are used to



determine m.p., and they relate to the SFI curve roughly as follows: Wiley m.p. corresponds to an SFI of 3; Mettler dropping point m.p. corresponds to an SFI of about 1.5; and the complete m.p. is at an SFI of 0.

Finally, it is emphasized that Table 8.1 gives typical physical specifications for a number of bakery shortenings: adjustments can and should be made to meet requirements for a particular product or equipment line. The values given are meant as guidelines, and a complete ingredient specification should be developed in consultation with shortening suppliers.

## REFERENCES

1. G. L. Hasenhuettl, "Fats and Fatty Oils," in the *Kirk-Othmer Encyclopedia of Chemical Technology*, 5th ed., vol. 12, Wiley, Hoboken, N.J., 2005.
2. W. R. Moore and R. C. Hoseney, *Cereal Chem.* **63**, 172–174 (1986).
3. N. B. Howard, *Bakers Dig.* **46**(5), 28–30, 32, 34, 36–37, **64** (1972).
4. J. C. Wootton, N. B. Howard, J. B. Martin, D. E. McOsker, and J. Holme, *Cereal Chem.* **44**, 333–343 (1967).
5. T. Crosby, "Fats and Oils: Properties, Processing Technology, and Commercial Shortenings," in F. J. Francis, ed., *Wiley Encyclopedia of Food Science and Technology*, 2nd ed., Wiley, New York, 2000.
6. J. L. Vetter, D. Blockcolsky, M. Utt, and H. Bright, *Tech. Bull. Am. Inst. Baking* **6**(10), 1–5 (1984).
7. J. L. Vetter, J. Zeak, and H. Bright, *Tech. Bull. Am. Inst. Baking* **10**(9), 1–6 (1988).
8. J. C. van den Enden, A. J. Haighton, K. van Putte, L. F. Vermaas, and D. Waddington, *Fette Seifen Anstrichm.* **80**, 180–186 (1978).
9. J. C. van den Enden, J. B. Rossell, L. F. Vermaas, and D. Waddington, *J. Am. Oil Chem. Soc.* **59**, 433–439 (1982).

# 8

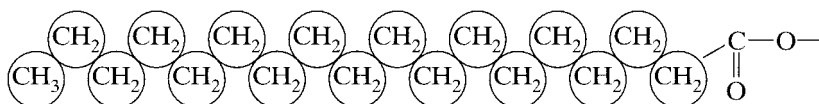
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## *Emulsifiers for the Food Industry*

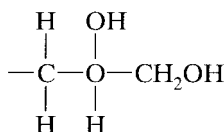
Clyde E. Stauffer

### 1. EMULSIFIERS AS AMPHIPHILES

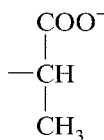
Surfactant is a coined word (from *surface active agent*) that is applied to molecules that migrate to interfaces between two physical phases and thus are more concentrated in the interfacial region than in the bulk solution phase. The key molecular characteristic of a surfactant is that it is amphiphilic in nature, with the lipophilic (or hydrophobic) part of the molecule preferring to be in a lipid (nonpolar) environment and the hydrophilic part preferring to be in an aqueous (polar) environment (Figure 1). By the word *preferring* it is actually meant that the thermodynamic free energy of the system is at a minimum when the lipophilic part is in an oil (or air) phase and the hydrophilic part is in water (1). If a surfactant is dissolved in one phase of an ordinary mixture of oil and water, some portion of the surfactant will concentrate at the oil–water interface, and at equilibrium the free energy of the interface (called interfacial or surface tension,  $\gamma$ ) will be lower than in the absence of the surfactant. Putting mechanical energy into the system (e.g., by mixing) in a way that subdivides one phase will increase the total amount of interfacial area and energy; the lower the amount of interfacial free energy per unit area, the larger the amount of new interfacial area that can be created for a given amount of energy



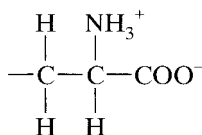
Lipophilic



Nonionic



Anionic



Amphoteric

Hydrophilic

**Figure 1.** Generalized amphiphilic molecular structure. The lipophilic portion is the long-chain fatty acid stearic acid.

input. The subdivided phase is called the discontinuous phase, and the other phase is the continuous phase.

As shown in Figure 1 surfactants have a lipophilic (fat-loving) and a hydrophilic (water-loving) part; for this reason they are sometimes called amphiphilic (both-loving) compounds. The lipophilic part of food surfactants is usually a long-chain fatty acid obtained from a food-grade fat or oil. The hydrophilic portion is either nonionic (e.g. glycerol); anionic (negatively charged, e.g., lactate); or amphoteric, carrying both positive and negative charges (e.g., the amino acid serine). Cationic (positively charged) surfactants are usually bactericidal and somewhat toxic, and they are not used as food additives. Examples of surfactants are monoglyceride (nonionic), stearyl lactylate (anionic), and lecithin (amphoteric). The nonionic surfactants are relatively insensitive to pH and salt concentration in the aqueous phase, while the functionality of the ionic types may be markedly influenced by pH and ionic strength.

Numerous books have been written on the subject of emulsions, but three good sources are Adamson (1), Larsson and Friberg (2), and Becher (3). They summarize and discuss all phases of emulsifier technology for the practical working scientist.

**TABLE 1. Food Systems Involving Interfaces (4).**

System	Continuous Phase	Divided Phase
Emulsion	Liquid	Liquid
Foam	Liquid	Gas
Sol (dispersion)	Liquid	Solid
Fog	Gas	Liquid
Aerosol	Gas	Solid

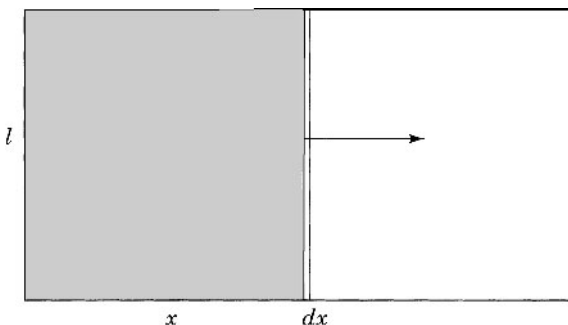
## 2. SURFACES AND INTERFACES IN FOODS

Table 1 lists five systems whose properties are influenced by interfacial tension. Usually emulsions are of the oil-in-water (o/w) type, where the continuous phase is polar (an aqueous solution of salts, sugars, proteins, etc.) and the discontinuous or divided phase is nonpolar (lipid, i.e., fat or oil). The suspension of whey in melted fat, used in margarine manufacturing, is a water-in-oil (w/o) emulsion. In foams, the discontinuous phase is usually air; the continuous phase may be either aqueous or lipid. The interface between the continuous aqueous phase of a dough and insoluble components (starch granules, gluten proteins) typifies a sol or dispersion. Fogs are usually found in processing steps (e.g., a spray application of an ingredient such as oil); aerosols are formed during spray drying of food ingredients. In these two instances, particle sizes are usually larger than sizes generally associated with the terms fog and aerosol.

## 3. SURFACE ACTIVITY

### 3.1. Energetics

*Surface Tension, Surface Free Energy.* Consider a soap bubble film contained in a rectangular wire frame (Figure 2). One side of this frame, of length  $l$ , is move-



**Figure 2.** Sketch of a wire frame with one movable side, containing an interior film.

able. The work necessary to move this side to the right by a distance  $dx$  is given by:

$$\text{work} = \gamma l dx = \gamma dA \quad (1)$$

where  $\gamma$  is the free energy of the water–air interface. The total surface free energy equals  $\gamma l x$ . The usual units for  $\gamma$  is milli-Newtons per meter (mN/m), which is numerically equal to the older (non-SI) units of dynes per centimeter or ergs per centimeter squared. The concept is general; the surface could be the interface between two condensed phases, e.g., water and oil, in which case the term is interfacial tension (free energy).

If the surface is curved, the radius of curvature plays a role. Given an air bubble of radius  $r$  the total surface energy is  $4\pi r^2\gamma$ . Decreasing the radius by the amount  $dr$ , decreases total surface energy by  $8\pi r\gamma dr = 4\pi r^2\gamma dr$ . This change must be balanced by a pressure increase,  $\Delta P$ , otherwise the bubble would be compressed to nothingness. This pressure difference times the change in surface area equals the change in total surface energy:

$$\Delta P 4\pi r^2 dr = 8\pi r\gamma dr \quad (2)$$

and

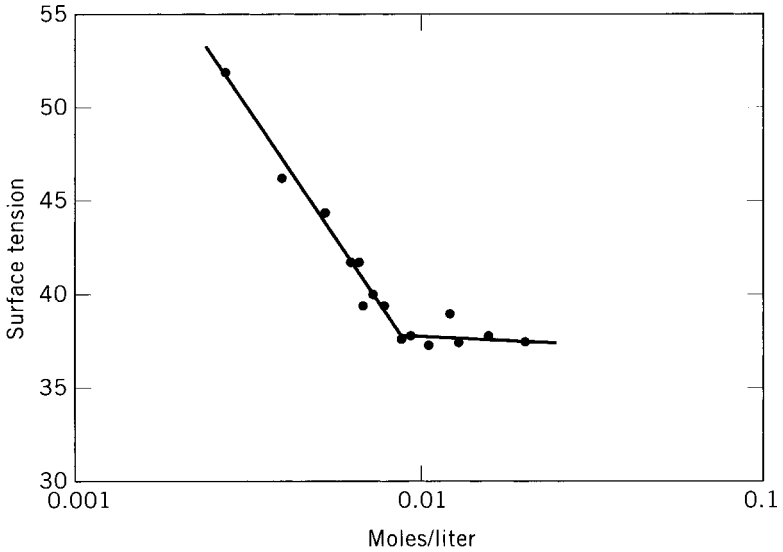
$$\Delta P = 2\gamma/r \quad (3)$$

Equation 3 indicates that the internal pressure of a small bubble is greater than that of a large bubble. This has practical consequences in aerated food systems. In a cake batter, for example, time-lapse photography shows that small bubbles (containing carbon dioxide) disappear and large bubbles increase in size (5). The carbon dioxide in the small bubbles dissolves into the aqueous phase because of a higher pressure, then enters the larger bubbles, which represent a region of lower internal pressure. Similar effects are expected in other foods where the continuous phase may act as a conduit for dissolved gases.

The surface tension of a solution of a surfactant is lower than that of the pure solvent. Surface tension is roughly a linear function of  $\ln(\text{surfactant concentration})$  up to the critical micelle concentration (CMC) (Figure 3). Above the CMC the thermodynamic activity of the surfactant does not increase with the addition of more surfactant, and the surface tension remains constant. Interfacial tension also decreases with the concentration of an emulsifier dissolved in one of the phases. In Figure 4 the decrease in  $\gamma$  does not level off, because the emulsifier (PGMS) does not form micelles in the organic solvent phase (heptane). The changes in the slope of the plot are attributed to changes in orientation of emulsifier molecules at the interface (7).

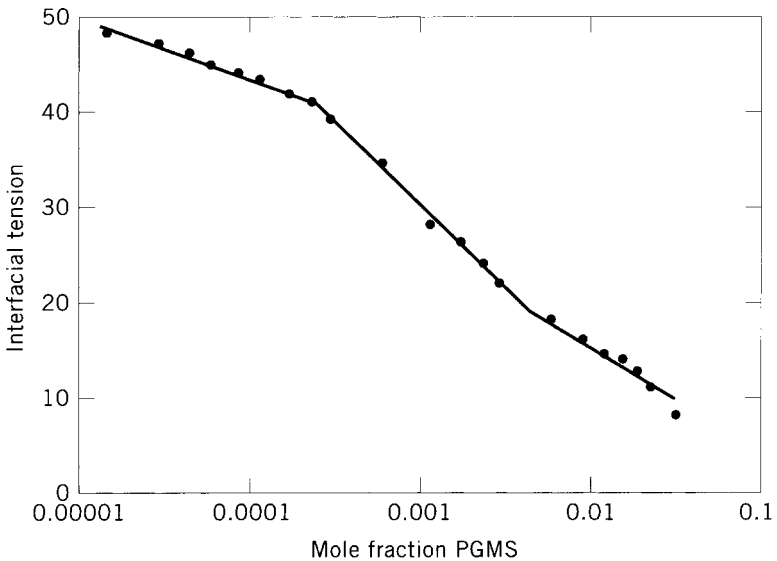
### 3.2. Concentration at the Interface

*Surface Excess of Emulsifier.* Surfactant molecules concentrate at the interface, with the lipophilic portion being in the nonpolar phase (air, organic solvent) and

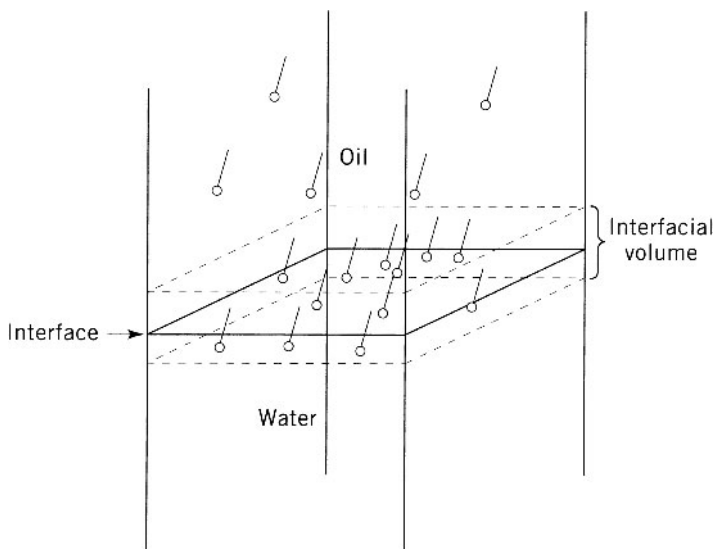


**Figure 3.** Surface tension of laurylsulfonic acid solutions (6).

the hydrophilic portion being in the polar (water) phase. This migration of some surfactant lowers the free energy of the total system. The result is a higher concentration of surfactant in the region that includes the interface (Figure 5). The difference between this concentration and the bulk concentration is called the surface excess,  $\Gamma$ .



**Figure 4.** Interfacial tension at the interface of water and solutions of propylene glycol monostearate in heptane (7).



**Figure 5.** Demonstration of the excess concentration of a surfactant at a water-oil interface.

The surface excess is calculated from a plot of  $\gamma$  versus the thermodynamic activity,  $a$ , of the surfactant:

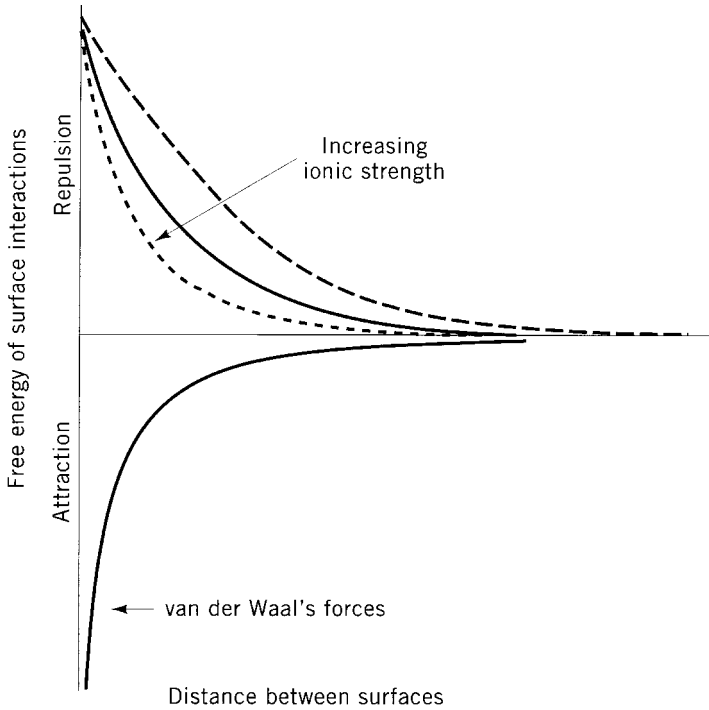
$$\Gamma = -(a/RT)(d\gamma/da) \quad (4)$$

For dilute solutions, surfactant activity equals concentration, and  $\Gamma$  is calculated from the plot of  $\gamma$  versus molar concentration.

When surfactant molecules concentrate at the interface, some solvent molecules are displaced, so the surface solvent concentration is lower than the bulk solvent concentration. The Gibbs convention defines the dividing line between the two phases so that the (negative) surface excess of solvent equals zero. Then equation 4 gives the surface excess of (say) laurylsulfonic acid at the air-water interface. When the actual interfacial concentration of surfactant is needed, the situation is more complicated. Methods for handling these complications have been discussed (1,7).

### 3.3. Interactions between Surfaces

When two surfaces approach each other, two forces exist: one repulsive and one attractive. Whether or not the surfaces touch and coalesce depends on the relative sizes of the two forces. This is equally true for liquids (e.g., oil droplets in an emulsion), solids (e.g., finely divided  $\text{CaCO}_3$ ), and films (air bubbles in a foam). The description of these interactions is stated in the DLVO theory (8).



**Figure 6.** Dependence of electrostatic potential ( $\psi$ ) and van der Waal's attraction as a function of distance between two surfaces.

*Electrical Double Layer.* Electrical repulsion exists when the surfaces carry net charges of the same sign and the continuous phase is water. For example, if an o/w emulsion is stabilized by an anionic surfactant, the oil droplets have a negative charge on their surface. Electrical repulsion then tends to keep the droplets from making contact. At the oil surface, the electrical potential is  $\psi_0$ , and the potential decreases as the square of the distance from the surface, because cations are attracted into the region, partially neutralizing the surface negative charge. This change is shown in Figure 6.

The rate of decrease of  $\psi$  is directly related to the ionic strength of aqueous phase. Ionic strength,  $\mu$ , is related to the concentration of individual salt ions and the square of the ionic charge ( $z$ ) of each ion:

$$\mu = \frac{1}{2} \sum c_i z_i^2 \quad (5)$$

Divalent ions are four times as effective as monovalent ions in decreasing  $\psi$ . Thus 0.25 M zinc sulfate, for example, is as effective as 1 M sodium chloride in promoting emulsion flocculation or coalescence. If gravity (creaming) is the only force bringing the droplets together, they will approach to a distance where repulsion



due to  $\psi$  is just balanced by gravitational effects, and the emulsion will then be stable.

*Attractive Forces.* Attractive forces, collectively called van der Waal's forces, exist between two oil droplets. Simplistically, these forces may be thought of as the attraction between oil molecules at the o/w interfaces that have lower energy when in contact with each other than when in contact with water. Several phenomena are involved: hydrophobic interactions and London dispersion forces are most commonly considered. These are effective as (roughly) the fourth power of the distance between the surfaces and are unaffected by ionic strength. The attraction due to van der Waal's forces is shown in Figure 6. Suspensions of solids (cellulose fibers, finely divided  $\text{CaCO}_3$ , etc.) are stabilized in the same way. Ionic surfactants are used that selectively adsorb to the solid surface, generating a  $\psi$  potential and making possible a stable suspension.

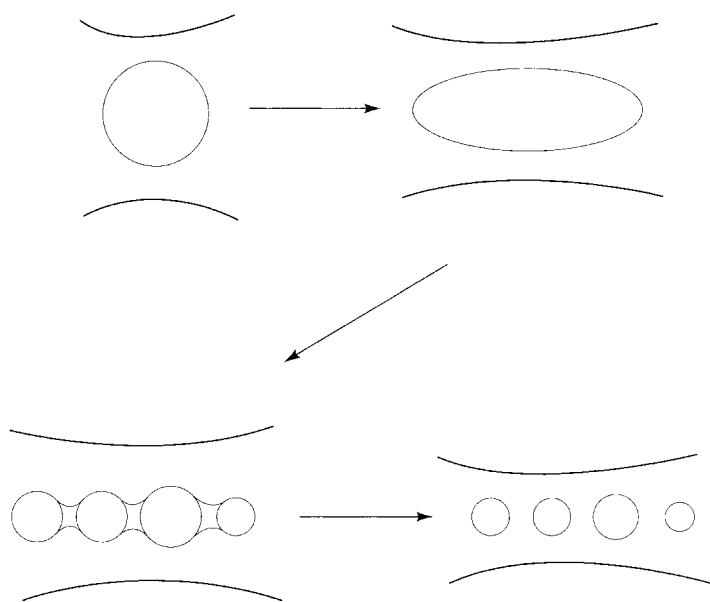
Drainage of foams is governed by similar principles. In a soap foam, for example, carboxylate molecules are concentrated at the air-water interface, and the surface has a net negative charge. Water drains from between two air bubbles until the ionic repulsion between the two surfaces equals the gravitational force on the water; at that thickness, the film becomes stable. The presence of salt in the water phase decreases the thickness of the final drained film, until the ionic strength is high enough to allow the surfaces to touch, when the foam collapses. Stabilization of protein foams (e.g., meringues) is due to a somewhat different mechanism (discussed below).

## 4. EMULSIONS

### 4.1. Formation

*Division of Internal Phase.* Simply adding oil to water does not result in emulsion formation. Input of mechanical energy subdivides the droplets of internal phase until they reach the final average droplet diameter, in the range 1–100  $\mu\text{m}$ . A cylinder of liquid whose length is more than 1.5 times its circumference is unstable and tends to break up into droplets. Mechanical stirring of an oil-water mixture forms drops of liquid that are then distorted into cylinders (along the lines of flow) and that break up into smaller droplets (Figure 7). The process is repeated until the droplets are so small they cannot be further distorted, and further subdivision ceases.

A suspended liquid drop forms a sphere, because this shape has minimum surface area (hence minimum interfacial free energy) for a given volume; area is related to the cube of droplet radius. Distortion is a flow shear effect, depending on droplet cross-section, related to the square of the radius. At large diameters, shear forces are greater than interfacial tension forces, droplets are distorted into cylinders, and subdivision occurs. Droplet radius decreases, until the interfacial tension forces balance (or exceed) shear forces, and further division stops. In emulsification experiments in which the amount of mixing energy is constant and  $\gamma$



**Figure 7.** Stages in subdivision of a liquid drop subjected to shear.

is changed by adding emulsifier, it is found that the average oil droplet diameter parallels  $\gamma$ , i.e., as more emulsifier is added,  $\gamma$  decreases and so does average droplet size. If  $\gamma$  is unchanged but mixing energy is increased, droplet size is also decreased. This is due to the change in the balance of shear and interfacial forces, allowing cylindrical distortion of smaller droplets. Equipment design that enhances shear is more effective at dividing droplets.

*Oil–Water Versus Water–Oil Emulsions.* If oil and water are vigorously shaken, they form a dispersion of water droplets in oil and oil droplets in water. When shaking is stopped the phases start to separate; small water drops fall toward the interface, and oil drops rise. The “emulsion” quickly breaks. Adding an emulsifier to the system changes the outcome; after standing, one phase becomes continuous, while the other remains dispersed. The nature of the emulsion is determined by the emulsifier. As a general rule, the continuous phase is the one in which the emulsifier is soluble. Thus sodium stearate promotes an oil-in-water (o/w) emulsion, while zinc distearate promotes a water-in-oil (w/o) emulsion. Several qualitative theories have been advanced to explain this empirical rule.

The oriented wedge theory states that the emulsifier at the interface is wedge shaped. The ionized end of a sodium soap has a wider (effective) radius than the hydrocarbon chain, hence the oil–water interface should be curved with the convex side oriented toward the water phase. This favors oil droplet formation, hence gives an o/w emulsion. The polar end of zinc distearate, on the other hand, is smaller than the two hydrocarbon chains, the interface is convex toward the water phase, and a w/o emulsion is formed.

A second theory considers the relative ease with which the two types of droplets can coalesce. Upon shaking, drops of both phases are formed. Sodium stearate ionizes, and the electrical potential hinders approach and coalescence of oil droplets; water droplets, on the other hand, experience no such hindrance and readily touch and coalesce. Zinc distearate, being un-ionized, does not interfere with the mutual approach of oil droplets, whereas van der Waal's forces favor subsequent coalescence. Thus the type of emulsion formed depends on the relative kinetics of oil-oil and water-water coalescence.

*HLB System.* The concept of hydrophilic-lipophilic balance (HLB) as a number for characterizing surfactants is an extension of the general rule of thumb stated above (4,9,10). The HLB scale as originally proposed ranged from 0 to 20, with the low end signifying an emulsifier that is much more soluble in oil than in water, and the high end meaning the reverse. A listing of HLB values for some food surfactants is given in Table 2. HLB is recommended as a guide for selecting emulsifier systems to give high emulsion stability to manufactured foods such as salad dressings, whipped toppings, and similar oil-water mixtures that must retain their emulsified character during a shelf life of up to 1 year.

The basic idea is that the HLB of a blend of two emulsifiers is equal to their algebraic sum, i.e., the weight fraction of *A* times its HLB plus the weight fraction of *B* times its HLB value. Boyd and co-workers (11) used a test system comprising equal volumes of water and Nujol (food grade mineral oil) and made blends of various Span (sorbitan fatty acid esters) and Tween (polyoxyethylene<sub>20</sub> sorbitan fatty acid esters) surfactants to cover the HLB ranges 8.5-16.5 (for o/w emulsions) and 2.0-6.5 (for w/o emulsions). After the emulsions were made under controlled conditions, the rate of oil globule coalescence was used to measure emulsion stability. It was found that o/w emulsions were most stable at an HLB of about 12 and w/o emulsions were most stable when HLB was about 3.5. In a practical system that includes other ingredients such as sugar, salt, protein, and other typical food components, the value for this optimum might shift, and a series of tests are necessary to

**TABLE 2. Hydrophilic-Lipophilic Balance.**

Surfactant	HLB
Sodium stearyl lactylate	21.0
Polysorbate 80 PE(20) sorbitan monooleate	15.4
Polysorbate 60 PE(20) sorbitan monostearate	14.4
Sucrose monostearate	12.0
Polysorbate 65 PE(20) sorbitan tristearate	10.5
Diacetyl tartaric ester of monoglyceride	9.2
Sucrose distearate	8.9
Triglycerol monostearate	7.2
Sorbitan monostearate	5.9
Succinylated monoglyceride	5.3
Glycerol monostearate	3.7
Propylene glycol monostearate	1.8

determine the exact blend of surfactants that gives the best results. As a rule of thumb, w/o emulsions are favored by HLBs in the 3–6 range, o/w emulsions are stabilized with HLBs in the 11–15 range, and intermediate HLBs give good wetting properties but not good emulsion stability.

*Microemulsions.* It is possible to make emulsions in which the diameter of the oil droplets is in the range 1.5–150 nm. The droplet size is less than the wavelength of visible light, and the emulsion appears transparent because no light scattering occurs. Several different strategies for making microemulsions have been tried (12), but a simple example using mineral oil and water demonstrates the principles involved. With pure liquids,  $\gamma$  is 41 mN/m, but the inclusion of 0.001 M oleic acid in the water phase reduces  $\gamma$  to 31 mN/m; a reasonably stable emulsion may be formed. Neutralization of the acid with 0.001 M NaOH lowers  $\gamma$  to 7.2 mN/m and gives a stable emulsion. Making the water phase 0.001 M in NaCl further lowers  $\gamma$  to less than 0.01 mN/m. This system will spontaneously form an emulsion; Brownian movement provides sufficient shear forces to elongate droplets into cylinders and cause further subdivision.

Spontaneous emulsions such as this one are frequently opalescent, because some particles have a diameter approaching the wavelength of light: 400 nm. Transparent microemulsions generally require a surfactant plus a cosurfactant, for example, acetyl monoglyceride plus hexanol. For use in food, various polyglycerol esters have shown some promise for making w/o microemulsions (13). The technology is promising but needs further refinement before it is readily applied to food systems.

## 4.2. Flocculation

*Creaming and Adhesion.* Oil droplets in an o/w emulsion float to the top, because the density of vegetable oil is about 0.91 g/mL, 0.08 g/mL less than that of water. The rate at which they rise depends on particle diameter. A drop having a 1  $\mu\text{m}$  diameter rises at a rate of 4 cm/day, while one with a 10  $\mu\text{m}$  diameter rises 4 m/day. Obviously, reducing the average droplet size reduces the rate of creaming. Fat globules in raw milk have an average diameter of 3  $\mu\text{m}$ ; after homogenization the average diameter is 0.5  $\mu\text{m}$ . In raw milk, the average flotation rate is 36 cm/day, while in homogenized milk it is 1 cm/day. Creaming brings the oil droplets closer together, and if contact is not prevented (say, by ionic repulsion) coalescence occurs. A simple creamed layer of oil drops is readily redispersed by inverting the container a few times.

In some circumstances the oil drops actually adhere to each other and are not readily redispersed. This occurs when the emulsifier is polymeric (e.g., protein, gum, or polyoxyethylene derivative). By one mechanism, different segments of a polymer molecule adsorb to the surfaces of two drops, thus forming a bridge that holds them together. Another mechanism obtains when polar parts of two polymer molecules (adsorbed to separate drops) approach each other and intertwine. This “tangle,” say of long polyoxyethylene chains, then holds the drops in proximity,

although they are not in actual contact. The creamed layer is said to be flocculated, although it is also stable against coalescence.

### 4.3. Stabilization and Coalescence

*Ionic Repulsion.* As discussed above, two surfaces carrying an identical net charge repel each other. The thickness of the electrical double layer (the region where  $\psi > 0$ ) is affected by ionic strength. As long as ionic strength is low, electrical repulsion is greater than van der Waal's attraction, and the droplets remain suspended. If, by addition of salt (particularly divalent or trivalent salts) the  $\psi$  potential is markedly suppressed, the surfaces can approach so closely that van der Waal's forces override repulsion, and the droplets can touch and coalesce. At some intermediate ionic strength, the two forces are approximately equal. There is actually a small free energy minimum, and the droplets will remain separated by about one droplet diameter (Figure 8). The practical conclusion to be drawn from this is as follows: if the emulsifier used is ionic in nature, the salt concentration of the aqueous phase markedly affects emulsion stability. Low salt concentration enhances stability, while high salt concentration increases flocculation and/or coalescence.

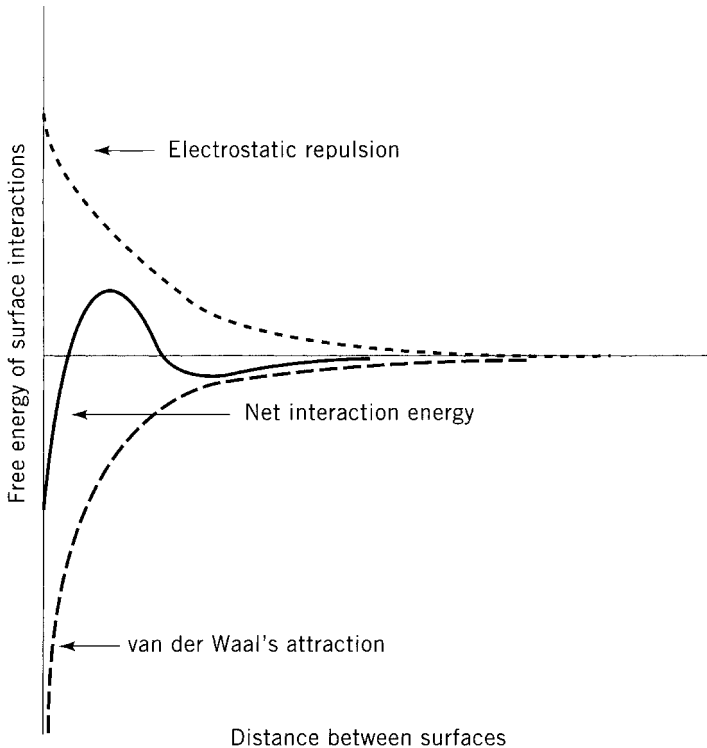
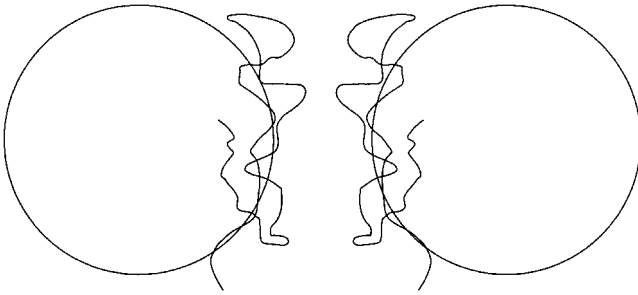


Figure 8. Net free energy of interaction between two droplets.



**Figure 9.** Hydrophilic polymeric surfactant at the interface in an oil-in-water emulsion.

*Steric Hindrance.* Another form of stabilization is relatively independent of ionic strength: the oil droplets are prevented from making contact by simple steric hindrance. This may take two forms, either an immobilized water layer at the interface or a solid interfacial film. Emulsion stabilization by proteins, gums, and polyoxyethylene derivatives occurs by the first mechanism. Hydrophobic parts of the stabilizers adsorb at the oil surface, but adjacent large hydrophilic segments are hydrated and form an immobilized layer on the order of 10–100 nm thick (Figure 9). As mentioned, these hydrated segments frequently interact to cause flocculation, while coalescence of the oil drops themselves is prevented. Such emulsions are frequently used as carriers for oil-soluble flavors, essences, and colorants.

The  $\alpha$ -tending emulsifiers such as propylene glycol monostearate are oil soluble. The emulsifier adsorbs at the oil–water interface, but under certain conditions (low temperature, presence of a free fatty acid) the emulsifier forms a solid interfacial film (Figure 10). While the oil droplets may make contact, the film prevents



**Figure 10.** The interfacial film formed by an  $\alpha$ -tending emulsifier. A water drop was suspended in vegetable oil containing 10% propylene glycol monoester; after a few minutes some of the water was withdrawn. Reprinted by permission from Ref. 14.

coalescence. The interfacial layer actually appears to be crystalline, with a well-defined melting point (15).

## 5. FOAMS

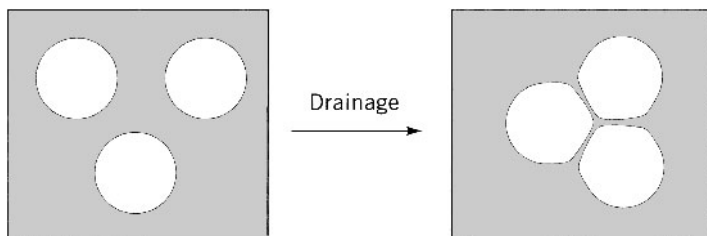
### 5.1. Formation, Film Drainage

For the purposes of this Chapter, air is a nonpolar medium. Surfactants concentrate at the air–water interface, with the hydrophobic portion extending into the gas phase. When the gas phase is finely divided, a foam is formed. In regard to the energetics involved, a foam is nearly identical to an o/w emulsion. The terminology is somewhat different, but the results are the same: a foam is stable or else the gas bubbles coalesce and the foam breaks. Rather than referring to “creaming,” a foam is said to “drain”; the effect is the same, with the water phase concentrating at the bottom and the dispersed phase concentrating at the top of the container. The volume fraction ( $\phi$ ) of gas in a foam is usually much higher than the  $\phi$  of oil in an emulsion. Whipping egg white, for example, may easily give a 10- to 15-fold expansion ( $\phi = 0.9\text{--}0.93$ ), while mayonnaise ( $\phi = 0.7\text{--}0.8$ ) is the food emulsion with the highest oil content.

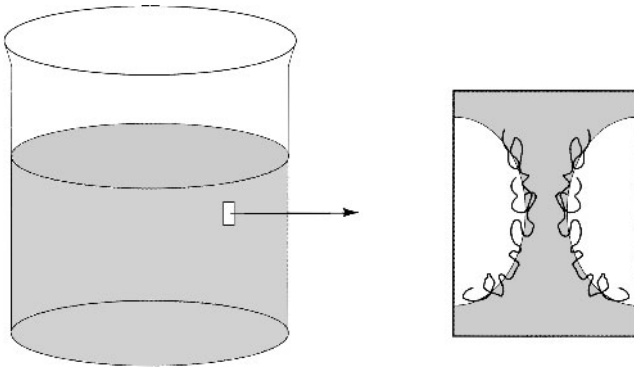
The mechanism of air incorporation and subdivision in a foam is the same as for an emulsion; large bubbles are elongated, and the unstable cylinders spontaneously divide. In a wet foam ( $\phi < 0.7$ ), the initial drainage of liquid from the regions between the bubbles is due to gravity and is governed primarily by viscosity. Figure 11 shows how the film between three bubbles meets at a  $120^\circ$  angle, after drainage. In three dimensions, four touching bubbles meet at the tetrahedral angle of  $109^\circ 28'$ . In the real situation, the bubbles assume the shape of regular polyhedra, but the contact angles remain fairly close to the ideal value. The thicker liquid in the corners, known as the plateau border, has a lower pressure relative to the straight contact films, and liquid moves into these regions. Drainage of a dry foam ( $\phi > 0.7$ ) is probably via these borders, connected throughout the foam.

### 5.2. Stabilization

Foam stability is governed by similar factors as emulsion stability. Thus in a soap foam the negative charges located at the air–water interface lead to repulsion as the



**Figure 11.** Drainage of liquid from between bubbles, forming the plateau border.



**Figure 12.** A protein foam, showing unfolded protein molecules at the air–water interface.

two surfaces of the film approach each other, and drainage stops when an equilibrium film thickness is reached. This thickness is influenced by the ionic strength of the aqueous phase; increasing the ionic strength gives foams of lower stability. Protein stabilizes foams by a combination of steric hindrance and surface viscosity. When egg whites are whipped, the protein molecules unfold, with the hydrophobic side chains entering the air phase and the hydrophilic chains remaining in the water phase. In Figure 12 the heavy lines in the magnified section represent unfolded albumin proteins adsorbed at the air–water interface. The portion of the proteins located in the aqueous phase hold water, preventing it from draining away from this region and hence preventing the air bubbles from coalescing and destabilizing the foam.

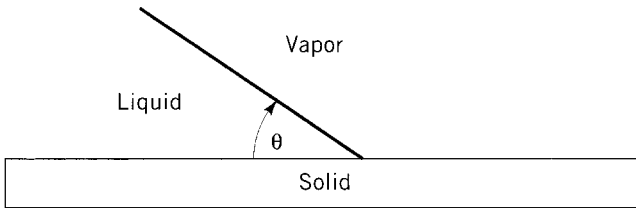
Film breakage is thought to be due to random fluctuations (e.g., Brownian movement) that momentarily bring the two surfaces into contact, allowing the air bubbles to merge. These fluctuations are minimized when the surface viscosity is increased. The addition of an alcohol to a soap solution (e.g., dodecanol added to sodium laurate) increases surface viscosity and also increases foam stability. Surface viscosity of some (but not all) protein solutions is quite high, and there is some correlation between this property and the ability of the protein to give a stable foam. Bulk viscosity does not correlate with stability of drained films, but if a wet foam is desired, increasing bulk viscosity (say, by adding a high viscosity gum) extends the usable life of the foam.

## 6. WETTING

### 6.1. Liquid Spreading on Solid Surface

As mentioned above, surfactants with HLBs in the 8–10 range are not good emulsion stabilizers but are good wetting agents. This property is useful in many circumstances: enhancing dispersion of dry mixes in liquid, improving spreadability of chocolate and cocoa-based coatings, and incorporation of dietary fiber materials





**Figure 13.** The contact angle between liquid, solid, and air phases.

in dressings. Qualitatively, a drop of water is placed on the solid surface. If the contact angle  $\theta > 90^\circ$  (Figure 13), the water does not spread; it is said that the solid is not wetted. If  $\theta < 90^\circ$ , the water spreads, and the solid is wetted.

The angle  $\theta$  is determined by the surface tension at the three interfaces involved:

$$\cos \theta = (\gamma_{SV} - \gamma_{SL})/\gamma_{LV} \quad (6)$$

The spreading coefficient is defined as:

$$S_{L/S} = \gamma_{SV} - \gamma_{LV} - \gamma_{SL} \quad (7)$$

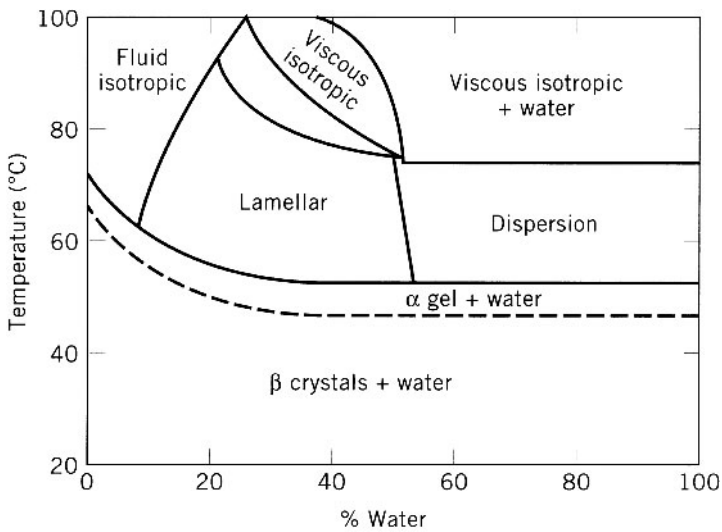
When  $S_{L/S} > 0$ , wetting occurs, and the liquid spreads. An efficient wetting agent is one that minimizes the surface tension of the air–water and solid–water interfaces, while leaving the air–solid surface tension unchanged. This is the situation, for example, when dry beverage powder is added to water. Sodium lauryl sulfate lowers the air–water and solid–water interfacial tensions and enhances dispersibility. In the absence of the surfactant, the contact angle at many of the (irregular) solid surfaces is  $> 90^\circ$ , and the powder with its entrapped air floats on the top of the water.

In chocolate coating, the liquid phase is an oil (cocoa butter). The addition of lecithin aids the wetting of solid cocoa particles by this oil, most probably by lowering  $\gamma_{SL}$ . This lowers the viscosity of the heterogenous mass as well as giving a smoother mouthfeel to the final product.

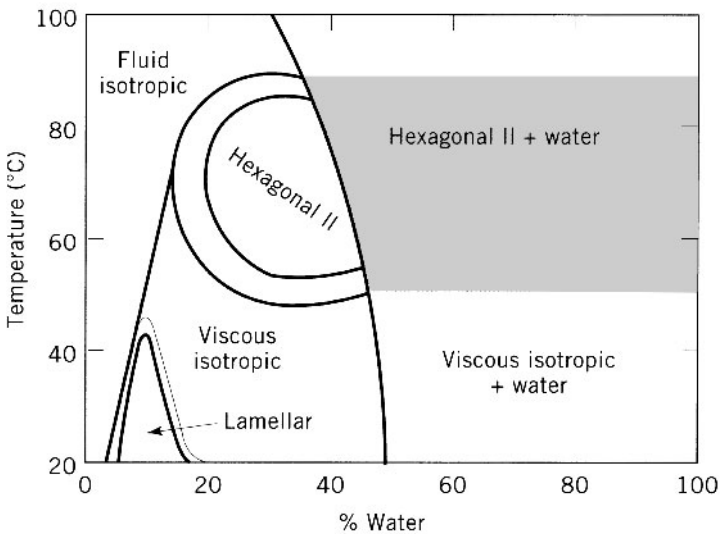
## 7. PHYSICAL STATE OF EMULSIFIER PLUS WATER

### 7.1. Phase Diagrams

Mixtures of surfactant and water form a number of different physical structures, depending on the surfactant: water ratio and the temperature. These mixtures are frequently opalescent dispersions, called liquid crystals, but are more properly termed mesophases. In publications on the subject these are usually shown as phase diagrams with temperature as the y-axis and percent water on the x-axis; the interior of the graph is divided into regions that represent the various



(a)



(b)

**Figure 14.** Phase diagrams of monoglycerides in water. (a) Refers to a distilled monoglyceride made with hydrogenated lard; (b) shows distilled monoglyceride made from sunflower oil (16).

mesophases (Figure 14) (16). Phase diagrams provide guidance to researchers who are trying to produce surfactant systems that are functional under use conditions. They are used to characterize detergent systems and industrial applications of surfactants as well as for food emulsifiers, which are the present concern.

### 7.2. Mesophase Structures

The main mesophase structures of interest in the baking industry are depicted in Figure 15 (16,17). A monoglyceride such as GMS crystallizes in bilayers, with the thickness of each bilayer being defined by the length of two monoglyceride molecules end to end. When heated in water, the crystals melt (the fatty acid chains gain thermal mobility and lose their ordered structure) and water begins to intrude between the bilayers along the plane defined by the glycerol head groups. Under the proper conditions of temperature and water content, this intrusion results in the formation of the *lamellar* mesophase. The thickness of the water layer may be from

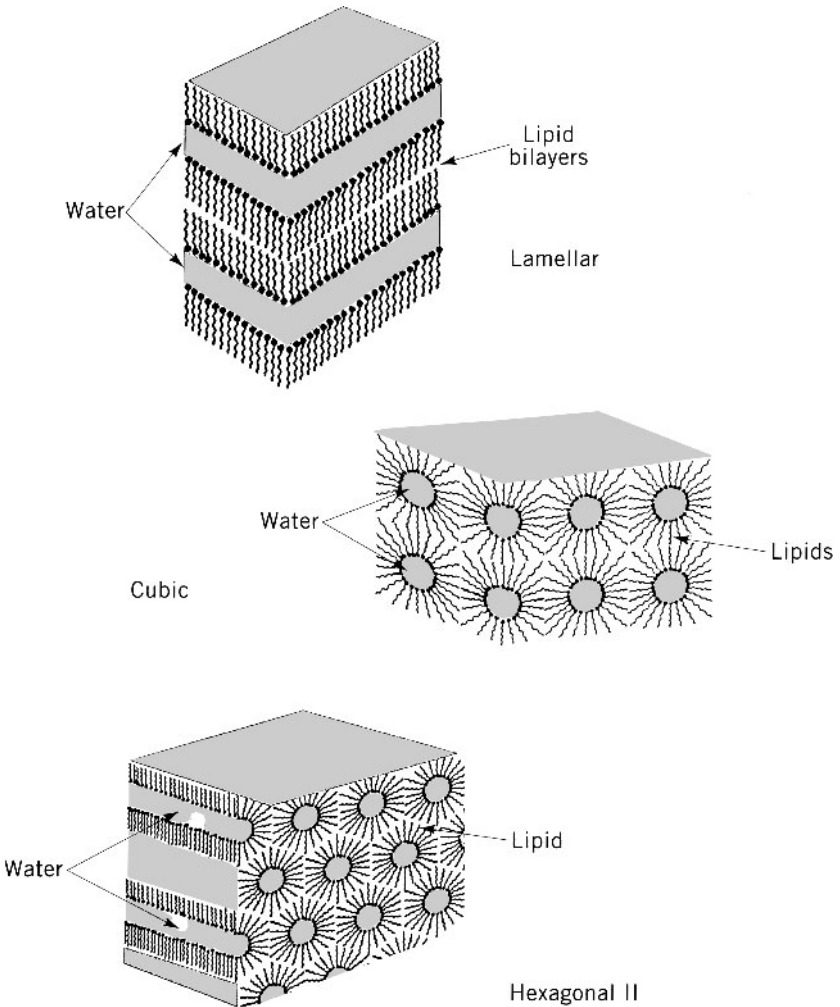


Figure 15. Three mesophasic crystalline structures found in studies of surfactants (16).

60 nm to 1.75  $\mu\text{m}$ , depending on various conditions (17), while the distance between the water layers due to the lipid bilayer remains relatively constant at around 400 nm. This material is rather fluid, but when the mixture is cooled, the lipid layers solidify in the  $\alpha$ -crystalline state, and the material becomes a gel with a lipid bilayer about 550 nm thick; the water layer also may increase in thickness, and some of the water may become free. This phase is of particular interest to bakers, because there is evidence that the lamellar mesophase is the most efficient in promoting the interaction between monoglyceride and starch, producing the antistaling effects (18). If this gel is cooled even further and allowed to come to equilibrium, all the water is expelled from the gel and the  $\alpha$ -crystalline layers of monoglyceride transform into the more stable  $\beta$ -crystalline form, yielding a suspension of  $\beta$  crystals in water. At higher water levels and within a limited temperature range, the lamellar mesophase is transformed into spherical multilamellar vesicles (liposomes), which is sometimes called a lamellar dispersion.

At higher temperatures and water concentrations, the system may shift into the cubic mesophase structure (see Figure 15). The water is present as spheres totally surrounded by monoglyceride. This phase has a high viscosity and is sometimes called viscous isotropic in the literature; the two terms refer to the same structure. In the presence of more water than can be accommodated in the internal spherical phase, one obtains a mixture of lumps of this cubic structure dispersed in excess water. With a saturated monoglyceride such as GMS, the lamellar structure is the main mesophase found under practical conditions, while with unsaturated monoglycerides this cubic phase is the predominant one at lower temperatures. At lower water concentrations, the spherical water "micelles" are farther apart, so the viscosity of the mixture becomes lower, approaching that of melted pure surfactant. This is the fluid isotropic mesophase, sometimes referred to as the L2 phase.

The third important mesophase structure is formed by unsaturated monoglycerides and also by most of the other surfactants of interest to bakers. The hexagonal structure (see Figure 15) consists of rods of the internal phase arranged hexagonally within a matrix of the external phase. In the case of monoglycerides and SSL, the internal phase is water, forming the hexagonal II structure as shown in Figure 15. With highly water-soluble surfactants of the polyoxyethylene type (polysorbates, ethoxylated monoglyceride), the internal phase is the lipophilic tail of the material and the external phase is water, giving the hexagonal I mesophase (16).

The lamellar mesophase of monoglyceride is stabilized if an anionic surfactant, such as SSL or a neutralized free fatty acid (soap), is part of the lipid fraction. The lipid-water interface takes on a negative charge, and electrostatic repulsion prevents the collapse and expulsion of water at the lower temperatures described above. As expected, this electrostatic stabilization can be counteracted by adding salts, and low concentrations (0.3%) in the aqueous phase will counteract the favorable effects of the anionic surfactant. Anionic monoglyceride derivatives, the succinate and diacetyltartrate esters, form lamellar mesophases under most conditions. This penchant is enhanced if the carboxyl group is partially neutralized so that the pH of a water dispersion of the surfactant is in the typical pH range for dough of 4–6.

Finally, the inclusion of a nonpolar triglyceride (oil) in the system tremendously complicates the phase diagrams. As an example, GMS forms a lamellar mesophase with water, as was noted above, but when soybean oil is added, the system converts into the hexagonal II structure (19). The ternary phase diagrams from combinations of flour lipids, water, and salt have been studied (20,21). The structures listed above were all found in some region of the phase diagram. The studies are interesting, but as of this writing the direct relevance to the practical application of surfactants in dough systems is not clear.

## 8. EMULSIFIERS FOR FOOD APPLICATIONS

The actual food emulsifier of commerce is seldom exactly like the organic chemical structures that are discussed in this section but is a mixture of similar compounds derived from natural raw materials. As a simple comparison, sodium chloride is one single, relatively pure chemical entity, accurately described by the formula NaCl. Glycerol monostearate, on the other hand, is made from a hydrogenated natural fat or oil, and the saturated fatty acid composition may well be something like 1% C<sub>12</sub>, 2% C<sub>14</sub>, 30% C<sub>16</sub>, 65% C<sub>18</sub>, and 2% C<sub>20</sub>, reflecting the chain length distribution in the source fat. In addition, the monoglyceride will be approximately 92% 1-monoglyceride and 8% 2-monoglyceride, which is in chemical equilibrium. Keep in mind, then, that the chemical structures shown here represent the major components in the commercial material and that related molecular species will also be present.

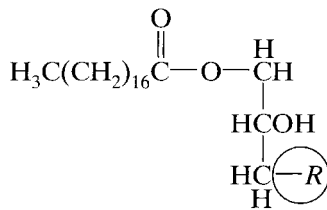
### 8.1. Monoglycerides and Derivatives

The manufacture and application of monoglycerides and derivatives have been reviewed by several authors (22–24). Roughly 18 million kg (40 million lb) of monoglyceride are used annually in the United States in yeast-raised goods for retarding staling (24). At least an equal amount finds its way into cakes, icings, and other applications. The third major use of monoglycerides is in the manufacture of margarine. Overall, this group of surfactants is the single most important one for food uses, representing about 75% of total emulsifier production.

The use of monoglycerides in baking first began in the 1930s, when “super-glycerinated shortening” became commercially available. Glycerin was added to ordinary shortening along with a small amount of alkaline catalyst, the mixture was heated, causing some interesterification of triglyceride with the glycerin, and the catalyst was removed by neutralization and washing with water. The resulting emulsified shortening contained about 3% monoglyceride and was widely used for making cakes, particularly ones containing sugar at high levels. The effectiveness of monoglyceride in retarding staling (crumb firming) in bread became known at about the same time, and bread bakers sought a more concentrated source of monoglyceride. This need was met by suppliers of plastic monoglyceride, which is made by altering the ratio of glycerin to fat to achieve a final concentration of 50–60% monoglyceride, with most of the remainder being diglyceride. When

industrial-scale molecular distillation processes became available it was logical to subject the plastic monoglyceride to this step, producing distilled monoglyceride containing a minimum of 90% monoglyceride, the rest of the mixture being diglyceride and small amounts of fatty acids, glycerol, and triglyceride. The next stage was to make a lamellar mesophase product from this distilled monoglyceride, adding some anionic surfactant (usually SSL) to stabilize the hydrated monoglyceride, which contains roughly 25% monoglyceride, 3% SSL, and 72% water. More recently, manufacturers have developed a powdered distilled monoglyceride in which the composition of the original feedstock fat is balanced between saturated and unsaturated fatty acids, so that the resulting powder is hydrated fairly rapidly during the process of dough mixing and is functional in complexing with gelatinized starch. Today, bakers use all three monoglyceride types (plastic, hydrated, and powdered distilled) with about equally good results.

The monoglyceride structure shown in Figure 16 is for 1-monostearin, also called  $\alpha$ -monostearin. If the fatty acid is esterified at the middle hydroxyl the compound is 2-monostearin, or  $\beta$ -monostearin. Both isomers are equally effective



<i>R</i>	Emulsifier
—OH	Glycerol monostearate (GMS)
—OOCH <sub>2</sub> CH <sub>2</sub> COO <sup>-</sup>	Succinyl monoglyceride (SMG)
$\begin{array}{c} \text{OH} \\   \\ \text{—OOCHCH}_3 \end{array}$	Lactylated monoglyceride (LacGM)
—OOCCH <sub>3</sub>	Acetylated monoglyceride (AcMG)
$\begin{array}{c} \text{OOCCH}_3 \\   \\ \text{—OOCCHCHCOO}^- \\   \\ \text{OOCCH}_3 \end{array}$	Diacetyl-tartrate ester of monoglyceride (DATEM)
—O(CH <sub>2</sub> CH <sub>2</sub> O) <sub><i>n</i></sub> H	Polyoxyethylene MG (EMG)
H	Propylene glycol monoester (PGME)

Figure 16. Structure of monoglyceride and several related compounds.

at retarding bread staling (24). In technical specifications, manufacturers usually give the monoglyceride content of their product as percent of  $\alpha$ -monoglyceride. The routine analytical method for monoglyceride (AACC method 58–45) detects only the 1-isomer; quantitation of the 2-isomer is much more tedious. The total monoglyceride content of a product is about 10% higher than the reported  $\alpha$ -monoglyceride content. In a practical sense, however, when various products are being compared for functionality and cost effectiveness, the  $\alpha$ -monoglyceride content is a useful number, since for all products this equals about 92% of the total monoglyceride present.

The fatty acid composition of monoglyceride reflects the makeup of the triglyceride fat from which it is made. Commercial GMS may contain as little as 65% stearate if it is made from fully hydrogenated lard, or as much as 87% stearate if it is made from fully hydrogenated soybean oil. The other major saturated fatty acid will be palmitic, and because complete hydrogenation (to iodine value of 0) is not practical, a few percent of unsaturated (oleic and/or elaidic) acid are also usually present. A typical commercial GMS will have an iodine value of about 5. Iodine values for powdered distilled monoglycerides are in the range of 19–36, and for plastic monoglycerides, a typical range is 65–75. The unsaturated fatty acids are a mixture of oleic, linoleic, and the *trans* isomers of these acids. The phase diagram of a highly unsaturated monoglyceride is quite different from that of a saturated one (see Figure 14), but the higher melting point of the hydrogenated monoglyceride makes its phase behavior much more like that of GMS than that of the sunflower monoglyceride. To the extent that mesophase behavior governs monoglyceride functionality, the plastic monoglycerides are quite adequate.

The derivatives of monoglyceride shown in Figure 16 are of two types: (1) dough strengtheners (SMG, EMG, and DATEM) and (2)  $\alpha$ -tending emulsifiers (LacGM, AcMG, and PGME). Details of the functionality of these two groups in baking are discussed below.

The succinate and diacetyltartrate half esters are fairly discrete compounds, produced by reacting the respective acid anhydride with monoglyceride in the presence of alkaline catalyst. The product of the manufacture of ethoxylated monoglyceride is somewhat more random in structure. Monoglyceride is treated with ethylene oxide gas under pressure in the presence of alkaline catalyst and at elevated temperatures. Ethylene oxide is polymerized via a series of ether linkages and also forms ether bonds with the free hydroxyl groups on monoglyceride. The average chain length comprises about 20 units ( $n = 20$  in Figure 16). Chains may be attached to hydroxyls at both the number 2 ( $\beta$ ) and number 3 ( $\alpha$ ) positions of the monoglyceride, although many more chains will be located at the  $\alpha$  position than at the  $\beta$  spot because of the difference in their chemical reactivities. The exact distribution of polymer chain lengths and distribution between  $\alpha$  and  $\beta$  positions are functions of reaction conditions, e.g., catalyst type and concentration, gas pressure, temperature, agitation, and length of reaction time.

The second group of monoglyceride derivatives, the  $\alpha$ -tending emulsifiers, find their main use in cake production. These emulsifiers are dissolved in the shortening phase of the cake formulation, and they contribute to the emulsification

of the shortening in the water phase as well as promoting incorporation of air into the fat phase. The particular property of these emulsifiers that makes them valuable in liquid shortening cakes is that they form a solid film at the oil–water interface, not only stabilizing the emulsion but also keeping the lipid phase from preventing air incorporation (protein-stabilized foam formation) during cake batter mixing.

### 8.2. Sorbitan Emulsifiers

When the sugar alcohol sorbitol is heated with stearic acid in the presence of a catalyst, two reactions occur: sorbitol cyclizes to form the five-membered sorbitan ring and the remaining primary hydroxyl group is esterified by the acid. The resulting sorbitan monostearate (Figure 17) is oil soluble, with a rather low HLB value, and

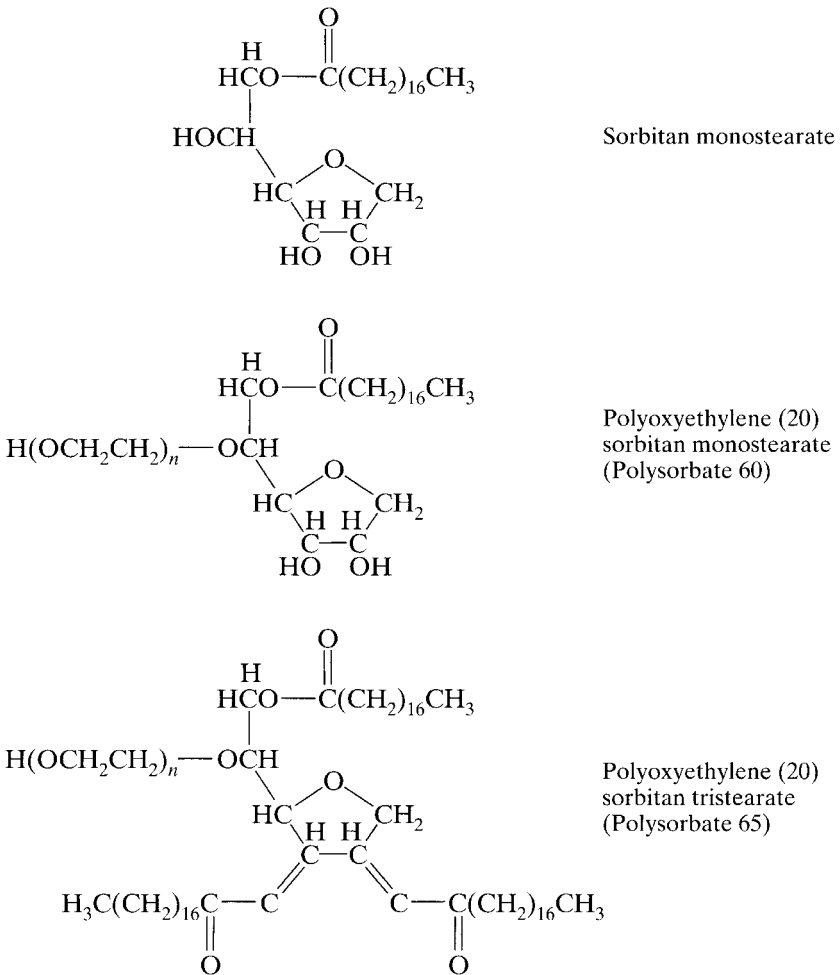


Figure 17. Structures of food-grade sorbitan derivatives.



it is the only one of the many sorbitan esters presently approved for food use in the United States. Other sorbitan esters of importance are the monooleate and the tristearate. Any of the three esters may be reacted with ethylene oxide to give polyoxyethylene derivatives (see Figure 17). The monostearate derivative is known as polysorbate 60, the tristearate is polysorbate 65, and the monooleate is polysorbate 80. The remarks made in connection with EMG regarding the length and location of the polyoxyethylene chains apply to these compounds. The average number of oxyethylene monomers is 20 ( $n = 20$ ), and in the case of the monoesters, chains may be located on more than one hydroxyl group of the sorbitan ring (with the triester, of course, only one hydroxyl group is available for derivatization).

Sorbitan monostearate finds use as a good emulsifier for making icings that have superior aeration, gloss, and stability characteristics. It is also used as part of the emulsifier system in whipped toppings and in coffee whiteners. The polyoxyethylene derivatives have found more acceptance, with the monostearate polysorbate 60 being the most widely used of the group. At a level of 0.25% (flour basis) the ability of polysorbate 60 to strengthen dough against mechanical shock is greater than that of SMG and about equal to those of EMG and SSL. Polysorbate 60 has also been used in fluid oil cake shortening systems, generally in combination with GMS and PGMS.

### 8.3. Anionic Emulsifiers

In addition to SMG and DATEM, some other anionic surfactants that have been tried as dough strengtheners are shown in Figure 18. Presently SSL is the one most widely used in the United States; sodium stearyl fumarate did not find acceptance, and sodium lauryl sulfate is used mainly as a whipping agent with egg whites.

Lactic acid, having both a carboxylic acid and a hydroxyl function on the same molecule, readily forms an ester with itself. In commercial concentrated solutions

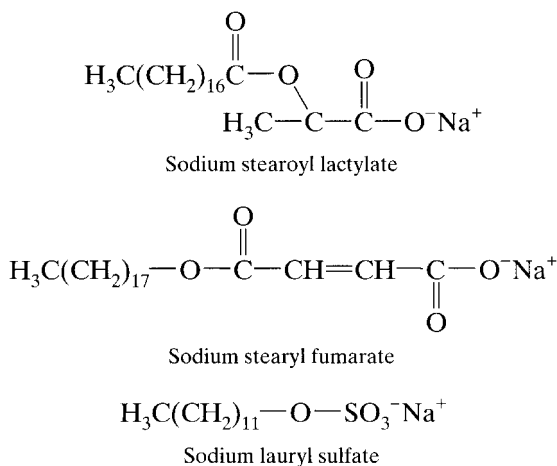


Figure 18. Three food-grade anionic surfactants.

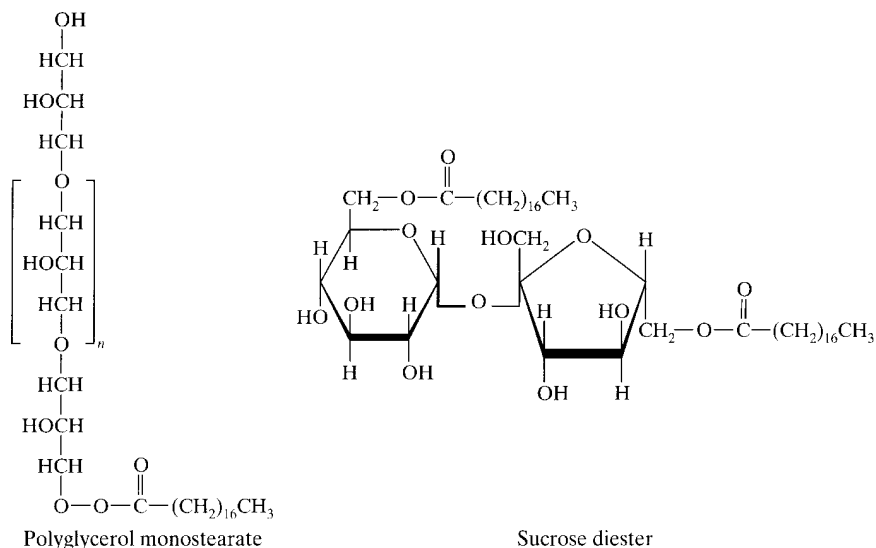
almost all the acid is present in this polylactylic form, and to get free lactic acid it must be diluted with water and refluxed for a period of time. When stearic acid is heated with polylactic acid under the proper reaction conditions and then neutralized with sodium hydroxide, a product having the structure shown in Figure 18 is obtained. The monomer lactic acid shown represents the predominant product; the dilactylic dimer is also present as well as lactic trimers and tetramers (25). As with all compounds based on commercial stearic acid derived from hydrogenated fats, some percentage of the fatty acid is palmitic, with small portions of myristic and arachidic acids also being present. Sodium stearyl lactylate is readily water soluble, but the calcium salt is practically insoluble. In this respect, it mimics a soap e.g., sodium stearate is water soluble but calcium stearate is oil soluble. Either form may be used, depending on the details of the intended application, but as a dough strengthener, the sodium salt is more commonly used. As a stabilizer for hydrated monoglyceride the sodium form is used, because ionization in the water layer is necessary.

Stearyl fumarate is a half ester of fumaric acid with stearyl alcohol (octadecanol). Although stearyl fumarate might be expected to have dough-strengthening properties similar to those of SSL, this was not found to be so in practice, and the product was not a commercial success. Stearyl fumarate is still approved by the FDA for use in bread.

The third structure shown in Figure 18 is sodium dodecyl sulfate (SDS), often used by research workers for solubilizing proteins. It is a sulfate ester of the  $C_{12}$  alcohol dodecanol. Commercially, this alcohol is produced by reduction of coconut oil, and the resultant mixture is called lauryl alcohol (from lauric acid, the predominant fatty acid in coconut oil). The alcohol portion of sodium lauryl sulfate is a mixture of chain lengths, the approximate composition being 8%  $C_8$ , 7%  $C_{10}$ , 48%  $C_{12}$ , 20%  $C_{14}$ , 10%  $C_{16}$ , and small amounts of longer chains. In bakeries the most common use of sodium lauryl sulfate is as a whipping aid. The compound is added to liquid egg whites at a maximum concentration of 0.0125%, or to egg white solids at a level of 0.1%. It promotes the unfolding of egg albumin at the air-water interface and the stabilization of the foam.

#### 8.4. Polyhydric Emulsifiers

*Polyglycerol Esters.* Polyglycerol esters (Figure 19) have a variety of applications as emulsifiers in the food industry. The polyglycerol portion is synthesized by heating glycerol in the presence of an alkaline catalyst; ether linkages are formed between the primary hydroxyls of glycerol. In Figure 19  $n$  may take any value, but for food emulsifiers the most common ones are  $n = 1$  (triglycerol),  $n = 4$  (hexaglycerol),  $n = 6$  (octaglycerol), and  $n = 8$  (decaglycerol), remembering that in all cases  $n$  is an average value for the molecules present in the commercial preparation. The polyglycerol backbone is then esterified to varying extents, either by direct reaction with a fatty acid or by interesterification with a triglyceride fat. Again, the number of acid groups esterified to a polyglycerol molecule varies around some central value, so an octaglycerol octaoleate really should be understood as an



**Figure 19.** Surfactants based on polyglycerol and sucrose.

(approximately octa)-glycerol (approximately octa)-oleate ester. By good control of feedstocks and reaction conditions, manufacturers manage to keep the properties of their various products relatively constant from batch to batch.

The HLB balance of these esters depends on the length of the polyglycerol chain (the number of hydrophilic hydroxyl groups present) and the degree of esterification. For example, decaglycerol monostearate has an HLB of 14.5, while triglycerol tristearate has an HLB of 3.6. Intermediate species have intermediate HLB values, and any desired value may be obtained by appropriate blending, as described earlier. The wide range of possible compositions and HLBs makes these materials versatile emulsifiers for food applications.

**Sucrose Esters.** Sucrose has eight free hydroxyl groups, which are potential sites for esterification to fatty acids. Compounds containing six or more fatty acids per sucrose molecule have been proposed for use as noncaloric fat substitutes under the name Olestra; this material acts like a triglyceride fat and has no surfactant properties. Compounds containing one to three fatty acid esters, on the other hand, do act as emulsifiers and are approved for food use in that capacity. They are manufactured by the following steps:

1. An emulsion is made of fatty acid methyl ester in a concentrated aqueous sucrose solution.
2. The water is removed under vacuum at elevated temperature.
3. Alkaline catalyst is added, and the temperature of the dispersion is raised slowly to 150°C under vacuum, distilling off methanol formed on transesterification.
4. The reaction mixture is cooled and purified.

**TABLE 3. Sucrose Ester-Surfactants (26).**

Manufacturer's Designation	Percent Monoester	Percent Diester	Percent Triester	Percent Tetraester	HLB
F-160	71	24	5	0	15
F-140	61	30	8	1	13
F-110	50	36	12	2	11
F-90	46	39	13	2	9.5
F-70	42	42	14	2	8
F-50	33	49	16	2	6

The degree of esterification is controlled by the reaction conditions, especially the sucrose: methyl ester ratio, and the final product is a mixture of esters (Table 3). The HLB value of a particular product is smaller (more lipophilic) as the degree of esterification increases, as would be expected.

### 8.5. Lecithin

Lecithin is a by-product of the processing of crude soybean oil; it is the “gum” that is removed during the degumming step of oil refining. The crude gum is treated and purified to give the various commercial lecithin products that are available to the food processor today (27,28). Oil-free lecithin is a sticky plastic material, but blending with half its weight of soy oil sharply reduces the viscosity to give the product known as fluid lecithin. The crude material is dark, almost black (mainly because of the high temperatures during processing), so it is bleached to give a more acceptable light brown color. Treatment with up to 1.5% hydrogen peroxide gives the product known as single-bleached lecithin, and further addition of up to 0.5% benzoyl peroxide yields double-bleached lecithin (27). Reaction with hydrogen peroxide at even higher levels, plus lactic acid, hydroxylates unsaturated fatty acid side chains at the double bond (yielding, e.g., dihydroxystearic acid from oleic acid). Hydroxylated lecithin is formed, which is more dispersible in cold water than the other types and is more effective as an emulsifier for o/w emulsions.

Figure 20 shows the structure of the main surface-active components of lecithin. The phosphatidyl group is a phosphate ester of diglyceride. The fatty acid composition of the diglyceride is similar to that of the basic oil so a number of different fatty acids are found, not just the stearic and oleic acids depicted. Little phosphatidylserine is present in soybean lecithin, and the other three derivatives are found in approximately equal amounts. Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) are amphoteric surfactants, while phosphatidylinositol (PI) is anionic. The HLB values of the three species are varied, with PC having a high HLB, PE an intermediate HLB, and PI having a low value. The HLB of the natural blend is in the range of 9–10, and emulsifier mixtures having values in this range will tend to form either o/w or w/o emulsions, although neither type is highly stable. On the other, hand intermediate HLB emulsifiers are excellent wetting agents, and this is a major application for lecithin.

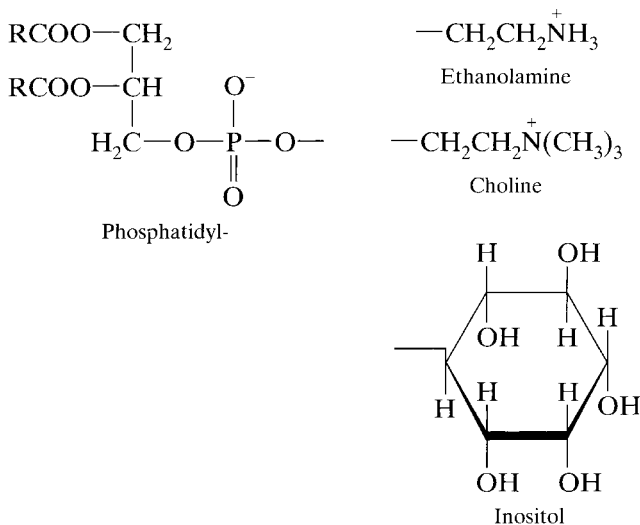


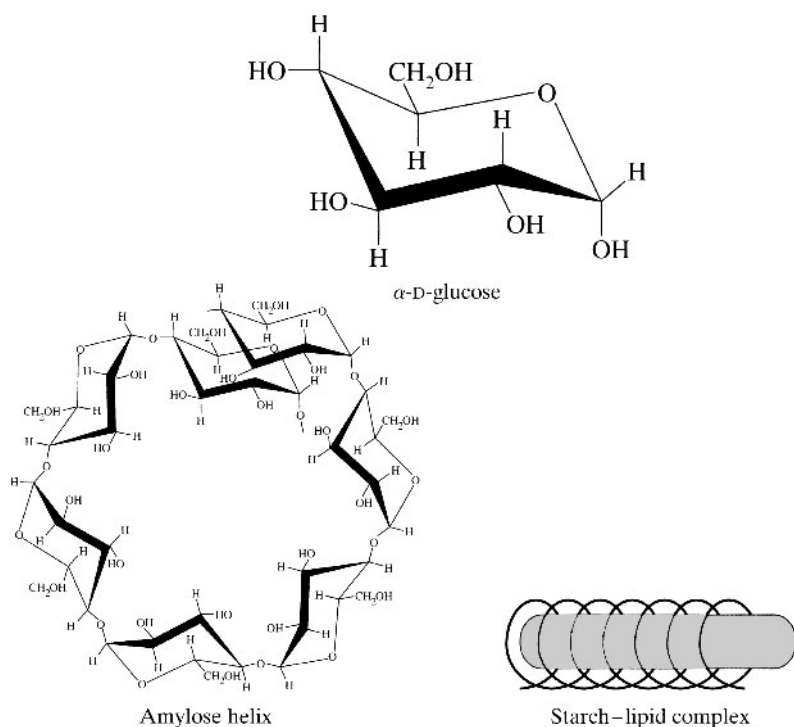
Figure 20. Lecithin structure and components.

The emulsifying properties of lecithin can be improved by ethanol fractionation (29). Phosphatidylcholine is soluble in ethanol, PI is rather insoluble, and PE is partially soluble. Adding lecithin to ethanol gives a soluble and an insoluble fraction. The phosphatide compositions of the two are (1) ethanol soluble, 60% PC, 30% PE, and 2% PI; and (2) ethanol insoluble, 4% PC, 29% PE, and 55% PI (30). The soluble fraction is effective in promoting and stabilizing o/w emulsions, while the insoluble portion promotes and stabilizes w/o emulsions. At the present time several European companies are using this process to produce industrial food-grade emulsifiers.

## 9. INTERACTIONS WITH OTHER FOOD COMPONENTS

### 9.1. Starch

Starch molecules are polymers composed of  $\alpha$ -D-glucopyranosidyl residues joined primarily by 1,4 acetal linkages (1,6 linkages occur at the branch points in amylopectin). Glucopyranoside is a six-membered ring, not flat, but puckered in what is called the “chair” configuration (Figure 21). The bond angles from each carbon are such that the hydrophilic hydroxyl groups project outward to the side of the plane of the ring, while the hydrogen atoms project either above or below this plane; the perimeter of the ring is hydrophilic, while the two faces are hydrophobic. The bond angle of the  $\alpha$ -1,4 acetal linkage is such that the starch chain coils to form a helix, with about six residues per turn (see Figure 21). It is difficult to draw the details of this helix, but from molecular models it is apparent that the plane of the residue ring



**Figure 21.**  $\alpha$ -D-glucose, one turn of the helix formed in linear portions of starch, and the complex between a linear alkane and a starch helix.

lies parallel to the wall of this helix and the hydrogen atoms on carbons 3 and 5 project into the interior of the helix. The result is a hollow cylinder that has a hydrophilic outer surface and a hydrophobic inner surface. This inner space is about 45 nm in diameter, and straight-chain alkyl molecules such as stearic acid will fit into it (see Figure 21), as will other molecules, e.g., iodine. The blue color of an iodine–starch complex is caused by the presence of iodine in this nonpolar environment; iodine dissolved in chloroform is blue, but in water, it is brown. The complex of amylose with *n*-butanol crystallizes much more readily than does amylose alone, and it has been used to separate amylose from amylopectin.

The *n*-alkyl portion of emulsifiers such as glycerol monostearate form a complex with helical regions of starch, a phenomenon that is thought to be responsible for the ability of GMS to retard starch crystallization in bread crumb, slowing the process of staling. Several workers have measured the stoichiometry of complex formation by using various methods. Some of these studies are mentioned later in connection with the discussion on bread staling; here three typical reports are summarized.

Legendijk and Pennings (31) heated amylose or amylopectin with excess monoglyceride, cooled the heated material, and measured the amount of uncomplexed

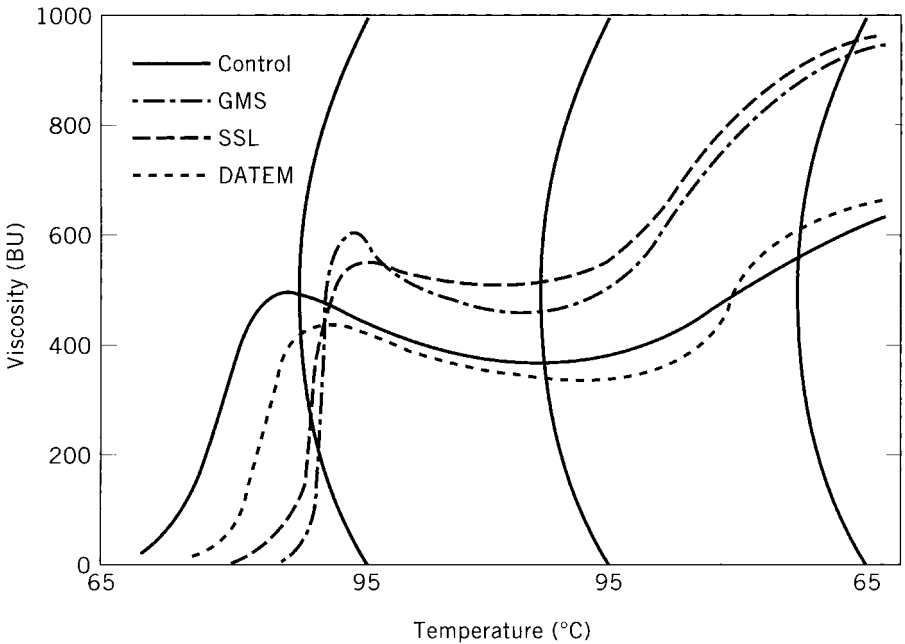
monoglyceride. With amylose, the maximum complexation occurred with monopalmitin, with both longer and shorter saturated fatty acid monoglycerides reacting to a lesser extent. If the average molecular weight of amylose is taken as 150 kDa, then 1 mole of amylose bound 10.6 moles of monomyristin (23.4 mg/g starch), 16 moles of monopalmitin (37.4 mg/g), 14 moles of monostearin (33.9 mg/g), 10.4 moles of monoolein (24.6 mg/g), and 5.1 moles of monolinolein (12.2 mg/g). These results show the effect of unsaturation in the alkyl chain; the *cis* double bond makes the chain bend so that it is more difficult to form a complex with the supposedly straight cavity of the amylose helix. Amylopectin bound increasing amounts of the saturated monoglycerides, the weight ratios being 5 mg monopalmitin, 8.3 mg monostearin, and 11 mg monoarachidin per gram of amylopectin.

Batres and White (32) reacted monoglyceride with amylopectin at 60°C, isolated the precipitate that separated on cooling, and analyzed this complex. They found significantly more interaction than Legendijk and Pennings, obtaining values of 370 mg of monomyristin, 580 mg of monopalmitin, and 250 mg of monostearin per gram of amylopectin. While there were procedural differences between the two studies, these differences would not seem to be enough to account for such large discrepancies. Nevertheless, it is clear that monoglyceride complexes with amylopectin, probably by forming clathrates with the helices in the linear portions of the molecule.

Eliasson and Ljunger (33) reported that the cationic surfactant cetyl trimethylammonium bromide (CTAB) slowed down the rate of formation of amylopectin crystallites in gelatinized waxy maize starch, as measured by differential scanning calorimetry (DSC).

Krog (34) reacted an excess of amylose with various surfactants at 60°C, removed the precipitated complex after cooling, and measured the amount of uncomplexed amylose remaining in the supernatant. Taking as a standard ratio 5 mg of emulsifier per 100 mg amylose he calculated the amylose complexing index (ACI), which is a measure of the amount of amylose precipitated by 5 mg of the surfactant. Monomyristin had an ACI of 100, with monopalmitin and monostearin giving values of 92 and 87, respectively. Unsaturated fatty acid monoglycerides gave results of about 30, several other stearate esters (of propylene glycol, sorbitan, or sucrose) yielded ACIs in the range of 10–25, but sodium stearyl lactylate had an ACI of about 75. The low results with the stearate esters were thought to be due to the inability of these materials to form the particular lamellar mesophases that promote the interaction of the alkyl chain with the amylose helix. Several papers from his research group have appeared since 1971, relating the complex formation between emulsifiers and starch to bread making and antistaling properties. The overall picture is that under the right conditions, the straight-chain hydrophobic portion of emulsifiers will complex with helical sections of amylose and amylopectin as shown in Figure 21, and this complexation has advantageous consequences for bakery foods.

Surfactants modify the gelatinization behavior of starch. Figure 22 shows the changes in amylograph gelatinization curves for wheat starch caused by the inclusion of 0.5% of various emulsifiers (35). Of the three emulsifiers shown, DATEM is



**Figure 22.** Amylograph curves obtained with wheat starch and various emulsifiers (35).

the least interactive, raising the swelling temperature by about 5°C but not changing the viscosity of the gelatinized starch. Glycerol monostearate is the most effective, raising swelling temperature by about 18°C and also increasing the paste viscosity. Sodium stearoyl lactylate is less effective in regard to inhibiting swelling, but it increases paste viscosity to about the same extent as does GMS. Monolaurin effectively prevents gelatinization of potato starch when it is heated for one hour at 64°C (36). Sodium stearoyl lactylate and GMS reduce the extent of solubilization of starch molecules on heating in excess water (37). At 85°C, 10% of the starch from the control sample was soluble, but in the presence of 2% SSL or GMS, only 1.7% of the starch dissolved. Measures of swelling of the samples followed the same pattern. The interaction between emulsifier and starch takes place at the surface of the granules, and the starch-surfactant complex apparently serves to stabilize the granule, retarding water penetration and swelling as the temperature is raised.

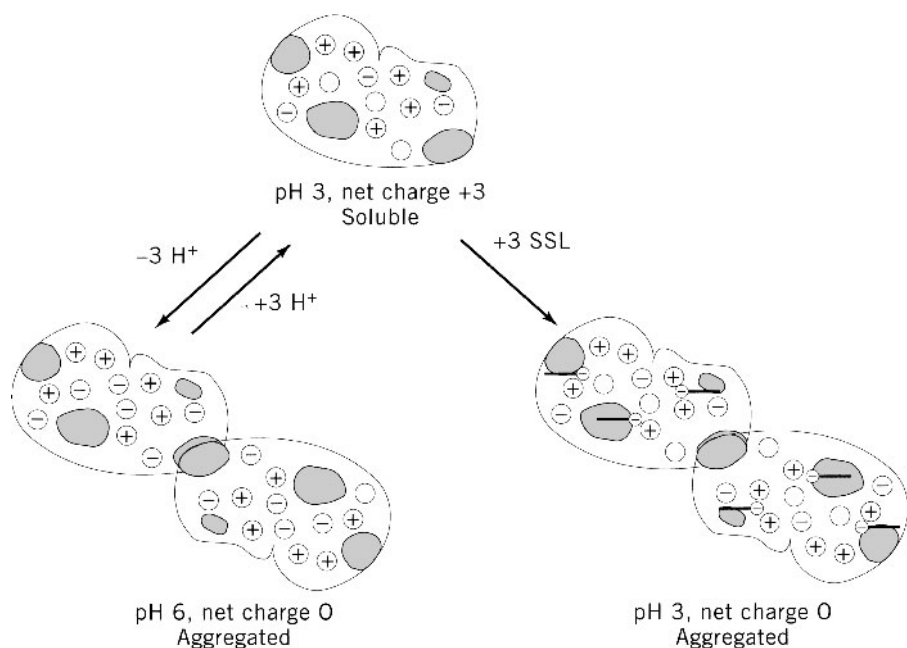
## 9.2. Protein

Some of the amino acid side chains in proteins are hydrophobic, generally buried in the interior of the folded protein molecule but exposed if the protein is unfolded. Sometimes these hydrophobic regions are partially exposed even in the native folded protein, and they are often referred to as hydrophobic patches on the protein surface. The lipophilic parts of surfactants interact with these hydrophobic regions,



sometimes contributing to unfolding (denaturation) of the protein and further binding of surfactant. Much work has been done on the binding of surfactants (sodium dodecyl sulfate in particular) to soluble proteins such as bovine serum albumin (38) and also on interactions of lipids with food proteins (39,40). The adsorption of protein at the oil–water interface is frequently altered by the presence of surfactant and vice versa. As a general rule, surfactant contributes to protein unfolding, enhancing interfacial absorption and emulsion stabilization. Sodium lauryl sulfate promotes unfolding of egg white albumin, thus increasing its ability to form a stable foam. Given the disparate nature of proteins found in various foods, generalizations about the effects to be expected by adding a surfactant to the food system are tenuous at best.

Wheat gluten protein contains about 40% hydrophobic amino acids and interacts with lipid-type materials such as the surfactant SSL. Figure 23 depicts schematically the effect of this interaction on gluten characteristics. The addition of acid to a flour–water dough solubilizes some of the protein. Dough pH (about 6) is roughly the isoelectric point of the gluten proteins. As the pH is lowered many of the anionic carboxylates are protonated, i.e., become unionized, and the protein molecule takes on an overall positive net charge. At pH 6 the hydrophobic patches can interact, and the protein aggregates via hydrophobic interaction (38). At pH 3, the net positive charges cause the molecules to repel each other, and solubilization occurs. This situation has many similarities to that of emulsified oil droplets stabilized by



**Figure 23.** A model for the aggregation of gluten molecules affected by protons or by sodium stearyl lactylate.

an ionic surfactant, where the surface charge prevents droplet contact and coalescence. As in that case, salt represses the electrostatic repulsion, and protein aggregation is favored. Most dough strengtheners are anionic surfactants, and when the lipophilic tail of the surfactant binds to the protein hydrophobic patches, it incorporates this negative charge into the complex, moving the overall charge closer to zero and promoting aggregation in the dough (see Figure 23). Salt and SSL have similar effects on dough mixing properties. Salt suppresses the electrostatic repulsion while SSL neutralizes it; the final effect in both cases is the same, namely hydrophobic aggregation of the gluten protein and an increase in dough strength.

An excess amount of surfactant can solubilize proteins, presumably by additional adsorption to the surface and generation of a large net charge at the surface of the protein molecule. This is the basis for estimation of protein molecular weight by gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE). Such effects are usually found only at surfactant concentrations well in excess of those normally found in foods, and have more use in laboratory investigations than in ordinary applications.

## 10. SOME FOOD APPLICATIONS

The use of emulsifiers in foods is regulated in nearly all countries. Table 4 lists emulsifiers approved in the United States (by the Food and Drug Administration) and in Europe (by the European Economic Community). Several emulsifiers that have FDA sanction are not legally usable in Europe and vice versa. In some cases, specific limits are placed on the amount of emulsifier that a manufacturer may include in a food; in other instances the limitation is “that amount sufficient to produce the intended functional effect.” If there is any question the appropriate regulation (in the United States, chapter 21 of the *Code of Federal Regulations*, 21 CFR) should be consulted. (See also Ref. 41.)

One emulsifier, triethyl citrate, is approved only for use as a whipping agent for egg whites, and this is also the main use for sodium lauryl sulfate. Dioctyl sodium sulfosuccinate is used only as a wetting agent for various dry desert and beverage bases. Some emulsifiers may be classified as good for “general use,” whereas others are emulsion promoters or stabilizers in certain limited applications or have effects such as the “dough conditioners” discussed above.

### 10.1. Baked Goods

*Bread Antistaling.* Monoglycerides added to the dough delay bread staling of baked bread during storage. During baking, starch gelatinizes when the internal temperature reaches a temperature of 60–65°C. The amylose (a linear polymer having a molecular weight of 100–200 kDa) is partially solubilized in the aqueous matrix of the dough. Amylopectin is a highly branched structure with a molecular weight of 10–40 MDa, that exists in the native starch granule as microcrystalline regions connected by amorphous segments of the molecule. During gelatinization, these

TABLE 4. Regulatory Status of Emulsifiers.

Emulsifier	U.S. FDA (21 CFR)	EEC Number
Monoglycerides and diglycerides (GRAS)	182.4505	E 471
Succinyl monoglyceride	172.830	—
Lactylated monoglyceride	172.852	E 472
Acetylated monoglyceride	172.828	E 472
Monoglyceride citrate	172.832	E 472
Monoglyceride phosphate (GRAS)	182.4521	—
Stearyl monoglyceride citrate	172.755	E 472
Diacetyl-tartrate ester of monoglyceride (GRAS)	182.4101	E 472
Polyoxyethylene monoglyceride	172.834	—
Propylene glycol monoester	172.854	E 477
Lactylated propylene glycol monoester	172.850	—
Sorbitan monostearate	172.842	E 491
Polysorbate 60	172.836	E 435
Polysorbate 65	172.838	E 436
Polysorbate 80	172.840	E 433
Calcium stearyl lactylate	172.844	E 482
Sodium stearyl lactylate	172.846	E 481
Stearyl lactic acid	172.848	—
Stearyl tartrate	—	E 483
Sodium stearyl fumarate	172.826	—
Sodium lauryl sulfate	172.822	—
Dioctyl sodium sulfosuccinate	172.810	—
Polyglycerol esters	172.854	E 475
Sucrose esters	172.859	E 473
Sucrose glycerides	—	E 474
Lecithin (GRAS)	184.1400	E 322
Hydroxylated lecithin	172.814	E 322
Triethyl citrate (GRAS)	182.1911	—

microcrystallites “melt” but remain connected. Bread fresh from the oven has an extremely soft crumb. Upon cooling the amylose begins to crystallize (retrograde) and is essentially all crystalline within 24 h. The amylopectin microcrystallites begin to reform, but much more slowly; the half-life for this recrystallization is on the order of 2–3 days at room temperature. The progressive firming of bread crumb during storage is closely connected with this amylopectin retrogradation process (42).

As discussed above, linear alkyl chains such as the fatty acid moiety of monoglycerides can form a complex with helical segments of the solubilized starch molecules. The recrystallization of amylose is apparently little affected by complexation with monoglycerides. Differential scanning calorimetry (DSC) studies of day-old bread crumb show that all the amylose seems to be present in a crystalline state. Complex formation with monoglyceride by the short side chains of amylopectin, however, markedly slow the rate of retrogradation. The half-life for reformation of amylopectin crystals (measured by DSC) is increased twofold to threefold when

1% (relative to flour weight) glycerol monostearate (GMS) is incorporated in the dough. The rate of increase of crumb firming (measured as resistance to compression in an Instron or similar apparatus) is also halved by the addition of 1% GMS.

In bread or roll production monoglyceride is added at a level of 0.5–1% of the flour weight. There is some evidence that formation of the starch–monoglyceride complex occurs best when the monoglyceride is present as the lamellar mesophase. For this reason, many bakers prefer to use hydrated GMS, stabilized in this form. Plastic monoglycerides and diglycerides (roughly half  $\alpha$ -monoglyceride) and hydratable distilled monoglycerides are also used, with about equally good antistaling results. It is probable that these latter types hydrate during dough mixing and processing, forming the lamellar mesophase, which can then react with gelatinizing starch during baking. In any event, about 1%  $\alpha$ -monoglyceride is usually the upper level used, balancing antistaling properties versus cost.

*Dough Strengthening.* As discussed, some anionic and polyoxyethylene surfactants increase the ability of a proofed bread dough to withstand mechanical shocks during transfer from the proof box to the oven. These are termed dough strengtheners or dough conditioners. The ones in common use in commercial bakeries are sodium stearoyl lactylate (SSL) and diacetyl tartrate esters of monoglycerides (DATEM). Less frequently calcium stearoyl lactylate (CSL), ethoxylated monoglyceride, and polysorbate 60 are used. DATEM may be used at any level in accordance with good manufacturing practices; the practical upper level is 1% (flour weight basis). The use of the other dough strengtheners is limited to no more than 0.5% of the flour weight. Sometimes bakery suppliers sell a combination ingredient, containing both a softener (monoglyceride) and strengthener, slightly simplifying weighing out ingredients for each batch of dough.

*Cake.* A plastic shortening intended for making cakes contains 3–4%  $\alpha$ -monoglyceride. This may be produced by superglycerination or by addition of monoglyceride to the melted oil before it is plasticized. The primary function is to aid subdivision of air bubbles in the shortening phase during mixing, giving more nucleation of leavening gases during baking and improved grain and volume in the finished cake. A secondary function is to stabilize the dispersion of sugar in the fat phase, allowing the incorporation of more sugar than flour in the batter (so-called high ratio formulas).

A liquid oil is not well suited to entrapping air bubbles, but it was found that the addition of  $\alpha$ -tending emulsifiers to the oil before it was incorporated into the cake mix allowed the production of one-stage, high ratio cakes with good volume, fine grain, and excellent keeping qualities. The  $\alpha$ -tending emulsifiers solidify in a stable  $\alpha$ -crystalline (waxy) form. The main examples in commercial use today are acetyl monoglycerides (AMG), lactyl monoglycerides (LMG), and propylene glycol monoesters (PGME). A number of other emulsifiers have also been used, including polysorbate 60, stearoyl lactic acid, and sucrose esters, but most commercial cake emulsifiers now offered by suppliers are based on PGME and/or AMG. At concentrations above a certain level, these emulsifiers form a solid film at the oil–water interface (see Figure 10). The addition of a second surfactant may enhance film

formation; a mixture of PGME and stearic acid (80:20) is a stronger film former than pure PGME at the same weight concentration.

Cake mixes made with oil containing an  $\alpha$ -tending emulsifier may be used in a one-stage method, i.e., the dry mix is placed in a bowl, liquid is added, and the batter is mixed at low speed (to blend ingredients) and then at high speed to incorporate air. Air incorporation is primarily as a foam stabilized by protein contributed by flour, milk, and egg whites. The presence of oil inhibits foam stabilization by a protein (e.g., a trace of oil makes it almost impossible to beat egg whites into a meringue), but the solid film at the oil–water interface effectively encapsulates the oil during air incorporation, thus preventing destabilization.

*Icings.* Many icings used in the bakery are aerated w/o emulsions. The simplest example is a butter cream icing, which contains butter, sugar, and egg whites. This icing has a limited stability, losing air rather readily on standing. Improved versions use an emulsified shortening. A typical emulsified plastic shortening for icing manufacture might contain 1–2% monoglyceride and up to 0.6% polysorbate 60. This emulsifier system improves the air-holding properties of the semisolid water-in-fat suspension; the icing retains its eye appeal and good eating qualities for several days.

## 10.2. Dairy-type Emulsions

*Whipped Toppings.* Numerous formulations of whipped toppings exist in which the butterfat of ordinary whipping cream is replaced with a fat having a melting point lower than 38°C, but a rather steep solid fat content (SFC) curve (palm kernel oil and hydrogenated coconut oil are frequently used). The aqueous phase contains milk protein, either sodium caseinate or whey, and sugars. About 5% of an  $\alpha$ -tending emulsifier (PGME, LMG, AMG) is also used. Since the fat phase is around 25% of total weight, the concentration of emulsifier in the lipid phase is up to 17%. This is more than sufficient to form the solid interfacial film mentioned earlier. Thus the soluble milk proteins are able to form a stable foam when the emulsion is whipped.

*Margarine.* This spread, originally formulated as a less-expensive butter substitute, is now made with a variety of partially hydrogenated vegetable oils and often presented as a “healthy” spread, largely because of the presence of some polyunsaturated long-chain fatty acids and the absence of cholesterol. Standardized margarine contains at least 80% fat, and is an w/o emulsion. The fat phase contains 0.1–0.3% monoglyceride, sometimes with the addition of 0.05–0.1% lecithin,  $\beta$ -carotene (for color), and vitamins A and D (if included). The fat is melted, then the aqueous phase (water, flavors, salt, sometimes whey solids) is dispersed in a large agitation tank. The emulsion is then chilled (to solidify the fat) and worked in a scraped-surface heat exchanger. During the working step, the average water drop size is reduced to 2–4  $\mu\text{m}$ . The finished product is solid, and this fat crystal matrix prevents water drop coalescence. The rheological properties (solidity, spreadability) of margarine are governed by the properties of the fat used. With certain fats, the crystals tend to convert from  $\beta'$  crystals (which confer a smooth

texture) to  $\beta$  crystals (giving a sandy texture). This may be counteracted by the inclusion of sorbitan monostearate or monoglyceride citrate in the fat phase. Monoglyceride citrate at a level of 0.1–0.2% also counteracts the tendency of margarine to spatter when it is used as a frying fat in the kitchen.

*Ice Cream and Mellorine.* Ice cream contains no less than 10% milk fat and is basically a mixture of cream, milk, sugar, and flavors that, after emulsification, is frozen (21 *CFR* § 135.110). Emulsification of the milk fat is primarily due to casein proteins present in the milk plus the natural interfacial film present on the milk fat globules. In “economy” ice creams up to 0.1% of emulsifier is sometimes added, primarily to improve stiffness, dryness, and texture in the final product. GMS and polysorbates 65 and 80 are the ones most often used.

Mellorine is similar to ice cream, but contains at least 6% of fat other than milk fat (usually vegetable oil). Milk protein is, again, the major emulsifier, but 0.1% of polysorbates 65 and/or 80 may also be used.

*Coffee Creamer.* Emulsions of vegetable oil in an aqueous phase, including caseinates, are sold (as pasteurized liquid or as a spray-dried powder) as substitutes for cream for addition to coffee. For the liquid form, an emulsifier system of some combination of polysorbate 60, polysorbate 65, and sorbitan monostearate is used at a concentration of up to 0.4%. The spray-dried whitener uses the same emulsifier system, at a level of 1–3% of the dried powder. In both cases, sodium caseinate is probably the major stabilizer of the o/w emulsion.

### 10.3. Salad Dressings

*Mayonnaise and Starch-based Dressings.* Egg yolk is the only emulsifier allowed in the making of mayonnaise. This food is an o/w emulsion, in which the internal phase constitutes a minimum of 70%, and sometimes up to 82%, of the total volume. At this level of  $\phi$ , the emulsion may invert (i.e., become an w/o emulsion) if it is subjected to unfavorable stresses, in particular excess shear in the colloid mill used to subdivide the oil droplets, and if the emulsifier is inadequate for the job. Egg yolk contains about 16% protein (much of it lipoprotein), 35% total lipid, and 10% phospholipids. The protein and phospholipids are both surface active, and readily coat the oil–water interface. The protein–phospholipid combination forms an elastic film that stabilizes the emulsion. Mayonnaise manufacture involves two steps: (1) formation of a rather coarse emulsion of oil in the aqueous phase (egg yolk, vinegar, spices) and (2) reduction of oil droplet size by passage through a colloid mill. If all the internal phase is present as spheres of the same diameter, the maximum theoretical  $\phi$  is 0.74. In mayonnaise, the spheres have a range of diameters, so the small spheres can fit into the spaces between larger drops, allowing  $\phi$  to be greater than this theoretical limit. The amount of emulsifier must be sufficient to coat all the additional interface formed during drop size reduction. If the clearance in the colloid mill is too small (shear is too large), the oil drops become too small, the interface area is greater than can be covered by emulsifier, and the mayonnaise inverts.

The oil used for mayonnaise manufacture must remain liquid even during storage at refrigerator temperatures. If some portion of the oil solidifies, the fat crystals disrupt the surface film and the emulsion breaks. The oil is usually winterized, i.e., it is held at a low temperature for 1 or 2 days and the solid fraction is removed. Polyglycerol esters may also be added to the oil to inhibit fat crystal formation to prevent this problem.

A less expensive alternative (spoonable salad dressing) contains 40–50% oil, and contains gelatinized modified starch as the stabilizing agent. After treatment in a colloid mill, the emulsion is cooled; the starch gels and the semisolid matrix prevents oil droplet coalescence. Up to 0.75% of emulsifiers (polysorbates, mono-glyceride citrate, or DATEM) may be included in the formulation to enhance emulsification of oil during manufacture of the first-stage emulsion.

*Pourable Dressings.* Numerous pourable dressings (e.g., French, Italian, Thousand Island) contain 30–40% oil in an aqueous phase of vinegar, spices, flavorings, and stabilizers. For the most part, the stabilizers are gums of various sorts that increase the viscosity of the aqueous phase and decrease the rate of oil coalescence when the dressing is shaken before being poured on the salad. Polysorbate 60 may be used at up to 0.3% of the total dressing weight to enhance the ease of oil dispersion on shaking.

## REFERENCES

1. A. W. Adamson, *Physical Chemistry of Surfaces*, Wiley-Interscience, New York, 1967.
2. K. Larsson and S. E. Friberg, eds., *Food Emulsions*, Marcel Dekker, Inc., New York, 1990.
3. P. Becher, *Encyclopedia of Emulsion Technology*, Marcel Dekker, Inc., New York, 1985.
4. W. C. Griffin and M. J. Lynch in T. E. Furia, ed., *Handbook of Food Additives*, 2nd ed., CRC Press, Inc., Cleveland, Ohio, 1972.
5. A. R. Handleman, J. F. Conn, and J. W. Lyons, *Cereal Chem.*, **38**, 294–305 (1961).
6. J. W. McBain and L. A. Wood, *Proc. R. Soc. London*, **A174**, 286–293 (1940).
7. C. E. Stauffer, *J. Colloid Interface Sci.*, **27**, 625–633 (1968).
8. E. J. W. Verwey and J. T. G. Overbeek, *Theory of the Stability of Lyophobic Colloids*, Elsevier Science Publishing Co., Inc., New York, 1948.
9. J. D. Dziezak, *Food Technol.*, **42**(10), 172–186 (1988).
10. *Food Additives*, Technical literature, Danisco USA, New Century, Kansas, 2003.
11. J. Boyd, C. Parkinson, and P. Sherman, *J. Colloid Interface Sci.*, **41**, 359–370 (1972).
12. M. El-Nokaly and D. Cornell, eds., *Microemulsions and Emulsions in Foods*, American Chemical Society, Washington, D.C., 1991.
13. M. El-Nokaly, G. Hiler Sr., and J. McGrady in Ref. 11.
14. J. C. Wootton, N. B. Howard, J. B. Martin, D. E. McOsker, and J. Holme, *Cereal Chem.*, **44**, 333–343 (1967).
15. E. S. Lutton, C. E. Stauffer, J. B. Martin, and A. J. Fehl, *J. Colloid Interface Sci.*, **30**, 283–290 (1969).
16. N. Krog, *Cereal Chem.*, **58**, 158–164 (1981).

17. N. Krog and A. P. Borup, *J. Sci. Food Agr.*, **24**, 691–701 (1973).
18. N. Krog and B. Nybo Jensen, *J. Food Technol.*, **5**, 77–87 (1970).
19. N. Krog in R. B. Duckworth, ed., *Water Relations of Foods*, Academic Press, Inc., New York, 1975.
20. T. L.-G. Carlson, K. Larsson, and Y. Mieziš, *Cereal Chem.*, **55**, 168–179 (1978).
21. T. L.-G. Carlson, K. Larsson, K. Mieziš, and S. Poovarodom, *Cereal Chem.*, **56**, 417–419 (1979).
22. H. Birnbaum, *Bakers Dig.*, **55**(6), 6–18 (1981).
23. D. T. Rusch, *Cereal Foods World*, **26**, 111–115 (1981).
24. W. H. Knightly, *Cereal Foods World*, **33**, 405–412 (1988).
25. J. Birk Lauridsen, paper presented at the AOCS Short Course on Physical Chemistry of Fats and Oils, Hawaii, May, 11–14, 1986.
26. C. E. Walker, *Cereal Foods World*, **29**, 286–289 (1984).
27. B. F. Szuhaj, *J. Am. Oil Chem. Soc.*, **60**, 258A–261A (1983).
28. B. F. Szuhaj, *Lecithins: Sources, Manufacture & Uses*, American Oil Chemists' Society, Champaign, Ill., 1989.
29. W. Van Nieuwenhuysen, *J. Am. Oil Chem. Soc.*, **53**, 425–427 (1976).
30. O. L. Brekke in D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soy Oil Processing and Utilization*, American Soybean Association, St. Louis, Mo., 1980.
31. J. Lagendijk and H. J. Pennings, *Cereal Sci. Today*, **15**, 354–356, 365 (1970).
32. L. V. Batres and P. J. White, *J. Am. Oil Chem. Soc.*, **63**, 1537–1540 (1986).
33. A.-L. Eliasson and G. Ljunger, *J. Sci. Food Agric.*, **44**, 353–361 (1988).
34. N. Krog, *Starch/Stärke*, **23**, 206–210 (1971).
35. N. Krog, *Starch/Stärke*, **25**, 22–27 (1973).
36. K. Larsson, *Starch/Stärke*, **32**, 125–133 (1980).
37. K. Ghiasi, R. C. Hosenev, and E. Varriano-Marston, *Cereal Chem.*, **59**, 81–85 (1982).
38. C. Tanford, *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, John Wiley & Sons, Inc., New York, 1973.
39. E. Dickinson in Ref. 12.
40. B. Ericsson in Ref. 2.
41. L. Somogyi, "Food Additives," in *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 12, John Wiley & Sons, Inc., Hoboken, New Jersey, 2005.
42. C. E. Stauffer, *Functional Additives for Bakery Foods*, Van Nostrand Reinhold, New York, 1990.



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## *Frying of Foods and Snack Food Production*

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### **1. INTRODUCTION**

Frying of food is an old and traditional means to prepare food quickly and enjoy the preparation for culinary delight. Fried foods are tasty because of the fried food flavor and the pleasant mouth feel produced by the oil absorbed in the fried food. This increases the salivation process that helps release of oil the soluble flavors from the fried food, making it very appealing to the taster. Fried food is popular in North and South America, Mexico, Europe, India, China, Japan, Malaysia, and elsewhere.

The frying technique has improved over the years and has evolved from kitchen frying to large-scale industrial frying. Coated products, par-fried products with or without seasoning, have become popular across the world. The fried products are packaged and distributed for sale. Innovation of the packaging technology has enabled the fried food industry to distribute the products that maintain freshness over an extended period.

## 2. FRYING PROCESS

This is a process where the food is dehydrated by using the thermal energy from a bed of hot oil. The oil is heated either by electrical or gas heating. Frying process can be divided into three major categories, such as (1) home frying, (2) restaurant or food service frying, and (3) industrial frying.

Home frying is the traditional frying procedure where the food is either deep-fried or skillet fried. Frying in a skillet is done with a shallow bed of oil in the skillet, heated to the frying temperature, which is maintained manually by the cook by adjusting the heat. The food is fried until the food attains the desired color and texture. In the deep frying process, the food is placed in a bed of hot oil. The food to be fried can be sliced potatoes, seasoned mixed vegetables mixed with some binders, batter coated vegetables, fish, and so on. Deep fryers vary in sizes, such as (1) the small countertop fryers, (2) floor-model restaurant or food service fryers, (3) industrial batch or kettle fryers, and (4) continuous industrial fryers. In each type of these fryers, the food is fried to the desired moisture content and the crust is allowed to develop a brown to dark-brown color to achieve proper flavor, texture, and appearance. Home-fried foods are consumed soon after frying, whereas the industrially fried products are packaged and distributed for sale.

Although home frying is on the decline in the United States, it is widespread in many countries where the people are still serving home-cooked meals to their families.

The freshly fried foods are consumed almost immediately by the customers at the restaurants. This practice is carried out even during the rush hours of lunch or dinner. At the fast food restaurants, the food is fried on demand. However, in the food service establishment, fried foods are generally prepared in larger amounts ahead of the rush hour and are kept warm either by a warm oven or by holding them under an ultraviolet lamp.

Restaurants, fast food restaurants, and the food services use a wide range of precooked or par-fried products. This includes products such as (1) French fries and (2) batter-coated and par-fried fish, chicken, meat patties, cheese sticks, vegetables, and so on. These products are taken out of the freezers and placed directly in the bed of hot oil in a fryer without thawing.

## 3. TYPES OF RESTAURANT FRYERS

The principal categories of restaurant fryers are (1) countertop fryers, (2) floor-type fryers, (3) pressure fryers, and (4) turkey fryers.

### 3.1. Countertop Fryers

These fryers are suitable for frying small quantities of food at a time. The fryers may have a single or a dual basket (see Figure 1). In a dual-basket fryer, the oil capacity may vary from 15 to 30 pounds (6.8–9.1 kg) per pan. These fryers have



(a)



(b)

**Figure 1.** Countertop fryers: (a) Gas fired and (b) electrically heated.

a heat capacity that ranges from 25,000 to 45,000 BTUs (6300 to 11,343 CHUs)/hr. These fryers are suitable for restaurants where a small amount of food is fried at a time and less frequently. An electrical heating element, immersed in the oil, or a gas-fired burner under the pan heat the oil up to 400°F (204.4°C). A thermostatic “on-off” control device controls the oil temperature in the fryer. The control device



**Figure 2.** Countertop pressure fryer.

automatically shuts off the heat when the fryer is left idle for several minutes and allows the oil to cool down to 200°F (93.3°C). The oil remains at this temperature until frying is resumed.

Countertop pressure fryers (see Figure 2) are especially suitable for frying batter-coated fish or shrimp in small restaurants and as experimental fryers for research work. These fryers have a single pressure setting and an automatic shut-off device.

### 3.2. Floor-Model Fryers

Restaurants and food services use floor-model fryers (see Figure 3). These fryers are significantly larger than the countertop fryers. The oil holding capacity widely varies in these fryers, which ranges from 40 to 90 pounds (18.2 to 40.9 kg.). The heat capacities of these fryers range from 90,000 to 200,000 BTUs/hr (22,680 to 50,400 CHUs/hr). The fryers have single or dual baskets. Some have split frying pans with individual temperature controls. The oil is heated either by gas or electricity. Most of these fryers are equipped with sufficient thermal capacities to



**Figure 3.** Floor-model restaurant fryer.

**TABLE 1. Oil Temperature Profile During Frying French Fries.**

Steps	Fryer oil temperature
Initial fryer oil temperature	375 +/- 2°F (190.6 +/- 1.1°C)
Fryer oil temperature	Dropped immediately to <330°F (<165.6°C)
Fryer oil temperature after:	
1 min. and 30 sec.	330°F (165.6°C)
2 min. and 45 sec.	345–350 (173.9–176.7°C)
Temperature after recovery	375 +/- 2°F (190.6 +/- 1.1°C)

(Total Fry Time/Batch: 2 min. and 45 sec., Quantity /batch = 3/4 pound)

provide rapid heat recovery to the oil in order to respond to the high demand at the fast food restaurants.

Electrically heated fryers have the heating elements immersed in the oil like in the countertop fryers. The oil temperature in the fryer is thermostatically controlled. The fryer has an on-off power switch that turns on power either to the gas control system or to the electrical heating elements. The control system generally has two operating modes, namely, (1) standby and (2) high heat (or fry). The fryer is kept on the standby mode when the demand is low, such as after the lunch rush or in late evening.

A typical restaurant fryer operates at 335–375°F (168–190.6°C). A measured amount of food is placed in the basket. The basket is lowered into the oil, which is already heated to the desired temperature. The oil temperature in the fryer drops immediately, and then it gradually recovers. The recovery time for the oil temperature within a specific time is critical for French fries, coated vegetables, and so on to achieve the desired product texture, flavor, and appearance. However, the recovery time for the fryer oil temperature is especially critical for chicken, fish, and other meat products, where the interior of the product must reach a specific temperature to prevent food-borne diseases associated with the meat and poultry products.

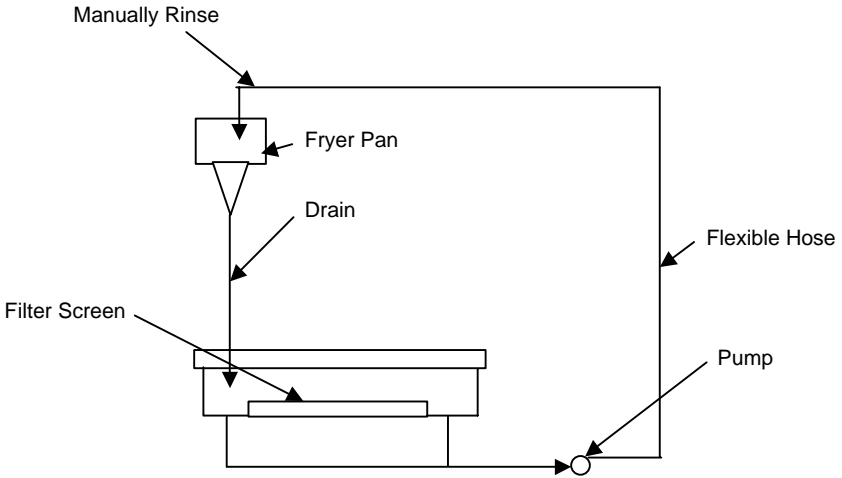
The typical oil temperature profile during frying French fries is shown in Table 1. The frying test was conducted in a countertop twin fryer (Model 5301A, Star Manufacturing International, Inc., Hobson Lane, Smithville, TN). Each pan had an oil capacity of 15 pounds. The total thermal capacity of the fryer was 45,000 BTUs (11,364 CHUs) per hour (1).

Par-fried coated chicken, when fried in the same fryer, produced the oil temperature profile that is shown in Table 2.

**TABLE 2. Fryer Oil Temperature Profile During Frying Par-Fried Chicken.**

Steps	Fryer Oil Temperature
Initial fryer oil temperature	360 +/- 2°F (182.2 +/- 1.1°C)
Fryer oil temperature	Dropped immediately to <330°F (<165.6°C)
Temperature after 4 min.	350°F (176.7°C)
Product interior temperature	160°F (71.1°C)
Temperature after recovery	360°F (182.2 +/- 1.1°C)

(Total Fry Time/Batch: 4 min., Quantity /batch = 1 pound)



**Figure 4.** Filter system for a restaurant fryer.

Most floor-type fryers have V-shape frying pans. The narrow lower part, which is called the cool-zone, allows the accumulation of the crumbs and reduces the scorching of the food during frying. This helps maintain good food color and reduces the development of burnt or scorched flavor even with an extended period of frying.

It is necessary to filter the fryer oil at some regular frequency. This is done to remove the particulate matter that accumulates in the oil, which makes the oil become dark. The product begins to exhibit a burnt odor or flavor if these particles are left in the oil. The accumulated particles can also act as catalysts for oil degradation, increasing the risk of fire hazards at the restaurants.

Some floor-model fryers have built-in filters. These filters are made of stainless steel mesh. Usually, a layer of filter paper is used on the screen. A built-in pump circulates the oil through the screen for several minutes. The filtered oil is used to flush the residual solids in the fryer pan, and the filtered oil is put back in the fryer pan and stored until the next operation. The oil is filtered at least once every day. For some products, it is necessary to filter the fryer oil twice or three times daily because of the heavy accumulation of crumbs.

External filters are also used to filter the fryer oils. These filters are similar in principle to the built-in filters. Operation of these filters is hazardous because of the high oil temperature and manual handling of the hot oil. Burns from the hot oil is one of the common accidents in the fast food restaurants. Figure 4 shows the schematic diagram for an external filter.

### 3.3. Pressure Fryers

Pressure fryers are used for frying chicken, meat products, and breaded shrimp. These fryers are equipped with lids that are tightly held to maintain pressure

**TABLE 3. Applications of the Pressure Fryers.**

Operating Pressure (PSIG)	Operating Temperature °F (°C)	Type of Food to be Fried
5	220 (105)	Fish, Breaded shrimp, Soft textured vegetables
8–10	235 (113)	Rice pudding, Custard
15	250 (121)	Every type of food

inside the fryer. The pressure can range from 5 to 15 PSIG (pounds per square inch, gauge, or 0.35 to 1.06 kg/cm<sup>2</sup>). Initial design of these fryers allowed only one pressure setting on the fryer. In 1917, the U.S. Department of Agriculture (USDA) established the maximum operating pressure for pressure fryers to be 15 PSIG (1.06 kg/cm<sup>2</sup>) for the safety of the operators at the food establishments. The later designs included a jiggle-top load setting on the fryer allowing the operator to change the operating pressure in the fryer. These fryers have three pressure settings such as, (1) low pressure (5 PSIG), (2) intermediate pressure (8–10 PSIG), and (3) high pressure (15 PSIG). Table 3 lists the typical applications for these fryers.

Par-fried foods as well as other products require different pressure settings. Manufacturers of these fryers indicate the recommended pressure settings for the various types of products to be cooked in their fryers.

### 3.4. Benefit of Pressure Fryers

Pressure frying is less time consuming. Pressure fried products appear to have better flavor because of the following factors.

- The volatile flavor components, which are normally stripped from the food during conventional frying, are retained in the confines of the fryer.
- Some of these delicate flavor components are claimed to penetrate into the food during frying. This improves the flavor of the product compared with the conventional frying process.
- The oil does not deteriorate in a pressure fryer as rapidly in a conventional fryer. This is because the oil is not in contact with air during frying.

A good example of a pressure fried product is the Original Recipe of Kentucky Fried Chicken (KFC), which is fried in pressure fryers. It is claimed by KFC that the subtle volatile flavor components are better retained in the product due to enclosed pressure frying. This is a reasonable claim because some of the volatile flavor components are stripped from the food by the evaporating water during frying and released into the kitchen, during conventional frying. The pressure fryers are available in countertop as well as floor models (see Figure 5).



**Figure 5.** Pressure fryer (floor model). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

### 3.5. Turkey Fryers

Frying the whole bird has become popular. This is done in the fryer that is referred to as a turkey fryer (see Figure 6). These fryers can fry a whole bird (turkey or chicken) in 6 minutes or less. The heating system has a temperature regulator and a thermal capacity of 150,000 to 200,000 BTUs (37,800 to 50,400 CHUs)/hr.



**Figure 6.** Turkey fryer. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)



The heat source can be either gas or electrical. The heat is applied at the bottom of the pan, which contains the oil. The bird is lowered into the bed of hot oil in a stainless steel basket, and the lid is closed. At the end of frying, the basket is raised, the excess oil is allowed to drain into the frying vessel, and the bird is removed.

The fryer is equipped with the components as listed: (1) a stainless steel stock pot, (2) a vented lid, (3) a perforated basket (or rack) for the poultry, (4) a grab hook on the basket, (5) thermometer, (6) a gas cooker, and (7) a seasoning injector to inject oil and seasoning into the bird.

### 3.6. Fryer Operation in a Restaurant

Depending on the product, the restaurant fryers are operated at 335 to 375°F (168–190.6°C). During slow hours, the fryers are maintained on standby mode to keep the oil temperature relatively low. The oil is always maintained at the frying temperature during rush hours.

In a fast food restaurant the following operating mode is common:

1. The fryers are turned on first thing in the morning.
2. The restaurants are very busy during lunch and dinner but the rest of the time they are slow.
3. The oil is kept hot during the rush hours.
4. The oil is filtered once/day, or sometimes two or three times a day.
5. The fryer is cleaned at night, and refilled with the used oil.
6. A small amount of fresh oil is added for make-up.

## 4. SAFETY ISSUES

### 4.1. Pressure Fryers

Consumers try to use a pressure cooker as a pressure fryer. This practice should be avoided because of the following reasons:

- A pressure cooker **IS NOT** a pressure fryer.
- A pressure fry pan **IS NOT** a pressure fryer.

It is important to note that pressure cookers are designed to cook foods under pressure, and they are safe when they are used for cooking products. However, when they are used as pressure-fryers, small amounts of oil come in contact with the gasket and damage it. It eventually can melt the gasket, causing a burst of hot oil out of the pan. This can injure people or can even cause a fire. Therefore, pressure cookers are not recommended for pressure frying.

## 4.2. Turkey Fryer

Serious accidents have been reported regarding turkey fryers. The Consumer Product Safety Commission (CSPC) has issued the following safety guidelines for the use of turkey fryers.

Since 1998, the CSPC has reported 75 incidents that involved fires, flames, or burns associated with turkey fryers. Twenty-eight of these incidents were reported for the year 2002. Turkey fryers are becoming popular. These fryers can be seen in many fast food restaurants, frying either whole turkey or chicken. Therefore, the users of this type of fryer must be aware of the safety tips offered by the agency. The detail of this report can be found on the website of the agency.

## 4.3. Floor-Model Fryers

Restaurant fires are common. This generally starts when one or more of the following conditions exist:

1. The oil in the fryer is used far beyond its good operating life.
2. Smoke coming out from the oil is noticed at the top of the fryer.
3. The safety devices have failed to operate.

The thermostatic control on the fryer automatically switches off the source of heat when the temperature in a fryer pan exceeds a preset temperature, which is typically set at 400°F (204.4°C). In addition, every fryer has a high-temperature protection device, known as the high-temperature cutoff (normally set at 425°F or 218.3°C). The purpose of the high-temperature switch is to cut off the source of heat if the thermostatic control fails to turn off the source of heat when the oil temperature exceeds 400°F (204.4°C). This is to protect the fryer oil from reaching a very high temperature and cause a fire because of overheated oil. There is another safety device used to protect against fire in the event the high-temperature cutoff device fails to function. The system for the gas-fired fryer operates in the following manner.

When the fryer oil temperature rises beyond control because of the failure of the high-temperature cutoff device, a certain amount of fire retardant (powder) is discharged on top of the fryer to create a blanket to cut off the oxygen supply to the hot oil. Simultaneously, the main gas supply line gets automatically turned off by a solenoid valve on the gas line set to be shut off in the event the fire retardant is discharged.

The main circuit breaker is tripped in an electric fryer using a temperature sensor connected to the fire retardant system.

## 5. IMPROVING RESTAURANT FRYER OPERATION

It is possible to maintain high quality in fryer oil and that of the fried food in a restaurant by applying the following procedures:

1. Remove all gums and deposits from the frying pan and the heating elements every night by scrubbing thoroughly using a scrub brush.
2. Inspect the fryer pan and look for spots with discoloration. These indicate hot spots caused by localized heat. The pan and the heating element should be replaced if hot spots are noticed.
3. Fresh oil must be added to the fryer at regular intervals, e.g., every 2 hours.
4. The fryer oil must be filtered at least once or twice every day.
5. Treat the oil every day with proper treating agents that are commercially available, which does not form soap in the oil.
6. Develop a plan to discard the fryer oil, based on product taste and flavor.

## 6. MEASURING OIL QUALITY IN THE FRYER

It is difficult to decide on oil discard. The restaurant manager or a senior employee decides when the oil from a fryer should be discarded.

Traditionally, the restaurants have used oil color or the foam height in the fryer to decide on oil discarded. However, both approaches are subjective and can lead to either premature discarding of the oil or the use beyond its limit for optimum product flavor and taste.

Various physical and chemical quick test devices are available to check oil quality in a fryer (2). Unfortunately, many of these devices have not gained popularity because of the following reasons:

1. They are either difficult to operate or costly.
2. The restaurants also undergo a very high rate of turnover of the kitchen personnel.
3. The kitchen personnel do not always feel comfortable with the test kits. Therefore, testing oil at a restaurant is a major challenge.

A few instruments to measure oil degradation have recently been introduced. They mostly measure the oil decomposition products commonly referred to as polar compounds. Researchers are testing these instruments to determine their applicability for evaluating fryer oil quality in the restaurants.

The USFDA has not imposed any regulation on frying oil quality at restaurants or in the industrial frying operations. The USDA requires the fryer oil to be discarded when the free fatty acid (FFA) in the oil exceeds 2%.

Most countries in Europe and Chile have adopted some form of regulations to control the quality of frying oil at the restaurants. Most of these countries have decided that the oil reaches the end point when the polar material in the oil exceeds a certain level. This level is somewhat different for different countries. This regulation compels the restaurant personnel to learn the techniques of testing fryer oil for quality and to determine when to discard it. Table 4 lists the regulatory limits on the restaurant oil quality (3).

**TABLE 4. Regulatory Guidelines on Restaurant Fryer Oil in Various Countries.**

Country	Max. Fry Temp. °C	Smoke Pt. Min. °C	Acid Value MAX. (%)	FFA Max (%)	Polars Max (%)	Ox. FA Max (%)	DPTG Max (%)	Viscosity Max mPA's
Austria	180	170	2.5	—	27	1	—	37
Belgium	180	—	—	—	25	—	10	—
Chile	—	170	—	2.5	25	1	—	—
Czech Rep.	—	—	—	1	<25	—	<10	—
Finland	—	170	2.5	25	—	—	—	—
France	180	—	—	—	25	—	—	—
Germany	170	—	—	—	24	0.7	—	—
Hungary	180	180	2.0	—	<25	—	—	—
Iceland	190	—	—	—	—	—	—	—
Italy	180	—	—	—	25	—	—	—
Japan	—	170	2.5	—	—	—	16	—
The Neth.	180	—	—	—	—	—	—	—
Portugal	180	—	—	—	25	—	—	—
S.Afric	—	—	—	—	<25	—	16	—
Spain	—	—	—	—	25	—	—	—

FFA FFA

DPTG dimeric and polymeric triglycerides

Viscosity for Liq. Oils 27

Various testing devices recommended as well as practiced in this area can be divided into the following groups.

## 7. PHYSICAL TESTING DEVICES

### 7.1. Noninstrumental

The early test methods used to determine the point of discard for the oil in a restaurant fryer are as follows:

- Oil color
- Clarity
- Foam height

Color wands and color strips were promoted to determine the oil quality in a restaurant fryer. These did not provide any practical help to the restaurant personnel because a universal color standard does not apply for all types of oil. Sometimes, cottonseed oil, corn oil, and palmolein turn darker than soybean or sunflower oil in a restaurant type fryer, even though the oil quality is perfect for frying foods. In such cases, the restaurant operator may discard the good quality fryer oil just because the color may appear dark to the restaurant operator.

Oil clarity diminishes as the oil is used. KFC introduced a visual tester that they called the "visibility tester." It is a stainless steel rod attached to a shiny silver disk at the end. The rod has a linear scale showing three indented marks, which are used to judge shortening quality. The shiny disk is lowered into the oil with the depth of

insertion monitored on the rod. A penlight is held next to the rod, turned on, and the disk is observed. The oil is discarded if the disk is not visible at a certain depth.

The above subjective test methods provided very little practical value and are not used at many restaurants.

## 7.2. Instrumental

Several inventors have developed instruments for measuring fryer oil quality. Unfortunately, some of them are too complex to operate, whereas others can be applied in monitoring the oil quality in restaurants. These instruments are as follows:

- FRI-CHECK
- Viscosity Meter
- Food Oil Sensor
- Oil Condition Sensor
- 3M PCT tester
- FOM (Food Oil Meter)
- OptiFry

The Fri-Check instrument, designed by Dr. Christian Gertz of Hagen, Germany, is a device that measures the viscosity and density of the fryer oil, which correspond to its degradation as determined by concentration of the polar material and polymers in oil. This instrument consists of an electronic box with a removable steel tube. The tube is filled with the test oil. The oil is maintained at a constant temperature. A cylindrical metal piece is carefully released from the top of the tube filled with the test oil. The amount of time required by this object to fall a measured vertical distance through the tube is measured. The amount time is dependent on the density and viscosity of the oil. Because the density and viscosity of the oil increase with the formation of polar materials and polymers during frying, the “falling-time” can be related to the degree of oil decomposition. In actual tests, the FRI-CHECK data correlated well with percent polar material and polymers in the oil. The system is relatively simple and can be used for monitoring degradation of used frying fats in the restaurants.

The scientists at Leatherhead Food Research (United Kingdom) developed an instrument to measure viscosity of oil. The principle of its operation is similar to that of a tuning fork. Dampening of the vibration of the tuning fork-like device depends on the viscosity, and resonance depends on the density of the surrounding fluid. Although the instrument was simple to operate, the actual determination of oil quality was difficult for the restaurant operators.

An instrument, known as the Food Oil Sensor, has been marketed by the Northern Instrument Company since the late 1970s. The instrument measures the change in the value of the dielectric constant in frying fat. This value increases as the oil is degraded because of the formation of polar material in the oil. The instrument is simple to operate and is used by many researchers to study fryer oil quality in the

test laboratory. However, the instrument is prone to error, if the sample cup is not properly cleaned after every use. Any amount of oil left in the sample cup is likely to polymerize and give erroneous readings in the subsequent tests. This makes the instrument less suitable for restaurants because the fryer operators are not used to pay such attention to instruments.

An oil condition sensor has recently been introduced by the Tech Town co. to monitor the oil quality in frying. This also operates on the principle of conductivity of the oil, which increases as the oil is degraded during frying. This instrument is relatively new, and the author does not have any information on its long-term performance.

The 3M Company (Europe) introduced an instrument called the Polar Compound Tester or PCT 120. This instrument operates on the principle of polarity developed in the oil with frying. Although the instrument is easy to use and provides good information, some restaurant operators find it difficult to use.

Dresser Instruments of Stratford, CT, under the ebro brand has introduced a handheld instrument called the Food Oil Meter (FOM-200). This instrument can repeatedly measure polar compounds within seconds in hot oil to directly monitor oil quality in a fryer. The results obtained from the FOM-200 correlates within  $\pm 2\%$  of the official IUPAC method 2.507/DFG method III-3b for polar compounds and can store many characteristic oil curves for reference. The instrument is being used by several researchers in their frying studies and fast food franchises to better schedule filtering aids additions and oil replacements in order to improve overall food quality.

OptiFry from MirOil co. (also known as Oil Process Systems of Pennsylvania) can measure the increase in polar compounds in the fryer oil compared with a reference value for the corresponding fresh oil. Measurement with this instrument show excellent correlation with the laboratory test values generated at the institute for research of the German Health Ministry on cereals, potatoes, and lipids for consumer protection, nutrition, and agriculture. It is possible to establish a program for fryer oil quality management with the help of this instrument and combining the data with the consumer acceptability of the product. One can maintain a relatively uniform oil quality by applying the technology offered by the company.

## 8. CHEMICAL TESTS

1. Oxifrit—Merck
2. Fri-Test—Merck
3. ACM/PCM\*—Mir-Oil add location
4. TPM\*\*, FFA\*\*, WET\*\*—Test Kit Technologies - location
5. AV<sup>+</sup> Check—Advantek add location
6. Shortening Monitor-3M—USA

\* ACM—alkaline contaminant material; PCM—polar component material

\*\* TPM—total polar material; FFA—free fatty acid; WET—water emulsion titratable.  
+ Acid value.

The Merck Company of Darmstadt, Germany developed the OXIFRIT & FRIT-EST to measure oil quality in frying operations, especially in restaurants. The Fritest measures the alkali color number to indicate oxidized fatty acid (OFA) and the Oxifrit Test measures oxidation products in the fryer oil. Both tests are colorimetric and use a solvent-based reagent system. The test methodology is too complicated for the restaurant personnel.

The ACM and PCM tests were developed and patented by Libra Laboratories (USA). Oil Process Systems of Allentown, PA also known as Mir-Oil, owns the patent and distribution rights for the testing devices. The ACM test measures alkaline contaminant materials, which include soaps. The PCM measures polar contaminant materials (accumulated polar compounds). These tests provide results that could be used at the restaurants. However, the test units are relatively expensive and the restaurants tend to avoid using them after the initial introduction of the device.

Test Kit Technologies has been manufacturing and marketing rapid tests for measuring FFA, TPM, and WETs for over a decade. The initial units used the solvent technology and color cards, similar to MirOil and Merc tests. The subsequent development included the Gel-in-tube Instant Chemistry (GiTIC). In this method, hot and filtered fryer oil without filtration suspended material is added directly into the tube containing a gel. The gel melts, and the components from the gel react with the oil, producing color that can range from light green to deep blue. The tube, while it is still hot, is inserted in a colorimeter that provides a reading on the oil sample in the tube. The reading provides information on TPM, FFA, and WETs, also known as surfactant in the oil. In the GiTIC technique, hot oil samples are collected from the fryer, filtered, and added directly to the gel. The oil melts the gel, and the components of the gel react with the oil to producing a color. While still hot, the tube is placed in a small colorimeter and a reading is obtained. The reading may be directly related to oil quality and used for monitoring oil oxidation. The TPM test is a single-phase test, whereas the FFA and WET tests are two-phase, producing a colored bottom layer, all of which are read by an instrument. The users must establish their own color limits to reflect the oil quality standards for their product. This instrument is simple, but the cost of the tubes can be prohibitive for many restaurants. In addition, the restaurants are not trained to develop their own oil quality criteria for discarding the oil as required by this instrument.

The 3M Company of St. Paul, MN introduced the Shortening Monitor, which consists of a white strip of paper, measuring  $0.3 \times 3.75$  inches, which has four blue bands across it. The strips are used as a dip test to measure accumulated free fatty acids in the oil. The tests were developed to provide users, especially those in the fast-food industry, with an inexpensive means to objectively measure FFA in the cooking oil. This test might be suitable for restaurants because the FFA in restaurant fryers tend to run high and might be effective to predict the imminence of reduced smoke point in the fryer oil.

Advantec manufactures and distributes test strips for measuring the acid value (AV) in the fryer oil. The product is marketed in Asia through Ajinomoto. This is similar to the 3M test-strip and monitors acid value in the oil. The operator places a plastic strip with an indicator on the tip into the cooking oil. The color indicator

changes from dark blue to a light olive green color depending on the acid value of the oil. The color chart goes as follows:

Color	Acid Value, %
Blue	0
Dark Green	0.5
Green	1.0
Light Green	2.0
Olive	3.0
Very Light Olive	4.0

This test strip can be a useful for restaurants. For industrial fryers, it has limited usefulness.

## 9. FILTRATION AND TREATMENT OF OIL

The oil from the fryer must be cleaned routinely to obtain good fried food quality. Oil filtration can be done through an external filter where the oil is collected in an external tank with a filter screen at the bottom (see Figure 4). A filter paper or filter cloth is used on top of the filter screen. Using a pump, the oil from the pan is circulated through the filtration chamber. The fryer pan is also rinsed with the clean oil, and then the filtered oil is put back into the fryer pan. This process removes most of the particulate matter accumulated at the bottom of the pan (cool zone) during frying. This can help reduce the burnt color and flavor in the fried food. Some fryers have built-in filters.

The impurities in the oil can be removed more effectively by treating the oil with treatment powders. Some of these materials remove the soap and polar compounds from the oil. This can significantly improve the fry life for the oil.

However, there are commercial products that remove free fatty acids by chemically reacting with an alkali. This increases the amounts of soap in the treated oil, which reduces its stability. The presence of high amounts of soap in the fryer oil can exhibit the following phenomena:

- Foaming in the fryer, increasing oil oxidation and reducing its fry life.
- Can develop an unpleasant flavor in the fried food.
- Can change the appearance of the fried food.
- According to the regulatory guidelines in many countries, including the United States, the presence of soap, created in this manner in frying oil may constitute adulteration.

It is recommended that one must select the treatment powder that does not chemically react with the FFA, but it removes the oil impurities by a physical adsorption process.



The restaurant owner or manager must review the test data from the company that manufactures the treatment chemicals and make sure that the soap content in the oil after treatment is not higher than before the treatment. The treatment temperature for oil is also critical, especially if the material contains citric acid. Citric acid is a metal chelator and is expected to remove the excess metal ions that are chemically transferred into the fryer oil during frying. Citric acid breaks down if the oil temperature is above 284°F (140°C). Fryer oil, transferred to the filter sump, can be very hot (300°F/149°C). This can break down citric acid at this high temperature and make it ineffective.

The restaurant fryers do not have oil cooling capability. The operators do not normally check the oil temperature before the treatment material is added to the oil. Thus, one can expect less than desirable results from the oil treatment.

The flavor of the fried food improves with the oil treatment, when appropriate treatment material is used. However, one needs to look at the overall economics. The cost of oil treatment material, the recommended frequency of treatment, the dosage of the treatment material, and the savings on oil must be checked carefully to justify the use of the treatment material for treating the oil.

## 10. INDUSTRIAL FRYING

Industrial frying is one of the major segments in the frying industry. Fried food in the United States and other countries are prepared and marketed in various forms. The most popular of these products is the salty snack food segment. This segment consists of a wide variety of products such as potato chips, tortilla chips, corn chips, extruded products, pretzels, fried or roasted nuts, and so on. Out of all these products, potato chips has been known to the people for a long time.

### 10.1. United States

Although the fried snack foods have been enjoyed by people from many countries around the world and for centuries, the only documented introduction of potato chips can be found in the United States (4). According to the record of the Snack Food Association (United States) modern-day potato chips started as a joke at the resort in Saratoga Falls in 1853.

The railroad magnate Commodore Cornelius Vanderbilt was dining at the resort one evening when he sent the fried potatoes back to the kitchen because they were too thick. George Gum, the cook on duty decided to thinly slice potatoes, deep fry, and salt the fried chips. The chips were thin, crispy, and salty. What was meant to be a joke turned out to be the birth of the modern-day potato chips. The product was an instant hit. The Saratoga Chips became a fad with the resort's socialite patrons. Soon the recipe spread throughout the eastern region of the country. Subsequently, it grew in this country and in Europe. The packaging materials were introduced to package and deliver the product. The fryer operation evolved from its kitchen and sink stage to kettle frying and eventually to the modern-day fryers with

sophisticated designs. The Snack Food Association celebrated the one hundred fiftieth birthday of potato chips in 2003 at Saratoga Falls.

Today's leader, Frito-Lay Inc. of Texas, has brought about the most dramatic evolution in the American snack food industry. In 1932, Mr. Elmer Doolin of San Antonio started the Fritos brand Corn Chips, and Mr. Herman W. Lay started to sell potato chips to the stores in Nashville, TN. In September of 1961, just 29 years after the two companies began their ventures, the Frito Company and H. W. Lay Company merged to become the Frito-Lay Company. In 1965, Frito-Lay and Pepsi-Cola merged. Today, Frito-Lay is the leading snack food producing company in the world. The company sells numerous salty snack food products that include potato chips, corn chips, extruded products, nuts, and a variety of other products.

## **10.2. United Kingdom**

Potato chips are one of the popular and oldest savory-snacks sold in the United Kingdom. It is believed that Sir Walter Raleigh and Sir Francis Drake brought potatoes to England from Peru in 1570. Soon the crop expanded into various parts of Europe, and by the end of the eighteenth century, potatoes were available almost everywhere in Europe including the United Kingdom.

A man named Frank Smith started his potato chip plant and sold the product in the early 1900s. The chips were sold in bags made of grease-proof paper.

## **10.3. Germany**

Frank Flessner and his wife Ella started their Stateside Potato Chip Company in Germany in 1951. The chips were made at home, packaged in glassine bags, and delivered to the U.S. Army base in Germany. The U.S. soldiers were his primary clients. By 1961, the company had established two manufacturing plants.

It is difficult to obtain the history of the snack food industry or the list of salty snack products in other countries because of the lack of documented information. For example, Indians of the Indian subcontinent consume at least 300 different varieties of salty snacks that are fried in vegetable oils. These products contain grains, pounded rice, nuts, vegetables, raisins, legumes, coconut, and seasoning to suit the palates of the people in the various parts of the country. Although these products have been used for centuries, no one can determine the date or the place of origin of most of these products. This market has advanced from a cottage industry to the manufacturing sector in the past three decades. A large number of these products are being exported to the United Kingdom, the United States, Canada, and many European countries.

There are various types of fried snack foods that are prepared and sold fresh or packaged and distributed through retail channels like super market, convenience stores, restaurants, food services, vending machines, and so on. Most common fresh fried products are as follows:

1. Donuts
2. Beignets
3. Varieties of meat and vegetable savory products.
4. Popcorn

The beignets and the savory products are consumed soon after they are prepared. Popcorn and donuts can be stored for a day or two.

There is a long list of packaged fried food products. These are both salty as well as products seasoned with sweet and tasty seasonings. Most common salty fried foods are as follows:

1. Potato chips—plain or seasoned.
2. Tortilla chips.
3. Fabricated products.
4. Extruded corn products—seasoned (salty) and coated (sweet).
5. Batter-coated products.
6. Par-fried products, e.g., French fries, fish, chicken, cheese, vegetables, and so on.
7. Fried nuts—salty, seasoned, or coated (sweet).

Potato chips and tortilla chips have become household names in North America. Corn products are not as common in Europe. Fabricated products can be produced from pellets or from sheeted material made from a preprocessed and formulated mixture of ingredients. The most commonly known products of this category are Pringles made by Procter & Gamble co. and Lay's Stax introduced recently by Frito-Lay. Products made from formulated sheets as well as from the pellets are sold as salted as well as seasoned with various seasonings. These products are now popular in most countries.

Batter-coated fried foods have become popular in the restaurant and domestic uses in the United States. Flash frying has been used to deliver good stable batter-coated products. These are coated shrimp, fish, corn dogs, and fried chicken and are popular restaurant items. All of these products are manufactured on large scales and distributed in frozen state. The frozen products are fried directly out of the freezer and served.

Meat products, such as chicken-fried steaks, are manufactured on large scales and distributed to the restaurants in the frozen state. Frying of a whole bird, such as turkey or chicken, has become popular in the United States and Europe.

Par-frying of French fries, chicken, and vegetables has grown to become a multi-billion-dollar business because of the convenience and low cost to the food services and restaurants.

Potato chips sales are the highest in the snack food industry. The next big seller is the tortilla chip. Table 5 lists the sales figures for the major snack food products in the United States (5).

**TABLE 5. Salty Snack Food Sales in the United States During 2003.**

Segment	Dollar Sales (Millions)	% Change	Pound Sales (Million)	% Change
Potato Chips	6,037.8	+1.5	1,815.1	-0.3.
Tortilla Chips/ Tostatda	4,529.4	+1.1	1,523.6	+0.2
Corn Snacks	873.1	-2.0	254.1	-2.6
Pretzels	1,255.6	-2.0	564.5	-6.4
Pork Rinds	645.2	+37.4	107.8	+36.8
Snack Nuts				
Seeds	2,347.3	+15.9	648.0	+12.5
Corn Nuts				
Micro Wave Popcorn	1,30.5	+2.8	456.2	-1.8
Ready to Eat Pop Corn	445.3	-3.9	109.5	-3.6
Cheese Snacks	1,181.2	10.9	371.5	+7.1
Meat Snacks	3,404.3	+14.4	159.2	+14.7
Variety Pack	320.8	-3.9	76.3	-1.6
Other	2,062.5	+2.0	437.8	+4.2
<b>Total</b>	<b>23,472.0</b>	<b>+4.5</b>	<b>6,525.6</b>	<b>+0.2</b>

One can appreciate the size of the snack food market in the United States from these sales figures. The low oil and no oil category of snack foods, which began to flourish in the late eighties through the nineties, have shown a significant decline in their demand. The fat-free product made from olefin (Olestra) has also shown a significant decline in volume since its introduction and its initial growth. The sales for pork rinds, cheese snacks, nuts, and the meat snacks increased significantly in 2003 primarily because of the Atkins diet program. The sales of the traditional snack foods declined. The snack food market showed an overall increase of 4.5% in sales (dollars). This could be attributed to the increased sales of the nuts, meat snacks, and other high-price products.

The latest challenge in the fried food industry is to produce products with low *trans*-fats. This requires oils that are not hydrogenated but stable under the frying conditions.

The technology in the frying industry has evolved greatly from the early days of kitchen frying to highly automated industrial frying. Innovative packaging techniques have helped the industry to deliver products with longer shelf life. Frying equipment development has also allowed the industry to manufacture products with specific traits that were not possible in the past. There has been significant improvement in the oil heating techniques to obtain longer life for the frying oil.

## 11. THE PURPOSE OF FRYING FOODS

Frying process is the quickest way to dehydrate foods and make the product palatable. Fried food has better product appeal than baked or cooked products because of the unique traits of fried products, as listed below:

- Flavor
- Texture
- Appearance
- Taste/Mouth feel
- Fried food aroma
- Pleasant after-taste
- Overall satisfaction or acceptability

## 12. DIFFERENCE BETWEEN THE FRYING AND OTHER COOKING METHODS

Frying is a very distinct process because of the reasons listed below:

1. The dehydration in frying is very rapid. It can vary from 10 seconds up to a few minutes, depending on the type of product and the frying process.
2. Extreme difference between the fryer oil and the product helps develop the crust and texture of the fried food.
3. The fried food absorbs a substantial amount of oil during frying that imparts some unique taste and flavor to the product.
4. Fully fried products, such as salty snacks lose practically of the moisture because the final product contains less than 1.5% moisture.
5. Par-fried products, such as batter-coated fish, chicken, meat patties, and so on; the surface coating is dehydrated, whereas the interior is not. For chicken and meat, it is important for the center of the food to reach certain temperature (discussed later) for food safely, whereas the fish remains frozen inside the fried crust. It is important to retain most of the moisture in these products to maximize the yield and reduce the dryness of the product for the subsequent frying process.

## 13. WHAT HAPPENS DURING FRYING?

The food undergoes a simultaneous process of heat and mass transfer as well as many chemical reactions during frying. This is a complex process as summarized below:

1. Hot oil in the fryer begins to heat the surface of the food. The heat penetrates into the food through the surface.
2. The moisture from the interior of the food begins to migrate to the surface in the form of water vapor and escapes to the space above the bed of oil.

3. The surface temperature of the product increases, producing browning and crispness in the product. The degree of browning of the fried food is affected by sugars and additives in the food as well as the frying temperature. Browning of the food surface can be controlled by adjusting the frying temperature, frying time, and sometimes with certain additives such as dextrose, coloring, or leavening (to get a lighter surface color).
4. Some changes take place in the appearance, size, and shape of the products during frying.
5. Excess oil from the food surface is removed by draining the oil or in some cases with the help of a centrifuge.
6. Fresh oil is added to the fryer either automatically or manually to replace the oil carried out by the product.
7. The oil chemistry changes because of hydrolysis, oxidation, and polymerization. Changes in the oil quality can significantly affect the storage stability of the fried product. Extreme deterioration of the oil quality in the fryer may even affect dehydration, degree of browning, and loss of coating from the coated products.

## **14. TYPES OF INDUSTRIAL FRYERS**

There are two main types of industrial fryers. They are as follows:

1. Batch fryers
2. Continuous fryers

Vacuum fryers are less common but used to fry products where the product is required to maintain most of its natural color. This is mostly used for frying sliced fruits and certain vegetables.

### **14.1. Batch Fryers**

These fryers resemble the so-called restaurant fryers, except they are much larger in size, capable of frying several hundred pounds of product per batch. Figure 7 shows the picture of a typical batch fryer.

These fryers are used for small-scale production or for frying very specialty-type products such as kettle-fried Hawaiian-style potato chips. The oil is placed in a large pan where it is heated by gas burners, located under the pan. In certain installations, the oil is heated in an external heat exchanger. In this case, the oil is continuously taken out of the fryer, circulated through an external oil heater, and returned back into the fryer pan. The former method of heating the oil is known as the “Direct Heating” system, and the latter is called the “Indirect Heating” system. The external oil heaters can be either steam heated or gas fired. The steam heat



**Figure 7.** Batch fryer (Courtesy of Heat & Controls Co.).

exchangers are generally shell and tube type, with the oil passing through the tubes and steam on the jacket side. Moderate pressure (180–200 PSI, 17.59–19.4 kg/cm<sup>2</sup>) steam is used. The gas-fired heaters are of different designs. In some of the heaters, the oil in tubes is exposed directly in the path of the flu gas from the burner. The others use the hot flu gas from the burners to heat air, which in turn heats the oil in tubes. A level control maintains the oil level, and a temperature control device maintains the temperature of the oil in the pan.

The operating sequence in batch frying is as follows:

1. The oil temperature is allowed to reach the desired steady state condition.
2. The product is added at specified amounts into the pan.
3. The oil temperature drops sharply and then recovers at the point when the product has reached the desired end-point moisture content.
4. The product is stirred either with a manual stirrer or with an automatic stirrer.
5. The product is removed from the fryer with a takeout conveyor.
6. The product is sometimes centrifuged to remove the excess oil on the surface.
7. The product is then seasoned and packaged.

## 14.2. Continuous Fryers

A continuous fryer is used for large-scale production of fried snacks. The oil is heated in a straight or in a horseshoe-shaped pan with temperature and level controls. The product is fed into the fryer at one end, and the fried product is taken out from the opposite end by a takeout conveyor. The internal construction of the fryer varies greatly with the type of product fried.

Like in batch fryers, the oil in a continuous fryer is heated either in direct or indirect oil heaters.



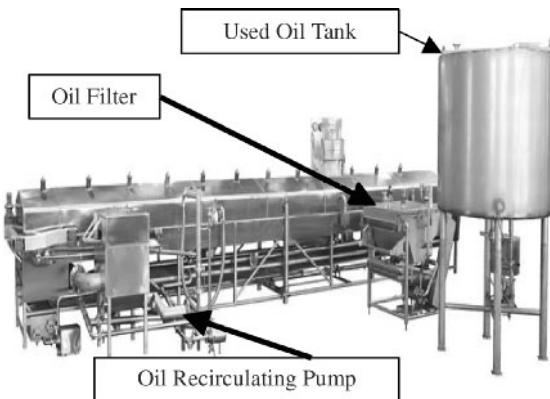
**Figure 8.** Direct fired fryer (Courtesy of Heat & Control Co.). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

Figure 8 shows the picture of a direct heat fryer, and Figure 9 shows the picture of an indirect fryer.

### 14.3. Vacuum Fryers

A vacuum fryer is used to fry fruits or vegetables where it is important to retain the original product color with minimum browning. These fryers generally do not have high production capacity. They are also very expensive. Most commonly used fryers of this category are batch type. Continuous vacuum fryers are extremely costly and are hardly used. These fryers have several distinctive features, such as:

- The fryer is operated under vacuum. Typical operating pressure is  $<100$  mm of mercury.
- The frying is conducted at roughly  $250^{\circ}\text{F}$  ( $121.1^{\circ}\text{C}$ ). The food can be dehydrated at this temperature under vacuum.



**Figure 9.** Continuous fryer with direct heat (Courtesy of Heat & Control Co.). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)





**Figure 10.** Multi-zone fryer (Courtesy of Heat & Control Co.). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp.>)

- The food is placed in a basket.
- The basket is inserted and placed in the vacuum chamber above the surface of the oil.
- The vacuum is applied.
- The basket is lowered into the hot oil at an approximate temperature of 250°F.
- Frying begins, and the oil temperature drops (as described in a batch fryer).
- The oil is circulated continuously through an external heater.
- The oil regains the temperature when the moisture content of the product reaches the predetermined value.
- The vacuum in the fryer is broken slowly.
- The fryer is opened, and the product basket is taken out.
- The excess oil is allowed to drain, and the product is cooled before packaging.

#### 14.4. Multizone Fryer

These fryers have become popular because of their capability to maintain a more uniform oil temperature and more controlled dehydration of the product. The oil is heated in an external heater and is pumped at several predetermined locations in the fryer. Figure 10 shows the picture of this type of fryer.

This type of fryer may cause more rapid oil degradation than a conventional fryer of similar capacity because of the following reasons:

- The average oil temperature in the fryer is higher than in a conventional fryer. This increases oil degradation.
- Without very careful design, the fryer tends to hold a higher volume of oil than a conventional fryer of equal capacity. This increases the oil turn over time, causing more rapid oil degradation.

## 15. CRITERIA FOR FRYER SELECTION

It is important that a fryer selected for frying a given product must meet all requirements to deliver the desired product attributes. At the very outset, it may appear that the fryer is selected on the basis of the following criteria:

1. The type of product to be fried.
2. Physical property of the food to be fried, i.e., whether the food floats or sinks or expands in the fryer.
3. The volume or rate of production needed.

Many snack products tend to float in the fryer, and therefore, they need to be submerged into the oil with a mechanical submerger. Potato chips, tempura-coated products, are prime examples of this type of products. Certain products float on the oil surface, and the frying is conducted in this fashion, such as donuts. Others are coated products, and therefore, they need different designs for the feed belts. Chicken-fried steaks, French fries, nuts, and so on are good examples of this category of products. These products do not float. Products like pellets expand many folds in the fryer and must be fully submerged. Batter-coated products, such as tempura products, batter-coated fish, and so on also expand and need to be submerged during frying.

Thus, based on the above discussions, it is critical for one to know whether the food product is going to sink or float in the fryer. For a “semibuoyant” product, the fryer needs the following features:

1. Only a main (bottom) conveyor if the product sinks.
2. A submerger conveyor if the product is buoyant. This keeps the product submerged in the oil during frying while the main conveyor carries the product through the fryer. For those products that change buoyancy during frying, the fryers must have a main and submerger conveyor.

In addition, it is important to consider the following criteria for the selection of a fryer:

1. Finished product characteristics, such as appearance, texture, and so on.
2. Desired production capacity.
3. Thermal load (also referred to as heat load) required.
4. Desired maximum oil turnover time.
5. Fryer accessories needed.
6. A fines-removal system needed.
7. Ease with which the fryer can be sanitized.
8. Ease of performing the required maintenance on the system.
9. Required emission control for certain geographic locations.

10. Type of oil heater, e.g.,
  - a. Direct heating
  - b. Indirect heating
11. Technical support from the fryer manufacturer.

## 16. COMPONENTS IN AN INDUSTRIAL FRYING SYSTEM

An industrial frying system consists of a number of components. These include:

1. The preparation system.
2. The fryer.
3. Conveyors for product feed to the fryer and take out.
4. Salt applicator.
5. Seasoning applicator.
6. Toasting oven for tortilla chips.
7. Equilibrator (Cooler) for tortilla chips.
8. Extruder for the extruded products.
9. Stirrers for batch fryers.
10. Optical sorters for product color monitoring.
11. Oil filter.
12. Oil recirculation pump and piping.
13. Oil heating system for direct and indirect heated fryers.
14. Product conveying system to the filling machines.
15. Conveyors and submerger in the fryer.
16. Specially designed conveyor that dredges the frying pan to remove the accumulated solids or sludge from coated products.
17. Paddle wheels for potato chip fryer.
18. Metal detectors.
19. Filling/packaging machine.
20. Nitrogen flush (if used) at the filling machine.
21. Oil temperature controller.
22. Oil level controller.
23. Process alarm for any process upset.

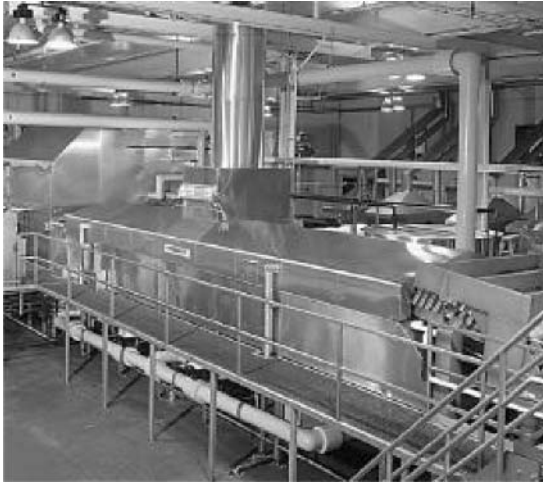
Thus, one can see that the fryer is probably the center piece for the entire frying system, and the other peripheral units provide the capability for the fryer to make the right product. Some of the processes for making fried products are described below.



*Figure 11. High-speed potato slicer (Courtesy of Urschel Co.).*

## 17. CONTINUOUS POTATO CHIP PROCESS

1. Potatoes are washed with clean, cold water to remove the mud, sand, and so on from the surface. These extraneous materials not only show adverse effects on the product quality, but also they damage the oil in the fryer during the process.
2. Rocks and stones are removed in a destoner.
3. A mechanical or a steam peeler is used to remove most of the peel from the surface of the potatoes.
4. The potatoes pass through an inspection table where the defective potatoes are manually removed and all large tubers are cut into smaller pieces.
5. Peeled potatoes are held submerged in a bed of cold water before they pass through the slicers. This operation protects the potato surface from browning.
6. A high-speed slicer, where the tubers are sliced to desired thickness for the product. Water is added into the slicer along with potatoes (see Figure 11).
7. The surface starch from the slices is removed in a washer to minimize darkening of the oil because of charring of the starch. This also protects the potato chips from turning brown and from developing a burnt flavor. There are different types of washers, such as drum washers or high-speed washers. In the former type, the slices are washed in a perforated drum, rotating over a bed of water that is continuously replenished. The speed washers are becoming more common because of their simplicity.
8. A high-speed draining cum feed belt feeds the slices to the fryer in a single layer. This minimizes the amount of water entering the fryer along with the feed and provides better frying in the free-fry zone. This also reduces formation of clusters where several chips form clumps (see Figure 12 for potato chip fryer).



**Figure 12.** Potato chip fryer (Courtesy of Heat & Control Co.).

9. In the free-frying zone, potato slices come in contact with the highest oil temperature (360°F or 182.2°C) that produces maximum initial dehydration.
10. The slices (Chips) then enter a zone where their forward-flow along the length of the fryer is controlled by a set of paddle wheels. The rate of rotation (RPM) and the intermeshing of the adjoining paddles are set carefully by the fryer manufacturer.
11. The chips then pass through a zone where a submerger holds the product under the oil surface. This completes the last amount of dehydration of the product.
12. A takeout conveyor carries the fried chips out of the fryer. These conveyors are specially designed to drain the excess oil from the chips. The total fry-time is around 2 min., 45 sec. to 3 minutes.
13. Moisture in the fried chips is around 1.5%.
14. At the end of the fryer, the oil temperature drops by 40°F (22.2°C) to 50°F (27.8°C) as compared with the inlet temperature of 360°F or 182.2°C.
15. The oil leaves the fryer, passes through a filter screen, and is pumped through an external heat exchanger to heat the oil back to 360°F or 182.2°C.
16. The heated oil enters the fryer at the product inlet end through a manifold that distributes the oil evenly across the width of the fryer pan.
17. The salt applicator is normally situated near the discharge end of the fryer, but precautions are taken to prevent the salt being blown into the fryer oil because of the draft created by the air flow system at the plant. Salt in the fryer oil can degrade the oil rapidly (6, 7).
18. Seasoning is applied in a tumbler. Most seasonings come preblended with salt. Therefore, the seasoned products do not require additional salt.

19. The finished product is packaged mostly in metallized bags with nitrogen flush for long shelf life.
20. The bags are packaged in cases and sent to the warehouse for distribution.
21. The product quality is checked after frying, after applying the seasoning, and after it is filled in the bags.
22. The quality of fryer oil is checked at a regular frequency.

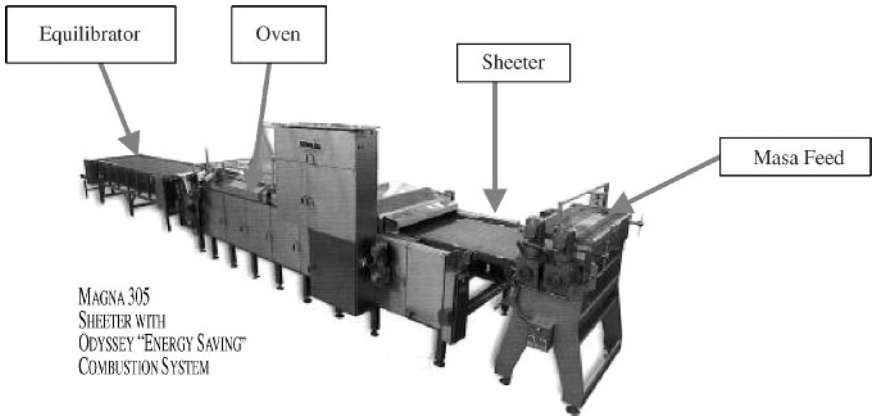
### **17.1. Continuous Tortilla Chip Frying System**

The main ingredient in tortilla chips is corn. The corn is used in two different ways:

1. Freshly cooked corn
2. Corn flour (masica)

#### **17.1.1. Freshly Cooked Corn**

1. Corn is cooked in a vat or in a continuous cooker with a certain amount of lime (calcium hydroxide), which softens the outer shell of the corn and adds some flavor of the product.
2. Excess lime from the surface of the cooked corn is removed by washing the corn in a tumbler using water spray.
3. The corn is then ground in a stone (or stainless steel) grinder to make dough called the masa. The degree of grinding is controlled carefully to obtain the right product characteristics.
4. Water is added in the grinder (also known as mill) to provide an approximate water content of 50% for the masa.
5. The masa is then sheeted and cut to specific size and shape.
6. The raw chips are passed through a gas-fired toaster oven where the temperature is around 640°F (338°C). The chips lose some moisture and develop some toast-points on the surface. This gives the pleasant toasted flavor in the finished product. The degree of toasting is carefully controlled. Overtasting can produce burnt flavor in the product. On the other hand, undertasted chips develop lower corn flavor after frying.
7. The chips from the toaster-oven then pass through an equilibrator. This is a chamber with open sides. The chips pass through a series of special conveyor belts. The object is to achieve an even distribution of moisture in the chips. This also makes the chip color and texture more uniform. Moisture of the chips at this stage is around 40% for masa made from cooked corn and 30% to 35% for the chips made from dry corn masica. (See Figure 13 for the tortilla system before frying). Temperature of the chips reaches below 100°F (38°C) in the equilibrator.
8. The product is then fried in either in a horseshoe-shaped fryer or a straight-through fryer (see Figure 14). The frying oil temperature is approximately 350°F (177°C). The delta-T is 10°F (5.6°C). The value of delta-T in this case



**Figure 13.** Tortilla chip system (Courtesy of Cara Herra Inc.). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)



**Figure 14.** Tortilla chip fryer (Courtesy of Heat & Control Co.). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

is much lower than in the potato chip process because of the lower moisture removal required by the oil.

9. Frying time is very short compared with potato chips. It is typically less than a minute.
10. The fried chips contain approximately 1% moisture.
11. The product removal, salting, and the seasoning application techniques are very similar to those in the potato chip operation.
12. The seasoned product is filled mostly in clear bags. Some manufacturers use metallized bags with nitrogen flush.

## 18. EXTRUDED PRODUCTS

There are many extruded snack products that are fried, seasoned, and packaged for distribution. One of the popular products is extruded corn chips, made either from the cooked corn or from masa, and is extruded through extruders. The product is



**Figure 15.** Corn chip fryer (Courtesy of Heat & Control Co.).

added directly into the fryer and is generally fried at a higher temperature than either tortilla chips or potato chips. These fryers have shallow frying pans. Figure 15 shows a typical corn chip fryer.

### 18.1. Frying Coated Products

These products are batter coated and par-fried for the distribution to the restaurants, food services and the super markets. The products are frozen in blast freezers using liquid nitrogen immediately after frying. The products are shipped in freezer-trucks and then stored in freezers at the destination (storage temperature  $-5$  to  $-10^{\circ}\text{F}$  ( $-21$  to  $23^{\circ}\text{C}$ ). The products are taken out of the freezer and fried without thawing them.

Various products in this category are as follows:

- Fish
- Chicken
- Beef
- Cheese sticks
- Vegetables

**18.1.1. Fish** The fish arrives at the plant in frozen packages. The product is cut to sizes or the pieces separated. They are coated with batter and dusted with the mixture of starch, dextrose, flavoring leavening (for tempura), and so on. Batter coating and dusting process is repeated more than once to get better adhesion of the coating. The fish inside is still frozen.

Frying is done at a temperature of  $380^{\circ}$  ( $193.3^{\circ}$ )– $390^{\circ}\text{F}$  ( $198.9^{\circ}\text{C}$ ) for sufficient time to set the outer coating. The fish inside is still frozen. The product is then frozen in a blast freezer at  $-25^{\circ}\text{F}$  ( $-32^{\circ}\text{C}$ ) to  $-30^{\circ}\text{F}$  ( $-34^{\circ}\text{C}$ ).

One category of fish product is fully cooked. This is sold for cooking in microwave ovens. In this case, the temperature in the interior of the fish must reach  $145^{\circ}\text{F}$  ( $62.8^{\circ}\text{C}$ ) and then frozen like the others.



Chicken and the beef patties are fully cooked before they are frozen. The interior of the product must reach a certain temperature to prevent the growth of pathogens; these are shown below:

Chicken (Boneless)	160°F (71.1°C)
Chicken with bones <sup>†</sup>	185°F (85°C) to 195°F (90.6°C)
Beef patties	145°F (62.8°C)

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<sup>†</sup>In New York City School System it is 205°F (96.1°C) to take out all redness from the outside of the bone and the marrow.

Cheese sticks and vegetable sticks are fried in a manner similar to that for the frozen fish. A hard crust is set without affecting the product inside.

All of these products are non-floating type except the tempura coated product. The tempura product fryer has a submerger to keep the product under the oil surface. The others are carried on conveying belt. All of these fryer conveyors have the bottom dredging capability. The sludge from the bottom of the frying pan is collected into a sump. The solids are separated in a series of paper and metal screen filters. The filtered oil is put back into the fryer continuously.

**18.1.2. Special Challenges in Frying Coated Products** Several products are easy to handle such as potato chips, tortilla chips, corn chips, and so on. On the other hand, the coated products are different and require more involved handling before and during frying.

Coated products have to go through the batter application and the dusting procedure. The product feed to the fryer is also specific to reduce any marriage (joining) of the individual pieces. The interior design of the fryer is critical for conveying the product through the fryer. There must be a submerger for the product that tends to float in the fryer. Accumulation of the fines can get burned and impart a dark color to the product and/or burnt flavor to the product.

It is important to “set” the outer surface of the coating in hot oil before the product touches the internal conveyor of the fryer. This zone is called the “free-fry” zone, which is located at the beginning of the fryer. This applies to coated products, potato chips, and various other fried products. This prevents sticking of the product to the fryer conveyor or to each other (clumping) before the product reaches the frying zone. The free-fry zone and the in-feed system vary with the product to be fried.

Both direct and indirect heated fryers can be used for frying coated products equipped with proper feed and conveyor systems.

## 18.2. French Fries

French fries are similar to fish or meat patties in physical behavior in that the product does not float. French fries are fried with and without the coating. The coated products have become popular. There are different cuts. The straight cut is most common. The other types of cut are Julienne and spiral.

French fries use potatoes with a high solid content. However, the potatoes contain 80–82% moisture. The process for making French fries is involved. The various steps are outlined below:

1. The potatoes are peeled and washed. French fries processors generally use steam peelers.
2. The washed potatoes are cut typically  $0.29 \text{ inch}^2$  ( $7.4 \text{ mm}^2$ ) cross section in water flume and using an in-line water knife.
3. The potato strands are washed to remove the surface starch as they are conveyed to a strainer. Excess water is drained through the strainer.
4. The raw potato strands are blanched in a two-stage blancher:
  - a. The first stage is maintained at  $165^\circ\text{F}$  ( $73.9^\circ\text{C}$ ). The product stays there for 7 minutes.
  - b. The second stage is operated at  $175^\circ\text{F}$  ( $79.4^\circ\text{C}$ ). The product stays there for approximately 3 minutes.
5. The blanched product is then soaked for 45–60 seconds in a vat containing 0.6% solution of sodium acid pyrophosphate (SAPP), solution maintained at  $145^\circ\text{F}$  ( $62.8^\circ\text{C}$ ).
6. The soaked product then passes through a dryer. This is operated in three sections, maintained at approximately  $83^\circ\text{F}$  ( $28.3^\circ\text{C}$ ). This is to achieve equilibration of the moisture in the product before it is fried.
7. The product is fried in a French fry fryer for 1 minute and 5 seconds, with an oil inlet temperature of  $365^\circ\text{F}$  ( $185^\circ\text{C}$ ).
8. The product is then conveyed into a freezer, directly from the fryer.
9. The freezer has two zones, the first zone is typically maintained at  $-14^\circ\text{F}$  ( $-25.6^\circ\text{C}$ ) and the second zone is maintained at  $-26^\circ\text{F}$  ( $-32.2^\circ\text{C}$ ). Typical residence time in the freezer is 20 minutes.
10. The product is collected in containers and stored at  $-10^\circ\text{F}$  ( $23.3^\circ\text{C}$ ). They are later packed in paper bags, with inner plastic lining.
11. The packed product is stored back in the same freezer, maintained at  $-10^\circ\text{F}$  ( $23.3^\circ\text{C}$ ).
12. Moisture content of the product at this point is approximately 64% to 65%, and the oil content is approximately 14% to 18%.

### 18.3. Comments on Par Frying Process

Par frying offers large-scale product and distribution capability. A par-fried product absorbs less oil than in a fully fried product. Lower oil pickup by the product increases the oil turnover time in the par-fried product fryer, causing high degradation to the oil. This produces high concentration of free radicals (oxidation products) in the fryer oil. The par-fried product absorbs the oil containing a high concentration of free radicals. These free radicals then catalyze the oxidative degradation process of the oil in the product during storage.

Subsequently, during final frying of the par-fried product, an exchange of oil takes place between the par-fried product and the frying oil at the finishing fryer. This increases the free radical concentration in the finishing fryer oil, causing a rapid oxidative breakdown of the frying oil. This is why the par-fried product develops an unpleasant oil flavor when the product is held in a package after finish frying. This oxidation in the product cannot be minimized even when the refried product is packaged in metallized bags with nitrogen flush. The product exhibits a fraction of the shelf life of the same product fried under normal process (not par fried and refried). Therefore, this process cannot be used for large-scale manufacture of par-fried salty snack food to distribute them to the final processors for refrying and sales.

## 18.4. Other Common Fried Snacks

**18.4.1. Donuts** Fresh donuts are popular and used for breakfast or snack. These are made from yeast-raised dough and fried in hydrogenated shortening to provide the taste and the mouth feel. Shelf stable donuts are sold in the super markets, convenience stores, or gas stations. These products are generally baked, instead of being fried.

**18.4.2. Fried Nuts** Peanuts, cashews, sunflower seeds, pumpkin seeds, and so on are sold in various forms, such as fried, dry roasted, coated, and glazed. The fried nuts absorb very little oil from the fryer. This makes the oil turnover time in a fryer very long. Oils used in this process must have good oxidative stability in order to have good product shelf life.

## 19. HEAT WAVE FRYERS

Figure 16 shows the picture of the heat wave fryer developed by Heat & Control Co. In this fryer, the individual product pieces travel in a miniature frying basket



**Figure 16.** Heat wave fryer (Courtesy of Heat & Control Co.). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

that is the part of a fryer conveyor. Hot frying oil gently cascades over the product being fried. This fryer has several advantages over the conventional fryers that are used for frying formed products, nuts, and so on. Because of the gentle treatment of the oil, there is significantly lower oil oxidation.

## **20. EVOLUTION OF THE FRYING INDUSTRY INTO DIVERSE PRODUCTS**

There has been a great deal of evolution in the frying industry from the early days of frying potato chips in kettles. The fryers have become larger and more sophisticated in terms of product-feed, internal construction, oil temperature control, and distribution.

Efforts have been made to reduce the volume of oil in the frying system in order to reduce the oil turnover time to preserve the quality of the oil.

Batch fryers have traditionally been used to produce smaller volumes and harder texture in the fried chips. New continuous fryers have successfully duplicated the harder texture of the kettle-fried chips, but at a much higher production rate.

Continuous fryers with very specialized design of the frying bed have been introduced to fry preformed chips with very low oil turnover time. These products require very short fry time, and the traditional design for the fryer for these products shows a high degree of oil degradation because of high oil turnover time.

Par-fried products, such as French fries, potato nuggets, chicken, chicken fried steaks, and so on are all par-fried products that are frozen immediately after frying and stored at  $-5^{\circ}\text{F}$  ( $-20.6^{\circ}\text{C}$ ) to  $-10^{\circ}\text{F}$  ( $-23.3^{\circ}\text{C}$ ). The product is distributed in freezer trucks. These products are fried directly from “Freezer to Fryer” without any thawing and served immediately at the restaurants, food services, and even at homes. These products have the great advantage of convenience and reduced cost. Products, such as par-fried French fries, chicken, and coated vegetables, require shortening with a fairly high level of solids, which can be achieved through the standard hydrogenation process as it is done in the United States and several other countries. Batter-coated fish-fillet is fried in either lightly hydrogenated oil or nonhydrogenated oil. The storage temperature of  $-5^{\circ}\text{F}$  ( $-20.6^{\circ}\text{C}$ ) to  $-10^{\circ}\text{F}$  ( $-23.3^{\circ}\text{C}$ ) helps protect the oil from rapid oxidation. This allows the food processors use nonhydrogenated oils.

Three countries, the United States, Canada, and the Netherlands are the major producers and exporters of frozen French fries. According to the Department of Commerce, U.S. Census Bureau, Foreign Trade Statistics, the United States exported nearly 316 million dollars worth of frozen French fries in 2002. The other par-fried frozen products are fish sticks, breaded shrimp, and par fried chicken.

## **21. LOW OIL SNACKS**

The consumers started to look for reduced fat in food including snack food in the eighties. The demand for reduced-fat and no-fat product was rising in the nineties,



**Figure 17.** Low-oil potato chip-making system (Courtesy of Heat & Control Co.).

but it tapered off and the demand has not increased. However, there is a market for reduced-fat snack food.

According to the Food & Drug Administration, the food can be called “Reduced Fat” if the oil content is reduced by 33 1/3% of the original (standard) level.

Low-oil potato chips are made using the following steps:

1. Potato chips are partially dehydrated in the fryer (moisture content 8–12%).
2. The partially dehydrated fryer then passes through a de-oiler, where some of the oil from the surface of the product is blown off by high-velocity steam, air, or nitrogen at high temperature.
3. The cooking of the chips is completed in the de-oiler.
4. The moisture content of the finished product is around 1.5%.
5. The finished product contains 1/3 less oil than the conventional potato chips.
6. The product is handled in the same manner as regular potato chips.

Figure 17 shows the picture of a low-oil chip-making system made by Heat & Control Co.

## **22. GENERATION OF FINES AND THEIR REMOVAL**

The fines accumulate in the fryer pan during frying. These are primarily carbohydrate material (can be proteinaceous material from poultry, meat, or fish). The crumbs get charred during frying. Thus, accumulation of fines can darken the fryer oil. This leads to darker colored product with burnt taste. This is why it is necessary to remove the fines from the fryer either continuously or at a certain frequency. The coated products generate a lot of fines accumulated at the bottom of the pan.

One can remove the fines from the fryer by using a continuous filtration system. Certain amounts of fines accumulate in the fryer even after using a continuous filter. Fryer sanitation removes the residual fines from the fryer. A continuous oil filtration



**Figure 18.** Rotary oil filter (Courtesy of Heat & Control Co.).

system is highly desirable for products that generate large amounts of fines during frying. Indirect-heat fryers with continuous and high oil flow make it easier to remove a large portion of the fines from the fryer via the continuous oil filtration system. However, with very high accumulation of crumbs or sludge, it is recommended to have a set of bottom-dredging bars that push the accumulated material from the fryer pan into a filter. The filtered oil is recycled through the fryer with appropriate system design.

Various types of filters are used to remove fines. Paper filters are not the best. They cause high oil loss and the hot oil is exposed to air for long. The fryer system generally contains a filter to remove the coarse material from the oil. Sometimes, this is augmented with a finer filter that removes the smaller particles from the fryer oil. In many applications, one needs to use a combination of a motorized and a centrifugal filter (or a rotary drum filter; see Figure 18).

The type of filter for a fryer should be carefully chosen based on the size, the amount, and the degree of hardness of the fines to be removed from the fryer oil. For products that produce heavy amounts of fines, such as breaded products, sugar infused products, and so on, a bottom-dredging system is used as described earlier. This is a specially designed conveyor system. The fryer manufacturer should be informed of the need for heavy fine removal during frying.

Sometimes, a continuous filtration system is used to clean the fryer oil. In this process, it is recommended to take out approximately 5% of the fryer oil, filtered through an in-line filter, and returned to the fryer. This system can be good, but in most cases, the flow of oil through the fryer is not uniformly distributed because of various factors. This allows fines to accumulate on the fryer pan at various spots and not be removed by the filter. In other words, a continuous filter of this type is as good as the system's capability to push all the fines through the filter, with minimum accumulation in the fryer.

In many operations, an oil treatment system is used. This takes out the fines and treats the oil to reduce free fatty acid, color, and oxidized material and soap from the fryer oil. This has been proven to improve the fry-life for the oil and extend product shelf life. The oil can be treated at the end of frying or during frying. In the former case, a batch system is used. In the latter case, a small amount (typically 5%) of the fryer oil is treated through a system.

There are many treatment agents that are commercially available for oil treatment. Although oil treatment can be beneficial, not every treatment material on the market is satisfactory. Some of them reduce the free fatty acid via acid/base reaction. The base metals in these materials are typically calcium and magnesium salts that react with the free fatty acid, forming calcium and magnesium soaps. These soaps in the oil cause rapid rise in free fatty acid and cause rapid oil oxidation. Therefore, one must make certain that the reduction of the free fatty acid is not accomplished via an acid/base reaction (8).

## 23. TERMINOLOGY USED IN INDUSTRIAL FRYING

### 23.1. Batch Versus Continuous Fryer

Both batch and continuous fryers have been discussed earlier.

## 24. FRYER CAPACITY

The fryer capacity is chosen on the basis of the production volume needed for the business. Various factors are taken into account to determine the actual capacity of a fryer, as outlined below:

1. The number of operating hours per day (8, 16, or 24).
2. Frying time needed for proper moisture and texture control of the product.
3. Frequency of fryer sanitation required.
4. Company's warehousing and distribution system.
5. Required product code date based on the shelf life and sales and distribution requirements.

The above information is used to determine:

1. The physical dimension of the fryer.
2. Belt width.
3. Belt loading, defined as:
  - a. Product in pounds per square-foot area of the belt
  - b. Pounds of product per foot of the belt
  - c. The number of pieces per linear foot

## 25. KEY POINTS IN DETERMINING THE FRYER SIZE

It is important to have the following information to size a given fryer.

1. Desired production rate in pounds per hour.
2. Physical dimension for the product.
3. The fry time required.
4. The belt loading, typically in pounds per square foot (kilograms per square meter).

### 25.1. Fryer Size

This indicates the number of pounds of product produced per hour in a given fryer. For example, PC 2000 means it is a continuous potato chip fryer that is capable of producing 2000 pounds of fried potato chips per hour under standard operating conditions.

### 25.2. Numbers Used to Designate the Physical Size of a Fryer

A number like 2410 indicates that the fryer pan (in a fryer) is 24'' wide and 10 feet long.

### 25.3. Cook Area

Cook Area = Fryer pan width  $\times$  cook length = square feet. In the above example, the fryer has a cook area of  $2'(24'') \times 10 = 20$  sq.ft.

### 25.4. Cook Time

It is the time that the product takes from the time it enters the fryer and when it goes out as fully cooked. This time is dependent on the type of product and the desired moisture content for the final product. This also depends on the fryer design and heat input among others.

### 25.5. Belt Loading

This is the weight of product per square foot of fryer surface. It is expressed as lb/ft<sup>2</sup> for products like chips or pieces/ft<sup>2</sup> for certain products such as meat patties or fish fillet with a specified weight per piece of product.

Example:

Desired Production Rate	= 3000 pounds/hr (1367 kg/hr)
Recommended Product Loading	= 1.5 pounds/ sq.ft (0.81kg/m <sup>2</sup> )
Cook Area Needed	= (3000 pounds/hr.) / (1.5 pounds/sq.ft X 60 min.)
	= 33.3 sq.ft. (2.97 sq.m)



## 25.6. Calculating the Fryer Length

Process (fry-time)	= 1 min.
Assume Fryer width	= 3.33 feet (1.01 m)
Fryer Length	= 33.3 sq. ft /3.33 ft = 10 feet
	= 2.97/1.01 = 2.95 m

## 25.7. Direct Heating

The oil is heated by the hot flu gas from the burner. The hot gas passes either through the tubes that are immersed in the oil or through a heat chamber directly located under the fryer pan.

## 25.8. Indirect Heating

Indirect heating is oil heated by hot thermal fluid that is heated in an external heater and then pumped through a bunch of tubes that are immersed in the oil.

## 25.9. External Heat Source

In this type of system, the oil from the fryer is pumped continuously through an external heater and returned to the fryer. The heater uses either high-pressure steam or gas-fired burners as the source of heat. The gas-fired heaters can be of direct or indirect heating type. In the direct heating type of a gas-fired heater, the oil passes through a bunch of tubes and the hot flu gas heats the oil from the outside of the tubes. The type of oil heating system can overheat the inner film of the oil inside the tube, damaging the oil. In an indirect heat system, the flu gas passes through a bunch of tubes. Ambient air is allowed to pass across these tubes when the air becomes heated. The heated air then heats the oil passing through a “tube bundle” (a number of tubes that are bundled together in the heat exchanger). This system is gentler to the oil film inside the tubes.

## 25.10. Oil Film Temperature

Fluid passing through a pipe forms a film along the inside of the tube. The thickness of this film decreases with increased flow rate of the fluid. Oil temperature at this film is significantly higher than the temperature at the center of the pipe or the mass average temperature of the oil passing through the tube. In direct gas-fired oil heaters, the oil film just inside the pipe wall can be heated to a very high temperature. This damages the oil, resulting in lower product shelf life, unless this film temperature can be controlled. In an indirect gas-fired heater, the film temperature can be maintained at a much lower value, providing better oil quality in the fryer.

## 25.11. Delta-T

This is the temperature difference between inlet (feed-end) and outlet (discharge-end) seen in the fryer when it is operated at the designed capacity. When the fryer is

idle, the temperatures at the inlet and outlet of the fryer are essentially the same (i.e., the Delta-T is Zero). A temperature differential is established between the inlet and the outlet of the fryer once frying resumes. For example, the delta-T is 40°F (4.4°C), when the oil inlet temperature is 360°F (182.2°C) and the outlet temperature is 320°F (160°C). The value of delta-T depends on the moisture content of the incoming feed and that of the final product. This temperature differential is critical to achieve the desired product characteristics (dehydration rate, amount of dehydration, development of texture and color, etc.).

In an indirect-heat fryer, this temperature differential can be designed into the system to a precise degree. By designing the oil flow through the fryer at a specific velocity range, the energy (heat) absorbed by the product at fryer inlet can produce a predictable temperature gradient (Delta-T) down the length of the fryer. Most direct-fired fryers have dual-control heat banks (either fire-tubes or thermal radiators) that can be set to different temperature set-points at each end of the fryers and can provide a fairly reliable Delta-T.

**25.12. Oil Turnover**

This is the theoretical time required by the product to pick up all the oil from the fryer filled to its designed level in the pan. This is expressed in hours. Sample calculation for oil turnover time is shown below:

Fryer requires 4000 lbs. of oil to fill to the designed capacity.  
 Pounds of finished product            2000/hr.  
 Oil content of the fried product        25%  
 Oil picked by the product                 $0.25 \times 2000 = 500$  lbs/hr.  
 Oil turnover time                             $4000$  lbs of oil/ $500$  lbs per hr.  
                                                            $= 8$  hrs.

Eight hours is considered desirable for designing a potato chip fryer. In actual operation, the turnover time is 9.5 to 10 hours. This is because the startup, shut-down, product changeover, and mechanical down time reduce the fryer utilization to 80–90%.

Oil turnover times for various fryers are listed below.

Type of Fryer	Actual Oil Turnover Time, Hours
Potato chips	9.5 to 11.0
Tortilla chips	6.5 to 8
Corn chips (Extruded Product)	4.0 to 5.0
Batch (Kettle) fryer	20 to 30
Restaurant fryer	18 to 20 days

**26. HEAT LOAD REQUIREMENT**

This is the theoretical amount of heat required to cook a product without considering system loses. This is expressed in BTU/lb or CHU/kg. The fryer

manufacturers can provide the net heat loads for individual products. These databases are treated as their trade secrets. No published values can be found in the literature.

The primary function of a fryer is to remove the moisture from the product being fried. The fryer oil supplies:

1. The heat of vaporization of the free water present in the food.
2. The sensible heat to the product to heat it up to the frying temperature.

The majority of the heat is needed for vaporizing the free moisture from the product. There are several areas in the process where thermal energy is lost. Therefore, the thermal capacity of the fryer must be in excess of the theoretical amount of energy required just for the dehydration and heating of the product. Following are the examples of the areas where heat is lost during frying and must be taken into account in designing the oil heating system:

- Heating the product feed from room temperature to the final fryer temperature.
- Dehydration of the fried product.
- Rate of dehydration of the product.
- Heating all physical equipment, including the piping, filters, and so on.
- Radiation heat loss from the fryer, furnace, and other ancillaries.
- Heat loss through the fryer exhaust.
- Heat loss through the gas heater flu.
- Thermal efficiency of the heat exchange system.

Heat load calculations are made to determine the actual heat load requirement for designing a fryer for a specific product and the production volume.

The typical heat required for various products are listed below:

Product	Frying Oil Temperature °F	Heat Requirement BTUs/lb
Potato Chips	370 (188°C)	4500 (1136 CHU)
Tortilla Chips	375 (190.6°C)	1500 (378.8 CHU)
Corn Chips	390 (199°C)	2250 (568.2 CHU)
Batter-Coated & Par-Fried Prod.*	375 (190.6°C)	350 (88.4 CHU)
Egg Rolls & Burritos**	355 (179°C)	200 (50.5 CHU)
Nuts	325 (163°C)	500 (126.3 CHU)
Fried Snack Pies	365 (185°C)	1000–1500 (252.5–378.8 CHU)

\*Heat is used only for setting the crust and not for cooking the fish inside.

\*\*This is only to fry the shell. The filling inside is precooked.

**Data: Courtesy of Sea Pack Corp.**

As one can see, these numbers vary greatly with the type of product. Potato chips require the highest amount of heat energy because of the high moisture content of the raw potatoes (around 80% moisture).

One must consider the following factors in order to estimate heat load for fryer design:

1. Temperature differential across the length of the fryer, known as “Delta-T”.
2. Recovery time or the response time for the heating system to maintain proper oil temperature.
3. Minimizing temperature overshoot.

**26.1. Example of Frying Heat Load Calculation**

Product type: 3-pass coated (pre-dust, batter, bread) Chicken Tender

Production rate:	4000 #/hr
BTU Requirement:	350 BTUs of heat per pound
Heat required:	$4000 \times 350 = 1,400,000$ BTUs/hour

The above-estimated thermal requirement must be corrected for the various sources of heat loss listed above.

**26.2. Recovery (Response) Time**

This represents the capability of the fryer to respond to increased frying load. This is influenced by several factors:

1. Available thermal energy beyond the amount required to fry the product at the designed rate of production with compensation for all sources of heat loss.
2. Type of heat exchanger used in the system.
3. Tube-fired (direct-fired) fryers have the slowest response rate. These systems take the longest time to heat up or cool down the oil in the fryer.
4. There is always a time lag between the time the set point is increased on the controller and the time the oil begins to indicate any change in temperature. This causes a 10°F (5.6°C) to 15°F (8.3°C) swing in the temperature of the fryer oil.
5. Temperature response is more predictable in a direct heat fryer heated by thermal fluid radiator. This is primarily because the system uses thermal fluid flow through the radiator to regulate the heat supply to the tubes. When the internal thermostat senses the need for a change in the heat requirement, a control valve regulates the flow of thermal fluid through the radiator. A temperature swing of 7°F (3.9°C) to 10°F (5.6°C) is common for this type of heating system.

6. Indirect heat external heat exchangers have the fastest response to the change of heat load requirement. It also has a temperature swing of  $+/- 2^{\circ}\text{F}$  ( $1.1^{\circ}\text{C}$ ). This can be further reduced by using continuous fresh oil makeup and precision oil flow.

## **27. AIR REQUIREMENT (FOR COMBUSTION)**

A combustion system requires air in two parts as described below:

1. Air needed for the primary combustion.
2. Air required for the secondary combustion.

The primary air for combustion is generally sufficient for complete combustion of the natural gas. However, complete combustion of the fuel (natural gas) is never attainable without adding a secondary stream of air to the initial product of combustion. This is called the secondary air for combustion.

Besides the total amount of air for combustion, the plant needs the supply of air for the room and replenish the air lost through the exhaust system. Therefore, the air handling system must be carefully designed so that there is no shortage of air for any of the systems mentioned above.

## **28. PRODUCT BULK DENSITY**

This is an important consideration for designing the fryer. For example, tortilla chips show some increase in specific volume after frying. However, the pellets expand tremendously in volume during frying. The internal design of the fryer must accommodate for this increase in volume. Product bulk density also impacts the size of packaging.

## **29. OIL QUALITY MANAGEMENT**

Oil quality is better maintained in a continuous fryer compared with a batch fryer. This occurs primarily because of the lower oil turnover time. However, the oil quality can become poor and produce a reduced shelf-life product if the oil is not properly handled even outside of the fryer. It is important to receive high-quality fresh oil, store them under nitrogen, and use them under proper conditions (discussed in the chapter on Frying Oils).

Most commercial frying operations monitor the FFA in the fryer oil to determine its quality. Based on the discussions in the chapter on frying oils, one should be able to appreciate that this is not the best measure for fryer oil quality. Some manufacturers analyze their fryer oils for para anisidine value, PM, and polymers. Others focus on FFA and PM analysis. There is no general rule for oils from fryers of every

type of product because the shelf-life prediction cannot be made for every product based only on one oil quality parameter.

The oil quality in frying is better managed by operating the fryer at its designed capacity and for long hours at a time. The fryer should be shut down only for sanitation at the end of the week's production, product changeovers, and unforeseen mechanical issues.

The fry life of the oil is further improved by timely and proper sanitation of the frying system. This involves washing the frying system with caustic or sanitation chemicals, rinsing, and neutralization of the residual soap or caustic left in the system after rinsing the fryer post the caustic wash.

### **30. FRYER SANITATION**

The hood and other inaccessible parts of fryer are equipped with spray nozzles as part of the cleaning-in-place (CIP) system installed by the fryer manufacturers. The CIP systems are common with equipment designed for operation in the plants processing meat, poultry, or fish where daily sanitation of the frying system is mandatory. However, the fryer pan, the piping, and the external oil heater must be washed with caustic solution or sanitizing chemical to remove the residual oil and crumbs left after the fryer is emptied.

Specific logistics for cleaning may vary depending on the type of fryer. For example, a direct-fired fryer with no oil recirculation will require more manual procedure compared with that with an oil recirculation pump because the pump can move the caustic solution through the entire system. Similarly, a kettle fryer will require manual scrubbing for sanitation, whereas a continuous potato chips fryer can use the recirculation pump to get the cleaning action.

Fryer sanitation is essential for maintaining the fryer oil quality. Any amount of residual oil as well as crumbs can catalyze the oil degradation reactions.

### **31. SUMMARY**

Fried food is an important segment of the food industry. There are two segments, namely, (1) the restaurant/food service sector and (2) the industrial packaged product sector. Both sectors use fryers that are designed to produce specific types of products. Par frying has become a significant part of the frying industry. This has provided the convenience and economic benefit to the restaurant and the food service sector.

The restaurants and food services consume the fried products soon after their preparation. Therefore, the shelf life of the product is not a significant factor for these products. The industrial products are sold in various types of packages that protect the products from becoming rancid during storage and distribution. The oil in the restaurant fryer degrades rapidly because of the constant exposure to high temperature and the extended hours of idle time. The continuous fryer

maintains better oil quality. However, frequent shutdowns, lack of sanitation, and overheating of the oil can cause rapid degradation. The kettle fryers are more gentle than the restaurant fryers on the oil but significantly poorer than the continuous fryers.

## REFERENCES

1. P. J., C. P. Su, and M. Gupta, in American Oil Chemists' Society Annual Meeting, Kansas City, Missouri, Abstract in AOCS program book, 2003, pp. 45–46.
2. Richard F. Stier, Consulting Food Technologist, personal communication.
3. D. Firestone, "Regulatory Requirements for the Frying Industry," in M. K. Gupta, P. J. White, and Warner, eds., *Frying Technology and Practices*, AOCS Press, Champaign, IL, 2004.
4. America Snack Food Association website.
5. *Snack Food & Wholesale Bakery*, **91**: SI-5 (2002).
6. M. M., Blumenthal, *Optimum Frying: Theory and Practice*, Libra Laboratories, Inc., Piscataway, New Jersey, 1987.
7. M. M., Blumenthal, *Food Technol.*, **45**:68 (1990).
8. C. K. Chow, and M. K. Gupta, Treatment, Oxidation and Health Aspects of Fats and Oils: Technological Advances in Improved and Alternative Sources of Lipids, Blackie Academic & Professional, 1994, p. 328.

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## *Fats and Oils in Feedstuffs and Pet Foods*

**Edmund E. Lusas and Mian N. Riaz**

Fats and oils are used for many purposes in animal feeds and pet foods, including the following:

- Increasing caloric density of feeds (by about 2.25 times that of similar dry weights of proteins or carbohydrates).
- Improving feed palatability and appearance.
- Reducing total feed intake, increasing feed efficiency, and minimizing feed costs.
- Increasing blood glycogen levels and endurance in working animals like horses and sled dogs.
- Lowering the heat of reaction during digestion and metabolism—important for comfort and productivity of large animals in hot weather.
- Delaying digestion of feedstuffs beyond the rumen by use of inert forms of fats and coatings.
- Providing needed molecular structures through dietary essential fatty acids (EFA) and phospholipids.
- Improving appearance of skin and hair, and prevention of dermatitis.
- Modifying fatty acid profiles in “designer food” animal products.



Carrying fat-soluble vitamins and color compounds.

Binding heat-sensitive flavorings, vitamins, medications, and “instant gravy” mixes to pet foods and feeds after extrusion or drying.

Improving dispersion of dry mixes, e.g., lecithin in calf milk replacers.

Preventing segregation of mixed feeds.

Reducing dustiness of feeds and feeding operations and of grain elevator dust.

Lubricating feed processing machinery.

Nutritional values of fats and oils differ with fatty acid composition, with the species, age, physiological stage, environment of the animal, and adequacy of the overall diet. Care should be exercised in regard to the following:

Avoid overtaxing the fat-handling capabilities of digestion systems of the respective species, especially young animals and ruminants.

Avoid contamination of fats, oils, and oil-bearing materials with toxic or unwholesome constituents.

Handle oil-bearing feedstuffs with active antinutritional or toxic constituents, including mycotoxins, properly and legally.

Avoid excessive levels of fat that interfere with making cohesive pellets (more than 4%) or restrict the desired puffing of starches and development of lamellar soy protein textures (more than 6%) in extruded feeds.

Edible animal fat in the United States can be rendered only in food grade plants under inspection of the U.S. Department of Agriculture (USDA) (1). The majority of tallows and greases used domestically in animal feeds and pet foods are feed grade. The National Renderer’s Association (NRA) describes rendering as a process that

heats raw animal by-products to release fat (71.1–82.2°C; 160–180°F) and remove moisture (115.6–126.7°C; 240–280°F). Ninety percent of the fat is removed from the protein by presses, leaving approximately 10% in the protein meal. Fat quality is determined by hardness, color, moisture, impurities, stability, and free fatty acids (FFA) content.

In the United States, inedible fats are called *tallows* if, after saponification in the American Oil Chemists’ Society’s titer test (AOCS Cc 12-59), solidification occurs above 40°C (104°F), and *greases* if solidification occurs below this temperature (1). The demarcation temperature varies among countries and 38°C (100.4°F) is used by some. Throughout this chapter, the term *tallow* refers to both tallows and greases, which also may contain vegetable oils, recycled by renderers.

*Fat* and *oil* usually refer to semisolid and liquid forms of triacylglycerols (triglycerides), respectively, although the use of these terms is not consistent throughout industry. In this chapter, the term *fats* means the group of commercial lipids (mono-, di- and triacylglycerols, fatty acids, and phospholipids), whether in solid or liquid

state. In endorsement of the movement to improve the value of by-products of various agribusiness operations, the term *coproduct* is used throughout this chapter, except where established regulations are being quoted.

Individuals who are not fats' and oils' chemists may benefit from the following orientation. Polyunsaturated fatty acids is abbreviated as PUFA. Essential fatty acids (EFA) are required by the host animal but are not synthesized and are available only through the diet. The notation C18 indicates a fatty acid chain, 18 carbons long. C18:0 and C18:3 identify the number of unsaturated ("double") bonds. C18:0 represents a completely saturated fatty acid, 18 carbons long, in this case, stearic acid. C18:3 represents a fatty acid, 18 carbons long and with 3 unsaturated sites, but does not indicate the location of the double bonds.

Linolenic acid (9,12,15-octadecatrienoic acid; IUPAC convention) is also 9,12,15-C18:3, meaning an 18-carbon fatty acid with double bonds occurring after the 9, 12, and 15 carbons counted from the carboxyl end of the chain; this is the major linolenic acid isomer and also is called  $\alpha$ -linolenic acid. Another C18:3 fatty acid that has received considerable interest from nutritionists in recent years is 6,9,12-octadecatrienoic acid ( $\gamma$ -linolenic acid), actually a reduced member of the linoleic family. The symbol  $\Delta$  has been retained from the Geneva naming convention for limited use here. For example, a  $\Delta 5$  desaturase enzyme installs a double bond after the fifth carbon, counting the carboxyl carbon as number one. When working with polyunsaturated fatty acids, sometimes it is more convenient to count from the methyl end. The terms omega ( $\omega$ ) and  $n$  are sometimes used for this purpose. The notation C18:2 $n$ -6 signifies linolenic acid, an 18-carbon chain fatty acid with two unsaturated positions, the first one occurring after the sixth carbon atom counting from the methyl end. (Linolenic acid is represented by C18:2 $n$ -3.) Throughout this chapter, the all-cis forms of fatty acids are intended unless identified otherwise.

## 1. HISTORY

### 1.1. Evolution of Uses

Occasional feeding trials of extracted fats and oils to animals have been reported in the United States since about the 1890s when the first state agricultural experiment stations were established. However, significant quantities did not become available at prices affordable for volume feeding until the late 1940s. Feeding applications in the 1950s and 1960s focused on high energy broiler rations and on improving palatability of dry pet foods. Research on feeding fats and oils to swine and ruminants grew rapidly in the 1970s and 1980s, and was extended to aquaculture species in recent years.

### 1.2. Demands for Better Feeds and Foods

Improvements in genetics and biotechnology manipulations of metabolic systems, including feeding of growth-stimulating hormones (bovine somatotropin, BST, and

porcine somatotropin, PST), have developed animals with increased capabilities for producing milk and meat. Increasing the caloric and amino acid intakes to realize these abilities are difficult with traditional feedstuffs, and calorie-dense and nutritionally balanced feedstuffs are increasingly sought.

Concerns by the public about the amounts and types of fats in the human diet have led to increased demands for poultry, fish, and leaner red meats. The latter need is being met by hand and mechanical trimming until animal genetics and feeding practices are developed to produce lean animals directly. Various snack and convenience food manufacturers, and fast food shops, have changed from frying in tallow to frying in vegetable oils. Currently, rendered animal fats and inedible recycled tallows are in surplus in the affluent countries and are sold at prices that place them among the lower cost sources of concentrated metabolizable energy for feed uses.

The types of fat fed affect the fatty acid composition of animal tissues (meat) and products (milk and eggs) as well as the fat metabolism in consumers of these products. This has led to the “designer foods” concept—animal products with fat compositions intentionally altered by feeding for human health benefits (2). Although nutritionists are not fully in agreement on optimum fatty acids profiles, designer eggs with increased contents of linolenic and other *n*-3 PUFA fatty acids are being marketed. The question of which fats or fractions are suitable for achieving the desired designer foods is materializing and may dominate feeds applications research for the next decade or longer.

### 1.3. Feeding of Whole Oilseeds

Oilseed producers have recognized that it may be more profitable to process and feed whole oilseeds on the farm, rather than sell them into the elevator–oil mill–refinery infrastructure and purchase extracted protein meals, tallows, and spent restaurant yellow greases (mixtures of animal fats and vegetable oils). Technologies have been developed to minimize the effects of natural toxic and growth-inhibitor components in raw cottonseed, soybeans, and canola seed. Feed processors and livestock feeders can now switch between using in-seed and extracted fats and oils, depending on price. Both options must be considered in a comprehensive review of feeding fats.

## 2. INFORMATION SOURCES, AUTHORITIES, AND OBLIGATIONS

### 2.1. Early Technology Transfer

The modern feeding of commercial animals and pets has become a quantifiable, information-based science with alternative choices and accompanying responsibilities. The exploration and codifying of animal nutritional requirements and feedstuff nutrient compositions have long been ongoing processes. Major contributions to transferring knowledge have been made by the 22 editions of *Feeds and Feeding*, published from 1898 to 1956 (by W.A. Henry and later by F.B. Morrison). This

bible was succeeded in 1978 by *Feeds and Nutrition—Complete* (3). Updated information is published in the more compact *Feeds and Nutrition Digest* (4). Other feeding and feedstuffs reference books also have been published during the years (5,6). The newer editions of books on feedstuffs and feeding management are more guarded in generalized statements about nutrients metabolism than publications of a decade ago. This is probably because different metabolic pathways are being found between species as well as alternative pathways within the same species.

## 2.2. National Research Council Nutrient Requirements

The developing knowledge about nutrient requirements of different species in various life stages has been monitored, interpreted, and summarized by expert subcommittees of the Committee on Animal Nutrition of the Board on Agriculture of the National Research Council (NRC). Various editions of *Nutrient Requirements* are currently available for beef cattle (7), cats (8), dairy cattle (9), dogs (10), fish (11), goats (12), horses (13), mink and foxes (14), nonhuman primates (15), poultry (16), rabbits (17), sheep (18), and swine (19). Each publication contains recommendations for various life stages of the respective species, a literature review and bibliography of feeding trials, and tables of ingredients commonly fed to that species. Respective nutrients composition, digestibility, and metabolizable energy values are available in the more recent editions. Gradually, the older general nutrients composition references, including the *United States–Canadian Tables of Feed Composition* (20), are being replaced by species-specific information.

Some of the recent *Nutrient Requirements* publications, notably for dairy cattle (8) and horses (13), include computer discs with interactive programs for calculating nutrients requirements based on age, weight, sex, physiological stage, and work or performance of the animal. Various compendiums of feedstuff compositions and animal requirements, based on NRC publications, are published periodically, including the annual *Feedstuffs Reference Issue*.

## 2.3. Association of American Feed Control Officials Regulations

NRC nutrient requirements are advisory and have no regulatory status. The Association of American Feed Control Officers (AAFCO) establishes the official definitions and names of feed ingredients, nutritional and labeling requirements for dog and cat foods, regulations on medicated feeds, labeling practices, and other requirements for selling feeds. AAFCO official ingredient names must be shown in listings on the product package, tag, or delivery invoice, in order of diminishing content for fixed (“closed”) formula products and percentage-wise for open formula mixed feeds. Uniform regulations have been developed to expedite interstate commerce in feedstuffs, but each state also has reserved the right to establish independent regulations and interpretations. All commercial feedstuffs must be registered directly with the feed control official in each state where sold. AAFCO definitions and regulations are updated and published annually in the *Official Publication*. Copies can be obtained by contacting local feed control officials.

Ingredients and regulations continuously evolve. For example, the 1994 *Official Publication* includes the first listing and temporary status of feed grade “hydrolyzed sucrose polyesters”; these are nutrient-containing coproducts from the manufacture of low calorie fat substitutes. The establishment of an ingredient definition in the *Official Publication* is not an endorsement of its efficacy for specific nutritional objectives, but rather affirmation of its general safety under the conditions of intended use within the scope of current expert knowledge.

## 2.4. International Feed, AAFCO Ingredients, and FDA Numbers

Ingredients in NRC publications are identified by their international feed numbers (IFN) and names, currently developed and maintained by the Feed Composition Data Bank (FCDB) at the National Agricultural Library in Beltsville, Maryland. Ingredient definitions in the *Official Publication* use AAFCO numbers and also include the IFN. Several different IFN specifications and commercial quality trading grades for a similar material may qualify under the same AAFCO name. Some AAFCO ingredients (essentially isolated chemicals) also carry FDA identity numbers under the Food Additives Amendment of the Code of Federal Regulations (21 CFR).

## 2.5. Formulating Feeds for Minimum Ingredients Costs

The published tables of animal nutrient requirements and feedstuffs composition are crude approximations across the industry at best. A formulator usually will be more effective if he or she applies local experience in feeding animals of similar genetic origin under the environmental conditions at hand and relies on actual analyses of the ingredients received or the historical performance of the specific supplier—if such information is available.

Modern techniques for measuring metabolism and instrumental analysis have enabled rapid estimations of animal needs and nutrient availability in specific feedstuffs. Uniformity in feedstuffs, despite origin, is being advanced by global trade, harmonization of product definitions, and trading standards and by total quality management (TQM) programs like the ISO 9000 movement in Europe. The availability and decreasing costs of desktop and notebook computers in the last decade has made the calculation of least-cost feed formulas by linear programming available to many feed formulators. These factors, coupled with instantaneous knowledge of costs and availability of alternative feedstuffs, regionally and globally, through on-line communication networks are rapidly exhausting the undiscovered bargains in feedstuffs. In the future, profits in animal feeding are increasingly likely to result from private knowledge about (1) responses of animals with known genetic abilities, (2) ensuring animal health and comfort in extreme climates and weather conditions, and (3) improved effectiveness in feeding management practices. Claims of improved feed conversion abilities and improved health status already are being made for genetically controlled broilers and swine. Computer programs are now able to formulate feeds at least cost on the basis of digestible, metabolizable, or

net energy, protein, crude fiber, ash, essential amino acids, and specific vitamins and minerals for different growth stages of selected species. Formulas also may be flexed for specific EFA and triacylglycerol structures in the future.

## 2.6. Consequences of Feedstuffs Contamination

The consolidation of feeds processing and rendering into fewer but bigger installations has also set up the conditions for large and widespread economic losses when mistakes or accidents occur. One example is the chick edema and death problems in the early to mid 1970s that were traced to feeding fats and fatty acids contaminated with polychlorodibenzo-*p*-dioxins (21, 22) and polychlorophenols (23) possibly of herbicide origin. This led to purchase specifications that tallows be guaranteed free of chick edema factors by renderers or distributors.

Another example is the 3-year outbreak of polybrominated biphenyls (PBB) poisoning that occurred in Michigan during 1973–1976 from a one-time accidental mislabeling of a toxic fire retardant and its inclusion in an animal feed concentrate mix. An unknown quantity of meat, dairy products, and rendered materials entered the food and recycling chains before the problem was recognized and the involved farms quarantined. By 1978, an estimated 8 million of Michigan's 9.1 million residents had detectable levels of PBB in their bodies (24). Later studies continued to find PBB in samples of serum, body fat, and breast milks of most Michigan residents (25).

Finally, dairy cows fed whole cottonseed that contains aflatoxins, at levels higher than the 20 ppb permitted by FDA, can transfer them to milk in amounts greater than the 0.5  $\mu\text{g}/\text{kg}$  FDA action level (26). State and national programs for monitoring aflatoxin content in milk have been implemented as the feeding of whole cottonseed to cattle has increased and expanded to states not producing cotton. Even nontoxic substances like polyethylene packaging materials, which melt at rendering temperatures can cause nuisance problems by solidifying at 80°C and forming lumps that clog fat application spray nozzles (27).

## 3. AVAILABILITY, CHARACTERISTICS, AND COMPOSITION

### 3.1. Supply and Uses of Feeding Fats

*Stability of Obligatory Coproducts Supplies.* Many fats and oils are generated as obligatory coproducts of other endeavors, with the supply relatively inelastic to price change. Examples include (1) slaughtering and dressing of animals and poultry, with hand trimming of the fatty tissues at the packing plant or in-store meats department; (2) disposal of spent frying oils from restaurants, skimmings from grease traps, and dead animals from pastures and feedlots—all necessary for sanitation, public health, and environmental interests; (3) production of high protein content soybean and fish protein meals; (4) growing of cotton fiber for domestic and world markets, with cottonseed as a coproduct; and (5) development of the

TABLE 1. U.S. Sources of Rendered Animal Fats and Oil (1).

Source	Number Slaughtered (or Kilogram of Product)	Fat Produced	
		Percent of Total	Metric Tons
Steers and heifers	28,000,000	37.3	1,527,000
Cull cows and bulls	7,000,000	3.9	159,000
Market pigs	83,000,000	21.3	874,000
Cull sows and boars	5,000,000	2.2	91,000
Broiler	5,200,000,000	8.7	355,000
Turkey	242,000,000	1.4	57,000
Dead stock	(1,636,000,000)	5.2	213,000
Restaurant grease	(1,023,000,000)	16.7	682,000
Miscellaneous	—	3.3	136,000
Total domestic inedible	—	100.0	4,125,000

domestic corn starch and sweeteners industry, which has generated enough corn germ to make corn oil the second major oil currently produced in the United States.

Because of these situations, the production of the major portion of feed grade fats and some oil-bearing feedstuffs is likely to continue regardless of their market prices. However, future prices will reflect (1) domestic competition between feed, oleochemicals, and detergent industries needs; (2) opportunities to ship into global markets; and (3) abilities to import lower cost fats like palm oil and stearin.

*Sources of Fats, Oils, and Tallows.* The total world production of fats and oils is estimated at 76.2 million MT. It consists of 59.2 million MT of edible vegetable fats and oils (soybean oil, 16.9 million; palm oil 11.5 million; rapeseed and canola oil, 9.1 million; sunflower seed oil, 7.6 million; cottonseed oil, 4.2 million; peanut oil, 3.4 million; coconut oil, 2.9 million; olive oil, 2.1 million; and palm kernel oil, 1.5 million), butter fat, 5.3 million; total marine oils, 1.1 million; and total tallows and greases, 7.0 million (28).

Approximately 4.1 million MT of inedible animal fats are rendered in the United States annually (Table 1). The major sources, in order of decreasing tonnage, are beef packing, pork packing, spent restaurant fats, and broiler and turkey processing. Only about 5% of the total supply of inedible fat is recovered from dead stock (1).

*Use of Domestically Produced Tallows.* Currently, approximately 35% of domestically produced inedible tallow is exported, leaving about 2.7 million MT available for domestic use (1). Rouse (29) reported that domestic use of inedible tallow increased by 63%, from 0.81 million MT in 1950 to 1.3 million MT in 1991. In 1950, about 72% of the available domestic inedible tallow (0.58 million MT) was used in making soap and hardly any in animal feeds. With the development of synthetic detergents, the use of edible tallows in soap making dropped to 0.15 million MT or 12% of the total in 1991, and animal feeds rose to using about 62% of the domestic supply.

The largest use for inedible fats worldwide is in animal feeds. Domestic use in various feeds is shown in Table 2. Approximately 56.2% is used in broiler and turkey feeds, and another 2.7% in feeding poultry layers (29). It has been estimated

**TABLE 2. Estimated Use of Fats and Oils in Domestic Animal Feeds in 1993 (29).**

Type of Feed	Fat Used	
	Percent of Total	Million Metric Tons
Broilers	34.8	591
Turkeys	21.4	364
Pet foods	16.0	273
Swine	10.7	182
Beef cattle	5.4	91
Dairy cattle	4.8	82
Layers	2.7	45
Veal	2.1	36
Fish	2.0	34
Total	100.00	1,698

that inclusion of 3–4% fat in all feeds, a level favored by some nutritionists and feeders, would use about 2.5 million MT of fats in domestic animal feeds (1).

### 3.2. Definitions of Fats and Related Products Used in Feeding

Industrywide definitions for feed fats products are still being developed. USDA and NRA definitions of rendering and tallows and greases were already discussed.

*NRA Recommended Standards and Definitions.* AFOA grading standards for tallows and greases for industrial uses are shown in Table 3 (1). Typical analyses

**TABLE 3. American Fats and Oils Association Grading Standards for Tallow and Grease (1).**

Grades	Titer (Min. °C)	FFA (Max. %)	FAC Color (Max.)	Specifications (R & B, Max.)	MIU <sup>z</sup> (%)
Edible tallow	41.0	0.75	3	None	<sup>b</sup>
Lard (edible)	38.0	0.50	<sup>c</sup>	None	<sup>b</sup>
Top white tallow	41.0	2	5	0.5	1
All-beef packer tallow	42.0	2	None	0.5	1
Extra fancy tallow	41.0	3	5	None	1
Fancy tallow	40.5	4	7	None	1
Bleachable fancy tallow	40.5	4	None	1.5	1
Prime tallow	40.5	6	13–11B	None	1
Special tallow	40.0	10	21	None	1
No. 2 tallow	40.0	35	None	None	2
"A" tallow	39.0	15	39	None	2
Choice white grease	36.0	4	13–11B	None	1
Yellow grease	<sup>d</sup>	15	39	None	2

<sup>a</sup> Moisture, insolubles, unspecifiables.

<sup>b</sup> Moisture, maximum 0.20%; insoluble impurities, maximum 0.05%.

<sup>c</sup> Lovibond color 5.25-inch cell, maximum; 1.5 REd. Lard Peroxide Value 4.0 ME/K maximum.

<sup>d</sup> Titer minimum, when required, to be negotiated between buyer and seller on a contract by contract basis.



**TABLE 4. Typical Analyses of Feed Grade Fats (30).**

Fat Source	Titer (°C)	FAC Color (Maximum)	MIU <sup>a</sup> (%)	Iodine Value	FFA <sup>b</sup> (Maximum %)	Percent Fatty Acids		
						Satu- rated	Unsat- rated	Lino- leic
FGF (for all feeds)	34–38	37	2	55	15	44	56	10
FGF (for milk replacers)	38–41	9	1	45	5	50	50	4
All-beef tallow	38–42	7	1	40	5	56	44	2
All-pork fat	32–38	37	2	58	15	36	64	12
All-poultry fat	28–35	19	2	65	15	28	72	20
Butter fat	28–35	—	—	32	—	63	37	2
Vegetable fat (palm oil)	28–36	—	2	53	—	42	58	10

<sup>a</sup> Moisture, insolubles, and unsaponifiables.

<sup>b</sup> Free fatty acids.

of feed-grade fats of different origins are shown in Table 4 (30). Quality specifications suggested by the NRA for commonly traded feeding fats are shown in Table 5 (1). The NRA additionally suggests the following.

Feeding fats pass a minimum of 20-h active oxygen method (AOM) test.

Blended feeding fats contain only tallow, grease, poultry fat, and soapstocks; any other products should be included only with the knowledge and approval of the purchaser.

All fat products must be below tolerances for toxic chemicals and pesticide residues; certification is available from most renderers; fats used in poultry rations must be free of the chick edema factor; and all fats should be devoid of contaminants such as heavy metals.

**TABLE 5. National Renderer's Association Suggested Fat Quality Standards for Feeds (1).**

Standard	Tallow	Choice White Grease	Yellow Grease	Hydrolyzed A/V Blend
Total fatty acids (%)	90	90	90	90
Free fatty acids (%)	4–6	4	15	40–50
FAC color	19	11A	37–39	45
Moisture (%)	0.5	0.5	1.0	1.5
Impurities (%)	0.5	0.5	0.5	0.5
Unsaponifiables (%)	0.5	0.5	1.0	2.5
Total MIU (%)	1.0	1.0	2.0	4.0
Iodine value	48–58	58–68	58–79	85
AOM (h)	20	20	20	20

Fats for poultry rations should not contain cottonseed soapstock or other coproducts.

Changes to new sources of fats, especially in ruminant and swine feeds and pet foods, should be gradual due to potential differences in palatability from previous sources (1).

No standards exist for polyethylene in fats; the practical way to remove it is by filtering tallow at low temperatures using special filter aids; most customers can use tallow containing up to 30 ppm polyethylene, and a few up to 150 ppm (31).

Maximum pesticide residues tolerances are 0.5 ppm for DDT, DDD, and DDE; 0.3 ppm for Dieldrin; and 2.0 ppm for PCB (31).

Some buyers also have a rate of filtration (ROF) specification, which is defined as the milliliters of tallow at 110°C (230°F) that will pass through a filter paper in 5 min under specified test conditions; a commonly sought value is 35–40 ROF; the test identifies fats that may give processing difficulties such as slow filtration, emulsion, and foaming (31).

Poultry fat is rendered mainly from poultry offal collected in packing plants but may include renderings from mortalities, hatchery rejects, and condemned or unmarketable parts of birds. Much of the rendered poultry fat and meal is produced and recycled in integrated growing–processing plant operations, and only limited amounts are available in the open market.

*AAFCO Definitions—Fats and Oils.* Many feed ingredients are coproducts of agribusinesses and are of secondary interest. With some exceptions, little is done currently to process them further, and they are sold as made. The AAFCO defines feed fats as follows.

*Animal fat* (AAFCO number 33.1) is obtained from the tissues of mammals and/or poultry in the commercial processes of rendering or extracting. It consists predominantly of triacylglycerol esters of fatty acids and contains no additions of free fatty acids or other materials obtained from fats. It must contain, and be guaranteed for, not less than 90% total fatty acids, not more than 2.5% unsaponifiable matter, and not more than 1% insoluble impurities. Maximum free fatty acids and moisture must also be guaranteed. If the product bears a name descriptive of its kind or origin (e.g., beef, pork, or poultry), it must correspond thereto. If an antioxidant is used, the common name or names must be indicated, followed by the words *used as a preservative*. Includes IFN 4-00-409 (animal poultry fat).

*Vegetable fat, or oil* (33.2) is the product of vegetable oil origin obtained by extracting the oil from seeds or fruits that are commonly processed for edible purposes. It consists predominantly of glyceride esters of fatty acids and contains no additions of free fatty acids or other materials obtained from fats. It must contain, and be guaranteed for, not less than 90% total fatty acids, not more than 2% unsaponifiable matter, and not more than 1% insoluble impurities. Maximum free fatty acids and moisture must also be guaranteed. If the product bears a name descriptive of its kind or origin (e.g., soybean oil or cottonseed oil) it must correspond

thereto. If an antioxidant is used, the common name (or names) must be indicated, followed by the words *used as a preservative*. Includes IFN 4-05-077 (vegetable oil).

*Hydrolyzed fat, or oil, feed grade* (33.3), is obtained by the fat-processing procedures commonly used in edible fat processing or soap making. It consists predominantly of fatty acids and must contain, and be guaranteed for, not less than 85% total fatty acids, not more than 6% unsaponifiable matter, and not more than 1% insoluble impurities. Maximum moisture must also be guaranteed. Its source must be stated in the product name, e.g., hydrolyzed animal fat or hydrolyzed animal and vegetable fat. If an antioxidant is used, the common name (or names) must be indicated, followed by the words *used as a preservative*. Includes IFN 4-00-376 (animal fat hydrolyzed) and IFN 4-05-076 (vegetable oil hydrolyzed).

*Ester, feed grade* (33.4), is the product consisting of methyl, ethyl, and other nonglyceride esters of fatty acids derived from animal and/or vegetable fats. It consists predominantly of the ester and must contain not less than 85% total fatty acids, not more than 10% free fatty acids, not more than 6% unsaponifiable matter (2% for methyl esters), and not more than 1% insoluble matter. Its source must be stated in the product name, e.g., methyl ester of animal fatty acids or ethyl ester of vegetable oil fatty acids. Methyl esters must contain not more than 150 ppm (0.015%) free methyl alcohol. If an antioxidant is used, the common name or names must be indicated, followed by the words *used as a preservative*. This feed fat includes FDA regulation 573.640:

IFN 4-00-377 (animal fatty acids of ethyl ester).

IFN 4-00-378 (animal fatty acids of methyl ester).

IFN 4-00-379 (animal fats of nonglyceride ester).

IFN 4-12-240 (vegetable fatty acids of ethyl ester).

IFN 4-05-075 (vegetable fatty acids of nonglyceride ester).

IFN 4-05-074 (vegetable fatty acids of methyl ester).

*Fat product, feed grade* (33.5), is any fat product that does not meet the definitions for animal fat, vegetable fat or oil, hydrolyzed fat, or fat ester. It must be sold on its individual specification, which will include the minimum percentage of total fatty acids, the maximum percentage of unsaponifiable matter, the maximum percentage of insoluble impurities, the maximum percentage of free fatty acids, and moisture. The above listed specifications must be guaranteed on the label. If an antioxidant is used, the common name or names must be indicated, followed by the words *used as a preservative*. Includes IFN 4-00-414 (animal vegetable fat product).

*Corn endosperm oil* (33.6) is obtained by the extraction of oil from corn gluten. It consists predominantly of fatty acids and glycerides and must contain not less than 85% total fatty acids, not more than 14% unsaponifiable matter, and not more than 1% insoluble matter. If an antioxidant is used, the common name or names

must be indicated, followed by the word *preservative*. Includes IFN 4-02-852 (maize endosperm oil and FDA Reg. 8.322).

*Vegetable oil refinery lipid, feed grade* (33.7), is obtained in the alkaline refining of a vegetable oil for edible use. It consists predominantly of the salts of fatty acids, glycerides, and phosphates. It may contain water and not more than 22% ash on a water-free basis. It is to be neutralized with acid before use in commercial feed. Includes IFN 4-05-078 (vegetable oil refinery lipid).

*Calcium salts of long-chain fatty acids* (33.14) are the reaction products between calcium and long-chain fatty acids of vegetable and/or animal origin. They shall contain a maximum of 20% lipid not bound in the calcium salt form and the percent total fat shall be indicated. The unsaponifiable matter (exclusive of calcium salts) shall not exceed 4% and moisture shall not exceed 5%. If an antioxidant is used, its common name must be indicated on the label. Before conducting an assay for total fats, hydrolysis of the calcium salts should be performed to liberate the lipid fraction.

*Hydrolyzed sucrose polyesters, feed grade* (T33.15) is the product resulting from acid hydrolysis of sucrose polyesters, such as olestra, to make them digestible. It shall consist predominantly of fatty acids and contain, and be guaranteed for, not less than 85% total fatty acids, not more than 2% sucrose polyesters (hex ester and above), not more than 2% unsaponifiable matter, and not more than 2% insoluble impurities. Maximum moisture must also be guaranteed. Its source must be stated in the product name (e.g., hydrolyzed animal sucrose polyesters, hydrolyzed vegetable sucrose polyesters, or hydrolyzed animal and vegetable sucrose polyesters). If an antioxidant is used, the common name must be indicated, followed by the words *used as a preservative*. This definition was proposed in 1993 and is tentative at this time.

*Fish oil* (51.8) is the oil from rendering whole fish or cannery waste. It includes IFN 7-01-965 (fish oil).

*Salts of volatile fatty acids* (60.73) is a blend containing the ammonium or calcium salt of isobutyric acid and the ammonium or calcium salts of a mixture of 5-carbon acids/isovaleric, 2-methyl-butyric, and *n*-valeric. The contained ammonium or calcium salts of volatile fatty acids shall conform to the specifications in 21 CFR 573.914. It is used as a source of energy in dairy cattle feed. The label of the product shall bear adequate directions for use, including statements expressing maximum use levels. For ammonium salts of volatile fatty acids, not to exceed 120 g per head per day thoroughly mixed in dairy cattle feed as a source of energy, and for calcium salts of volatile fatty acids, not to exceed 135 g per head per day thoroughly mixed in dairy cattle feed as source of energy. Includes FDA Reg. 21 CFR 573.914.

*Soy phosphate or soy lecithin* (84.10) is the mixed phosphatide product obtained from soybean oil by a degumming process. It contains lecithin, cephalin, and inositol phosphatides, together with glycerides of soybean oil and traces of tocopherols, glycosides, and pigments. It must be designated and sold according to conventional descriptive grades with respect to consistency and bleaching. Includes IFN 4-04-562 (soybean lecithin).

*AAFCO Definitions—Fat-associated Vitamins and Essential Compounds.* Fat-associated feed ingredients include sources of natural or synthetic fat-soluble vitamins. AAFCO defined vitamin A sources include

- 90.3, vitamin A oil (IFN 7-054-141).
- 90.14, vitamin A supplement (IFN 7-05-1244).
- 90.25, vitamin A acetate [IFN 7-05-142, FDA Reg. 582.5933 (GRAS)].
- 90.25, vitamin A palmitate [IFN 7-04-143, FDA Reg. 582.5936 (GRAS)].
- 90.25, vitamin A propionate (IFN 7-26-311).

Defined vitamin D sources include

- 90.4, vitamin D<sub>2</sub> supplement (IFN 7-05-149).
- 90.7, cholecalciferol (D-activated animal sterol) (IFN 7-00-408).
- 90.8, ergocalciferol (D-activated plant sterol) (IFN 7-03-728).
- 90.15, vitamin D<sub>3</sub> supplement (IFN 7-05-699).
- 96.3, irradiated dried yeast, irradiated dried yeast (IFN 7-05-524).

Defined vitamin A and D sources include

- 90.1, cod liver oil (IFN 7-01-993).
- 90.2, cod liver oil with added vitamins A and D (IFN-7-08-047).
- 90.25, herring oil (IFN 7-08-048).
- 90.25, menhaden oil (IFN 7-08-049).
- 90.25, salmon oil (IFN 7-08-050).
- 90.25, salmon liver oil (IFN 7-02-013).
- 90.25, sardine oil (IFN 7-02-016).
- 90.25, shark liver oil (IFN 7-02-019).
- 90.24, tuna oil (IFN 7-02-024).

Vitamin E is a biological antioxidant necessary to prevent cell membrane damage. It is essential for proper growth, hormone functions, and proper muscle and nervous system activity. Vitamin E also retards fats and oils oxidation. Defined sources are as follows.

- 90.12, vitamin E supplement (IFN 7-05-150).
- 90.25, tocopherol [IFN 7-00-001, FDA Reg. 582.5890 (GRAS)].
- 90.25,  $\alpha$ -tocopherol acetate [IFN 7-18-777, FDA Reg. 582.5892 (GRAS)].
- 90.25, wheat germ oil (IFN 7-05-207).

Choline (bilineurine) is a lipotropic factor in the metabolism of fatty acids in the liver of fish, poultry, and swine. It is a nonspecific source of biologically active

methyl groups and is essential in the synthesis of acetylcholine (the main chemical in transmission of nerve impulses), for building and maintaining cell structure, and for prevention of perosis (slipped tendon) in poultry (3). Choline also is a precursor for phosphatidylcholine, which is active in absorption of lipids in the small intestine and for their transport. AAFCO defined sources include the following.

- 90.25, choline chloride [IFN 7-01-228, FDA Reg. 582.5252 (GRAS)].
- 90.25, choline pantothenate (IFN 7-01-229).
- 90.25, choline xanthate (IFN 7-01-230, FDA Reg. 573.300).
- 90.17, betaine (hydrochloride or anhydrous), IFN 7-00-722; this is sometimes included in diets as a methyl group donor and partial replacement for choline.
- 90.25, inositol (myo-inositol, meso-inositol, *i*-inositol) [IFN 7-09-354, FDA Reg. 582.5370 (GRAS)]; this is a lipotropic growth factor for some animals.
- 6.12, taurine (IFN 5-09-821, FDA Reg. 573.980), this is a sulfonic amino acid; the bile salts of most mammals are a mixture of taurine and glycine conjugates but are exclusively taurine conjugates in the cat; deficiencies have led to central retinal degeneration and blindness in cats; it is normally found in muscle flesh; supplementation is recommended in kitten and cat diets that might not contain sufficient proteins of animal origin; it is listed as a required nutrient for cats by the NRC (8) and in the AAFCO's *Official Publication*.

*AAFCO Definitions—Chemical Preservatives (Antioxidants).* Antioxidants are used to retard oxidation of unsaturated fatty acids in fats, oils, and fish and meat meals, and to slow the destruction of fat-soluble vitamins, especially vitamin E. Their presence must be identified on the label when used. Use of the following compounds is permitted alone or in combination at levels not to exceed 0.02% of the fat present.

- 18.1, butylated hydroxyanisole (BHA) (IFN 8-01-044, FDA Reg. 582.3169); either the full name or BHA may be used in the ingredients listing.
- 18.1, butylated hydroxytoluene (BHT) (IFN 8-01-045, FDA Reg. 582.3173); either the full name or BHT may be used in the ingredients listing.
- 18.1, tertiary butyl hydroxyquinone (TBHQ) (IFN 8-04-829, FDA Reg.); in the informal review process.
- 18.1, thiodipropionic acid (IFN 8-04-830, FDA Reg. 582.3109).
- 18.1, dilauryl thiodipropionate (IFN 8-04-789, FDA Reg. 582.3280).
- 18.1, distearyl thiodipropionate (IFN 8-01-792, FDA Reg. 582.3280).
- 18.1, propyl gallate (IFN 8-03-308, FDA Reg. 582.3660).
- 18.1, resin guaiac (IFN 8-03-909, FDA Reg. 582.3336); same as guaiac gum, may be used at 0.1%, or equivalent preservative 0.01%, in fats and oils.
- 18.1, ethoxyquin (FN 8-01-841, FDA Reg. 573.380); may be used not to exceed 0.015% in or on feed.

18.1, tocopherols (IFN 7-05-0348, FDA Reg. 582.3890); no statutory maximum exists for tocopherols, but their use should conform to good manufacturing practices.

The formulator needs to know the properties of each antioxidant. For example, most of the antioxidants are easily distilled by heat or steam. Propyl gallate will form strong purple complexes with iron. The preservative effects of antioxidants also can be enhanced by addition of metal chelating agents like citric acid. Furthermore, many crude feedstuffs of plant origin, including soybean meal, crude soybean oil, and lecithin, contain a variety of natural quinone-type compounds with beneficial antioxidant properties that do not require labeling.

*AAFCO Definitions—Special-Purpose Products.* Fat-based or associated special-purpose products include the following.

85.5, diacetyl tartaric acid esters of mono and diglycerides of edible fats or oils, or edible fat-forming fatty acids (IFN 8-07-248, FDA Reg. 582.4101); used as emulsifying agents.

85.5, ethoxylated mono and diglycerides, (FDA Reg. 172.834); used as emulsifiers.

85.5, lecithin (IFN 8-08-041, FDA Reg. 582.1400); used as stabilizer.

85.5, methyl glucoside coconut oil ester (IFN 8-09-346, FDA Reg. 573.660); used as surfactant in molasses.

85.5, mono and diglycerides of edible fats or oils, or edible fat-forming acids (IFN 8-07-251, FDA Reg. 582.4505); used as emulsifying agents.

85.5, monosodium phosphate derivatives of mono and diglycerides of edible fats or oils, or edible fat-forming fatty acids (IFN 8-07-252, FDA Reg. 582.4521); used as emulsifying agents.

*AFIA Definitions.* The American Feed Industry Association definitions include the following.

*Animal and vegetable fat blends, feed grade,* are blends of rendered animal fats, cooking fats, crude vegetable fats, by-products of fat splitting and hydrolyzed fats in any combination that have been processed under good manufacturing practices. They shall be of a quality suitable for use as an animal feed ingredient. Typical analyses include total fatty acids, 93%; free fatty acids, 40%; moisture, 1.0%; impurities, 0.4%; and unsaponifiables, 3.5%. Fatty acid profiles and iodine values should be consistent from one load to another. Metabolizable energy claims should be substantiated by research data. Fat should be stabilized with an acceptable feed- or food-grade antioxidant and at levels recommended by the antioxidant manufacturer. Fish oil and fish oil by-products should be avoided in other fowl, unless agreed on between the purchaser and the supplier. Fats must be within the pesticide and industrial chemical tolerances set by federal and state agencies. Blends for poultry should pass the color test for fat acceptance (a modification of the Liebermann-Burchard test), an indicator of the presence of the chick edema

factor. This is not a specific test for the chick edema factor, and results must be carefully evaluated. Physical properties are color, brown to black; odor, typical and not rancid; density, 7.5 lb/gal. animal and vegetable fat blends are primarily used as sources for energy in livestock and poultry feeds, especially for broilers, turkeys, and cattle. Blended fats are also added to dairy and swine feeds. These are not defined by the AAFCO, but tentative definition has been requested. Must be declared on mixed feed labels as animal fat and vegetable fat (31).

*Acidulated cottonseed soapstock* is the product obtained from the complete acidulation and thorough setting of soapstock, which itself is the by-product obtained from the alkali refining of cottonseed oil. It is sold on a basis of 95% total fatty acid content. If it falls below 85% total fatty acid content, it may be rejected. It should not have more than a total of 6% unsaponifiable plus insoluble matter. Typical analyses are total fatty acids (TFAs), 90% and moisture, 2.5%. The amount of gossypol present in acidulated cottonseed soapstock is variable and difficult to determine with existing methods of analysis. Physical properties include dark brown to greenish black color, appearing as an oily mixture. It has a slightly sour odor with a pH of 4. It is soluble when cold and is a pumpable liquid at 49°C (120°F). It is used as a feed-grade fat for ruminants. Caution must be exercised when used with other species due to the gossypol content. It is not to be used with layer rations. It qualifies under AAFCO 33.3, IFN 4-17-942 or IFN 4-05-076 (31).

*Acidulated soybean soapstock* is the product obtained from the complete acidulation and thorough setting of soapstock, which itself is the by-product obtained from the alkali refining of soybean oil. It is sold on a basis of 95% total fatty acid content. If it falls below 85% total fatty acid content, it may be rejected. Typical analyses are TFAs, 90%; moisture, 1%; and iodine value 125. In practice, soybean soapstock may be found in combinations with other vegetable oil soapstocks. The buyer should determine if cottonseed soapstock is present, as it may contain gossypol, which is detrimental in nonruminant feeds. Physical properties are medium brown color; odor somewhat typical of soybeans, slightly nutty; solid when cool; and liquid and pumpable at 38–44°C (100–110°F). It qualifies under AAFCO 33.3, IFN 4-17-893 (31).

### **3.3. Definitions of Extracted and Whole Seed Products**

*Mechanically Extracted Meals.* Solvent extracted oilseed meals typically contain less than 1.5% residual fat unless the gums (hydrated phosphatides) or soapstock have been added back to the meal before the desolventizer-toaster or meal dryer. Mechanically extracted (expeller or screw-pressed) meals can contain 4–9% oil, which can be a significant calorie source in animal feeds. Fat contents of extracted meals are not part of the definition, although typical analyses are shown below.

*AAFCO Definitions—Extracted Products.* The words *mechanical extracted* are not required when listed as an ingredient in a manufactured feed.



- T24.10, cottonseed meal, mechanical extracted; contains anticaking agent; will replace definition 24.10 if adopted (IFN 5-02-045; cottonseeds meal mechanical extracted 36% protein); a typical NRC composition (9) is 4.6% oil ether extract (EE), and dry matter basis (dmb).
- 71.1, linseed meal, mechanical extracted (IFN 5-16-280; flax seeds meal mechanical extracted); NRC: 6.0% oil EE, dmb.
- 71.9, peanut meal, mechanical extracted and solvent extracted (IFN 5-03-649; peanut seeds without coats meal mechanical extracted); NRC: 6.3% oil, EE, dmb.
- 71.25, rapeseed meal, mechanical extracted (IFN 5-03-870: rape seeds meal mechanical extracted); NRC: 7.9% oil EE, dmb.
- 71.130, safflower meal, mechanical extracted (IFN 5-04-109: safflower seeds meal mechanical extracted); NRC: 6.7% oil EE, dmb.
- 71.210, sunflower meal, dehulled, mechanical extracted (IFN 5-04-738: sunflower seeds meal without hulls meal mechanical extracted); NRC: 8.7% oil EE, dmb.
- 71.220, sunflower meal, mechanical extracted (IFN 5-27-477: sunflower seeds meal mechanical extracted.)
- 84.60, soybean meal, mechanical extracted (IFN 5-04-600: soybean seeds meal mechanical extracted); NRC: 5.3% oil EE, dmb.

*Full-fat Soybeans.* AAFCO definitions do not exist for whole oilseeds per se. Considerable variations in composition occur between species, location and weather during crop maturation. Soybeans typically contain 19% oil at 92% total solids and 21% oil at 100% total solids (dmb). Definitions for full-fat soybean products are as follows.

- 84.1, ground soybeans are obtained by grinding whole soybeans without cooking or removal of any of the oil; includes IFN 5-04-596 (soybean seeds ground).
- 84.11, heat-processed soybeans are the result of heating whole soybeans without removing any of the component parts; they may be ground, pelleted, flaked, or powdered; the maximum pH rise using the standard urease testing procedure should not exceed 0.10 pH units; they must be sold according to their crude protein, crude fat, and crude fiber content; includes IFN 5-04-597 (soybean seeds heat processed).
- 84.15, ground extruded whole soybeans are the result of extrusion by friction heat and/or steam of whole soybeans without removing any of the component parts; the meal must be sold according to its crude protein, fat, and fiber content; includes IFN 5-14-005 (soybean seeds extruded ground).

The AFIA (31) has further elaborated on quality factors and feed applications of whole (full-fat) soybeans. Whole soybeans must be properly heated to provide optimum protein nutrition for critical animals, especially poultry, swine, lambs, and calves as well as pets and fur-bearing animals.

Underheating of whole soybean may fail to destroy the trypsin inhibitor and reduce urease and lipase activity, resulting in low protein efficiency for critical feeds. An underheated soybean meal greatly increases the need for vitamin D to prevent rickets in turkey poults. Overheating of whole soybeans tends to inactivate or destroy the essential amino acids lysine, cystine, and methionine and possibly others (31).

Laboratory tests such as urease activity, protein dispersibility index (PDI), nitrogen solubility index (NSI), thiamine, and water absorption have been found valuable in monitoring daily production for protein quality. But biological chick and/or rat assays are the only reliable means currently available for predetermining the nutritional value of whole soybean protein; they must be conducted periodically to verify results of chemical tests (31). If whole soybeans are to be used in a mixture containing 20% or more soybean meal, 5% or more urea, and 20% or more molasses, or an equivalent mixture, and exposed to hot, humid storage conditions, it is advisable that the urease activity of the whole soybeans not exceed 0.12 increase in pH (31). Extruded or roasted soybeans properly treated for cattle to increase bypass protein should have urease values of less than 0.05 pH rise. A urease rise of 0.05–0.20 is an indication of proper treatment for swine and poultry.

Whole soybean may be fed to gestating swine (ground form) and to mature ruminants (whole or rolled form). However, feeding whole soybeans to all other livestock will result in reduced performance and is not recommended. Extruded soybeans may be fed to all livestock, especially swine and poultry. Extruded soybeans fed to lactating dairy cattle increase by-pass protein but most often will reduce butterfat. Roasted soybeans may be fed to all livestock, especially mature ruminants. Roasted soybeans fed to lactating dairy cattle will increase by-pass protein and slightly increase butterfat.

Poultry and swine will perform as well on pelleted rations containing extruded or roasted soybeans as on isocaloric, isonitrogenous ration containing soybean meal and soybean oil. The improved performance from pelleting with rations containing extruded soybeans is primarily because of increased ration nutrient density. The improvement with rations containing roasting soybeans is primarily because of increased fat digestibility (the oil vesicles are ruptured to allow the oil to be more available for digestion). Bulk densities are whole soybeans, 737–769 kg/m<sup>3</sup> (46–48 lb/ft<sup>3</sup>); whole soybeans, ground, 384–545 kg/m<sup>3</sup> (24–34 lb/ft<sup>3</sup>); extruded soybeans, 384–497 kg/m<sup>3</sup> (27–31 lb/ft<sup>3</sup>); and roasted soybeans, 720–753 kg/m<sup>3</sup> (45–47 lb/ft<sup>3</sup>) (31).

*Cottonseed and Cottonseed Products.* Whole cottonseed and its oil-bearing (screw press/expeller) meals can be economically attractive sources of oil and protein but must be used properly. Currently, approximately 35–40% (1.5 million tons) of the domestic cottonseed crop is fed whole to adult ruminants (primarily dairy cattle) annually. Whole cottonseed is not defined by AAFCO but is identified as IFN 5-01-614; it is called feed-grade cottonseed by the National Cottonseed Products Association's trading rules (32). It consists of the entire seed of the cotton plant after the cotton fibers have been removed by ginning at a fiber:seed ratio

of approximately 1:1.65. Naked (Pima-type) cottonseed exists, and operations have been installed between the gin and the oil mill to intercept the seed and remove the linters for other uses. Whole cottonseed is sold in three grades: prime feed-grade cottonseed (moisture, 13% maximum; free fatty acid in oil, 3% maximum; crude protein and crude fat, 34% minimum, dmb); delinted prime feed-grade cottonseed (lint, 5% maximum; moisture, 13% maximum; free fatty acid in oil, 3% maximum; crude protein and crude fat, 34% minimum, dmb); and feed-grade cottonseed, off quality. Fuzzy (“white,” “with lint”) whole cottonseed contains approximately 21% fat at 92% total solids and naked seed contains about 23% fat at the same moisture level. Rolling (flaking) naked seed before feeding reduces whole seed passage through dairy cattle (33). Bulk density for undelinted cottonseed is 288–401 kg/m<sup>3</sup> (18–25 lb/ft<sup>3</sup>) and 401–561 kg/m<sup>3</sup> (25–35 lb/ft<sup>3</sup>) for delinted seed.

Cottonseed is added to feeds for ruminant animals as a source of highly concentrated energy and rumen undegradable protein and fiber. It typically is fed whole—not ground or pelleted—although a process for extruding whole seed and soybeans was patented in 1993 (34). Whole cottonseed contains 0.5–1.2% gossypol, a yellow-green pigment that is toxic to monogastric animals and young calves, lambs, and kids whose rumens are not yet functional. It can be fed to cattle, sheep, and other ruminant animals due to bacterial detoxification in the rumen. Care should be exercised in feeding so ruminal detoxification abilities are not overstressed, resulting in gossypol toxicity. The typical feeding rate is 2.3–3.6 kg (5–8 lb) whole cottonseed per day per cow. “Free” gossypol in cottonseed products can be deactivated (“bound,”) either during processing or by use in conjunction with iron (ferrous) salts. Levels of gossypol typically present in common cottonseed products, and tolerances in feeds by selected species, are presented in Tables 6 and 7, respectively (35).

**TABLE 6. Analyzed Gossypol Levels in Common Cottonseed Products (35).**

Product	Gossypol	
	Percent Total	Percent Free
Cottonseed kernel	0.39–1.7 <sup>a</sup>	0.39–1.4 <sup>a</sup>
Whole cottonseed	—	0.47–0.63 <sup>b</sup>
Delinted whole cottonseed	—	0.47–0.53 <sup>b</sup>
Cottonseed meal		
Screw press	1.02 <sup>a</sup>	0.02–0.05 <sup>a</sup>
Prepress solvent	1.13 <sup>a</sup>	0.02–0.07 <sup>b</sup>
Direct solvent	1.04 <sup>a</sup>	0.1–0.5 <sup>a</sup>
Solvent (expander process)	—	0.06–0.1 <sup>b</sup>
Cottonseed hulls	—	0.06 <sup>a</sup>
Glandless whole cottonseed	0.01 <sup>a</sup>	—

<sup>a</sup>Dry matter basis.

<sup>b</sup>As-fed basis.

**TABLE 7. Reported Effect and No Effect Levels of Free Gossypol (35).**

Class of Livestock	Free Gossypol Intake (ppm)	
	Effect	No Effect
Ruminants		
Preruminant calves	—	100 <sup>a</sup>
Young lambs	824 <sup>b</sup>	—
Mature dairy cows	1076 <sup>b</sup>	—
Nonruminants		
Yearling horses	—	115 <sup>a</sup>
Weanling horses	—	348
Catfish	—	900 <sup>b</sup>
Tilapia	—	1800
Rainbow trout	1000 <sup>a,c</sup>	250 <sup>a,c</sup>
Shrimp	—	170 <sup>b</sup>

<sup>a</sup>As-fed basis not reported.<sup>b</sup>Dry matter basis.<sup>c</sup>Fed-as gossypol acetic acid.

The AFIA (31) advises that (1) 200 ppm (0.02%) dietary free gossypol does not affect egg production, (2) 50 ppm (or 150 ppm with addition of FeSO<sub>4</sub> at 4 parts iron to 1 part gossypol) avoids (green) egg yolk discoloration during refrigerated storage, and (3) up to 150 ppm free gossypol (or 400 ppm with addition of FeSO<sub>4</sub> at 1 part iron to 1 part free gossypol) can be used for growing broilers. Levels of 100 ppm free gossypol (or higher levels, but no more than 400 ppm FeSO<sub>4</sub> added at a weight ratio of 1 part iron to 1 part free gossypol) can be used for growing and fattening pigs.

Jones (36) reviewed the natural antinutrients of cottonseed protein products—gossypol and the cyclopropenoic fatty acids (CPFA; malvalic and sterculic acids). The CPFAs participate in forming the pink color complex in the Halphen reaction, a test specific for the admixture of cottonseed oil with other oils and fats. They also inhibit  $\Delta^9$  desaturase, an enzyme that converts stearic acid into oleic acid, and thus increase hardness of fats from animals (e.g., pig backfat and lard) raised or finished on feedstuffs containing high levels of polyunsaturated oils like corn. Feed industry practice is to limit cottonseed lipids to no more than 0.1–0.2% in the diet of laying hens to avoid pink discoloration of egg whites and alterations of the vitelline membrane that cause pasty yolks.

*Other Whole Oilseeds.* Various oilseeds have been fed whole, or dehulled, when available for feed at competitive prices or in grades substandard for extraction but still wholesome for feeding. Examples include safflower (*Carthamus tinctorius*), fat content 35%, dmb; and *oil-type sunflower seed* fat content 44%, dmb. Currently, interest is high in feeding whole canola seed (41–46% oil) in Canada and northern European countries. “Double-zero” strains of *Brassica napus* (rapeseed, oilseed rape, swede rape, and Argentine rape) and *Brassica campestris* (turnip rape, oil

**TABLE 8. Fatty Acid Composition of Common Feed Animal Fats, Fish Oils, and Vegetable Oils.<sup>a</sup>**

Lipid Source	IFN <sup>b</sup>	Percentage of Total Fatty Acids																
		14:0	16:0	16:1	18:0	18:1	18:2 <i>n</i> -6	18:3 <i>n</i> -3	18:4 <i>n</i> -3	20:1	20:4 <i>n</i> -6	20:5 <i>n</i> -3	22:1	22:5 <i>n</i> -3	22:6 <i>n</i> -3	Σ <i>n</i> -6	Σ <i>n</i> -3	<i>n</i> -3: <i>n</i> -6
<b>Animal fat</b>																		
Beef tallow	4-08-127	3.7	24.9	4.2	18.9	36.0	3.1	0.6	—	0.3	—	—	—	—	—	3.1	0.6	0.19
Pork fat	4-04-790	1.3	23.8	2.7	13.5	41.2	10.2	1.0	—	1.0	—	—	—	—	—	10.2	1.0	0.10
Poultry fat	4-09-319	0.9	21.6	5.7	6.0	37.3	19.5	1.0	1.1	0.1	—	—	—	—	—	19.6	1.0	0.05
<b>Fish oils</b>																		
Anchovy		7.4	17.4	10.5	4.0	11.6	1.2	0.8	3.0	1.6	0.1	17.0	1.2	1.6	8.8	1.3	31.2	24.0
Cod liver	7-01-994	3.2	13.5	9.8	2.7	23.7	1.4	0.6	0.9	7.4	1.6	11.2	5.1	1.7	12.6	3.0	27.0	9.0
Capelin	7-16-709	7.9	11.1	11.1	1.0	17.0	1.7	0.4	2.1	18.9	0.1	4.6	14.7	0.3	3.0	1.8	12.2	6.78
Channel catfish, cultured		1.4	17.4	2.9	6.1	49.1	10.5	1.0	0.2	1.4	0.3	0.4	—	0.3	1.3	12.7	3.2	0.25
Herring, Atlantic	7-08-048	6.4	12.7	8.8	0.9	12.7	1.1	0.6	1.7	14.1	0.3	8.4	20.8	0.8	4.9	1.4	17.8	12.71
Herring, Pacific		5.7	16.6	7.6	1.8	22.7	0.6	0.4	1.6	10.7	0.4	8.1	12.0	0.8	4.8	1.0	15.7	15.7
Menhaden	7-08-049	7.3	19.0	9.0	4.2	13.2	1.3	0.3	2.8	2.0	0.2	11.0	0.6	1.9	9.1	1.5	25.1	16.73
Redfish		4.9	13.2	13.2	2.2	13.3	0.9	0.5	1.1	17.2	0.3	8.0	18.9	0.6	8.9	1.2	19.1	15.92
Salmon, sea caught		3.7	10.2	8.7	4.7	18.6	1.2	0.6	2.1	8.4	0.9	12.0	5.5	2.9	13.8	2.1	31.4	15.00
<b>Vegetable oil</b>																		
Canola	4-06-144	—	3.1	—	1.5	60.0	20.2	12.0	—	1.3	—	—	1.0	—	—	20.2	12.0	5.94
Coconut	4-09-320	16.8	8.2	—	2.8	5.8	1.8	—	—	—	—	—	—	—	—	1.8	0.0	0.0
Corn	4-07-882	—	10.9	—	1.8	24.2	58.0	0.7	—	—	—	—	—	—	—	58.0	0.7	0.01
Cottonseed	4-20-836	0.8	22.7	0.8	2.3	17.0	51.5	0.2	—	—	—	—	—	—	—	51.5	0.2	0.0
Linseed	4-14-502	—	5.3	—	4.1	20.2	12.7	53.3	—	—	—	—	—	—	—	12.7	53.3	4.2
Palm		1.0	43.5	0.3	4.3	36.6	9.1	0.2	—	0.1	—	—	—	—	—	9.1	0.2	0.02
Peanut	4-03-658	0.1	9.5	0.1	2.2	44.8	32.0	—	—	1.3	—	—	—	—	—	32.0	0.0	0.0
Safflower	4-20-526	0.1	6.2	0.4	2.2	11.7	74.1	0.4	—	—	—	—	—	—	—	74.1	0.4	0.0
Soybean	4-07-983	0.1	10.3	0.2	3.8	22.8	51.0	6.8	—	0.2	—	—	—	—	—	51.0	6.8	0.13
Sunflower	4-20-833	—	5.9	—	4.5	19.5	65.7	—	—	—	—	—	—	—	—	65.7	0.0	0.0

<sup>a</sup>Adapted with permission from Ref. 11. A dash indicates that the measurement was taken but no values were detected.

<sup>b</sup>International feed number.

turnip, and Polish rape), which contain greatly reduced levels of erucic acid and thioglucosinolates, are used (37). Canola is the name coined for low-erucic acid rape (LEAR), compared with the traditional high-erucic acid rape (HEAR), in Canada where the crop was developed.

Factors to be considered before feeding whole oilseed include fiber content, natural toxic components, and fungal contaminations. The high hull/fiber content of many oilseeds often restricts their use as ruminant feeds. Almost every oilseed has one or more recognized toxic or antigrowth components. Fortunately, most are labile to processing by dry or moist heat. The only exception is sunflower seed, but animal growth still is improved by heating before feeding (38).

### 3.4. Compositions of Fat Sources Used in Feeding

*Fatty Acid Compositions of Tallows and Fish and Oilseeds Oils.* Fatty acid compositions of tallows and fish and vegetable oils used as feed ingredients are shown in Table 8 (11). Profiles for palm oils are not shown; they typically are chill crystallized and fractionated into oleins and stearins and can vary greatly in fatty acid compositions. The principal fatty acids of major marine oils are shown in Table 9 (39).

*Lecithins.* Soybean lecithin is identified as AAFCO 84.10; IFN-4-04-562, and lecithin as AAFCO 87.5, IFN 8-08-041, 21 CFR 582.1400 GRAS in the AAFCO's *Official Publication*. Lecithin is also known as "gums" or "phospholipids" when hydrated and recovered wet from oil. Commercial crude, dried, soybean lecithins are standardized before trading to meet the NOPA specifications for fluid natural lecithin, containing 62% minimum acetone insolubles, 1% maximum moisture, 0.3% maximum hexane insolubles, 32 maximum acid value (AV), 10 maximum Gardner color, and 150 poises maximum viscosity at 25°C (77°F). This is usually accomplished by adding fatty acids and oil to ensure fluidization. The crude lecithin may then be deoiled, fractionated by alcohols and other solvents, and granulated for specific applications (40–42).

**TABLE 9. Principal Fatty Acids of Major Marine Oils of Commerce (g/100 g).<sup>a,b</sup>**

FA	M	SM	P	C	H	A	CL	MA	HM	NP	S	SA
C14:0	9	7	8	7	7	9	3	8	8	6	1	7
C16:0	20	15	18	10	16	19	13	14	18	13	16	15
C16:1	12	10	10	10	6	9	10	7	8	5	7	8
C18:1	11	15	13	14	13	13	23	13	11	14	16	9
C20:1	1	3	4	17	13	5	0	12	5	11	10	15
C22:1	0.2	2	3	14	20	2	6	15	8	12	14	16
C20:5	14	17	18	8	5	17	11	7	13	8	6	9
C22:6	8	10	9	6	6	9	12	8	10	13	9	9

<sup>a</sup>Reprinted with permission from Ref. 39.

<sup>b</sup>M, menhaden; SM, specially processed marine oil (menhaden); P, pilchard; C, capelin; H, herring; A, anchovy; CL, cod liver; MA, mackerel; HM, horse mackerel; NP, Norway pout; S, sprat; SA, sand eel.

**TABLE 10. Composition of Polar Lipids in Deoiled Lecithins of Various Species<sup>a</sup>**

Compound	Soybean (%)	Cottonseed (%)	Corn (%)	Sunflower (%)	Rapeseed (%)	Peanut (%)	Rice Bran (%)
Phosphatidylcholine (PC)	29–39	34–36	30	13–27	16–24	49	20–23
Phosphatidylethanolamine (PE)	20–26	14–20	3	15–18	15–22	16	17–20
Phosphatidylinositol (PI)	13–18	—	16	7–8	8–18	22	5–7
Phosphatidylserine (PS)	5–6	7–26	1	—	—	—	—
Phosphatidic acid (PA)	5–9	—	9	—	—	—	—
Phytoglycolipids (PGL)	14–15	—	30	—	—	—	—
Other phospholipids	12	—	8	—	—	—	—

<sup>a</sup>Reprinted with permission from Ref. 39.

The major lecithin sold domestically in commercial quantities is extracted from soybeans. Corn and sunflower seed lecithins are available in limited amounts. Canola is being reviewed as a lecithin source in countries that do not grow significant quantities of soybeans. Lecithins may be added to feeds in crude or refined forms, remain as residuals in solvent- or mechanical-extracted oilseed meals, be returned to oilseed meals as extracted gums or soapstocks at combined solvent extraction-oil refinery operations, or simply be native to an oilseed fed whole.

As the shortest carbon chain members, the polar lipids in lecithins are traditionally depicted in the *sn*-3 triacylglycerol position. The major polar lipids and fatty acids in deoiled lecithins from various oilseed species are presented in Tables 10 and 11, respectively (44, 45). In the trade, the term *lecithin* sometimes is used interchangeably with *phosphatidylcholine*, and *cephalin* is interchanged with *phosphatidylethanolamine*.

**TABLE 11. Composition of Fatty Acids in Deoiled Lecithins of Various Species<sup>a</sup>**

Fatty Acid	Soybean (%)	Cottonseed (%)	Corn (%)	Sunflower (%)	Rapeseed (%)	Peanut (%)	Rice Bran (%)
Myristic (C14:0)	0–2	0.4	—	—	—	—	—
Palmitic (C16:0)	21–27	31.9	17.7	11–32	18–22	12–34	18
Palmitoleic (C16:1)	7–9	0.5	—	—	—	—	—
Stearic (C18:0)	9–12	2.7	1.8	3–8	0–1	2–3	4
Oleic (C18:1)	17–25	13.6	25.3	13–17	22–23	30–47	43
Linoleic (C18:2)	37–40	50.0	54.2	42–69	38–48	27–36	34
Linolenic (C18:3)	4–6	—	1.0	—	7–9	—	2
Arachidic (C20:0)	0–2	—	—	—	—	—	—
Total gossypol	—	9.1	—	—	—	—	—
Free gossypol	—	0.02	—	—	—	—	—

<sup>a</sup>Reprinted with permission from Ref. 39.

## 4. DIGESTION METABOLISM AND FATS FEEDING REQUIREMENTS

### 4.1. Comparative Fat Digestion Systems

An orderly system does not exist for discussing the use of fats over the breadth of domesticated animals; thus a survey approach is used here. Metabolism is the sum of processes by which nutrients are handled in the living organism. Digestion is the process of reducing foods into smaller particles and finally to compounds that are absorbed for physiological processes. The relative nutritional value of a component is the product of its concentration in the feedstuff times its digestibility coefficient. Current research on fat metabolism is intensive, and new information is continuously reported; the reader is referred to technical journals for the latest details and concepts.

Within a period of several hundred years, people have learned how to breed selectively for broad-breasted turkeys and low-profile dachshund dogs, to get eggs from chickens and milk from cows the year around, and to feed cooked oilseeds and cereals to carnivores like dogs and cats. Although meat, milk, and egg production have been increased, the animals remain limited by their primordial digestive and metabolic systems, which must be respected in feeding management. Currently, recommended levels of fat supplementation often result in doubling the level already consumed by the respective species, with the existing digestive and metabolic system expected to handle about equal parts of indigenous and supplemented fats.

Short-chain fatty acids consist of 6 or less carbons; medium-chain fatty acids, 6–10 carbons; and long-chain fatty acids, 12–24 carbons (45). Considerable interest exists in the medium-chain fatty acids and triacylglycerols (MCTs), especially in young animal nutrition and human medical applications (46). These compounds are quickly catabolized or elongated in everyday nutrition, but will be mentioned only briefly here. Most of the storage triacylglycerols found in nature consist of long-chain fatty acids, called long-chain triacylglycerols (LCTs).

Familiarity with the comparative differences between digestive systems helps identify the opportunities and limitations in feeding fats to various species. Animals have been classified as (1) *carnivores*, flesh eaters who derive their nutrients and energy primarily from proteins and fats (dogs, cats, and mink among the mammals); (2) *herbivores*, vegetarians who depend entirely on plant materials (cattle, goats, sheep, and horses); and (3) *omnivores*, flesh and plant consumers (humans and swine). Carnivores have short digestive tracts and require concentrated food sources. Herbivores have relatively long digestive tracts, with additional structures like rumens and enlarged cecum-colons for microbial fermentation of fiber. The digestive tracts of omnivores are intermediate in length, complexity, and efficiency in using plant matter (3–6).

Birds, a major nonmammalian group without teeth for mastication, but with adaptations for reducing the size of their food, typically are described as *avians*; however, carnivores, herbivores, and omnivores exist among birds. Fish, who do not chew their food, must rely on strong digestive enzymes.



Lipids occur in cell walls, cellular cytoplasm, and in fat storage cells in animals. In contrast, specialized fat storage cells per se do not exist in plants or seeds. Rather, fatty acids are found in surface waxes that reduce the loss of moisture from leaves, stems, fruits, and seeds of plants; in seed cell walls and cytoplasm; and as stored triacylglycerols in dispersed spherical organelles in cells of seed embryos (48).

Fat digestion is a two-step process. The first step is gaining access to fats through proteinaceous matrixes of flesh, matrixes consisting mainly of nonstructural carbohydrates and proteins in seeds and fruits, or more complex matrixes, including structural carbohydrates (fiber). The second step is splitting the triacylglycerols into fatty acids and absorbable components and bringing these into the bloodstream by various pathways.

*Overview of Digestion Systems.* The basic digestive system can be described as *monogastric* (one stomach) consisting, sequentially, of the following:

A *mouth* for biting off and chewing food and for admixing saliva containing enzymes to initiate hydrolysis of carbohydrates and fats.

An *esophagus* for conveying the digesta.

A *stomach* (pH 1.5–3.5), where many proteins are brought below their isoelectric point (e.g., the curdling of casein), proteolytic enzymes are introduced, and protein-based matrixes are further reduced by churning; gastric lipases may also be introduced into the digesta.

A *small intestine*, where the fat is emulsified by bile salts; the triacylglycerols are hydrolyzed at the *sn-1* and *sn-3* positions by pancreatic lipases at near-neutral pH to produce fatty acids and *sn-2*-monoacylglycerols; and these products are absorbed by several alternative processes.

A *colon* (large intestine), where water is absorbed, and fermentation of remaining nutrients may occur.

A *rectum* for holding the extracted digesta.

An *anus* or vent for discharge of feces.

In all species, the small intestine is the main site for simultaneous hydrolysis of fats, proteins, and carbohydrates by selective enzymes and absorption of the resulting nutrients. It consists of three sequential sections: duodenum, jejunum and ileum, each with villi and mucosal linings. During the process, the pH of the digesta is raised from that of the stomach, to near neutrality over the length of the small intestine. In swine, the pH profile is as follows: stomach, 2.4; proximal duodenum, 6.1; distal duodenum, 6.8; proximal jejunum, 7.4; distal jejunum, 7.4; and ileum, 7.5. In sheep, the profile is abomasum, 2.0; proximal duodenum, 2.5; distal duodenum, 3.5; proximal jejunum, 3.6; distal jejunum, 4.7; and ileum, 8.0 (48). Several types of contractive and peristaltic actions mix and move the digesta down the intestine. The lower pH at the proximal duodenum of ruminants plays a critical part in fatty acid reabsorption. Hydrolysis of triacylglycerols by pancreatic lipase

starts at the duodenum, but different absorption sites in the three sections are reported for the various species.

In ruminants (cattle, sheep, goats), the digesta is intercepted after the esophagus and processed in a four-section stomach before being returned to the basic digestive system at the small intestine. Fermentation is conducted by bacteria, protozoa, and fungi at near neutrality in the rumen. Fiber is digested to produce two- to 4-carbon acetic, propionic and butyl volatile fatty acids (VFA), which are absorbed through the rumen wall. Maximization of acetic VFA is preferred. Breaking down the fiber matrixes makes the nonstructural carbohydrates, proteins, and fats accessible to microbial enzymes. Within the capacity of the system, the triacylglycerols are hydrolyzed to fatty acids, the glycerol is metabolized, the short-chain fatty acids are absorbed through the rumen wall, and the polyunsaturated long-chain fatty acids are biohydrogenated and allowed to proceed to the small intestine for absorption (3–6).

But the overfeeding of high-grain (carbohydrate) rations can result in a buildup of lactic acid and a reduction of rumen pH to 4–5, resulting in conditions uninhabitable by rumen bacteria. This arrests normal fermentation, and the animal goes off feed with acute digestive problems, at the minimum. Part of this problem can be offset by feeding sodium bicarbonate or other buffers. Ammonia also is lost from proteins digested in the rumen, and the amino acid profiles of microbial protein typically are lower in quality than those of the original animal or oilseed protein supplements. Excessive free oil coats the fiber and interferes with its digestion and with the fermentation process. The need to get more nutrients into dairy cattle to increase milk production, despite the natural physiological limitations of the rumen, has led to the development of various commercial bypass, or escape, proteins and fats. These products are not accessible to rumen bacteria and are digested later in the monogastric part of the digestive system.

The nonruminant herbivores (horses, rabbits, guinea pigs) have a functional cecum and enlarged colon, where microbial fermentations are conducted on the digesta after the small intestine. Capabilities for absorption of selected microbe-digested products, primarily VFA and simple nitrogenous compounds, are extended to the large intestine for these animals. Most mammals have at least rudimentary cecums, but these usually are not efficient sites for fiber digestion due to limited size. Although the fiber matrix is not attacked until after initial digestion and absorption of accessible nonstructural carbohydrates, proteins, and lipids is completed, considerable metabolic energy is still generated and absorbed as VFA. Rabbits and guinea pigs practice coprophagy (the eating of their own feces) to increase the absorption of essential amino acids, vitamin K and the B complex vitamins produced in the cecum (3, 4). Horses also will coprophage if fed protein-deficient diets.

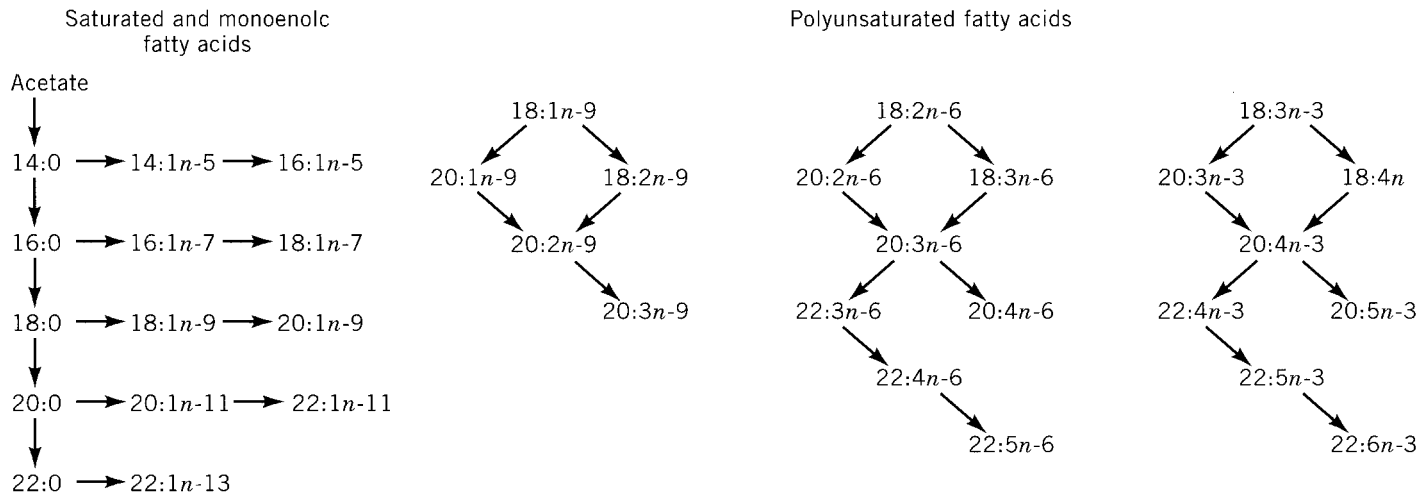
*Fatty Acids Synthesis, Elongation, and Desaturation.* The main objective of feeding fats to animals is to provide a concentrated energy source, not to have the fat stored in the tissues. Recognized EFA requirements are no more than several percent of dry matter at the most, but the critical roles they play in maintaining the metabolic machinery has attracted the majority of current research on dietary fat utilization.

New (*de novo*) fatty acids are synthesized from two-carbon acetyl units produced during metabolism. Two enzyme complexes, acetyl-coenzyme A carboxylase and fatty acid synthetase, work in concert to build up fatty acid chains, two carbons at a time, until released by the complex. The primer in plants and animals is essentially a two-carbon acetyl group and the fatty acid chains have even numbers of carbons. If the primer is a three-carbon propionate group, odd-number carbon chains result. Odd-number fatty acids are common in microbial lipids and also are synthesized *de novo* from propionic VFA by rumen bacteria and deposited in adipose tissue. The length of the fatty acid synthesized depends on the tissue. Palmitic acid is produced in the liver and adipose tissue, and shorter-chain fatty acids are also produced in the mammary glands (49).

Palmitic acid produced by fatty acid synthetase is lengthened (or shortened), two carbons at a time, by (1) the mitochondria, which use acetyl-CoA and NADH, or NADPH for reduction, or (2) the microsomes, which use malonyl-CoA and NADPH, but in a different pathway from the cytosolic enzyme fatty acid synthetase. Palmitic acid is elongated to stearic acid and desaturated to oleic acid (*cis* 9-C18:1) by the acyl-CoA  $\Delta 9$  desaturase complex. All saturated long-chain fatty acids melt above body temperature range (lauric acid, 44°C; myristic acid, 58.5°C; palmitic acid, 63.6°C; and stearic acid, 69.5°C) and must be combined with polyunsaturated fatty acids in the triacylglycerols to ensure fluidity of body lipids. Desaturation is necessary to maintain fluidity of adipose tissue and of phospholipids in membranes and to enable elongation and conversion of the major dietary essential fatty acids (linoleic and linolenic) into even longer and more unsaturated fatty acids (50).

PUFA are required for synthesis of prostaglandins, cell wall lipids, and various other chemical structures. But, unlike plants, the vertebrate animals do not have enzymes able to insert double bonds between the 9-carbon and the terminal methyl group in C18 fatty acids. It has been known for many years that linoleic acid is the precursor for arachidonic acid synthesis and must be obtained through the diet. Unfortunately, early rat studies led to the mistaken belief that animals are able to interconvert linoleic acid to all other EFA. Gradually, it became apparent that there are at least four families of polyunsaturated fatty acids (*n*-7, palmitoleic; *n*-9, oleic; *n*-6 linoleic; and *n*-3 linolenic). Given the shortest member of each polyunsaturated C18 family, fatty acids can be elongated and desaturated to as many as 22 carbons and six double-bond sites, with various essential intermediate compounds also produced. A schematic diagram of pathways for synthesis of highly unsaturated fatty acids (HUFA) in fish is shown in Figure 1 (41). Elongation and desaturation is done by the same enzyme complex, and contemporary pathway diagrams show products of two alternative routes, desaturation first or elongation first. However, only products of the elongation first pathway are reported in typical fish oil analyses. The names of selected HUFA are shown in Table 12.

Since animals are able to desaturate up to, but not including, the C12 position, products of the *n*-7 (palmitoleic acid) and *n*-9 (oleic acid) families and the lesser-known *n*-5 myristoleic (9-C14: 1*n*-5) family are interesting but not dietary essential. However, *n*-6 and *n*-3 fatty acids must be provided in food or feed to enable



**Figure 1.** Pathways for synthesis of saturated and polyunsaturated acids. Reprinted with permission from Ref. 42.

TABLE 12. Members of Polyenoic Fatty Acid Families.<sup>a</sup>

Series	Unsaturated	Common Name	IUPAC Nomenclature
Palmitoleic (family)			
C16:1 <i>n</i> -7	9-16:1	Palmitoleic acid	9-Hexadecenoic acid
C18:1 <i>n</i> -7	11-18:1	<i>trans</i> -Vaccenic acid	11-Octadecenoic acid
C18:2 <i>n</i> -7	8, 11-18:2	—	8,11-Octadecadienoic acid
Oleic acid (family)			
C18:1 <i>n</i> -9	9-18:1	Oleic acid	9-Octadecenoic acid
C18:2 <i>n</i> -9	6, 9-18:2	Octadecadienoic acid	6,9-Octadecadienoic acid
C20:2 <i>n</i> -9	8, 11-20:2	Eicosadienoic acid	8,11-Eicosadienoic acid
C20:3 <i>n</i> -9	5, 8, 11-20:3	Eicosatrienoic acid	5,8,11-Eicosatrienoic acid
Linoleic acid (family)			
C18:2 <i>n</i> -6	9, 12-18:2	Linoleic acid	9,12-Octadecadienoic acid
C18:3 <i>n</i> -6	6, 9, 12-18:3	$\gamma$ -Linolenic acid	6,9,12-Octadecatrienoic acid
C20:3 <i>n</i> -6	8, 11, 14-20:3	Dihomo- $\gamma$ -linolenic acid	8,11-14-Eicosatrienoic acid
C20:4 <i>n</i> -6	5, 8, 11, 14-20:4	Arachidonic acid	5,8,11,14-Eicosatetraenoic acid
C22:4 <i>n</i> -6	7, 10, 13, 16-22:4	Adrenic acid	7,10,13,16-Dicosatetraenoic acid
C22:5 <i>n</i> -6	4, 7, 10, 13, 16-22:5	—	4,7,10,13,6-Dicosapentaenoic acid
Linolenic acid (family)			
C18:3 <i>n</i> -3	9, 12, 15-18:3	$\alpha$ -Linolenic acid	9,12,15-Octadecatrienoic acid
C18:4 <i>n</i> -3	6, 9, 12, 15-18:4	—	6,9,12,15-Octadecatetraenoic acid
C20:4 <i>n</i> -3	8, 11, 14, 17-20:4	—	8,11,14,17-Eicosatetraenoic acid
C20:5 <i>n</i> -3	5, 8, 11, 14, 17-20:5	EPA	5,8,11,14,17-Eicosapentaenoic acid
C22:5 <i>n</i> -3	7, 10, 13, 16, 19-22:5	Culpadonic acid; DPA	7,10,13,16,19-Docosapentaenoic acid
C22:6 <i>n</i> -3	4, 7, 10, 13, 16, 19-22:6	DHA	4,7,10,13,16,19-Docosahexaenoic acid

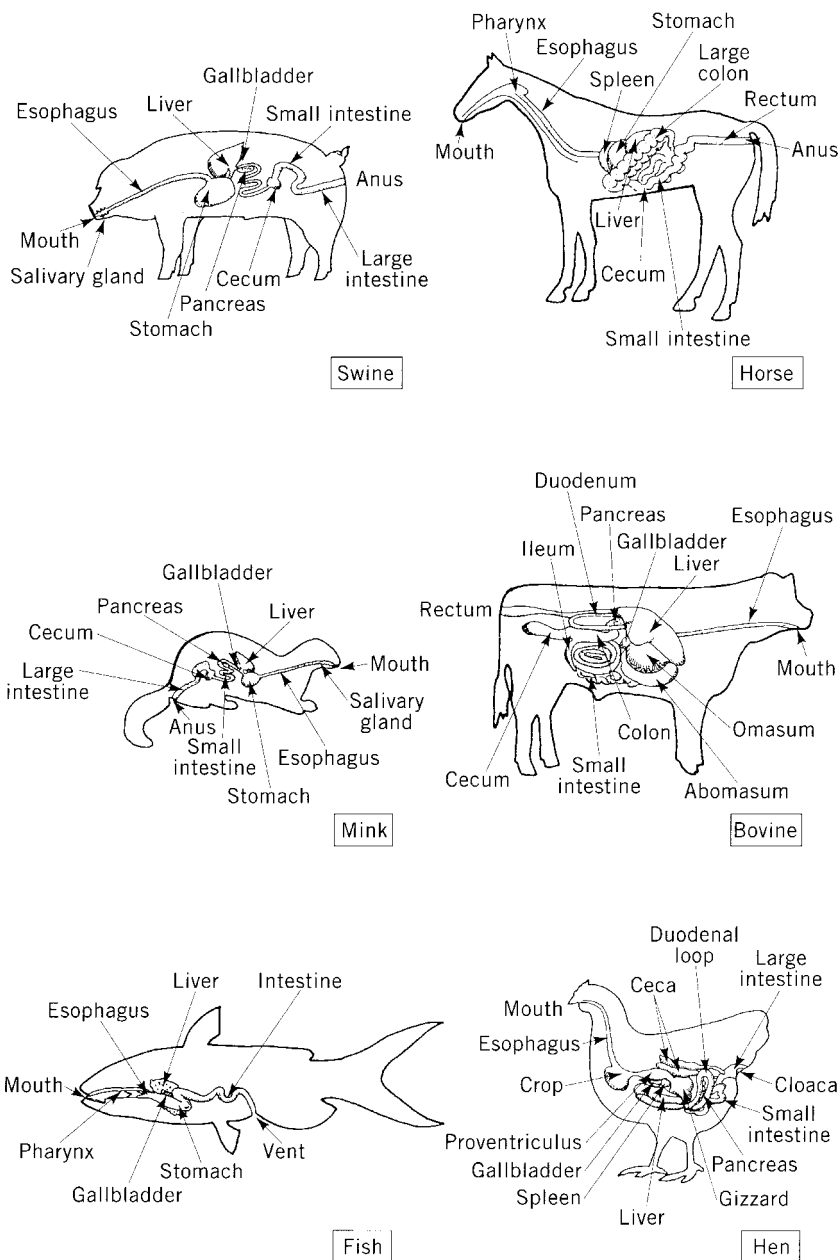
<sup>a</sup>All are *cis* isomers, unless noted.

synthesis of their other family members. Linoleic and linolenic acids are plentiful in oilseeds and typically the least-cost sources, respectively.

Complete metabolic pathways may not be operational in certain animal species and in human fat metabolism disorders. Some animals require linoleic and arachidonic acid supplementation, although both are members of the *n*-6 family. Some carnivorous fish require eicosapentaenoic acid (EPA; 5,8,11,14,17-20:5*n*-3) and docosahexaenoic acid (DHA; 4,7,10,13,16,19-22:6*n*-3) rather than linolenic acid (9,12,15-18:3*n*-3) alone.

*Nonruminant Mammalian Systems.* Fat digestion is described in greater detail in the following sections. The digestive systems of monogastric animals (swine, mink, and fish), a ruminant (bovine), a nonruminant herbivore (horse), and an avian (hen) are shown in Figure 2 (4).

In neonate, suckling mammals, short- and medium-chain fatty acids are preferentially split at the *sn*-3 triacylglycerol position by oral and gastric lipases and are absorbed in the stomach, while the long-chain fatty acids are hydrolyzed at the *sn*-1 and *sn*-2 positions and by pancreatic lipases and are absorbed in the small intestine (50, 51). With growth, the neonate fat digestion system becomes less active, and is replaced by the small intestine-pancreatic lipase pathway. But residual oral and gastric lipase activities and direct absorption of short-chain fatty acids in the



**Figure 2.** Digestive systems of representative animals. (1) Monogastrics with nonfunctional cecums: swine (omnivore), mink (carnivore), and catfish (omnivore). (2) Nonruminant herbivore with functional cecum and colon: horse. (3) Ruminant: bovine. (4) Avian : hen. Reprinted by permission from Ref. 4.

stomach continue at reduced levels in some species and individuals. These factors help explain why young animals use long-chain fatty acids less efficiently and have inspired the use of medium-chain coconut oil fatty acids in human and calf milk replacers.

The digestion of triacylglycerols in adult nonruminant mammals has been described as initiated in the mouth by lingual lipase released in the saliva at the base of the tongue (52). Up to 6% of the fatty acids are hydrolyzed and initiate emulsion formation in the stomach. The digesta (called chyme at this location) is released from the stomach slowly into the duodenum to ensure complete mixing with the bile salts and emulsification. Lipolysis occurs by association of pancreatic lipase and co-lipase at the surface of the bile salt-stabilized emulsion. Amphipathic molecules (fatty acids, *sn*-2 monoacylglycerols, and lysolecithins) are produced and associate with the bile salts to form water-soluble micelles from which absorption occurs.

Short-chain fatty acids and some medium-chain fatty acids are absorbed directly through the mucosa in the small intestine and are transported to the portal circulation and liver. In contrast, the long-chain fatty acids and 2-monoacylglycerols are first incorporated into bile salt micelles and transferred across the microvilli membranes. Then the fatty acids are activated into acyl-CoA esters and resynthesized into triacylglycerols. Resynthesis is favored if the *sn*-2-monoacylglycerol is unsaturated. The resulting hydrophobic triacylglycerols are coated with amphiphilic compounds (phospholipids—mainly phosphatidylcholine, cholesterol, and apoproteins) to form chylomicrons. Chylomicrons also can be produced by other pathways, including exocytosis (51), also called pinocytosis (4). The chylomicrons enter the lymphatic system as chyle, pass through the thoracic duct, and enter the blood circulation in the jugular vein (52). Digestibility of fats depends on the unsaturated: saturated (U:S) ratio of fatty acids in the total diet. Digestibility is high (85–92%) at ratios above 1.5, but decreases linearly below U:S of 1.5 (52). This also helps explain the often seen improvements in digestible energy in tallow-vegetable oil blends. Differences in digestibility of various fats between species have been reported.

**Ruminants.** Ruminants (cattle, sheep) offer many exceptions to the basic one-compartment stomach digestive system model. The first is a four-compartment stomach (located ahead of the small intestine): the rumen, reticulum, omasum, and abomasum. Food in the mouth is mixed with saliva, which provides enzymes, bicarbonate and phosphate buffers, and lubrication, and passes through the esophagus into the atrium ventriculi, a convex area formed by the rumen and reticulum. The reticulum has a honeycomb-like interior that serves as a collection point for foreign objects and as an organ for digestion. The rumen is essentially a fermentation vat, operating at pH 6.2–6.8, and is inhabited by bacteria and protozoa that are later swept down the digestive system as food. Fats deposited in their cells become available to the animal through nonrumen digestion. The omasum contains numerous tissue-like leaves that help grind the food and is also believed to reabsorb water from the partially digested food. The abomasum, with a pH of 1.6–3.2, functions essentially like a true stomach (3, 4).

Young ruminants initially function as young monogastric mammals. In calves, nursing stimulates a reflex closing of the esophageal groove, formed by muscular folds of the rumen and reticulum, and results in conveying milk from the esophagus directly to the abomasum. There, it is curdled by acid and rennin action, and the fat is digested by saliva and gastric lipases. The rumen starts developing as the young animal consumes increasing amounts of solid food; it is often considered to be operating at 2 months of age. Mature rumen size in lambs and goats is reached at 8 weeks, and at 6–9 months in cattle (3, 4). Rumen microorganisms are able to detoxify limited amounts of compounds, such as free gossypol in whole cottonseed and cottonseed meal, and reduce the effects of trypsin inhibitor in raw soybeans. Despite possible economic pressures for early feeding of cottonseed products to young animals, it is critical that the rumen first be operating.

Not having upper incisor teeth, cattle break off plant stems between their tongue and upper dental plate when grazing or eating dry hay, and swallow the mouthful without chewing. Bulk feed in feedlots is also swallowed by similar action. The feed is later regurgitated as soft lumps called boluses; the “cud” is chewed and again swallowed. This crushing action, after initial soaking, enhances breaking down the typical fibrous matrix. Microbes in the rumen rapidly digest carbohydrates and cellulose, to produce VFAs, which are absorbed through the rumen wall and can provide 60–80% of the animal’s energy needs from roughage alone (3).

The ether-extractable material in typical forages is 5–8%, of which about half is fatty acids occurring in monogalatosyldiacylglycerols and digalatosyldiacylglycerols. The fat content of most feeds is less than 5%, unless whole oilseeds or supplemental fats are added. In the rumen, fats are hydrolyzed to fatty acids and glycerol, which is metabolized immediately. Once in the unesterified form, 65–90% of the fatty acids are biohydrogenated, with the major products being stearic acid (C18:0) and vaccenic acid (*trans* 11-C18:1), a member of the palmitoleic (*n*-7) family. The presence of large amounts of free fatty acids and free oils and reduction of rumen pH by high grain feeding, increases the proportion of *trans* isomers formed. The saturated fatty acids are adsorbed to hydrophobic feed particles and are carried to the duodenum. The only esterified fatty acids reaching the duodenum are in the phospholipids of microbial cells. The fatty acids are dissociated from the feed particles by the detergent action of bile salts at the low pH of the proximal duodenum. In the absence of monoacylglycerols, lysolecithin (formed from the action of a pancreatic lipase on bile and microbial phospholipids) and oleic acid serve as amphipaths to form soluble micelles (52).

Factors favoring inhibition of microorganisms that digest cellulose and produce VFA and other compounds include the following (52):

- Feeding supplement fats at concentrations greater than 2–3% of feed dry matter.
- Degree of fatty acid solubility, with medium-chain (12- to 14-carbon) and unsaturated (vegetable oils, fish oils) being most inhibitory.
- Unesterified fatty acids are more inhibitory than triacylglycerols.
- Free oils are more inhibitory than those fed in whole seeds.



Factors minimizing inhibitory effects include:

Increasing roughage in the diet to induce more salivation results in increased buffer supply and rise in rumen pH; there is more surface area for fat absorption.

Removing the fatty acid or source from solution.

Ruminants are equal to or superior to nonruminants in their ability to digest saturated fatty acids, but unsaturated fatty acids fed conventionally have lower digestibility due to rumen biohydrogenation. Unsaturated fatty acids, fed in protected form, are digested equally in ruminants or nonruminants. Moderate levels of added fat (up to 3% of feed dry matter) are 85% truly digestible (52).

Although polyunsaturated fatty acids are biohydrogenated to stearic acid (C18:0) in the rumen, they are desaturated to oleic acid in the small intestine, mucosa adipose tissue, and mammary gland. Thus the ratio of C18:1 to C18:0 is greater in the fatty tissue and the milk lipids than in plasma triacylglycerols. Ruminant milk also contains 40–50% C4:0–C14:0 fatty acids by weight synthesized in the mammary glands from acetate (52).

Acetate is simultaneously produced and used (oxidized) by the gut, possibly accounting for up to 50% of the gut's energy requirement in ruminants. Propionate and butyrate produced in the rumen and/or hind gut fermentations (nonruminant herbivores) are extensively metabolized by the visceral tissues, so that only insignificant amounts reach the portal circulation. Plasma fatty acids, rather than acetate, are the major energy source for skeletal muscle energy in ruminants and other mammals (53).

*Avians.* Considerable specialization in digestive systems exists among avians. The focus here is on chickens, turkeys, and ducks (essentially omnivores) and ratites (herbivores). The flesh eaters are not discussed. The avian digestive system begins with the mouth and esophagus, which empty into the crop where feed is stored and soaked (see Figure 2). The feed then passes to the glandular stomach (proventriculus), where it is mixed with digestive juices, and then to a muscular organ (gizzard), which contains stones or grit to help crush and grind the feed. The digesta then moves through the small intestine where fat is digested and absorbed; to the ceca; the large intestine; and finally, the cloaca (3). Digestion, from the mouth to the cloaca, is rapid and takes about 2.5 h in the laying hen and 8–12 h in the nonlaying hen (4).

Following uptake, triacylglycerol resynthesis from long-chain fatty acids occurs in the small intestine mucosa of fowl by both the monoacylglycerol and glycerol-3-phosphate pathways. In contrast to mammals, the lymphatic system is poorly developed in poultry, and all nutrients are absorbed by the mesenteric portal system. The absorbed lipids are principally transported as triacylglycerols of the very low density lipoprotein fraction (VLDL). Lipoprotein synthesis in the liver is active in the laying hen. VLDL are present in the serum at high concentrations and appear to be the major lipid precursor for egg yolk synthesis (51, 54). Thus poultry and poultry products seem to be an almost direct way for transferring

selected fatty acids from feed into foods with little modification. Fats high in unsaturated fatty acids content are more readily absorbed by poultry. This partially explains the “extra caloric value” observed when vegetable oils are mixed with tallows in feeding broilers (55).

Ratites are flightless birds with heavily muscled legs for running and defense against enemies. The group includes the ostrich (*Sutruhio camelus*), emu (*Dromaius novaehollandiae*), common rhea and Darwin’s rhea (*Rhea americana* and *Pteronemia pennata*, respectively), Australian cassowary (*Casuaris*), and the kiwi (*Apteryx australis*). Although they are generally considered as zoo animals in the United States, there has been considerable interest recently in raising ostriches and emus for their hides, plumage, meat, and oil.

Ratites are equipped to subsist primarily on roughage in nature and have ceca of various activities. Emus have been reported to digest 35–45% of neutral-detergent fiber (NDF) in their diets (containing 26–36% NDF). This can contribute up to 63% of the standard metabolism and 50% of maintenance requirements for emus. Passage of feed through the gastrointestinal tract is rapid and  $4.1 \pm 0.2$  h has been observed (56,57). Relatively little has been published on the use of fat in raising ratites, with about 6% total being the norm.

*Fish.* It is estimated that at least 130 aquaculture species are being cultivated. Even so, today’s emerging industry already deals with far more species than the economic land animals and pets combined. Each species offers a potential challenge in unravelling a unique combination of metabolic pathways and nutrient needs. Fish are cold blooded, and are at one with their water environment in temperature and disposal of metabolic wastes.

Fish have been grouped into four classes: (1) *herbivores*, who eat green plants (carp, giant gourami, milkfish, perch, rabbit fish, tilapia, and Siamese gourami); (2) *detritus feeders*, who eat dead organic matter at the bottom of the pond, (mud carp); (3) *omnivores* (channel cat fish, common carp, gray mullet); and (4) *carnivores* (black carp, catfish, grouper, marble goby, salmon, sea bass, and trout). The relative intestine length of fish is shorter than that of land animals, but as with the land animals, carnivorous fish have shorter digestive tracts than the herbivores. The approximate ratios of intestine to body lengths of fish and land animals are trout, 1.0–1.5; carp, 2.0–2.5; dog and cat, 5; horse, 12; swine, 15; cattle, 20; and sheep, 30 (41).

Approximately 97% of the total fatty acids in fish oils consist of even-number carbon chains, but odd-number carbon chains and branched-chain odd-carbon acids are also present (58). Digestibility of fish fatty acids in poultry and swine feeds decreases with increasing chain length and increases with increasing unsaturation (59).

Green plants are able to synthesize the most complex lipids. As with land animals, fish function as gatherers and concentrators—in this case, of fatty acids that originated in plankton or may first have been cycled through lesser aquatic animals. Some carnivorous fish, in particular, have lost the ability to synthesize certain fatty acids and require supplementation. The marine food chain above 30°N latitudes is particularly rich in *n*-3 family fatty acids. In North Atlantic and Pacific fish,

eicosapentaenoic acid (C20:5 $n$ -3) and docosahexaenoic acid (C22:6 $n$ -3) account for about 90% of total PUFA, and linoleic acid (C18:2 $n$ -6) plus arachidonic acid (C20:4 $n$ -6) account for less than 2%. As a result, the  $n$ -6: $n$ -3 ratio is low ( $0.15 \pm 0.1$ ) compared with 0.38–0.93 in fish from Australian waters, where arachidonic acid is the major fatty acid in the southern latitude food chain. As the habitat moves farther south from 10°S Australia to 70°S Antarctica, the content of C20:4 $n$ -6 decreases in fish lipids, but C20:5 $n$ -6 increases. Among other documented observations is the fact that all desaturase enzymes are more active at lower (5°C) than higher (10°C) temperatures. A shift from C20:4 $n$ -6 to C22:6 $n$ -3 occurs in oil composition as fish adapt from freshwater to saltwater. The total lipids content is higher in cultivated fish than in wild fish and eels, but the total contents of C20:4 $n$ -6 and C18:3 $n$ -3 are higher in wild fish (60).

The feeding of soybean lecithin to cold-water fish and cold-water crustaceans—as a source of choline, inositol, ethanolamine, and PUFA—has been promoted in recent years. Levels as high as 7–8% of diet dry weight have been recommended (40, 41).

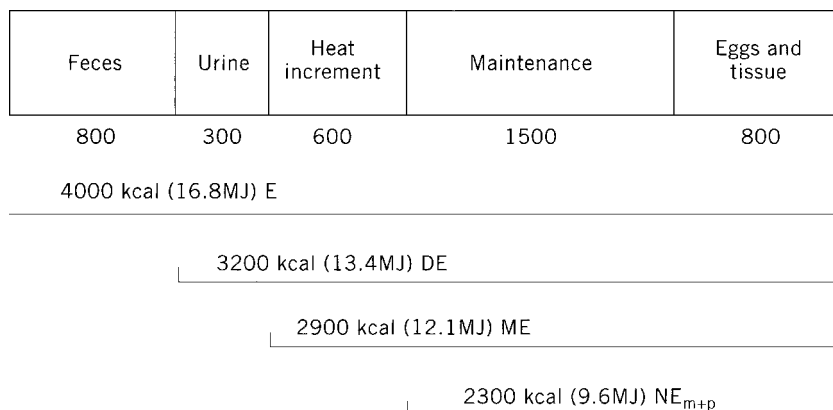
## 4.2. Energy Requirements and Availability

Feedstuffs provide water, energy, protein, mineral elements, and also organic chemical structures that the animal cannot make itself, and therefore are termed *dietary essential*. This group includes vitamins, certain amino acids, and certain fatty acids. All organic materials, including the essential structures, also have value as energy sources for muscular movement, body heat, synthesis of tissue, and storage nutrients, and metabolic reactions. The buildup of complex compounds requires energy, and their hydrolysis or breakdown gives up energy.

*NRC Definitions.* The majority of terms, used for describing energy assessments of feedstuffs have been defined by the NRC (61). A calorie (cal) is the heat required to raise the temperature of 1 g of water from 16.5 to 17.5°C, and is equivalent to 4.184 international joules (a term used for relating mechanical, chemical, and electrical energies as well as heat). A kilocalorie (kcal) consists of 1,000 calories, and a megacalorie (Mcal) equals 1 million cal, 1,000 kcal or 1 therm. The British thermal unit (BTU) is 252 calories, the amount of heat energy required to raise 1 lb of water 1°F, but is rarely used in nutrition. The joule equals  $10^7$  ergs (1 erg is the amount of energy expended to accelerated mass of 1 g by 1 cm/s) and was selected by Le Système International d'Unités (International System of Units) and the U.S. National Bureau of Standards as the preferred term for expressing all forms of energy (16). The kilocalorie is used here because it is the standard terminology used by nutritionists and industry in the United States.

Somewhat different terms and techniques are used between species for estimating or calculating different types of metabolic and production energies. The general relationships used in poultry are shown in Figure 3 (16).

*Gross energy* (E, GE) is the energy released as heat when a substance is completely oxidized to carbon dioxide and water. It is generally measured at 25–30 atmospheres of oxygen in a bomb calorimeter and is called the heat of combustion.



**Figure 3.** Disposition of dietary energy ingested by a laying hen. Reprinted with permission from Ref. 16.

*Apparent digestible energy* (DE) is the gross energy of feed consumed minus the gross weight of the feces. Birds excrete feces and urine together at the cloaca, and it is difficult to separate the feces and measure digestibility. DE values are used for many animals, but generally not with poultry.

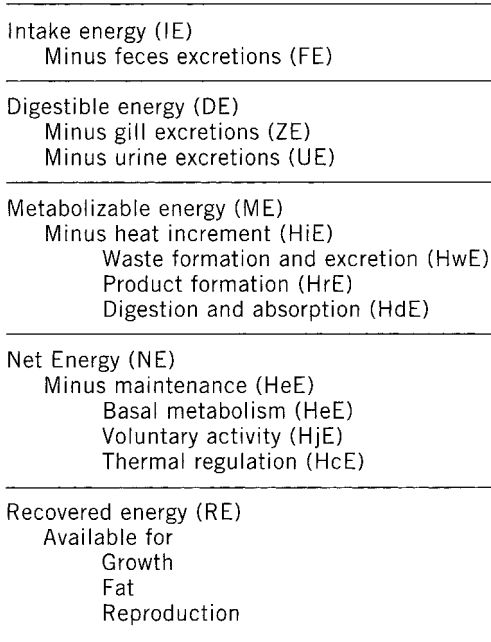
*Apparent metabolizable energy* (ME) is the gross energy of the feed consumed, minus the gross energy contained in the excreta (feces, urine, and gaseous products of digestion). A correction term for nitrogen retained in the body is usually applied to obtain ME<sub>n</sub>, the most common measure used in formulation of poultry feeds.

*True metabolizable energy* (TME) for poultry is the gross energy of the feed consumed minus the gross energy of the excreta of feed origin. A correction for retention of nitrogen is applied to give TME<sub>n</sub>. Most ME<sub>n</sub> values are determined by assays in which the test material is substituted for some ingredient of known ME value. When birds are allowed to consume the feed on an *ad libitum* basis, the ME<sub>n</sub> values obtained approximate the TME<sub>n</sub> values for most feedstuffs.

*Net energy* (NE) is metabolizable energy minus the energy lost to the heat of increment. NE may include the energy used for maintenance only (NE<sub>m</sub>) or for maintenance and production (NE<sub>m+p</sub>). Because NE is used at different levels of efficiency for maintenance or for production, there is no absolute NE value for each feedstuff. Productive energy, once a popular measure of the energy available to poultry from feedstuffs and an estimate of NE is seldom used (16).

Energy requirements can be partitioned in various ways, according to the needs for tending the species. For example, the DE requirement for the growing pig is the sum of its requirements for maintenance (DE<sub>m</sub>), protein retention (DE<sub>pr</sub>), fat retention (DE<sub>fr</sub>) and cold thermogenesis (keeping the body warm) (DEH<sub>c</sub>) (19) and is expressed as:

$$DE = DE_m + DE_{pr} + DE_f + DEH_c$$



**Figure 4.** Schematic presentation of fate of dietary energy for fish. RE is available for new tissue.

Net energy of lactation (NEL,  $NE_L$ ) is used for assessing energy requirements of dairy cattle (9). The relationships between different energy partitions for fish (11) are shown by a balance sheet in Figure 4. By this scheme, NE for fish is conceptually similar to  $NE_{m+p}$  for poultry, and recovered energy (RE) is equivalent to  $NE_p$ .

Gross (combustion) energies for carbohydrate, fat, and protein are 4.15, 9.40, and 5.65 kcal/g, respectively. Digestible and metabolizable energies have not been determined for all feedstuffs, and it is common nutrition practice to use 4 kcal/g for carbohydrates and proteins, and 9 kcal/g for estimating metabolizable energy (ME) values. However, calculated ME values for feed-stuffs generally overestimate actual feeding trial results.

*Analytical Techniques.* Lipids in feedstuffs typically are determined by Soxhlet extraction, using diethyl ether as solvent. But this technique also extracts waxes, chlorophyll, and other pigments as well as galactosides and other lipid-soluble materials, thus overestimating the quantity of lipid energy available to the animal. Fatty acid contents of ether extracts are 90% in triglycerides, 70–80% in cereal grains, and as low as 50% in forages (52). Because some insoluble salts of fatty acids are formed during digestion, it is important to use acidified solvents in determining fatty acid content of feces. The differences between conventional ether extract and an acidified ether extract of feces is often reported as “fecal soap.”

### 4.3. NRC Species Fat Feeding Considerations

The NRC subcommittees prepare extensive critical reviews when updating nutrient requirements for different species. Their reports probably have the broadest consensus of any available sources of nutritional information.

*Pet Foods.* Pet foods are a special consideration among the feedstuffs. Although formulated essentially from feed-grade ingredients, they are expected to have an anthropomorphic, or people foodlike, appearance and odor when used for house pets. Substantial quantities of pet foods are sold in discount chains and farm supply stores. “Professional” diets are also produced for dog breeders and trainers, guard services, police, and the military.

Dogs and cats are carnivores that have adapted to also eating cooked cereal carbohydrates and oilseed proteins. Cats are the more obligate carnivores, typically requiring higher protein content foods on a dry matter basis. Currently, they are the only domesticated animal for which a dietary essential level of taurine, an amino acid found mainly in meat, has been established.

Unlike the production-oriented economic animals (poultry, swine, cattle, fish, and shrimp) pets typically are allowed to live their full life cycles, and thus experience problems of aging, including kidney, vision, backbone, and hip failures, and obesity if food intake is not adjusted to the animal’s activity level. Special puppy, kitten, and life-cycle series pet foods have been marketed.

Pet foods are the main products, regulated by the Association of American Feed Control Officials, that reach urban and suburban households. AAFCO has chosen to establish its own nutrient requirements, rather than enforce the NRC nutrient requirements (8, 10). The following information was taken from the NRC recommendations (8, 10) and perhaps are still the most broadly examined and published consensus available on dog and cat nutrition.

*Dogs.* Far greater extremes in breed adult size and expected performance are seen among dogs than cats. Mature body weights range from 1 kg for the Chihuahua to 90 kg for the St. Bernard. Dogs are exposed to extreme temperatures and physical activities, like guard duty, pulling sleds, and hunting, not required of cats.

The breeds differ in the effectiveness of their coats to keep them comfortable over the range of temperatures encountered and the DE required to stay warm. Commercial dry dog foods usually contain 5.0–12.5% fat dmb. Nutritional deficiencies may be encountered in feeding high fat diets formulated without consideration for the higher energy value of fat. Dogs generally eat less of a high fat diet to maintain near normal energy intake, but in doing so, the daily intake of protein, minerals, or vitamins may be inadequate.

The apparent digestibility of fat by dogs varies from 80 to 95% when mixtures of plant and animal triacylglycerols are fed. Fatty acids are the primary source of energy for skeletal muscle during exhaustive exercise. Compared with a high carbohydrate diet, a high fat diet has been shown to lengthen the time to exhaustion of beagles on a treadmill by approximately 30% and to cause a greater elevation in plasma-free fatty acid concentration during exercise of sled dogs. Sedentary adult

dogs have a greater tendency for obesity when fed high fat diets *ad libitum* rather than high carbohydrate diets.

Linoleic acid is the only essential fatty acid recommended, and a minimum linolenic acid requirement has not been established. Signs of linoleic acid deficiency in puppies progress from coarse, dry hair to scaly skin, to lesions. The NRC recommends that a dog food contain at least 5% fat on a dry basis, including 2.7 g linolenic acid in the diet per 1,000 kcal ME, or 1% on the dry basis for diets containing 3.67 kcal ME/g) (10).

*Cats.* Mature body weights of domestic cats (*Felis domesticus*) range from 2 to 6 kg. Dry commercial cat foods usually contain 8–12% fat, and purified diets, containing 25–30% fat and 30–40% protein have been commonly fed. Fat adds to the palatability of diets, with cats having shown preference for beef tallow over butter and chicken fat, but no preference among beef tallow, lard, or partially hydrogenated vegetable oil. A diet containing 25% hydrogenated coconut oil has been reported to be unpalatable. Diets containing medium-chain triacylglycerols have been poorly accepted, and as little as 0.1% caprylic acid (C8:0) has caused diets to become unpalatable.

Cats have the capacity to tolerate and utilize high levels of dietary fat. Diets containing 25% fat have been selected over those containing 10–50%. Apparent digestibilities of 90% of fat (when fed at 10% of dry matter) and 97–99% (when fed at 25–50% of dry matter) have been reported. Fat in experimental diets has been raised to 64% of dry matter, without an increase in the proportion of fecal fat, indications of ketonuria, or significant pathological changes in the cardiovascular system.

Experiences in cat nutrition underscore the fallacy of assuming that metabolic pathways found in one species are automatically present in others. Early studies on metabolism of PUFA were conducted on rats, which have high  $\Delta 6$  and  $\Delta 5$  desaturase abilities to convert linoleic acid (18:2*n*-6) to the prostaglandin precursors dihomo- $\gamma$ -linolenic acid (20:3*n*-6) and arachidonic acid (20:4*n*-6), respectively. This led to the assumption that other species can desaturate polyunsaturated fatty acids equally well. Over a period of time, it was shown that cats are not able to convert 18:2*n*-6 to 20:3*n*-6 or 20:4*n*-6. The NRC currently recommends the inclusion of 5 g linoleic acid and 0.2 g arachidonic acid/kg diet dry matter.

Symptoms of EFA deficiency in cats include listlessness, dry hair coat with dandruff, poor growth, and increased susceptibility to infection. The effects of prolonged deficiencies include reduced feed efficiency without change in body weight, water loss through the skin, fatty livers, fat infiltration, and mild mineralization of kidneys as well as changes in blood platelet aggregation. Steatitis (yellow fat disease, a presumed vitamin E deficiency) has been often associated with consumption of high fish based diets, particularly canned tuna, red meat, and tuna oil. Supplemental vitamin E (D- $\alpha$ -tocopherol acetate) has been commonly included in diets containing tuna oils to prevent steatitis. A minimum of 30 mg  $\alpha$ -tocopherol/kg diet has been set for cats by the NRC (8). Compositions and metabolizable energies for fats from various sources, including animal organ meats, that might be used in cat foods are shown in Table 13 (8).

**TABLE 13. Fat, Fatty Acid, and Estimated Metabolizable Energy Contents of Oils for Potential Cat Feeding.<sup>a</sup>**

Common Name	International Feed Number	Dry Matter (%)	Ether <sup>b</sup> Extract (%)	Saturated <sup>b</sup> Fat (%)	Unsaturated <sup>c</sup> Fat (%)	Linoleic <sup>b</sup> Acid (%)	Linolenic <sup>b</sup> Acid (%)	Arachidonic <sup>b</sup> Acid (%)	ME (kcal/kg)
Bran oil	4-14-504	100.0	100.0	18.5	81.1	36.5	—	0	8047
Fat									
Swine (lard)	4-04-790	100.0	100.0	35.9	64.1	18.30	—	0.3–1.0	7850
Bacon	4-15-582	100.0	100.0	42.3	56.7	6.8	0.6	—	—
Beef	4-25-306	100.0	100.0	44.9	55.1	1.9	1.2	1.0	—
Lamb	4-24-921	100.0	100.0	52.1	47.9	2.4	2.4	—	—
Rabbit	4-24-923	100.0	100.0	43.3	56.7	19.9	9.4	1.8	—
Turkey	4-24-924	100.0	100.0	36.5	63.5	19.0	1.0	4.8	—
Brain, lamb	4-15-583	100.0	100.0	41.4	58.6	0.2	0.8	2.4	—
Kidney, lamb	4-15-584	100.0	100.0	45.4	54.6	6.1	3.0	5.3	—
Kidney, beef	4-15-585	100.0	100.0	56.3	42.7	3.6	0.4	1.9	—
Kidney, swine	4-15-586	100.0	100.0	43.5	56.5	8.7	0.4	5.0	—
Liver, beef	4-15-587	100.0	100.0	49.6	50.4	5.5	1.9	4.8	—
Liver, swine	4-15-588	100.0	100.0	41.6	58.4	11.0	0.4	10.7	—
Margarine									
Hard animal and vegetable oils	4-15-589	84.0	96.4	37.5	62.5	4.2	0.1	6.6	—
Hard vegetable oily only	4-15-590	84.0	96.4	38.2	61.8	9.1	0.5	0	—
Soft animal and vegetable oils	4-15-591	84.0	96.4	30.7	69.3	8.1	0.4	6.1	—
Soft vegetable oils only	4-15-592	84.0	96.4	33.1	66.9	19.3	1.8	0	—
Soft, polyunsaturated vegetable oils only	4-15-593	84.0	96.4	24.7	75.3	49.3	0.6	0	—
Offal fat, poultry	4-09-319	100.0	100.0	39.1	60.9	22.30	—	0.5–1.0	—
Oil									
Coconut	4-09-320	100.0	100.0	90.3	9.7	1.10	—	0	8047
Corn	4-07-882	100.0	100.0	12.3	87.7	55.40	1.6	0	8047
Fish, menhaden	7-08-049	100.0	100.0	40.0	60.0	2.70	—	20.0–25.0	—
Flax, common (linseed oil)	4-14-502	100.0	100.0	8.2	91.8	13.90	—	0	8047
Safflower	4-20-526	100.0	100.0	10.5	89.5	72.70	0.5	0	8047
Evening primrose	4-15-591	100.0	100.0	8.5–13.5	86.5–91.5	73.0	10.4	0	8047



**TABLE 13** (Continued)

Common Name	International Feed Number	Dry Matter (%)	Ether <sup>b</sup> Extract (%)	Saturated <sup>b</sup> Fat (%)	Unsaturated <sup>c</sup> Fat (%)	Linoleic <sup>b</sup> Acid (%)	Linolenic <sup>b</sup> Acid (%)	Arachidonic <sup>b</sup> Acid (%)	ME (kcal/kg)
Soybean	4-07-983	100.0	100.0	14.7	85.3	51.9	7.4	0	7283
Cotton seed	4-20-836	100.0	100.0	26.8	73.2	53.0	1.4	0	8047
Rapeseed low erucic acid	4-20-834	100.0	100.0	6.9	93.1	23.0	10.0	0	8047
Sunflower	4-20-833	100.0	100.0	10.4	89.6	65.7	—	0	8047
Tallow, animal	4-08-127	100.0	100.0	47.6	52.4	4.3	—	0.0–0.2	8343
White grease	4-20-959	100.0	100.0	—	—	1.1	—	—	—

<sup>a</sup>Reprinted with permission from Ref. 8

<sup>b</sup>Expressed as percent (by weight) of the ingredient on a dry basis (100% matter).

<sup>c</sup>Expressed as percent (by weight) of the total fatty acids in the ingredient as fed: fatty acids make up about 95% of the weight of triglycerides, assuming the average triglyceride contains one glycerol, one 16-carbon fatty acid, and two 18-carbon fatty acids: conversion factors for fat in brain, kidney, liver, and eggs were as recommended by Ref. 62.

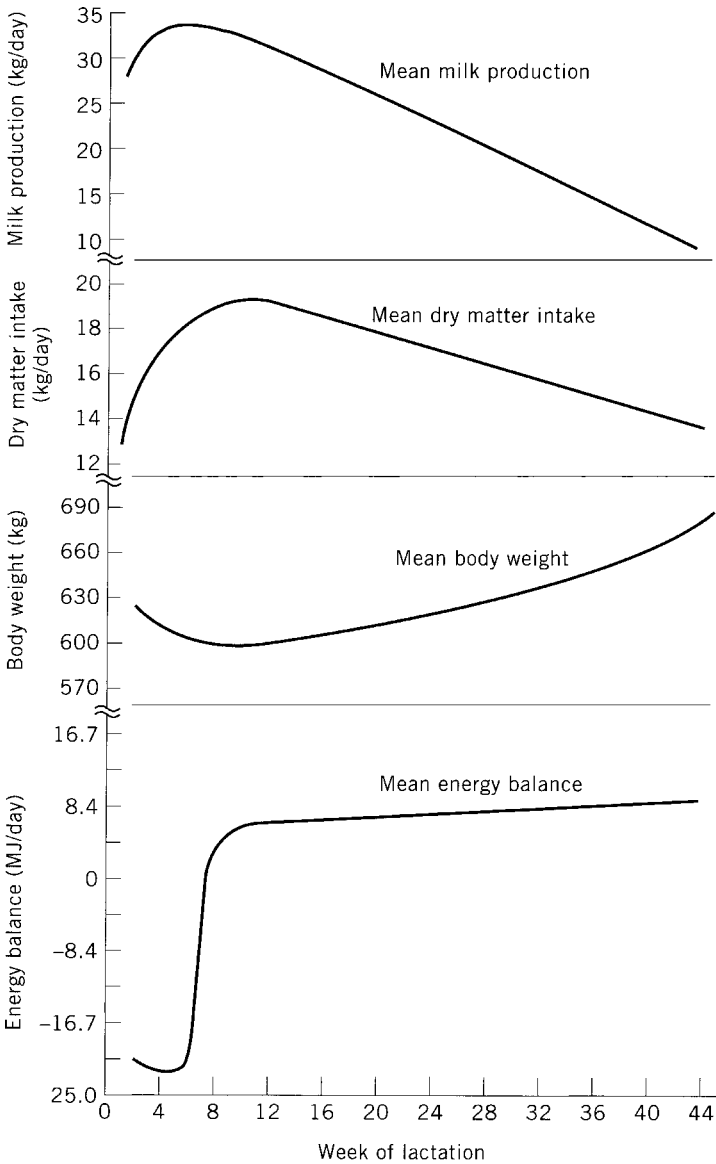
*Beef Cattle.* Nearly 50% of the tallow and grease produced domestically is of bovine origin, but only about 12% of the amount used in domestic feeds is consumed by the combined beef, dairy and veal industries. The principles of feeding fats to beef cattle, sheep, and meat goats are similar to those for dairy cattle. In recent years, dairying has essentially abandoned pastures and switched to intensive feedlot nutrition and animal health care practices. In contrast, beef cattle continue to be grazed on dispersed pastures and range-lands as the most profitable use of the land and might be finish fed in feedlots before marketing. Opportunities to observe individual animals and keep daily records on performance have not existed, and economics have not favored use of the concentrated feedstuffs common in dairying.

Although knowledge of bypass proteins have been applied in beef operations, there has been relatively little use of bypass fats or direct feeding of whole cottonseed, again perhaps because of economic considerations. However, beef cattle nutrition and feeding management currently are two of the more active research areas rejuvenated by the challenge of designer foods. The beef cattle industry has recognized that the ability to deliver leaner meats with a more favorable nutritional image, at a cost competitive to other meat, poultry, and fish alternatives, is critical to its future (7).

*Dairy Cattle.* Perhaps the greatest strides in modifying feedstuffs for use by any species has occurred with dairy cattle. The challenge is shown in Figure 5 (9). When a high yielding dairy cow calves, dry matter intake greatly declines and remains depressed on an average of 15% during the first 3 weeks of lactation. Body energy reserves are quickly mobilized, the animal falls into negative energy balance, and weight is lost. Peak milk production is reached in 4–8 weeks, but the animal does not start gaining weight until about week 10 when milk production has started to decline. In simple terms, full advantage is not taken of the animal's ability to increase and extend milk production because not enough nutrients are provided during the critical period. Various feeding strategies, including the use of protected fats as a high energy source, have been improvised in attempts to restore energy levels and maximize milk production.

The newborn dairy calf requires fat in its diet until the rumen becomes functional. About 10% fat in milk replacers is sufficient to supply the EFA, carry fat-soluble vitamins, and provide adequate energy for normal growth. Higher fat (15–20%) content milk replacers are recommended for colder climates and for veal production. Forages and grains usually contain less than 3–4% fat. Dairy cows can use approximately 0.45 kg fat/day, or 2–3% added fat in their diet.

Maximizing forage (fiber) intake is an important consideration in successful feeding of supplementary fat. Also, partial substitution of fat for starch and grain can correct the low milk fat syndrome caused by inadequate fiber and excessive grain diets. As much as 20–30% supplemental calcium and magnesium above recommended levels should be included to provide for calcium and magnesium soaps formed in the rumen when dairy cattle are fed unprotected long-chain fatty acids. Feeding unsaturated fats to dairy cattle is less desirable because of their inhibitory effects on rumen fermentation and digestion if the biohydrogenation



**Figure 5.** Relationships between milk production, dry matter intake, body weight, and mean energy balance of dairy cows during a typical lactation cycle. Reprinted with permission from Ref. 9.

ability of rumen microorganisms is exceeded. Animal fats, and blended animal-vegetable fat mixtures, have given the best results. The principles of feeding a maximum of about 0.5 kg fat/day, or to limit cottonseed to 2.5–3.0 kg/day/cow, have generally been successful.

An increase in long-chain fatty acids in the diet increases their secretion in the milk and inhibits synthesis of short- and medium-chain fatty acids in mammary tissue. Added dietary fat and whole cottonseed decrease the protein content of milk by about 0.1%. "Dry fats"—calcium salts of fatty acids that are 82% fat content—and prilled (beadlet-form) fats have been introduced in recent years. These products are free-flowing and easy to mix with the ration, do not coat the fiber, and are not biohydrogenated in the rumen. It has been suggested that cows can be fed up to 15% of their requirements as fat, the equivalent of 6–7% of total dry matter (9).

*Horses.* Horses are among the most visible nonruminant herbivores and have a highly active cecum–colon that enables microbial fermentation of fiber, but after opportunities for digestion and absorption of fat, proteins and nonstructural carbohydrates in the small intestine have been passed. Apparent prececal digestibilities of 71% for nonstructural carbohydrates have been reported for a high corn diet and 46% for a high alfalfa diet. Fermentation produces the VFA (acetic, propionic, isobutyric, isovaleric, and valeric acids). Approximately 7% of total glucose production (gluconeogenesis) from cecal propionate has been observed in ponies.

Body lipids are mobilized during exercise and are oxidized readily during strenuous exercise like galloping. Physically conditioned horses oxidize fat more efficiently than nonconditioned horses. Hyperlipidemia is an important clinical problem in small pony breeds. It is most common in mares in late gestation and lactation and occurs when the animal is in negative energy balance.

Horses accept added fat in the diet readily if it is not rancid. Corn oil was found to be preferred among different fats and mixtures evaluated at 15% of the diet. Increasing fat levels in the diet, in the form of rendered fat, has resulted in decreased feed consumption, early growth advantage for foals, and increased milk fat concentration. Horses fed a diet containing 12% fat (9% added corn oil) performed better and had higher blood glucose levels at the end of the ride than horses fed 3% fat. Rendered animal fat has been shown to spare muscle glycogen reserves. EFA requirements of horses are not known, and inclusion of 0.5% linoleic acid in the diet is recommended until more data are available (13).

*Swine.* Cold thermogenesis, the amount of energy required for the animal to stay warm, is important in swine nutrition. A total of 25 g (80 kcal of ME) of feed/day to compensate for each 1°C below the critical temperature has been recommended for 25–60-kg pigs during their growth period, and 39 g/ (125 kcal) for 100-kg pigs during their finishing period. It is further estimated that DE is reduced by 0.017% for each degree Celsius that the effective ambient temperature (EAT) exceeds the upper critical temperature of the animal (19).

The value of adding fat to the diet of weaning pigs is uncertain. Pigs cannot synthesize linoleic and arachidonic acids, which must be supplied in the diet. The linoleic acid requirement of 0.1%/kg diet is more than adequately met in typical corn–soy diets, and requirements have not been set for arachidonic acid. The nutritional value of fat as an energy source for pigs is influenced by its digestibility, quantity of fat consumed and its ME, and the environmental temperature in which the pigs are housed. Substitution of fat for carbohydrate calories in a diet for pigs

**TABLE 14. Characteristics and Metabolizable Energy of Various Fats Used for Feeding Poultry.<sup>a</sup>**

Sample	MIU <sup>b</sup> (%)	Free Fatty Acids (%)	Selected Fatty Acids, Percentage of Total Fatty Acids						Energy Content "As Fed"	
			16:0	16:1	18:0	18:1	18:2	18:3	kcal ME/kg	Methodology <sup>b</sup>
Animal tallow										
Beef	0.3	4.3	26.1	5.1	25.2	37.4	1.9	—	6,683–6,916	—
	—	—	35.4	2.7	36.5	24.5	0.9	—	7,268–7,780	ME <sub>n</sub> poult 10%
Commercial	3.5	1.6	22.0	2.7	15.8	47.6	8.7	1.9	7,628	—
	0.5	2.4	25.8	3.7	18.1	42.1	4.6	—	6,808–8,551	ME <sub>n</sub> poult 2–8 weeks
	2.2	4.8	26.9	3.3	17.4	41.5	7.5	0.1	6,020–7,690	ME <sub>n</sub> chick 10–20%
	4.1	6.0	19.9	1.5	14.0	47.2	12.7	1.7	6,060	—
	3.0	10.2	21.2	5.9	15.5	45.4	9.6	1.2	7,148	—
	4.0	15.5	22.0	3.6	13.1	49.6	8.4	1.7	6,258	ME <sub>n</sub> chick 9%
	3.6	16.5	22.5	3.0	16.0	47.9	7.0	1.6	6,709	—
	2.9	19.1	25.5	4.0	19.3	40.0	4.9	<0.1	6,633–9,353	ME <sub>n</sub> chick 2–6%
Soap stocks	5.9	65.1	36.2	0.9	9.6	44.1	8.2	—	4,900	—
Animal-vegetable blends										
Commercial	3.6	61.0	21.0	1.4	6.0	25.4	38.6	4.2	7,114–8,924	ME <sub>n</sub> poult 2–8 weeks
Commercial edible	—	—	21.1	2.1	16.2	41.3	10.3	0.6	9,360	—
Tallow, crude canola	—	—	16.8	2.2	10.3	47.6	12.1	4.6	8,710	—
Tallow, crude soy	0.8	13.6	19.8	1.6	10.3	34.4	29.9	6.3	7,660	ME <sub>n</sub> chick 10%
	0.9	2.6	19.0	1.7	10.7	34.3	27.8	3.8	8,110–8,820	ME <sub>n</sub> chick 10%
Tallow, refined corn	—	—	20.9	2.1	10.4	32.2	30.5	0.4	9,570	—
Tallow, refined soy	0.7	13.8	19.4	1.5	10.3	34.8	29.5	6.4	7,830	—
Tallow, vegetable oil soap stocks	1.5	49.2	24.7	2.3	9.6	34.6	21.9	0.5	8,490	—
Animal soap										
stock, soy soap-stock	1.7	68.7	23.9	0.5	6.9	34.1	32.6	—	5,834	—
Beef, crude soy	0.9	36.3	17.7	1.0	12.5	34.5	31.2	3.9	7,571	ME <sub>n</sub> chick 9%
	0.8	36.2	16.0	3.1	12.2	32.4	31.0	3.9	7,788	ME <sub>n</sub> chick 9%
Lard, crude canola	—	—	17.2	1.3	9.5	51.1	13.7	3.2	10,000	—
Canola oil										
Crude oil	—	—	4.9	0.4	1.9	61.0	18.8	7.7	9,210	TME 15%
Soap stock	—	—	9.9	0.4	4.8	52.4	22.4	7.5	7,780–8,930	ME <sub>n</sub> TME regression

Coconut oil										
24 oils, MCFA = 57%	—	—	8.2	0.4	3.0	5.7	1.8	—	—	—
Undefined, MCFA <sup>d</sup> = 34%	—	—	12.8	—	2.9	13.7	23.1	—	8,812	ME <sub>n</sub> chicks 9%
Corn oil										
Refined	—	—	12.2	0.5	0.7	24.7	60.5	1.4	9,639–10,811	ME <sub>n</sub> poults 10%
	—	—	12.4	0.1	1.9	26.9	57.0	0.7	9,870	TME 15%
Fish oil										
Menhaden	—	—	—	—	—	—	—	—	8,450	ME <sub>n</sub> chicks 4–12%
Hydrogenated	—	—	18.6	5.8	4.8	18.5	24.1	1.3	6,800	ME <sub>n</sub> chicks 9%
Lard										
Edible	—	—	28.7	2.1	19.6	40.9	8.7	—	9,114–9,854	ME <sub>n</sub> poults 10%
	—	—	28.9	2.2	16.9	38.0	9.7	0.2	9,390	TME 15%
	0.2	0.1	26.6	3.1	15.8	42.4	9.1	<0.1	9,926–10,236	ME <sub>n</sub> chicks 2–6%
	1.1	0.2	22.4	2.1	17.7	46.1	8.0	2.1	7,337	ME <sub>n</sub> chicks 9%
Palm oil										
Fatty acid composite	—	100	46.4	0.2	5.0	38.7	6.9	0.1	7,710	TME 15%
Refined oil	1.8	0.2	40.7	0.3	5.2	41.6	11.4	—	5,800	ME <sub>n</sub> chicks 9%
Used in cooking	1.8	1.0	38.0	1.5	5.5	44.3	9.0	—	5,302	—
Poultry fat										
Commercial	0.7	0.7	21.6	4.8	7.2	42.3	23.0	—	8,625–8,916	ME <sub>n</sub> TME chick 7%
	3.9	0.5	18.1	5.9	4.6	46.2	23.3	1.1	9,360	TME 7%
Soybean oil										
Crude	1.4	0.6	11.3	0.3	3.9	27.2	49.8	7.5	8,650–8,020	ME <sub>n</sub> chicks 10–20%
Dried gums	1.3	12.2	21.0	0.3	4.5	17.1	45.9	1.8	6,440	—
Crude	—	—	12.2	0.1	3.2	26.0	51.6	6.3	9,510	TME 15%
Refined	2.0	1.3	10.6	<0.1	3.9	25.1	52.1	7.0	9,687–10,212	ME <sub>n</sub> chicks 2–6%
	1.8	0.1	11.6	—	3.9	19.8	57.9	6.8	8,375	ME <sub>n</sub> chick 9%
Soap stocks	4.2	72.3	7.9	—	4.1	24.0	56.9	7.1	6,111	—
Used in cooking	4.0	1.1	28.5	—	5.0	35.8	28.0	2.7	6,309	—
Sunflower oil										
Refined	—	—	6.7	0.1	4.3	27.4	57.1	3.7	9,659	ME <sub>n</sub> chick 2–8%

<sup>a</sup>Reprinted with permission from Ref. 16.

<sup>b</sup>Moisture, ether insolubles, and unsaponifiable matter contents as a percentage of the fat.

<sup>c</sup>ME<sub>n</sub>, apparent metabolizable energy corrected for nitrogen retention; TME, true metabolizable energy using the rooster unless otherwise stated, and level(s) of fat used in the test diet. Some ME values are not corrected for nitrogen retention, particularly those before 1970.

<sup>d</sup>Medium-chain fatty acid contributions (C8:0 + C10:0 + C12:0).

maintained in a thermoneutral environment increases growth rate and decreases the ME required/unit of live weight gain. For pigs housed in a warm environment, voluntary ME intake increases by 0.2–0.6% for each additional 1% of fat added to the diet. This increase is thought to occur because of the reduced heat increment of fat compared to that of carbohydrate.

The age of the pig, chain lengths of the fatty acids, and the unsaturated: saturated (U : S) fatty acid ratio influence the apparent digestibility of short- or medium-chain fatty acids (14 carbons or less). The apparent digestibility of fatty acids of 14 carbons or less is 80–95%; and that of fats from diets containing a ratio of U : S fatty acids greater than 1.5 : 1 is 85–92%. Apparent fat digestibility decreases by 1.3–1.5% for each additional 1% of crude fiber in the diet. Digestible energy (DE) and metabolizable energy (ME) of different fats for pigs is estimated as lard, 7,860 and 7,750, respectively; poultry fat, 8,635 and 7,975; tallow, 8,200 and 7,895; corn oil, 7,620 and 7,350; and soybean oil, 7,560 and 7,280 (19).

*Poultry.* The science of nutrition economics is perhaps more advanced for chickens than for any other species. The most appropriate energy level in selecting diets is the one that results in the lowest feed cost per unit of product (weight gain or eggs). Depending on the relative cost of high energy grains and feed-grade fats, this may vary between low energy and high energy diets throughout the world. Fat is usually added to the feed for meat-type poultry to increase overall energy concentration and improve productivity and feed efficiency. The moisture, insoluble, and unspoonifiable compounds (MIU components) of rendered fats usually are of no value and act as diluents. Fatty acid chain length, extent of unsaturation, and nature of esterification all influence intestinal absorption. The percentage MIU and percentage digestibility combine to influence the  $ME_n$  value. All feed fats should be stabilized by an antioxidant to preserve unsaturated fatty acids.

Improved utilization of dietary fats has been shown to occur after 2–6 weeks of life for chickens, and is particularly evident with long-chain saturated fatty acids. When animal tallow is added to feed at a low level, it may be advantageous to blend it with a small amount of vegetable oil (55). The resulting  $ME_n$  values of blends are higher than can be accounted for on an arithmetic basis. Use of saturated fatty acids is improved by the presence of unsaturated fatty acids in the blend. In trials, fat utilization increased rapidly in the U : S ratio of 0–2.5, nearly reaching an asymptotic maximum at U : S of 4. For broilers, nearly 75% of the variation in fat utilization and  $ME_n$  was due to differences in the chemical composition of the fat fraction. High level fat feeding apparently increases intestinal retention time of feed and allows for more complete digestion and absorption of the nonlipid constituents. Adding fat to feed as an isogenic substitute for carbohydrate usually improves productive energy at the same level of  $ME_n$ . Characteristics and metabolizable energy contents of fats that have been fed to poultry are shown in Table 14. Equations for estimating metabolizable energy content of fats for feeding poultry are presented in Table 15 (16).

Fatty acid synthesis in fowl occurs primarily in the liver. The rate of fatty acid synthesis and deposit of body fat increase rapidly just preceding sexual maturity. Fat absorbed from the intestine is transported to the liver; the unsaturated fatty acids

**TABLE 15. Equations for Estimating Energy Value (kcal/kg Dry Matter) of Feed Ingredients from Proximate Composition for Feeding Poultry.**<sup>a,b</sup>

Ingredient	Prediction Equation
All fats and oils	$ME_n = 8,227 - 10,318^{(-1.1685)}$ [unsaturated : saturated ratio] $ME_n = 28,119 - 235.8 (C18:1 + C18:2)$ $- 6.4 (C16:0) - 310.9 (C18:0) + 0.726 (IV \times FR_1)$ $- 0.0000379 [IV (FR_1 + FFA)]^2$
Vegetable oils (free fatty acid <50%)	$ME_n = -10,147.94 + 188.28 IV + 155.09$ $FR_1 - 1.6709 (IV \times FR_1)$
Vegetable oils (free fatty acid >50%)	$ME_n = 1,804 + 29.7084 IV + 29.302 FR_1$
Animal fats (free fatty acid <40%)	$ME_n = 126,694 + 1645 IV + 838.4$ $C16:0 - 215.3 C18:0 + 746.61$ $FR_1 + 356.12 (FR_1 + FFA) - 14.83 (IV \times FR_1)$
Animal fats (free fatty acid >40%)	$ME_n = -9,865 + 194.11V + 300.1 C18 : 0$

<sup>a</sup>Reprinted with permission from Ref. 16.

<sup>b</sup> $ME_n$ , nitrogen-corrected metabolizable energy; IV, iodine value; C16:0, percent palmitic acid; C18:0, percent stearic acid; C18:1, percent oleic acid; C18:2, percent linolenic acid; FFA, percent fatty acid, calculated as oleic acid equivalents;  $FR_1$ , first fraction from a column chromatography separation that contains the practically unaltered triglycerides plus other apolar components.

are unchanged for the most part, but the saturated fatty acids, especially stearic acid may undergo desaturation to be converted to oleic acid. Elongation and further desaturation of 18 : 2n-6 and 18 : 3n-3 may occur in the liver. Most lipid in egg yolk is formed in the liver using fatty acid obtained from the diet or formed by *de novo* synthesis. Providing fats avoids the cost of synthesis and is more energy efficient than synthesis from carbohydrate. Directly employing dietary fat in the assembly of either body or egg lipids results in a fatty acid composition similar to that of the diet. Depot fat is most affected by the source of dietary fat and is more influenced by the vegetable oils having high proportions of polyunsaturated fatty acids than by saturated animal fats (16). The feeding of fish oil to chicks significantly improves growth and antibody production (63).

Linoleic acid (18 : 3n-6 and  $\alpha$ -linolenic acid (18 : 3n-3) are metabolically essential fatty acids, but linoleic acid is the only essential fatty acid for which a dietary requirement has been established. Characteristic EFA deficiency symptoms observed in poultry include an increased need for water and decreased resistance to disease. A dietary requirement for linoleic acid has been set at 1% of diet dry matter. No major special considerations are mentioned for turkeys, ducks, ring-necked pheasants, Japanese quail, and bobwhite quail (16).

*Fish.* Energy requirements in fish are lower than in animals that maintain constant body temperatures, thus leaving more of the net energy in production diets available for growth and maintenance. Proteins and lipids are highly available energy sources for fish, while the value of carbohydrates is variable among species. Nile tilapia and channel catfish (warm-water omnivorous species) digest more than 70% of the gross energy in noncooked starch; rainbow trout (cold-water carnivore) digests less than 50%. Extrusion processing and other forms of cooking increase



digestibility of starch for fish. Studies have shown extrusion-processed corn to have 38% higher DE for channel catfish than compression-pelleted corn; gelatinized starch has a 75% higher DE for rainbow trout than raw starch. The challenge in the coming years will be to learn how to make low cost carbohydrate feedstuffs usable in feeding fish and other aquaculture species.

Maintaining life takes priority over growth and other functions, and energy concentration is the first consideration in developing diets for fish. Protein usually is given first attention because it is more expensive than other energy-yielding diet components. Fish convert dietary protein to tissue as efficiently as warm-blooded animals. A dietary balance is then established between protein and energy, which may be expressed as carbohydrates—or as lipids in the case of the salmonoids.

In common with other vertebrates, fish cannot synthesize linoleic or linolenic acids *de novo* and vary considerably in abilities to convert 18-carbon unsaturated fatty acids to longer-chain, more highly unsaturated fatty acids of the same series. The EFA requirements for selected species of fish are presented in Table 16 (11). In general, freshwater fish require either linoleic acid (18:2*n*-6) or linolenic acid (18:3*n*-3) or both, whereas stenohaline marine fish (those unable to withstand a wide variation in water salinity) require dietary eicosapentaenoic acid (EPA, 20:5*n*-3) and/or docosahexaenoic acid (DHA, 22:6*n*-3).

Among the freshwater species, the ayu, channel catfish, coho salmon, and rainbow trout require 18:3*n*-3 or EPA and/or DHA. Chum salmon, common carp, and Japanese eel require an equal mixture of 18:2*n*-6 and 18:3*n*-6. Nile

**TABLE 16 Essential Fatty Acid Requirements of Fish<sup>a,b</sup>**

Species	Fatty Acid Requirement
Freshwater fish	
Ayu	1% linolenic acid or 1% EPA
Channel catfish	1–2% linolenic acid or 0.5–0.75% EPA and DHA
Chum salmon	% linoleic acid; 1% linolenic acid
Coho salmon	1–2.5% linolenic acid
Common carp	1% linoleic acid; 1% linolenic acid
Japanese eel	0.5% linoleic acid; 0.5% linolenic acid
Rainbow trout	1% linolenic acid; 0.8% linolenic acid; 20% of lipid as linolenic acid or 10% of lipid as EPA and DHA
Nile tilapia	0.5% linoleic acid
Zillii's tilapia	1% linoleic acid or 1% arachidonic acid
Striped bass	0.5% of EPA and DHA
Marine fish	
Red sea bream	0.5% EPA and DHA or 0.5% EPA
Giant sea perch	1% EPA and DHA
Striped jack	1.7% EPA and DHA or 1.7% DHA
Turbot	0.8% EPA and DHA
Yellowtail	2% EPA and DHA

<sup>a</sup>Reprinted with permission from Ref. 11.

<sup>b</sup>Linolenic acid, 18:3(*n*-3); EPA (eicosapentaenoic acid), 20:5(*n*-3); DHA (docosahexaenoic acid), 22:6(*n*-3); linoleic acid, 18:2(*n*-6); and arachidonic acid, 20:4(*n*-6).

tilapia and Zillii's tilapia require only 18 : 2n-6 for maximum growth and feed efficiency. Stripped bass cannot elongate 18 : 3n-3 and require 22 : 5n-3 and 22 : 6n-3. The EFA function as components of phospholipids in all biomembranes and as precursors of eicosanoids required for a variety of metabolic functions. Principal gross signs of EFA deficiency reported for various fish include fin rot, a shock syndrome, myocarditis, reduced growth rate, reduced feed efficiency, and increased mortality.

Lipids serve as an important source of dietary energy for all fish and to a greater extent for cold-water and marine fish, who have a limited ability to use dietary carbohydrates for energy. Increasing the lipid content of some diets has also been effective in decreasing protein requirements in some applications (11).

## 5. FAT UTILIZATION PRACTICES

### 5.1 Fat Selection Principles

Extensive reviews on feeding of fats to animals have been prepared (64, 65). It has been reported that feeding fats at high levels to ruminants decreases digestibility of protein and fat by overwhelming the fat absorption capacity (66). Fatty acid intake affected fatty acid digestibility quadratically.

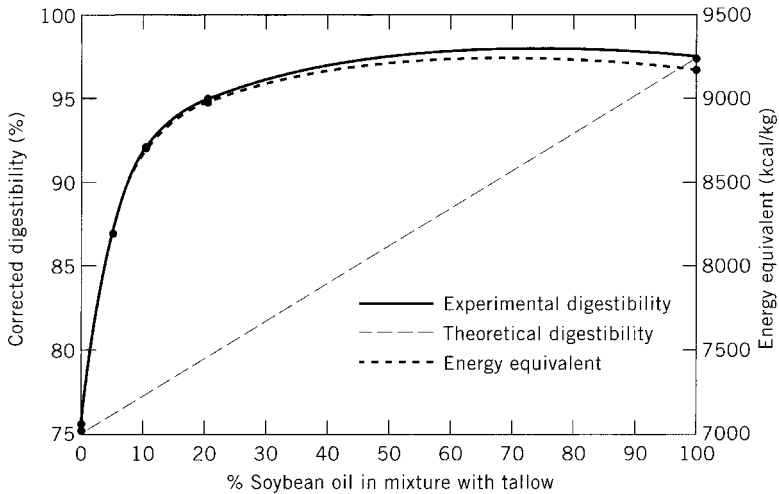
Sources and compositions of fatty materials, and characteristics of animals that will use them, were reviewed above. Approximately 3000–4000 citations on research in feeding fats to various species are available in computer-searchable databases, now approaching their twenty fifth year. The reader is referred to these for the role of fat in additional feedstuffs and animal species. Several principles stand out, which are discussed in the following paragraphs.

Fats are best fed back to the same species. Tallows are best used by cattle. Fish oils should only be used in levels that do not cause off-flavors in eggs, broilers, pork, or bacon. They are not recommended for cats and mink because of potential steatitis problems (67), unless the diet is adequately supplemented with vitamin E. Fish oils are valuable in growing fish, and are more effective in feeds if obtained from species whose metabolism focuses on the same HUFA family (*n*-6 or *n*-9) as the species cultivated.

In nonruminant species (poultry and swine), digestibility increases as chain lengths of fatty acids shorten and as polyunsaturation increases. Corrected digestibility and energy equivalent (the product of digestibility and heat of combustion) of beef tallow have been improved appreciably by adding up to 20% soybean oil (Figure 6) (55).

Fats not protected for "escape" or "bypass" will be altered if fed to ruminants. Within limits, triacylglycerols are hydrolyzed, and the polyunsaturated fatty acids are biohydrogenated by rumen bacteria enzymes.

Consistent performance in today's feed conversion–animal production industries requires consistent feedstuffs. In addition to providing products free of chick edema factors, pesticides, herbicides, polychlorinated biphenyls (PCBs), and other hazardous contaminants, the tallow and grease supplier is expected to deliver products with increasingly consistent fatty acid profiles. This requires appropriate



**Figure 6.** Synergistic effect of soybean oil on corrected digestibility and energy equivalent of beef tallow. Data from Ref. 55.

raw materials purchasing, classification, segregation, and blending abilities. Fat suppliers likely have users with different needs. Modern quality-control procedures for fats already have gone beyond iodine value (IV)—a measurement of unsaturation in the product—to gas liquid chromatography (GLC) for monitoring fatty acids profiles as well as potential contaminants.

Increasing use is being made of the peroxide value (PV, POV) as a means of screening feeding fats for potential toxic fat oxidation products. Fish larvae and fingerlings are the most sensitive to these compounds, but PV is also used for monitoring fat quality in feeding poultry and other animals. Peroxides are unstable compounds formed during oxidative deterioration of fats and oils and may not themselves be the toxic constituents. Thus a fat that has undergone oxidation may have reduced peroxide values but still contain significant quantities of toxic components. Despite these limitations, PV currently is preferred in practice over 2-thiobarbituric acid (TBA), diene value (DV), triene aldehyde value, *p*-anisidine value, and totox (two times the peroxide value plus the anisidine value).

As background, the AOCS active oxygen method (AOM) test uses 100 meq peroxide/1000 g sample as the end point for predicting hours of stability of a fat sample heated at  $97.8 \pm 0.2^\circ\text{C}$ , under controlled aeration. But, in food-grade applications, major buyers of frying oils typically specify 1 or 2 as the maximum allowable PV at receipt. In soy oil, 1.0–5.0 PV is considered low oxidation, 5.0–10.0 PV moderate oxidation, and 10+ high oxidation. The flavor of oil with a PV above 20 usually is so unacceptable that the product is of little interest.

Currently, PV generally is not included in industry product definitions or trading specifications, but increasingly appears in buyer purchase specifications. Oils with wide ranges of PV values have been reported fed to different species with varying toxic effects. The AOCS will table the AOM method as means for predicting the

oxidative stability of fats and oils in 1996, with endorsement of oil stability index (OSI) and other methods that estimate the time for an oil to reach the state of oxidation induction, instead of a specified PV value. Interest in the healthfulness of polar compounds formed in oils during deep fat frying of foods is also increasing and is likely to lead to reviews on use of spent restaurant frying oils in animal feeds. Anything that is written on this topic today is likely to become outdated rather quickly. It is recommended that readers check with the prevailing industry practices regarding measurement and use restrictions of oxidized and heated oils in feeding animals.

Processing fats and oils for feeds use includes:

Mixing liquid tallows, greases, and oils into dry feeds.

Coating (“enrobing”) extruded and pelleted feeds.

Extrusion of high fat content feeds.

Preparation of free-flowing granular fats for mixing into dry feeds.

Preparation of free-flowing rumen-protected fats.

Feeding whole cottonseed.

Extrusion of full-fat oilseeds.

Dry roasting of soybeans.

Not all decisions regarding the use of fats are technical or engineering-based. For example, liquid fats wick through kraft paper feed bags. In using a multiwalled bag, the fat barrier should be inside, next to the product. Some feed manufacturers prefer to use “harder” (higher melting point) fats to minimize this problem and possibly save on packaging costs. Undesirable fatty odors, and tendencies of products to oxidize (become “rancid”) during storage, also can be reduced using higher melting point fats.

## 5.2. Fat Storage and Application

*Storage Tanks.* There is no major reason for not making full use of permitted antioxidants and metal scavengers in feed fats. These ingredients should be added to fats, with thorough dispersion, as soon as they are made. Nitrogen blanketing, to form a cover of inert gas, is often used in storage and handling of edible fats. Costs of maintaining a nitrogen blanket may not be justified for storage of inedible fats, but at least every effort should be made to avoid mixing in air, including tight seals on the suction side of truck unloading pumps and bringing fat into tanks through internal down pipes with tips submerged or floating to avoid splattering of fat.

Fats must be liquid to be pumpable, whether for addition to feeds or for further processing. Heat accelerates all deterioration processes. Quality of the final product is improved by storing fats at temperatures slightly above the melting point (50°C; 121°F). They should then be heated as needed, by heat exchangers to 80°C (175°F), to improve mixing and absorption in feeds. In cold climates, or in wintertime, it may be necessary to heat to higher temperatures to avoid formation of “fat balls” when added to cold ingredients at the mixer.

Tanks typically are made of mild steel, but stainless steel may be required when continuously handling fatty acids, acidified soapstocks, or fats containing high levels of free fatty acids. Approved epoxide-type internal coatings are sometimes used. Moisture in the oil should be kept low; an increase in moisture content from 1 to 2% is reported to double the corrosion rate. The tank should be built and leveled so the bottom drains to the discharge port. An external thermostatically controlled jacket steam heating ring or electrical element should surround the tank near the bottom. Internal steam heating coils can develop unnoticed leaks and should be avoided. Some operators prefer to take oil from the tank slightly off the bottom, allowing water and precipitate to collect in the low spot. The installation of a side-mounted mixer may be desirable if the fat is of a quality that separates or forms precipitates on standing. The propeller should be of a size, speed, and placing that does not incorporate air into the fat. A side manhole is preferred for cleaning and inspecting the tank. The top of the tank should have a vent or vacuum release valve to avoid collapsing the tank when unloading, and equipped to avoid intake and condensing of moist air. Insulation of the tank is a good investment in almost any location. The tank should be enclosed by a containment dyke large enough to hold its contents in case of leakage.

If the installation is large enough, welded piping is preferred, and an expansion loop may be warranted if long horizontal runs are required. Galvanic corrosion always occurs between dissimilar metals and can be reduced by the use of bolted flanges and gaskets. Copper and brass valves should be avoided. All lines should be chased with steam-heated copper tubing or electrical heating wire, with thermostat controls to prevent hardening of tallows. Condensate traps should be included to drain the tubing. Unless the pipes are designed to drain completely, they should be blown down by compressed air to prevent solidification of fat when the installation is not operating. The fat should be filtered at the time of receipt, after storage, and before application to the product. Two parallel filters with crossover valves are preferred at each location to enable continued flow of fat while one is being cleaned. Characteristics of typical feed fats include specific gravities of 0.893 and 0.866 at 49°C (120°F) and 93°C (200°F), respectively, weights of 7.44 and 7.22 lb/gal, and viscosities of 24 and 8 centipoise, respectively (68, 69).

Most deliveries of feeding fats are made by insulated tank truck, which allows users without railroad sidings to be serviced, just-in-time deliveries, reduced numbers of local storage tanks, and rapid delivery while the fat remains hot. The industry and its contract haulers take pride in their equipment, and an inedible tallow tank truck looks little different from other milk or liquid ingredients trucks if kept clean.

*Liquid Fat Application to Mixed Feeds.* The typical operation consists of a horizontal batch dry feed mixer, equipped with fat addition ports. Hot fat is added to the batch after the dry ingredients have been mixed to minimize segregation of ingredients into fat balls. Some operators spray the fat, and others prefer to let it run into the mixer through small distributor lines or horizontal pipes with diagonal cuts. Much can be said in favor of the distributor line approach. Spray nozzles clog, even if equipped with screens. A spray fan pattern, that doesn't deposit fat on the sides of



**Figure 7** Insulated tanks for holding liquid fat at feeder lots. Note thermostatic controls for electrical heater (bottom center of each tank) and waterproof vinyl-coated covers. Courtesy of Griffin Industries, Cold Spring, Kentucky.

the mixer, must be chosen and the installation examined periodically to ensure the nozzles stay in alignment. Also, the production of spray mists cool the fat, reducing its temperature and wicking into the dry feedstuff.

In recent years, the addition of tallow has moved directly to dairy and beef feed lots. Small, heated, insulated tanks have been developed to receive and hold fat shipments (Figure 7). As described earlier, beef tallow is the most compatible fat for ruminants. It may be sprayed or flowed, at 2–3% of dry matter onto rations of chopped hay, other roughages, grains, or concentrates while in the mixer, or top-dressed on hay or other forages. Feed mixing operations like these require a positive displacement pump, like a Moyno or gear pump with variable-speed drive set to deliver a known amount of fat within a specific time period. As an alternative, mixers in feed mills can be mounted on load cells and equipped with controllers to stop the flow of fat when the desired weight has been added.

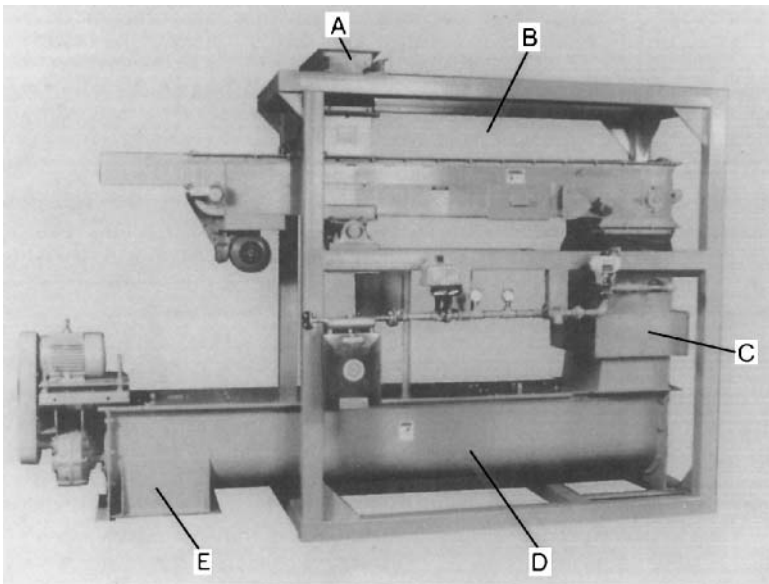
Hydrolyzed animal and vegetable fats, containing more than 90% fatty acids but no cottonseed oil, are available and used in feeding poultry. Their estimated metabolizable energy is 8360 kcal ME/kg.

**Fat Coating.** Historically, about 2–3% fat at the maximum could be added to feed ingredients before pelletizing and still obtain pellet integrity. About 6% fat, total, could be present in extruded feeds and retain expansion. (Levels of 10–12% fat can be included in extrusion premixes for “semimoist” pet foods, preserved by controlled-water activity, but these products typically are not expanded.) The upper limits of internal fat content have been raised in recent years by moist heat conditioning and holding (“ripening”) of starch-containing feedstuffs before pelleting and by more thorough precooking of starch in extrusion.

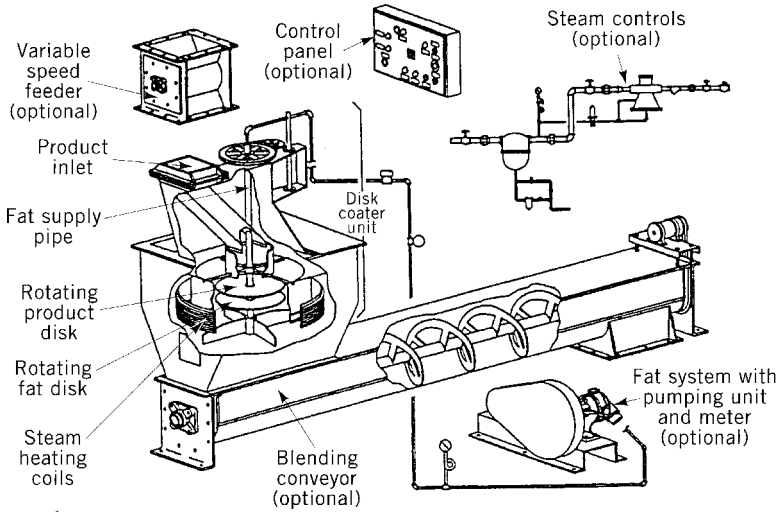
Fat coating of the pellet machine or extruded pellets improves animal acceptance of the feed because of surface fat aroma; binds heat-sensitive vitamin mixtures, flavorings, and rapidly hydrating “instant gravies” to dry pet foods; and enables addition of antioxidants, which steam distill easily and would be lost if included before extrusion. If emulsion-type coatings are used, they may be added to extruded feeds before the drying oven. Otherwise, the typical practice is to apply coating fats to warm pellets after the dryer, at 60–70°C (140–160°F) to enable the hot fat to soak partially into the product. The maximum amount of fat used for coating is about 5%. Cooling before packaging may be necessary to minimize wicking of liquid fat into the feed bag liner or container wall.

A slowly turning horizontal batch mixer, equipped with fat spray nozzles, can be used for coating small amounts of pellets or extruded collets. The mixing action should be gentle to avoid breakage of pellets. A simple continuous fat application system can be made by using a flat belt conveyor, straddled by a hopper and adjustable height gate to deliver a constant height (volume) of pellets. These are allowed to cascade off the end through a spraying chamber and then to continue through a slightly inclined barrel tumbler to finish distributing the fat.

Figure 8 shows a commercial fat applicator. The pellets enter through a hopper, are metered by a loss-in-weight belt feeder to cascade through a spray box, and then are mixed while being conveyed by mixing screw. A less expensive fat



**Figure 8.** Gravimetric fat coating–blending system. Granular product enters through chute or hopper (A). A constant feed rate is maintained by a gravimetric weigh belt feeder (B). Liquid fat falls through a spray chamber (C). Coating is completed by tumbling in continuous open screw conveyor (D). Product is discharged (E). A lower cost volumetric coating–blending system, with the gravimetric feeder replaced by an adjustable-flow drag feeder, is available. Courtesy of Hayes & Stolz, Ft. Worth, Texas.



**Figure 9** Disk-Coater fat coating system. Product is metered into a steam-coil heated chamber through a rotary lock feeder and is thrown off the upper rotating disk by centrifugal force. The particles fall through a thin spray of fat simultaneously thrown off the lower rotating disk. Mixing and absorption are continued in the takeaway conveyor. Courtesy of ASIMA Corp., Independence, Kansas.

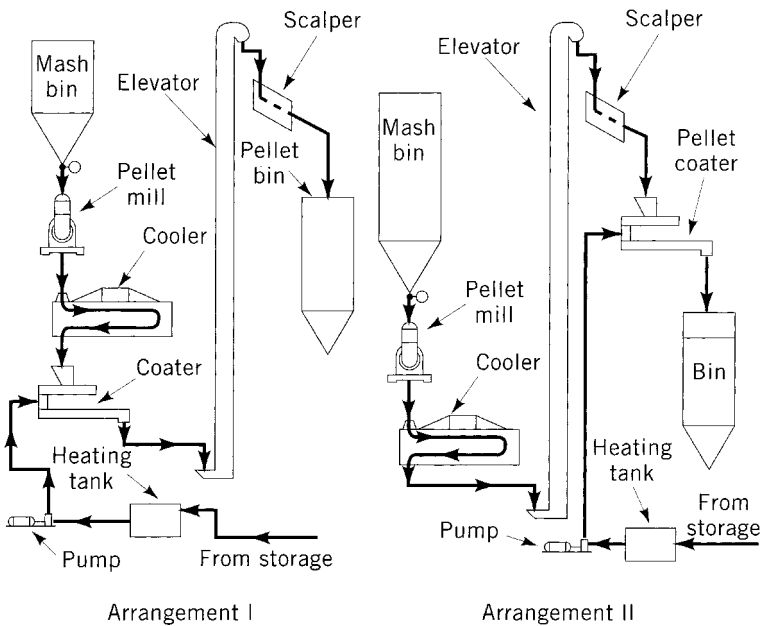
applicator, which uses a volumetric belt rather than a weight belt feeder, is also available from the same manufacturer.

A rotary pellet coating system is shown in Figure 9. Pellets and fat both are spun off separate rotating disks and mixed in a conveyor mixer. Elimination of spray nozzle clogging is claimed as the major advantage of this design.

Nozzles have the limitation of spraying only fat and fat-soluble materials alone. Dry materials must then be dusted onto the particles while sufficient liquid fat is at the surface to bind them. Another option is to add slurries of all coating ingredients to pellets passing through a continuous screw conveyor mixer. Fine particles often occur in the pellet stream, either from breakage, polishing of coarse edges, or disintegration of pellets. As shown in Figure 10, fine particles can be removed (scalped) from the stream either before or after fat coating.

**Extrusion of High-Fat Content Feeds.** Aeration (expansion) is required to produce floating fish feeds. In turn, this limits the amount of fat used in the extrusion formula. Calorie-dense feeds can be made if the ability to float in water is not a necessity. Dry pet foods containing 9–12% fat have been produced for many years. Trout and salmon have evolved as carnivores that obtain their caloric needs from either fat or protein. Special techniques have been developed to produce 20–25% fat content feeds on single-screw extruders and 25–30% fat content feeds on twin-screw extruders. (The twin-screw extruder has corotating, intermeshing, self-wiping double screws that essentially enable it to operate like a positive displacement pump. In contrast, fat-caused slippage between the screw and barrel limits the





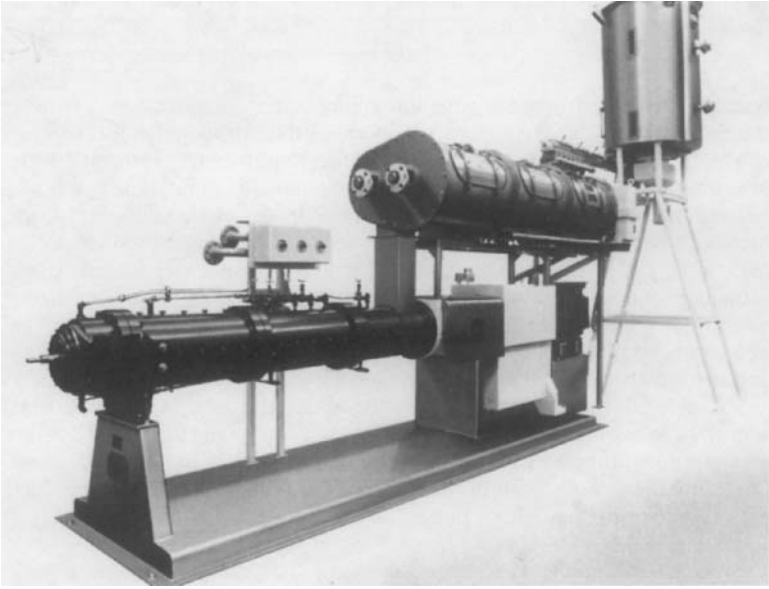
**Figure 10.** Two typical installation arrangements for coating feeds with liquid fat. Courtesy of Hayes & Stolz, Ft. Worth, Texas.

ability of single-screw extruders ability to push product against the resistance of steam locks and the die plate.)

Techniques used in making high fat content feeds include (1) using nonextracted cereals and oilseeds with full content of indigenous fats, (2) using starch-containing cereal fractions, (3) operating the extruder at moisture levels above 30% (well within starch gelatinization requirements, rather than the lower-moisture dextrinization range), (4) long-time (4–5 min) moistening and heating of cereals and other feed ingredients in the preconditioner, (5) use of special-design screw worm and barrel segments in single-screw extruders, (6) injection of steam into the extruder barrel to enhance starch cooking and formation of a starch-fat gel, and (7) pressure injection of fat into the screw barrel. After shaping, cutting, and drying, an additional 5% fat may be added by coating to obtain single-screw extruded feeds containing about 30% fat and twin-screw extruded feeds containing 35% fat (70).

A major advantage in extrusion is the ability to make various size feeds simply by changing the die plate. Particle sizes can range between 1.5 mm diameter for shrimp and eel larvae feeds, up to 2.5 cm (1 in.) for cattle feeds. One domestic manufacturer of commercial trout/salmon feeds offers products with the following particle diameters:

*Meal.* <0.600 mm (<U.S. mesh 30).



**Figure 11.** Large twin-screw extruder for producing pet foods and high fat content aquaculture feeds. Notice the differential diameter preconditioner (DDC). Machines available in sizes to 450 kw/600 hp, 15 MT/h. Courtesy of Wenger Manufacturing, Inc., Sabetha, Kansas.

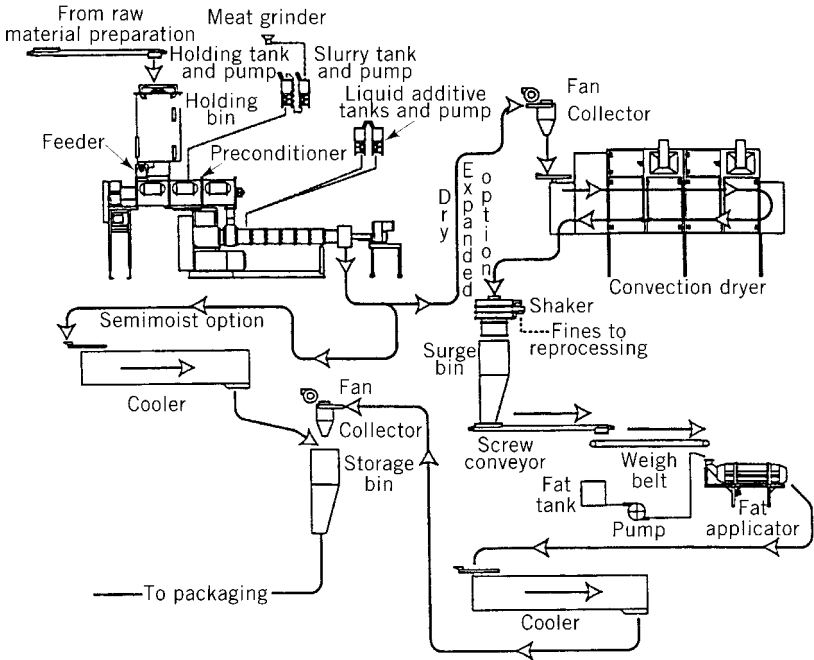
*Crumbles.* No. 1, 0.850 – 0.600 mm (U.S. mesh 20/30); no. 2, 1.18 – 0.850 mm (U.S. mesh 16/20); no. 3, 2.00 – 1.18 mm (U.S. mesh 10/16); and no. 4, 3.35 – 2.00 mm (U.S. mesh 6/10).

*Pellets.* 2.4 mm (3/32 in.), 3.2 mm (1/8 in.), 4.0 mm (5/32 in.), 4.8 mm (3/16 in.) (pigment available on request).

*Brood Pellets.* 6.4 mm (1/4 in.).

A large twin-screw extruder that is capable of making 6.3–15 MT of aquatic feeds and pet foods per hour and requires 450 kW (600 hp) is shown Figure 11. A process flow chart for making dry-expanded or semimoist pet foods and aquatic feeds is shown in Figure 12.

*Free-flowing Fats.* The objective in making free-flowing fats is to produce “dry” ingredients that can be readily mixed into feeds when rumen bypass is not required (primarily swine feed and milk replacers). Various mixtures of fats and cereal fractions, peanut and other hulls, and vermiculite were tried before the current spray-dried or chill-granulated products were developed. Choice white grease (including lard with its higher levels of unsaturated fatty acids) and coconut oil (containing medium-chain fatty acids) are often included. Milk or whole or delactosed whey solids, soybean protein, or corn syrup solids are included to help in processing the products. Typically, hardness of purchased fat (partially estimated by iodine value) increases with the percent fat content of the product. Products sold domestically currently include (1) 40% choice white grease and 7% protein from



**Figure 12.** Process flow sheet for making dry expanded or semimoist pet foods, and aquatic feeds. Courtesy of Wenger Manufacturing, Inc., Sabetha, Kansas.

nondelactosed whey; (2) 15% coconut oil, 45% edible lard, and 7% dairy protein; (3) 60% choice white grease and 7% dairy protein; (4) 80% edible beef tallow and 4% soy protein; (5) 80% coconut oil and 4% soy protein; (6) 80% choice white grease and 4% soy protein; (7) 80% animal fat, 4% dairy protein, and corn syrup solids; and (8) 90% animal fat. Lower fat content products (30% fat or less), spray-dried with skim milk, whey, or sodium caseinate, are available in addition to dry complete calf, lamb, and pig milk replacers.

**Rumen-protected Fats.** Rumen-protected fats are designed to be unavailable to rumen microorganisms, and pass to the stomach (abomasum) and small intestine for hydrolysis by pancreatic lipase and absorption. Free-flowing products are also desired. There is little advantage over the tallows, greases, and vegetable oils in using protected fats for nonruminants. Inertness in the rumen can be achieved by encapsulating the fat with a nondigestible protein, making the fat insoluble as a fatty acid-calcium soap, or by hydrogenation to raise the melting point of tallows (triacylglycerides) or fatty acids above the temperature of the rumen.

Scott and co-workers pioneered the development of rumen inert fats by demonstrating that the polyunsaturated fatty acids content of milk can be increased by feeding polyunsaturated oils encapsulated in formaldehyde-treated casein (71-74). Various types of domestically sold rumen inert fats are shown in Table 17. Palmquist and co-workers pioneered the feeding of calcium soaps to dairy cattle (75-77). Their work became the basis for Megalac, made from palm oil and stearin.

TABLE 17. Domestic Rumen Inert Fat Products.

Product	Company	Ingredient Composition	Fat (%)
Alifet	Alifet U.S.A	Hydrogenated tallow mixed with wheat starch and crystallized	95
Biopass	Bioproducts, Inc.	Hydrogenated long-chain fatty acids	98
Booster fat	Balanced Energy Co.	Tallow plus soybean meal treated with sodium alginate	95
Carolac	Carolina Byproducts	Hydrogenated tallow—prilled	98
Dairy 80	Morgan Mfg.	Hydrogenated tallow—prilled; contains some phospholipid, flavor, and coloring agents	92
Energy booster	Milk Specialities Co.	Relatively saturated free long-chain fatty acids—prilled fat	98–99
Megalac	Chuch & Dwight Co.	Calcium salts of palm oil	82

Calcium soaps dissociate as they move from the rumen into the acidic abomasum. pKa values have been estimated for the following calcium soaps: soy oil, 5.6; palm fatty acid distillate, 4.6; tallow, 4.5; and stearic acid, 4.5. Unsaturated fatty acids soaps are less satisfactory for maintaining normal rumen function because their pH of dissociation is higher (78). The feeding of calcium salts of long-chain fatty acids to goats significantly increased milk fat content from  $3.4 \pm 0.1\%$  to  $3.7 \pm 0.2\%$ , but the content of short- and medium-chain fatty acids decreased (79). Supplemental feeding of methionine, protected by coating with C14:0–C18:0 fatty acids and calcium carbonate, has been reported to increase the protein content of milk (80, 81).

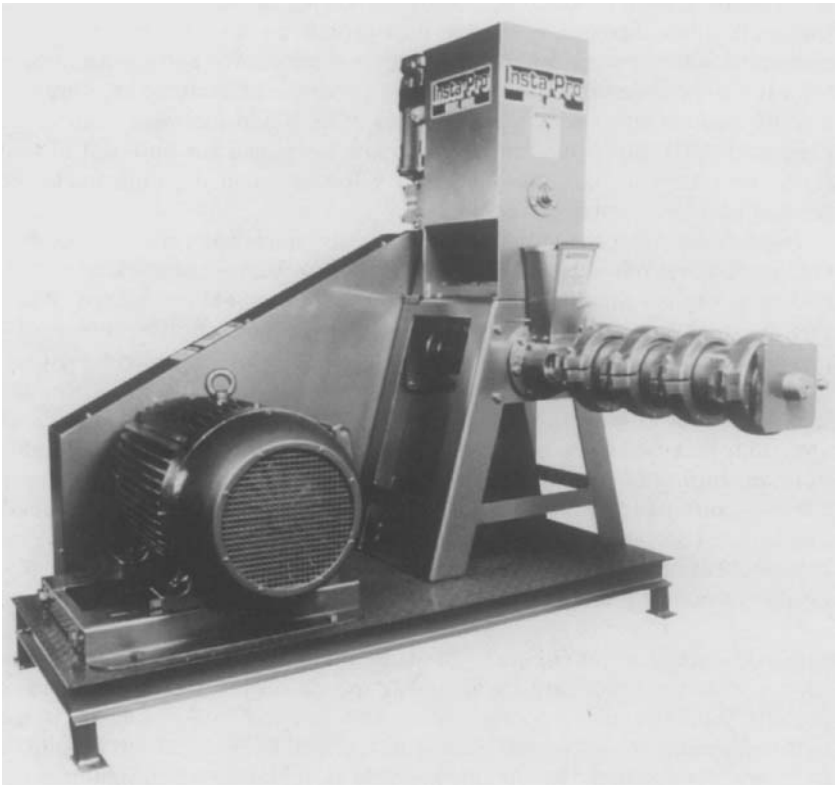
Preformed calcium soap is highly available to laying hens, with about a 99.2% availability. True metabolizable energy of calcium soap has been reported as 7,200 kcal ME/kg, estimated by regression and 8,140 kcal ME/kg calculated from retention (82).

*Feeding of Whole Cottonseed.* Whole cottonseed was one of the earliest forms of protected fats fed to ruminants, although its mechanism did not become apparent for many years. Feeding research on this crop was reported as early as 1890 in Mississippi (83) and 1894 in Texas (84). Because of its ready availability, cottonseed became the main source of edible oil domestically, from the 1890s until the late 1930s when soybean acreage increased rapidly. Competition and improvement in soybean and corn oil quality narrowed the price premium once enjoyed by cottonseed oil. Interest in feeding whole cottonseed was rekindled by a feasibility study by Stanford Research Institute in 1972. Currently, an estimated 35–40% of the cottonseed produced domestically is used for feeding dairy cattle. This practice has essentially spread throughout the country. Relative to other nutrient sources, cottonseed is worth enough to the dairy industry to outbid oil milling for the first 1.5–2 million tons produced in the United States even in low harvest years.

A typical response from feeding 3.6 kg (8 lb) cottonseed daily has been reported (85). Milk production rose by 4.9%, milk fat content by 15.3%, and total milk solids secreted by 1%, but milk protein content decreased by 6.4% compared

with a no-cottonseed diet. Other studies have had similar results (86). It also has been reported that feeding cottonseed decreases the proportion of medium-chain fatty acids (C6-16) in milk and increases stearic and oleic acids (87). Since substantial premiums were paid for butterfat at that time, the return through the increased value of fat in the milk made the feeding of whole cottonseed profitable.

In contrast, milk production and fat contents typically decreased in feeding studies of other oilseeds and liquid oils (88-90). Various studies were conducted to explain the different response in feeding whole cottonseed. While this question has not been answered, various researchers have noted that protein in cottonseed has a higher bypass property than soy protein. Furthermore, the kernel in fuzzy cottonseed is enmeshed in a porous cocoon and shell, which the cow crushes but does not disintegrate while chewing its cud. One study that used nylon bags and rumen fistula showed only 16% digestibility of linters during 24 h in the rumen, 0.0% protein digestibility in whole (non-chewed) cottonseed, and 64% protein digestibility in finely ground cottonseed. The linters cocoon and seed protein may act

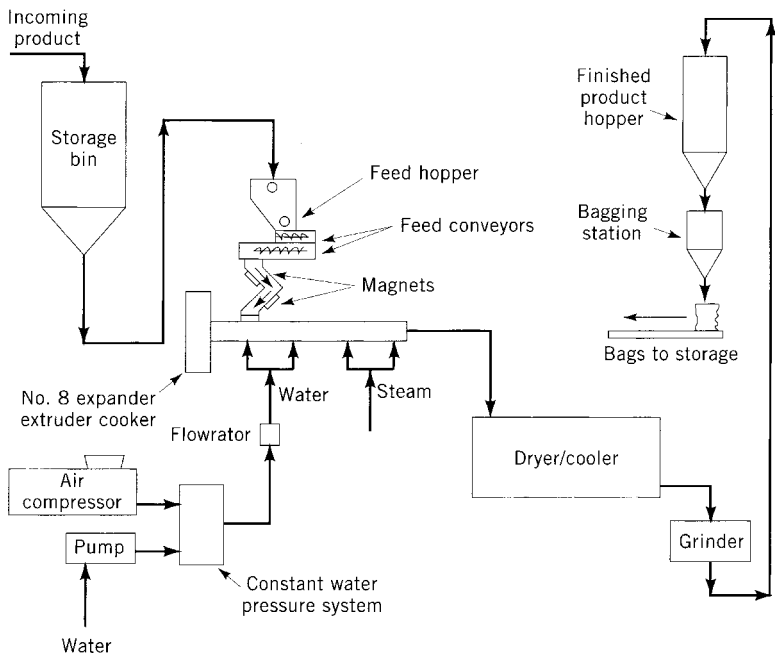


**Figure 13.** Dry extruder for making full-fat soybean meal and shaped extruded feeds; 272 kg (600 lb)/h. This machine also can be run by a farm tractor power take-off (PTO). Courtesy of InstaPro International, Inc., Des Moines, Iowa.

together to provide a physical bypass effect in the rumen, which shifts the major portion of the protein and fat digestion to the small intestine.

**Extrusion of Full-fat Oilseeds.** Because oil in oilseeds exists in a proteinaceous matrix, its usability by animals depends on the treatment given to protein. Whole soybeans contain about 18% fat, and 38% protein, with the best balanced essential amino acid profile available in significant quantities from one plant source. Ruminants are able to tolerate trypsin inhibitor (an antigrowth factor) to a limited degree; however, most of the trypsin inhibitor should be inactivated before the soybeans are fed to monogastric species, especially poultry and swine. Extruders are used to shred soybeans and inactivate trypsin inhibitor simultaneously. During this process, other toxic or anti-nutritional factors (hemagglutinins, goitrogens, and others) are also inactivated.

A farm-size dry extruder, with a capacity of 273 kg (600 lb) per hour, is shown in Figure 13. This model also can be run by the power take-off of a farm tractor. The manufacturer also makes larger units. A diagram of a processing line for making full-fat soybean meal by wet extrusion is shown in Figure 14. The manufacturer recommends that the machine be operated, using soybeans at 8–10% moisture content and a product exit temperature of 145°C (295–300°F). Metabolizable energies (kcal ME/kg) for growing poultry (3850), adult poultry (3960), swine (4180), and ruminants (3335) have been reported for full-fat soybean meal made by this machine (91).



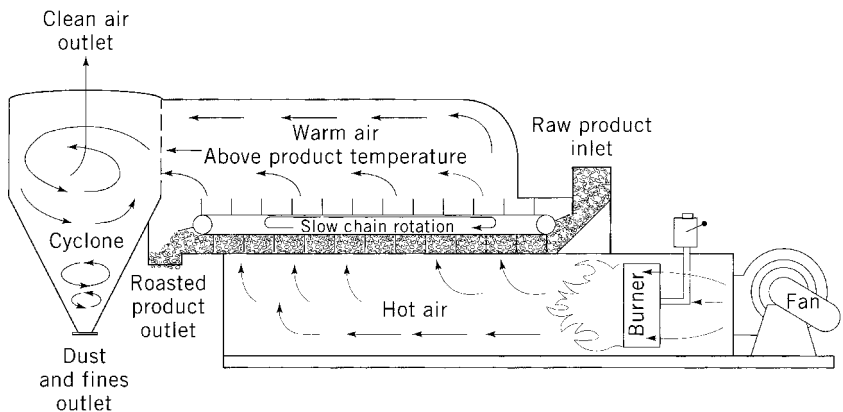
**Figure 14.** Process flow sheet for production of full-fat soybean meal. Courtesy of Anderson International Co., Cleveland, Ohio.

Almost a parallel relationship exists between deactivation of trypsin inhibitor and urease activity by heat. The latter component is often tracked because it is easier to analyze. Moisture plays a significant role in the rate of enzyme deactivation. For example, at 15% moisture content and an extrusion temperature of 135°C (275°F), approximately 12% trypsin inhibitor is inactivated with a corresponding urease activity of 1.0 pH units. But at 20% moisture content, 89% of the trypsin inhibitor is inactivated (urease activity 0.1 pH units). The protein efficiency ratio (PER) is 1.82 and 2.15 at these moisture levels, respectively (92).

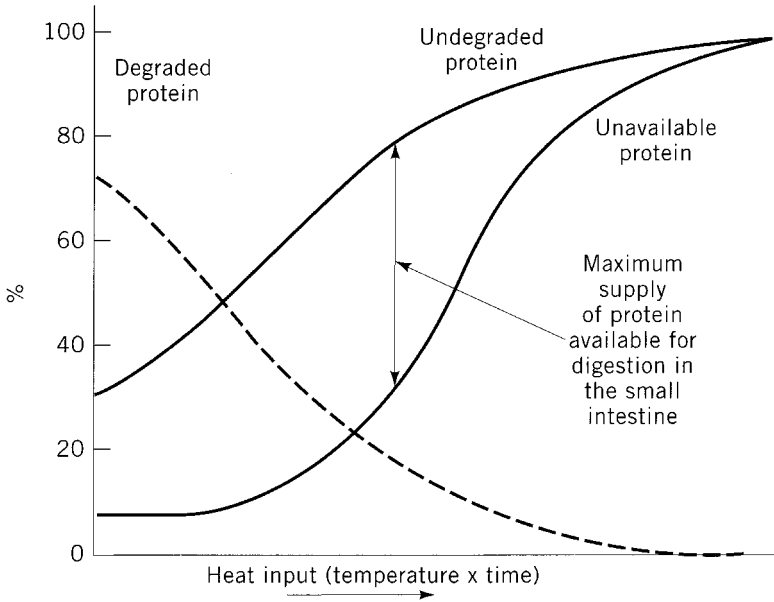
Extruders are also used for extrusion of secondary resources (coproducts rendering). Poultry mortalities, eggshells, feathers, shrimp heads, and various other meat and fisheries coproducts have been mixed with solvent-extracted soybean meal and extruded at sterilization temperatures for use as animal feeds. Significant amounts of fats can be recycled in this manner, while fresh and near the site of production (92).

Extrusion of whole double-O rapeseed, with 30–70% peas or 47–53% wheat meals, at 150°C (302°F) decreased trypsin inhibitors by 20–40%, total glucosinolates by 20–40%, and progointrin by 46–60%. Rapeseed lipids had apparent digestibilities of 70.1% and 80.5% in pigs 4 and 7 weeks of age, respectively, and were similar to corn (maize) oil (93).

**Dry Roasting.** Roasters are simple to operate and maintain and do not have parts that wear as rapidly as those of extruders. A diagram of a commercial unit is shown in Figure 15. Roasting of soybeans is done for two effects: deactivation of trypsin inhibitor, and increasing bypass properties of the protein. The manufacturer recommends a roaster exit temperature of 115–127°C (240–260°F) for feeding pigs and a final temperature of 149°C (300°F) plus 30 min hot holding for increased bypass. Roasted soybeans with a protein dispersibility index (PDI) of 9–11 have been deemed as optimally heated; PDI 11–14, as marginally underheated; and PDI above 14, as underheated (94).



**Figure 15.** Schematic drawing of a crop roaster adapted for soybeans. Courtesy of Sweet Manufacturing Co., Springfield, Ohio.



**Figure 16.** Effects of heating soybean on protein (and fat) bypass in cattle (98).

In one study, lactating cows were fed 8% soybean oil, either by direct addition of oil or as 50% coarsely ground raw soybeans (95). Both rations decreased content and yield of C10:0, C12:0, C14:0, C14:1, C16:0, and C16:1 fatty acids in milk fats but increased content and yield of C18:0, C18:1, and C18:2 fatty acids. Direct addition of soybean oil to the diet also reduced the percentage and yield of C6:0 and C8:0, while inclusion of raw soybeans increased the percentage of C4:0 and yields of C4:0 and C6:0 in the milk fat.

The feeding of roasted soybeans increases milk yield over the feeding of raw soybeans. But particle size has no apparent effect, indicating that whole or cracked roasted soybeans can be used as feed (96, 97).

It has been shown that heating soybeans reduces their protein degradability in the rumen and postpones digestion to the small intestine (98). However, the production of unavailable protein is also increased (Figure 16). The relative effects of feeding soybean meal, roasted soybeans, extruded soybeans, and raw soybeans on milk yield, protein, and fat content are shown in Table 18. Extruded soybeans gave the highest yield of 3.5% fat corrected meal (FCM) and was second to soybean meal in total protein produced. However, fat and protein contents of milk from extruded soybeans were lower than for the other treatments.

The effects of two commercial rumen inert fats and whole roasted soybeans on yield of milk and its fatty acid profile were evaluated (99). Fatty acid profiles of the three fat sources are shown in Table 19. Whole roasted soybeans produced more total and 3.5% FCM milk, total protein, and total fat than the two rumen inert fat sources (Table 20). However, Megalac produced the milk with the highest fat



**TABLE 18. Effect of Supplemental Protein and Fat on Feed Intake, Body Weight Change, Milk Yield, and Milk Composition (98) (<sup>a,b,c,d</sup> Means in the Same Row with Different Superscripts Differ at Given *p* Value).**

Item	Soybean Meal	Roasted Soybeans	Extruded Soybeans	Raw Soybeans
<b>Intake</b>				
DM, kg/d <sup>a</sup>	24.7 <sup>a</sup>	22.5 <sup>b</sup>	25.1 <sup>a</sup>	22.7 <sup>b</sup>
DM, % of BW <sup>a</sup>	4.01 <sup>a,b</sup>	3.83 <sup>a,b</sup>	4.10 <sup>a</sup>	3.74 <sup>b</sup>
CP, kg/d <sup>b</sup>	4.32	3.87	4.29	3.88
UIP <sup>c</sup> , kg/d	1.49 <sup>c</sup>	1.61 <sup>b</sup>	1.79 <sup>a</sup>	1.20 <sup>d</sup>
NEL <sup>d</sup> , Mcal/d	38.3	35.6	39.7	35.9
<b>Yield, kg/d</b>				
Milk <sup>a,e</sup>	36.0 <sup>b</sup>	37.5 <sup>a,b</sup>	39.0 <sup>a</sup>	35.9 <sup>b</sup>
FCM, 3.5% <sup>a</sup>	35.7 <sup>a,b</sup>	36.6 <sup>a,b</sup>	37.6 <sup>a</sup>	35.2 <sup>b</sup>
Protein <sup>a</sup>	1.16 <sup>a</sup>	1.06 <sup>b</sup>	1.14 <sup>a</sup>	1.06 <sup>a</sup>
<b>Milk Composition, %</b>				
Fat <sup>a,f</sup>	3.48 <sup>a</sup>	3.35 <sup>a,b</sup>	3.25 <sup>b</sup>	3.45 <sup>a</sup>
Protein <sup>a,g</sup>	3.10 <sup>a</sup>	2.95 <sup>b,c</sup>	2.89 <sup>c</sup>	3.00 <sup>b</sup>

<sup>a</sup>Means were covariately adjusted.

<sup>b</sup>No statistical analysis.

<sup>c</sup>Undegraded intake protein.

<sup>d</sup>Net energy lactation.

<sup>e</sup>Significant week × treatment interaction (*p* < .10).

<sup>f</sup>Significant week × treatment interaction (*p* < .01).

<sup>g</sup>Significant week × treatment interaction (*p* < .001).

content. Protein content in the milk decreased by about 0.1% for all fat sources. Fats of milks produced with Megalac (of palm oil origin) and Alifet (a hydrogenated tallow) contained more C16:0 and less C18:0, C18:2, and C18:3 than milk produced with roasted soybeans (Table 21). Feeding roasted soybeans whole apparently achieves fat bypass and milk production equivalent to rumen inert fats costing \$0.40–0.50/lb.

*Dust Control.* The use of soybean oil (100, 101) and inedible animal fats (102) to reduce dustiness in swine feeding operations, especially during months of low

**TABLE 19. Fatty Acid Profile of Fat Sources (99)<sup>a</sup>.**

Fat Source	C14:0 (%)	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)	Saturated <sup>b</sup> (%)	Unsaturated <sup>c</sup> (%)
Soybeans	—	12.5	4.5	22.0	52.9	8.13	17.0	83.0
Megalac <sup>d</sup>	1.8	50.9	4.1	35.1	8.1	—	56.8	43.2
Alifet <sup>e</sup>	3.6	26.7	37.2	31.7	0.8	—	67.5	32.5

<sup>a</sup>Percent by weight of fatty acids reported.

<sup>b</sup>Saturated fatty acids (C14:0, C16:0, C18:0).

<sup>c</sup>Unsaturated fatty acids (C18:1, C18:2, C18:3).

<sup>d</sup>Calcium salt of palm oil fatty acids.

<sup>e</sup>Hydrogenated tallow.

**TABLE 20. Effects of Fat Source on Milk Yield, Milk Composition, and Blood (99).**

Parameter	Treatment <sup>a</sup>			
	CTL	RSB	MG	AL
Milk yield (kg/d)	32.3	34.0	32.1	33.8
3.5% FCM <sup>b</sup> (kg/d)	31.6	33.8	32.6	32.5
Fat (%)	3.41 <sup>b</sup>	3.45 <sup>b</sup>	3.62 <sup>a</sup>	3.25 <sup>c</sup>
Protein (%)	3.01 <sup>a</sup>	2.95 <sup>a,b</sup>	2.92 <sup>b</sup>	2.91 <sup>b</sup>
Fat yield (kg/d)	1.10	1.18	1.16	1.11
Protein yield (kg/d)	0.96	1.00	0.94	0.99
Gross feed efficiency <sup>c</sup>	1.27 <sup>b</sup>	1.31 <sup>b</sup>	1.45 <sup>a</sup>	1.31 <sup>b</sup>
Blood plasma glucose (mg/100 mL)	70.7	69.8	70.3	69.9

<sup>a</sup>CTL, no fat supplement; RSB, roasted soybeans as fat supplement; MG, Megalac as fat supplement; AL, Alifet as fat supplement.

<sup>b</sup>3.5% FCM, kg/d = 0.432 kg milk + 16.2 kg fat.

<sup>c</sup>Gross feed efficiency values were calculated on an individual cow basis (kg 3.5% FCM/kg DMI).

humidity, have been reported. Particle counts and total dust on a mass basis have been reduced by 50–99% at levels of 2% oil. Fewer bacterial spores and respiratory problems, improved pig health and survival, and improved feed efficiency and daily gain have been claimed.

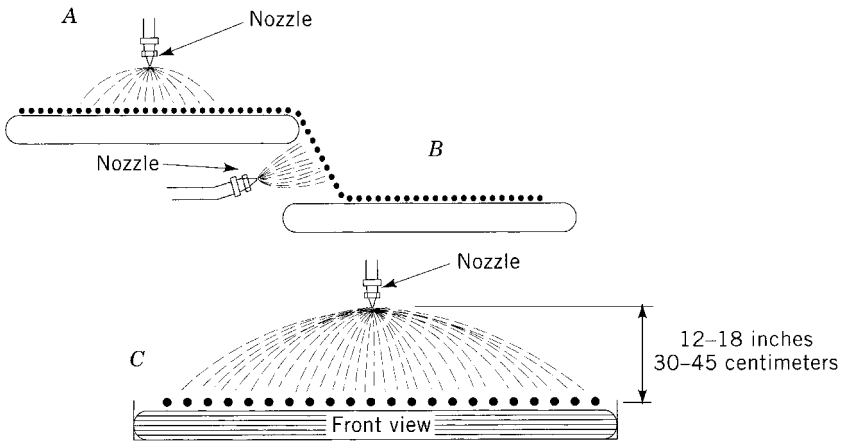
It is estimated that, each time grain is handled, about 0.15% weight is changed into dust, which potentially could reach explosive levels. The loss is equivalent to 1.5 kg/MT (3 lb/short ton). Use of 200 ppm (0.02%) of soybean oil sprayed onto the grain at the point of entry into commercial elevator and trading channels has

**TABLE 21. Effect of Dietary Fat Supplementation on Milk Fatty Acid Composition (99)<sup>a</sup> (<sup>A,B,C,D</sup> Means in the Same Row with Different Superscripts Differ ( $p < .001$ )).**

Fatty Acid	Treatment <sup>b</sup>			
	CTL	RSB	MG	AL
C4:0	2.5	2.5	2.5	2.6
C6:0	2.1 <sup>A</sup>	2.0 <sup>B</sup>	1.8 <sup>C</sup>	2.0 <sup>B</sup>
C8:0	1.5 <sup>A</sup>	1.4 <sup>B</sup>	1.2 <sup>C</sup>	1.3 <sup>B</sup>
C10:0	3.7 <sup>A</sup>	2.9 <sup>B</sup>	2.4 <sup>C</sup>	2.8 <sup>B</sup>
C12:0	4.5 <sup>A</sup>	3.2 <sup>B</sup>	2.9 <sup>C</sup>	3.3 <sup>B</sup>
C14:0	13.6 <sup>A</sup>	10.8 <sup>C</sup>	10.5 <sup>D</sup>	11.9 <sup>B</sup>
C16:0	37.1 <sup>A</sup>	27.2 <sup>C</sup>	37.4 <sup>A</sup>	32.8 <sup>B</sup>
C18:0	10.2 <sup>C</sup>	14.0 <sup>A</sup>	9.8 <sup>C</sup>	11.8 <sup>B</sup>
C18:1	20.2 <sup>B</sup>	27.3 <sup>A</sup>	26.2 <sup>A</sup>	26.7 <sup>A</sup>
C18:2	3.3 <sup>C</sup>	6.1 <sup>A</sup>	3.5 <sup>B</sup>	2.9 <sup>D</sup>
C18:3	1.5 <sup>C</sup>	2.2 <sup>A</sup>	1.5 <sup>C</sup>	1.9 <sup>B</sup>

<sup>a</sup>Fatty acid percent by weight.

<sup>b</sup>CTL, not fat supplemented; RSB, roasted soybeans as fat supplement; MG, Megalac (calcium salt of palm oil) as fat supplement; AL, Alifet (hydrogenated tallow) as fat supplement.



**Figure 17.** Recommended spraying procedures for applying soybean oil to reduce dust in grain. Oil may be sprayed on top of grain on conveyor (A) and/or on the underside of a cascade (B). The spray pattern (C) should be broad enough to cover the grain but not the sides of the conveyor. Courtesy of American Soybean Association, St. Louis, Missouri.

been claimed to suppress 99% of the dust that typically becomes airborne. The FDA and the U.S. Federal Grain Inspection Service have approved the application of refined soybean oil at this level. Suggested techniques for applying food-grade (refined) soybean oil by spraying onto flowing grain are shown in Figure 17. Application should be made at temperatures ( $10.0^{\circ}\text{C}$ ,  $50^{\circ}\text{F}$ ) where the oil flows freely (105).

### 5.3. Modification of Fatty Acid Profiles—Designer Foods

A recent survey of consumer attitudes and viewpoints found that the majority of U.S. consumers believe that natural substances in food can play a role in disease prevention (104). The designer foods movement to modify food components is broad and promotes increased uses of natural source phytochemicals; bioflavonoids; fiber, calcium, and/or vitamin-enriched milks and cereal products; probiotic yogurt, and isotonic beverages in addition to meat and eggs (105).

Fatty acid profiles of animals are automatically altered somewhat by the feed. Designer foods are modified for specific objectives. Chickens and swine readily translate fatty acids from feeds into meat and other products (106, 107). Cattle also have this ability, provided the fat is rumen inert (108).

It has been shown that the fatty acid profile of milk can be changed through feeding (109). Table 22 shows the fatty acids profiles of fat sources fed, and Table 23 shows the fatty acids profiles of the resulting milks. The pattern as seen by earlier researchers is repeated. Supplemental dietary fats reduce the level of medium-chain fatty acids but tend to increase the content of long-chain C18:0 and C18:1 fatty acids. Many of the researchers have also found that blood cholesterol levels increase in the presence of long-chain fatty acids.

**TABLE 22. Fatty Acid Content in Feed and Composition of Test Fats in a Feeding Trial on Fat Digestibility and Milk Fat Composition<sup>a</sup>.**

Fat Source	Fatty Acid (% of DM)	Fatty Acid (g/100 g)								IV
		C14:0	C16:0	C16:1	C18:0	Trans C18:1	Cis C18:1	C18:2	C18:3	
Basal diet	1.1	5.2	19.7	—	2.9	—	20.2	37.3	3.1	94
Animal-vegetable blend	88.7	0.7	21.7	5.1	5.1	—	34.9	28.6	1.5	92
Calcium soap	83.8	1.5	50.1	—	4.2	—	34.1	7.8	0.3	45
Hydrogenated animal fat	83.6	3.0	24.8	1.2	34.5	12.8	15.3	1.1	0.4	30
Saturated fatty acids	100	2.2	44.1	0.9	34.7	2.9	9.4	0.9	—	14
Tallow	81.0	2.8	23.7	2.8	20.9	4.0	37.7	2.0	0.4	45

<sup>a</sup>Reprinted by permission from Ref. 106.

As mentioned earlier, the lymphatic system is poorly developed in poultry. Absorbed lipids pass into the portal circulation and then are principally transported as triacylglycerols of the VLDL fraction. VLDLs are present in the serum at high concentrations and appear to be the major lipid precursor in the liver for egg yolk synthesis. Furthermore, fats high in unsaturated fatty acids are absorbed more readily than fats low in unsaturated fatty acids (51, 54).

Designer eggs with higher *n*-3 fatty acids content and lower saturated fatty acids and total fat contents have been in U.S. and Canadian supermarkets since 1992.

**TABLE 23. Effect of Dietary Fat Source and Level on Milk Fatty Acid Composition in a Feeding Trial<sup>a,b</sup> (<sup>A,B,C</sup> Values with Different Superscripts Differ ( $p < .05$ ); <sup>X,Y,Z</sup> Values with Different Superscripts Differ ( $p < .01$ )).**

Fatty Acid	Basal	Animal-Vegetable Blend	Calcium Soap	Hydrogenated Animal Fat	Saturated Fatty Acids	Tallow
C4:0	3.34	3.66	3.81	3.79	3.62	3.49
C6:0	2.70 <sup>A</sup>	2.40 <sup>AB</sup>	2.48 <sup>AB</sup>	2.53 <sup>AB</sup>	2.46 <sup>AB</sup>	2.34 <sup>B</sup>
C8:0	1.75 <sup>X</sup>	1.34 <sup>Y</sup>	1.35 <sup>Y</sup>	1.39 <sup>Y</sup>	1.41 <sup>Y</sup>	1.34 <sup>Y</sup>
C10:0	3.97 <sup>X</sup>	2.51 <sup>Y</sup>	2.57 <sup>Y</sup>	2.63 <sup>Y</sup>	2.72 <sup>Y</sup>	2.60 <sup>Y</sup>
C12:0	4.64 <sup>X</sup>	2.75 <sup>Y</sup>	2.84 <sup>Y</sup>	2.88 <sup>Y</sup>	3.03 <sup>Y</sup>	2.89 <sup>Y</sup>
C14:0	13.01 <sup>X</sup>	9.33 <sup>Y</sup>	9.54 <sup>Y</sup>	10.28 <sup>Y</sup>	10.10 <sup>Y</sup>	10.30 <sup>Y</sup>
C14:1	1.46 <sup>A</sup>	1.08 <sup>B</sup>	1.07 <sup>B</sup>	1.26 <sup>AB</sup>	1.26 <sup>AB</sup>	1.31 <sup>AB</sup>
C15:0	1.28 <sup>X</sup>	0.87 <sup>Z</sup>	0.84 <sup>Z</sup>	1.07 <sup>Y</sup>	1.06 <sup>Y</sup>	1.04 <sup>Y</sup>
C16:0	29.87 <sup>B,Y</sup>	26.45 <sup>C,Z</sup>	34.15 <sup>A,X</sup>	28.42	32.67 <sup>A,XY</sup>	28.41 <sup>B,C,Z</sup>
C16:1	1.68 <sup>Y</sup>	1.64 <sup>Y</sup>	1.64 <sup>Y</sup>	1.72 <sup>Y</sup>	1.99 <sup>X</sup>	1.80 <sup>XY</sup>
C17:0	0.60 <sup>Y</sup>	0.52 <sup>Y</sup>	0.39 <sup>Z</sup>	0.88 <sup>X</sup>	0.78 <sup>X</sup>	0.82 <sup>X</sup>
C18:0	9.05 <sup>B,YZ</sup>	11.50 <sup>A,XY</sup>	7.71 <sup>C,Z</sup>	11.68 <sup>A,X</sup>	9.86 <sup>B,XY</sup>	10.43 <sup>AB,XY</sup>
C18:1	17.22 <sup>C,Z</sup>	25.74 <sup>A,X</sup>	22.80 <sup>AB,XY</sup>	22.89 <sup>AB,XY</sup>	20.30 <sup>B,YZ</sup>	23.26 <sup>AB,XY</sup>
C18:2	2.24 <sup>B,XY</sup>	2.00 <sup>B,C,YZ</sup>	2.58 <sup>A,X</sup>	1.67 <sup>C,Z</sup>	1.74 <sup>C,Z</sup>	1.59 <sup>C,Z</sup>
C18:3	0.55 <sup>C,Z</sup>	1.16 <sup>A,X</sup>	0.63 <sup>C,YZ</sup>	0.72 <sup>B,C,YZ</sup>	0.62 <sup>C,YZ</sup>	0.91

<sup>a</sup>Reprinted with permission from Ref. 109.

<sup>b</sup>*n* = 12; numbers are in g/100 g of methyl esters.

Typically, they are produced by feeding poultry diets containing whole flax seed or flax seed oil or by eliminating animal coproducts and feeding poultry diets containing vitamin E, kelp, and canola oil (110). Use of deodorized seal blubber oil has also proven effective (111).

General experience in increasing the  $n-3$  lipids contents of eggs and poultry meat includes the following. The feeding of linseed oil, menhaden oil, and soybean oil to chickens has resulted in similar blood plasmas with VLDL and LDL lower than those fed chicken fat. The levels of  $C20:5n-3$  in tissue lipids of chickens fed linseed oil approached those of chickens fed menhaden oil. Chickens fed linseed oil had the highest levels of polyunsaturates in tissues while those fed chicken fat had the lowest. Chickens fed soybean oil maintained the highest levels of linoleic ( $C18:2n-6$ ) and arachidonic ( $C20:4n-6$ ) acids in tissue lipids, but linseed oil and menhaden oil resulted in reduced  $C20:4n-6$  content (112). Other researchers have also reported similar decreases in arachidonic acid, and increased levels of eicosapentaenoic ( $C20:5n-3$ ) and docosahexaenoic ( $C22:6n-3$ ) in the fatty acids of egg yolks from chickens fed menhaden oil (113–116).

Human trials of four eggs/day have shown that control eggs significantly increased plasma cholesterol levels but were unchanged by eggs with high  $n-3$  fatty acids contents. Mean plasma triglyceride concentration was decreased by  $n-3$  eggs but increased by control eggs. Both systolic and diastolic blood pressures were significantly decreased in one group of participants consuming  $n-3$  eggs, and systolic pressure was significantly decreased in a second trial. Blood pressure did not change when participants consumed control eggs (117).

## REFERENCES

1. NRA, *Rendered Animal Fats and Oils*, National Renderer's Association, Inc., Washington, D.C., July 1992.
2. National Research Council, *Designing Foods: Animal Product Options in the Marketplace*, National Academy Press, Washington, D.C., 1988.
3. M. E. Ensminger and C. G. Olentine Jr., *Feeds and Nutrition—Complete*, Ensminger Publishing Co., Clovis, Calif., 1978.
4. M.E. Ensminger, J. E. Oldfield, and W.E. Heinemann, *Feeds Nutrition Digest*, 2nd ed., Ensminger Publishing Co., Clovis, Calif., 1990.
5. D. C. Church, ed., *Livestock Feeds and Feeding*, 3rd ed., Prentice-Hall, Inc., Englewood Cliffs, N. J., 1989.
6. D. C. Church and W.G. Pond, *Basic Animal Nutrition and Feeding*, O&B Books, Inc., Corvallis, Oreg. 1978.
7. National Research Council, *Nutrient Requirements of Beef Cattle*, 6th rev., National Academy Press, Washington, D.C., 1984.
8. National Research Council, *Nutrient Requirements of Cats*, rev. ed., National Academy Press, Washington, D.C., 1986.
9. National Research Council, *Nutrient Requirements of Dairy Cattle*, 6th rev. ed., National Academy Press, Washington, D.C., 1989.

10. National Research Council, *Nutrient Requirements of Dogs*, rev. National Academy Press, Washington, D.C., 1985.
11. National Research Council, *Nutrient Requirements of Fish*, National Academy Press, Washington, D.C., 1993.
12. National Research Council, *Nutrient Requirements of Goats*, National Academy of Sciences, Washington, D.C., 1981.
13. National Research Council, *Nutrient Requirements of Horses*, 5th rev. ed., National Academy Press, Washington, D.C., 1989.
14. National Research Council, *Nutrient Requirements of Mink and Foxes*, rev. ed., National Academy Press, Washington, D.C., 1989.
15. National Research Council, *Nutrient Requirements of Nonhuman Primates*, National Academy Press, Washington, D.C., 1989.
16. National Research Council, *Nutrient Requirements of Poultry*, 9th rev. ed., National Academy Press, Washington, D.C., 1994.
17. National Research Council, *Nutrient Requirements of Rabbits*, 5th rev. ed., 2nd ed., National Academy Press, Washington, D.C., 1977.
18. National Research Council, *Nutrient Requirements of Sheep*, 6th rev. ed., National Academy Press, Washington, D.C., 1985.
19. National Research Council, *Nutrient Requirements of Swine*, 9th rev. ed., National Academy Press, Washington, D.C., 1988.
20. National Research Council, *United States–Canadian Tables of Feed Composition*, 3rd. rev., National Academy Press, Washington, D.C., 1982.
21. D. F. Fick, D. Firestone, J. Ress, and R. J. Allen, *Poultry Sci.* **52**, 1637 (1973).
22. C. Y. Pang, G. D. Phillips, and L. D. Campbell, *Can. Vet. J.* **21**, 12 (1980).
23. G. R. Higginbottom, J. Ress and A. Rocke, *J. Assoc. Off. Anal. Chem.* **53**, 673 (1970).
24. L. B. Brilliant and co-workers, and H. Price, *Lancet* **2** (8091), 643 (1978).
25. M. Barr Jr., *Environ. Res.* **12**, 255 (1980).
26. R. A. Frobish, B. D. Bradley, D. D. Wagner, P. E. Long-Bradley, and H. Hairston. *J. Food Protection* **49**, 781 (1986).
27. A. J. Howard in J. Wiseman, ed., *Fats in Animal Nutrition*, Butterworth & Co., London, 1984, pp. 483–494.
28. USDA, *World Oilseed Situation and Outlook*, USDA FAS Circular Series **FOP 6-93**, Foreign Agriculture Service, Washington, D.C., 1993.
29. R.H. Rouse, *Fat Quality—The Confusing World of Feed Fats*, Rouse Marketing, Inc., Cincinnati, Ohio, 1994.
30. NRA, *Pocket Manual*, National Renderers Association, Washington, D.C., 1993.
31. AFIA, *Feed Ingredient Guide II*, American Feed Industry Assoc., Inc., Arlington, Va., 1992.
32. NCPA, *Rules of the National Cottonseed Products Association, 1993–1994*, National Cottonseed Products Association, Memphis, Tenn. 1993.
33. C. E. Coppock and co-workers, *J. Dairy Sci.* **68**, 1198 (1985).
34. G. D. Buchs, U. S. Pat. 5,270,062 (Apr. 1, 1993).
35. S. D. Martin, *Feedstuffs* **62**(33), 14 (Aug. 6, 1990).

36. L. A. Jones in R. L. Ory, ed., *Antinutrients and Natural Toxicants in Foods*, Food & Nutrition Press, Inc., Westport, Conn., 1981, pp. 77–98.
37. F. Shahidi, ed., *Canola and Rapeseed*, Avi-van Nostrand Reinhold Co., Inc., New York, 1990.
38. H. E. Amos, *J. Dairy Sci.* **40**, 90 (1975).
39. A. P. Bimbo, *J. Am. Oil Chem. Soc.* **64**, 706 (1987).
40. G. R. List in B. F. Szuhaj, ed., *Lecithins, Sources, Manufacture and Uses*, American Oil Chemists' Society, Champaign, Ill., 1989, pp. 145–161.
41. J. W. Hertrampf, *Feeding Aquatic Animals with Phospholipids. I. Crustaceans*, Lucas Meyer, Co., Hamburg, Germany, 1991.
42. J. W. Hertrampf, *Feeding Aquatic Animals with Phospholipids. II. Fishes*, Lucas Meyer, Co., Hamburg, Germany, 1992.
43. *Lecithin—Properties and Applications*, Lucas Meyer Co., Hamburg, Germany, 1993.
44. J. P. Cherry and W. H. Kramer in Ref. 41, pp. 16–31.
45. National Research Council, *Recommended Dietary Allowances*, 10th ed., National Academy Press, Washington, D.C., 1989.
46. V. K. Babayan in J. Beare-Rogers, ed., *Dietary Fat Requirements in Health and Development*, American Oil Chemists' Society, Champaign, Ill., 1988, pp. 73–86.
47. M. I. Gurr in Ref. 28, pp. 3–22.
48. J. H. Moore and W. W. Christie in Ref. 28, pp. 123–149.
49. M. Enser in Ref. 28, pp. 23–52.
50. D. N. Brindley in Ref. 28, pp. 85–103.
51. C. P. Freeman in Ref. 28, pp. 105–122.
52. D. L. Palmquist in E.R., Ørskov, ed., *Feed Science*, Elsevier Science Publishing Co., Inc., Amsterdam, the Netherlands, 1988, pp. 293–311.
53. D. E. Pethick, A. W. Bell and E. F. Annison in Ref. 28, pp. 225–248.
54. C. C. Whitehead in Ref. 28, pp. 153–166.
55. D. Lewis C. G. Payne, *Br. Poultry Sci.* **7**, 209 (1966).
56. M. E. Fowler, *J. Zoo Wildlife Med.* **22**, 204 (1991).
57. R. M. Herd and T. J. Brown, *Physiol. Zool.* **57**, 70 (1984).
58. M. E. Stansby, H. Schlenk and E. H. Gruger Jr., in M. E. Stansby, ed., *Fish Oils in Nutrition*, Avi-van Nostrand Reinhold, New York, 1990, pp. 6–39.
59. J. Opstvedt in Ref. 28, pp. 53–82.
60. D. H. Greene in Ref. 59, pp. 226–246.
61. National Research Council, *Nutritional Energetics of Domestic Animals and Glossary of Energy Terms*, 2nd rev. ed., National Academy Press, Washington, D. C., 1981.
62. Ref. 8.
63. K. L. Fritsche, N. A. Cassity and S-C. Huang, *Poultry Sci.* **70**, 611 (1991).
64. D. L. Palmquist and T. C. Jenkins, *J. Dairy Sci.* **63**, 1 (1980).
65. D. L. Palmquist in Ref. 28, pp. 357–382.
66. D. L. Palmquist, *J. Dairy Sci.* **74**, 1354 (1991).
67. N. L. Karrick in Ref. 59, pp. 247–267.
68. R. E. Atkinson in Ref. 28, pp. 495–503.

69. D. E. Sayre in R. R. McElhiney ed., *Feed Manufacturing Technology III*, American Feed Industry Association, Inc., Arlington, Va., 1985, pp. 99–103.
70. *Process Description: Pet and Aquatic Food Production*, Wenger Manufacturing Co., Sabetha, Kans., 1993.
71. L. J. Cook, T. W. Scott, K. A. Ferguson, and I. W. McDonald, *Nature* **228**, 178 (1970).
72. T. W. Scott, L. J. Cook, and S. C. Mills, *J. Am. Oil Chem. Soc.* **48**, 358 (1971).
73. T. W. Scott and co-workers, *Aust. J. Sci.* **32**, 291 (1970).
74. T. W. Scott, P. J. Bready, A. J. Royal, and L. J. Cook, *Search* **3**, 170 (1972).
75. T. C. Jenkins and D. L. Palmquist, *J. Animal Sci.* **55**, 957 (1983).
76. T. C. Jenkins and D. L. Palmquist, *J. Dairy Sci.* **67**, 978 (1984).
77. D. L. Palmquist, T. C. Jenkins, and A. E. Joyner Jr., *J. Dairy Sci.* **69**, 1020 (1984).
78. P. S. Sukhija and D. L. Palmquist, *J. Dairy Sci.* **73**, 1784 (1990).
79. A. Baldi, F. Cheli, C. Coirino, V. Dell'Orto, and F. Polidori, *Small Ruminant Res.* **6**, 303 (1992).
80. D. J. Schingoethe and co-workers, *J. Dairy Sci.* **69**(Suppl. 1), 111 (1986).
81. D. P. Casper and D. J. Schingoethe, *J. Dairy Sci.* **69**(Suppl. 1), 111 (1986).
82. R. Rising, P. M. Maiorino, R. Mitchell, and B. L. Reid, *Poultry Sci.* **69**, 768 (1990).
83. E. R. Lloyd, *Feeding for Milk and Butter*, Bulletin **13**, Mississippi Agricultural Experiment Station, Mississippi State, 1890.
84. J. H. Connell and J. Clayton, *Feeding Milk Cows*, Bulletin **33**, Texas Agricultural Experiment Station, College Station, 1894.
85. J. E. Tomlinson and co-workers, *J. Dairy Sci.* **64**(Suppl. 1), 141 (1981).
86. M. J. Anderson, D. C. Adams, R. C. Lamb, and J. L. Walters, *J. Dairy Sci.* **62**, 1098 (1979).
87. E. J. DePeters, S. J. Taylor, A. A. Frank, and A. Aguirre, *J. Dairy Sci.* **68**, 897 (1985).
88. H. E. Amos in *Proceedings of the 1984 Georgia Nutrition Conference for the Feed Industry*, Atlanta, Georgia Agricultural Experiment Station, Athens, Ga., 1984, pp. 119–129.
89. H. E. Amos, *Feed Management* **35**(12), 26, 28, 32, 34 (1984).
90. D. L. Palmquist, *J. Dairy Sci.* **67**(Suppl. 1), 127 (1985).
91. M-J Kiang, *Dry Extrusion of Whole Soybeans*, Insta-Pro International, Des Moines, Iowa, 1993.
92. *Fullfat Soya Handbook*, American Soybean Association, Brussels, Belgium, 1983.
93. T. R. de Souza and co-workers, *J. Rech. Porcine France* **22**, 151 (1990).
94. L. D. Satter, J-T Hsu, and T. R. Dhiman paper presented at the Advanced Dairy Nutrition Seminar for Feed Professionals, Wisconsin Dells, Wisc., Aug. 18, 1993.
95. W. Steele, R. C. Noble, and J. H. Moore, *J. Dairy Res.* **38**, 43 (1971).
96. E. M. Tice, M. L. Eastridge, and J. L. Firkins, *J. Dairy Sci.* **76**, 224 (1993).
97. M. A. Faldet and L. D. Satter, *J. Dairy Sci.* **74**, 3047 (1991).
98. L. D. Satter, M. A. Faldet and M. Socha in *Symposium Proceedings: Alternative Feeds for Dairy and Beef Cattle*, National Invitation Symposium USDA Extension Service, in Cooperation with University Extension Conference Office, University of Missouri, Columbia, 1991, pp. 22–24.



99. T. R. Dhiman, K. Van Zanten, and L. D. Satter, personal communication, November 25, 1994.
100. R. M. Gast and D. S. Bundy, "Control of Feed Dusts by Adding Oils," ASAE Technical Paper No. 86-4039, Am. Soc. Ag. Engineers, St. Joseph, Mich., 1986.
101. A. J. Heber and C. R. Martin, *Trans. ASAE* **31**(2), 558 (1988).
102. E. R. Peon, Jr. and L. I. Chiba, *Tech. Newslett.* (141984) (1984).
103. *The Soybean Oil Dust Suppression System*, American Soybean Association, St. Louis, Mo., 1986.
104. K. L. Wick, L. J. Friedman, J. K. Brewda, and J. J. Carroll, *Food Technol.* **47**(3), 94 (1993).
105. M.E. van Elswyk, *Nutr. Today* **28**(2), 21 (1993).
106. N. Warnant, M. J. Van Oeckel, and Ch.V. Boucqué. *Meat Sci.* **44**, 125 (1996).
107. M. R. L. Scheeder, K. R. Gläser, B. Eichenberger, and C. Wenk. *Eur. J. Lipid Sci. Technol.* **102**, 391 (2000).
108. K. Nuernberg, G. Nuernberg, K. Ender, S. Lorenz, K. Winkler, R. Rickert, and H. Steinhart. *Eur. J. Lipid Sci. Technol.* **104**, 463 (2002).
109. D. L. Palmquist, D. Beaulieu, and D. M. Barbano, *J. Dairy Sci.* **76**, 1753 (1993).
110. B. Fitch-Haumann, *INFORM* **4**(41), 371 (1993).
111. M. Schreiner, H. W. Hulan, E. Razzazi-Fazeli, J. Boehn, and C. Iben. *Poultry Sci.* **83**, 462 (2004).
112. H. W. Phetteplace and B. A. Watkins, *J. Food Composition Anal.* **2**, 104 (1989).
113. Z-B Huang, H. Leibovitz, C. M. Lee, and R. Millar, *J. Agr. Food Chem.* **38**, 743 (1990).
114. H. W. Phetteplace and B. A. Watkins, *J. Agr. Food Chem.* **38**, 1848 (1990).
115. P. S. Hargis, M. E. van Elswyk, and B. M. Hargis, *Poultry Sci.* **70**, 874 (1991).
116. M. E. Van Elswyk, A. R. Sams, and P. S. Hargis, *J. Food Sci.* **57**, 342 (1992).
117. S. Y. Oh, J. Rye, C-H Hsieh, and D. E. Bell, *Am. J. Clin. Nutr.* **54**, 689 (1991).

## *By-Product Utilization*

M. D. Pickard

### 1. BY-PRODUCTS OF SEED PROCESSING

#### 1.1. Soybean

The by-products resulting from the processing, i.e., solvent extraction, of, soybean in the major producing countries—Argentina, Brazil, and the United States (1) are defatted protein meal and, to the extent that the beans are dehulled prior to extraction, hulls (2). A portion of the separated hulls may also be added back to the meal. Over 90% of the protein meal obtained from processing in the United States is used directly as livestock feed, after appropriate heat treatment (toasting) to inactivate trypsin inhibitors and other antinutritive factors (2, 3). Relatively minor quantities are milled into flour or grits, primarily for edible applications, or used in the preparation of protein concentrates and isolates having food, feed, and industrial applications (3).

*Utilization of Soybean Meal in Animal Feeds.* Soybean meal is the most extensively used of the oilseed meals and serves as a protein supplement for all classes of animals. It has become the standard to which all other protein sources are compared, and its quality, acceptance, and reputation are widely known (4). The meal contains from 44 to 50% crude protein and from 2500 to 2800 kcal of metabolizable energy per kilogram, depending on the amount of hull present and the species of animal being fed. Dehulling increases the metabolizable energy values by about 5% for cattle and 12% or more for pigs and poultry (5).

Soybean meal is an excellent protein supplement for lactating dairy cattle and for calves following weaning, as it is highly palatable and well digested. It also serves well as the supplemental protein in rations for growing and fattening cattle. However, in many ruminant feeding applications, soybean meal may not be cost competitive with other proteinaceous and nonprotein-nitrogenous ingredients (4, 5). Extensive research related to the development of "protected" or rumen bypass protein from soybean meal has resulted in the development of commercial products (4), which should increase the utilization of soybean meal in ruminant feeds.

Nearly 80% of the soybean meal consumed in the United States is fed to non-ruminants. Soybean meal is the most economic high-quality protein available to feed manufacturers; hence it assumes a dominant role (3, 4). Cereal-based rations for pigs and poultry may contain soybean meal as the only protein supplement, as a previous requirement for the inclusion of some animal or marine origin protein has been supplanted by the addition of lysine, methionine, and vitamin B<sub>12</sub>, where economics dictate (5).

Soybean meal is used effectively in the formulation of pet foods, particularly for dogs, where simple corn-soybean meal mixtures perform as well as complex diets containing high levels of animal protein, and at substantially lower cost (4). Rapid growth in aquaculture over the last 10–15 years has resulted in significant new opportunities for utilization of soybean meal in finfish and shrimp diets. Whether additional heat processing of soybean meal to further reduce levels of antinutritional factors or supplementation with lysine or methionine or both is beneficial to performance appears to be species dependent (4, 6).

*Utilization of Soybean Hulls.* Soybean hulls are high in cellulose but low in lignin and, therefore, highly digestible by ruminants (4, 7). In fact, the digestible energy content of soybean hulls, for ruminants, approaches that of grain (7). Consequently, soybean hulls may be used, to economic advantage, in high-forage diets in lieu of grain, with various additional functional advantages. For growing cattle and sheep, replacing grain with soybean hulls eliminates the risk of acidosis and reduces the negative effect of starch on fiber digestion. In the case of lactating cows or ewes, soybean hulls can replace a significant portion of the grain in a grain-forage diet with no reduction in fat content or milk yield (4, 7). Soybean hulls are seeing increasing use in human food as a source of dietary fiber. Fiber-enriched pasta and white bread appear to be the most popular vehicles for inclusion of soybean hulls at this time.

*Edible Products Derived from Soybean Meal.* The primary edible products derived from soybean meal/flakes are flour/grits (at 50% protein), protein concentrates (containing 65–70% or more of protein) and protein isolates (90% protein). The nutritional quality, availability, price, and functionality of these products has resulted in substantial usage in a wide variety of food and feed products. Given current trends in food consumption, strong growth in the use of soy protein products in foods appears assured (8, 9). A number of excellent articles on the manufacture of soy protein products exist; processing will not be described in detail here (8, 10–13).

Soybean flour and grits are essentially ground soybean meal/flake products differing primarily in particle size, both having received heat treatment appropriate to

their required functionality and expected end use. Flours and grits are primarily employed as fat and water binders in baked goods and pet foods and as raw material in the manufacture of soy sauce. Residual beany flavor and poor mouthfeel limit the use of soy flour and grits in many applications. Soy flours containing lecithin or various levels of fat (soybean oil) are also commercially available (8, 12, 13).

Protein concentrates with improved flavor and functionality relative to soy flour may be prepared from undenatured soy flour/flakes by a variety of processes including acid or alcohol leaching, alkaline extraction, heat denaturation followed by aqueous leaching, and membrane filtration. Products are ultimately spray or dispersion dried. Currently, acid- and alcohol-washed products dominate the marketplace and are available in a variety of functionalities for use wherever the nutritional, fat and water binding, emulsification, foaming, or viscosity modifying characteristics of casein or nonfat dry milk would otherwise be exploited. Typical applications of soy protein concentrates include comminuted meat products, baked goods, baby foods, cereals, milk replacers, pet foods, and snacks. The expanding market for meat extenders and substitutes employs extrusion-texturized soy concentrates that, when rehydrated, possess to a remarkable extent the chewiness and mouthfeel of meat (8, 10, 12, 13).

Protein isolates are produced from undenatured soy flour/flakes by dilute alkaline extraction and subsequent acidification of the protein extract to the isoelectric point, approximately pH 4.5. The precipitated protein curd is recovered by centrifugation, washed, slurried in water, and usually neutralized prior to being spray dried. Ultrafiltration or other protein recovery techniques may also be employed in isolate production. Soy protein isolates are relatively expensive but highly functional. Their binding, emulsification, foaming, and nutritional properties are exploited in a variety of products including comminuted meats, dairy-type products, and infant formulas (8, 11–13). Due to their inherent insolubility at acidic pH, pectin-aggregated soy isolate particles show potential as clouding agents in citrus beverages (14). Soy isolates may be modified by hydrolysis, addition of substituent groups, or texturization (spinning), thereby expanding their range of functionalities and applications (12, 13).

*Industrial Uses of Soybean Protein Products.* The historical, current, and potential industrial use of soybean proteins has been described recently in excellent fashion (15, 16). Consequently, this topic will be discussed only briefly here.

The early promise of plastics manufactured from acid- and formaldehyde-treated isolated soy protein was never realized, for economic and functional reasons. Soy flour has seen significant use in glues for plywood and other laminated wood products. These have been displaced by petroleum-based glues that exhibit superior microbial and water resistance. Textile fibers prepared from soy isolate or flour never saw commercial production due to poor wet strength and an unpleasant odor when wet. Soy protein isolate is currently used in paper-coating applications (15, 16).

Potential new uses for soy protein are driven primarily by environmental concerns and the search for new value-added uses for agricultural commodities. In addition, prices for petroleum-based polymers have increased relative to prices for

agricultural products (15–17). Examples of products currently under development include biodegradable plastics, edible soy protein films and soy protein–carbohydrate films intended to reduce packaging waste, and a soy flour-recycled paper composite material (sold under the trademark Environ) with the appearance of granite and the handling characteristics of hardwood. In addition, soy protein-based glues and adhesives and textile fibers, with functional characteristics superior to products from earlier efforts, are being developed (15, 16, 18, 19). Still another use is as a feedstock for fermentations.

## 1.2. Rapeseed/Canola

Rapeseed has become an important crop in the temperate zones of the world, with production in more than 30 countries on 5 continents. The productive capacity of the crop and the nutritive value of its protein have made rapeseed a leading potential source of food and feed protein ingredients. Oilseed rapeseed was grown in India over 3000 years ago, and at least 2000 years ago in China and Japan. It is not clear when rapeseed oil became a food oil in addition to its use as a fuel for lamp lighting and for soap and candles. Throughout most of the long history of this crop, the cake or meal was used as a fertilizer or soil conditioner, a practice that persists today in China and Japan (5).

The early nutritional experiences with the meal were probably not encouraging as the meal was unpalatable. Glucosinolates present in the meal release goitrogenic factors, such as oxazolidinethione, isothiocyanates, and thiocyanates, when hydrolyzed by the enzyme, myrosinase, also present in the seed/meal. These compounds interfere with iodine uptake and thyroxine synthesis by the thyroid gland. Under certain conditions, highly toxic nitriles may also be produced (5). Processing techniques that inactivate myrosinase and, therefore, prevent glucosinolate hydrolysis have become standard operating practice around the world (20, 21). Typically, seed containing 6–10% moisture is rapidly heated to 80–90°C. A second deterrent to the use of rapeseed meal as a nutritional supplement is its high fiber content. Most rapeseed varieties have a dark, hard seed coat containing a condensed polyphenol-based complex that contributes a substantial amount of fiber to commercial rapeseed meal (22).

The reduction of glucosinolate levels in rapeseed through extensive plant breeding programs has provided a major breakthrough in the utilization of rapeseed meal. The meal is becoming increasingly available as a protein supplement for animal nutrition as more of the genetically improved varieties, known as canola, are grown. Canola is a trademark name and a generic term to distinguish specific seed varieties containing less than 2% of erucic acid in the oil fraction and having a solid component containing less than 30  $\mu\text{mol/g}$  of glucosinolates. Successful feeding of canola meal still requires knowledge of its glucosinolate status as well as the age and class of animal involved (23, 24).

*Composition of Canola Meal.* Canola meal is an internationally traded commodity. Excellent summary information on canola meal composition has been developed to aid the animal feed industry (25). Canola meal contains 36–38% crude

protein and a favorable assortment of essential amino acids in its protein. It is higher in crude fiber, at 12%, than is soybean meal and consequently has lower metabolizable energy values (1900–2300 kcal/kg, depending on the species being fed). The crude fiber is largely in the hull fraction, which comprises 16–25% of the meal. Hulls are poorly digested, especially by nonruminants, and are largely responsible for the relatively low metabolizable energy values. Dehulling would improve metabolizable energy values, but hull utilization and loss of oil and cotyledon material in the hulls remains problematic (5).

*Use in Animal Feeds.* **Pigs.** Canola meal is a proven supplemental protein source in pig diets. Although poor palatability and reduced digestibility with young (6–20 kg) pigs prevent its use at levels higher than 25% of supplemental protein, research has confirmed that canola meal is a desirable supplemental dietary protein for growing, finishing, and reproducing swine. Canola meal can be used at 50–75% of the supplemental protein source in diets for growing (20–60 kg) pigs and as the sole supplementary dietary source for finishing pigs (60–100 kg) and dry and nursing sows (26).

**Poultry.** Canola meal is widely accepted for use in poultry diets. It is usually limited to 10% of the diet in layer feeds because of increased bird mortality at higher levels. Long-term studies of egg production show no effect of canola meal on the number of eggs produced per bird. However, there is a small decrease in egg size due to slightly lower feed intake. Canola meal and rapeseed meal have an interesting effect on brown-shelled layers. These birds are deficient in an enzyme responsible for the breakdown of trimethylamine. As canola and rapeseed have high levels of choline and sinapine (precursors of trimethylamine), the eggs of these layers have a fishy taint. For this reason, a maximum of 3% of canola meal may be used in the diets of brown-shelled egg layers. There is no concern about increased mortality at high canola meal inclusion rates for growing poultry such as broilers, turkeys, pullets, or waterfowl. When grower diets are appropriately balanced for energy and levels of digestible amino acids, canola meal can be effectively used as the major supplemental protein source (27, 28).

**Beef and dairy cattle.** Canola meal has gained widespread acceptance as a protein supplement in beef and dairy rations. Research has shown its effectiveness in a variety of production and management situations. Lactation trials have demonstrated that canola meal will maintain or slightly improve milk production relative to soybean meal-based rations. Improved milk production may in part reflect the amino acid content of the bypass protein fraction of canola meal. In beef cattle rations, animal performance has been shown to meet or exceed industry standards when canola meal is incorporated. Canola meal can be used as the sole protein supplement in rations for growing and finishing cattle (29).

*Use as Human Food.* Rapeseed, as a protein source for humans, has many obstacles to overcome. The glucosinolate and fiber contents require application of new processing technology (22, 30) to eliminate antinutritional qualities. Rapeseed flours, protein concentrates, and isolates are lower in protein but higher in crude fiber and ash contents than corresponding soybean products. Rapeseed flours are comparable to soybean flour in water adsorption and give much higher fat adsorption, oil

emulsification, and whippability values. Rapeseed flour viscoamylograph curves exhibit high viscosities but poor gelation properties. The utilization of rapeseed products may also be limited by green or brown colors in aqueous systems (31).

*Other Uses.* Rapeseed or canola meal has been used as a fermentation substrate. It has been included as an additive in compost for commercial button mushroom (*Agaricus bisporus*) production with good success (32). Canola meal has also been tested as a substrate for xylanase production by *Trichoderma reesei*. Results from this work indicate that the hydrolysis of canola meal by this enzyme system might be useful in converting this material to fermentable sugars that could be further processed to expensive end products such as solvents and chemicals (33).

### 1.3. Sunflower

*Sunflower Meal.* The vegetable oil extraction industry produces three types of sunflower meal: undehulled meal containing 28% protein and 25–28% fiber, partially dehulled meal containing 35–37% protein and 18% fiber, and double-dehulled sunflower meal containing 40–42% protein and 12–14% fiber. Thus, the composition of sunflower meal is dependent on the efficiency of the dehulling process (34).

The protein concentration and amino acid composition of sunflower meal also vary with the source of seed, and high-temperature processing may have a deleterious effect on its lysine content. Generally, however, sunflower meal exhibits a well-balanced amino acid composition with an essential amino acid index of 68, compared to 79 for soybean meal and 100 for whole egg (35).

The energy content of sunflower meal compares favorably with that of other oilseed meals and increases as the residual oil content increases and as the fiber content decreases. Sunflower meal also compares favorably with other oilseed meals as a source of calcium and phosphorus (36) and is an excellent source of water-soluble B-complex vitamins, namely nicotinic acid, thiamine, pantothenic acid, riboflavin, and biotin.

Sunflower meal contains the polyphenolic compound, chlorogenic acid, which results in a yellow-green coloration following oxidation in the presence of alkali. The production of protein isolates and concentrates from sunflower meal/flour would require the removal or inactivation of chlorogenic acid (35, 37).

*Use as Animal Feed.* Sunflower meal can be fed to all classes of livestock. Most sunflower meal is fed to ruminants and is comparable, nutritionally, to cottonseed meal. High levels of sunflower meal are used in dairy, beef, and sheep rations (5).

For swine, low-fiber sunflower meal is inferior to soybean meal as the sole source of supplemental protein. This inferior performance is the result of lower palatability and nutrient content. In swine rations, with 20–30% of the protein from sunflower meal, rates of gain are similar to soybean meal, but larger quantities of meal are required. Lysine supplementation reduces this requirement. Studies have indicated that sunflower meal can effectively replace 50% of the soybean meal in growing–finishing swine rations. Higher rates of utilization are possible as animals increase in weight because of the decreased requirement for essential amino acids (38).

Rations for laying chickens could incorporate low-fiber sunflower meal, to a level of 50% of the protein concentrate portion, without significantly reducing egg production (39). Higher levels of addition are possible with lysine supplementation but may cause egg staining due to the presence of chlorogenic acid in the meal (35). Metabolizable energy trials with laying hens yielded a value of 2205 kcal/kg (dry matter basis) for dehulled, solvent-extracted meal (38).

The use of sunflower meal is often limited by its availability. Adequate volumes, available on a sustained and consistent basis, would ensure a high utilization of sunflower meal in animal nutrition. Despite its dark appearance, lower energy, and higher fiber content, as compared to soybean meal, sunflower meal is a competitive product with potential for continued improvement through the use of tail-end screening to further reduce its fiber content (40).

*Use in Human Foods.* Confectionery sunflowers have a history of use in the snack trade and the trend continues (41). The roasted seed has a pleasant nutty flavor. Dehulled and roasted sunflower kernels can be used as a nut substitute in many confectionery and bakery formulas. Physical and organoleptic analysis of color, flavor, texture, and acceptance indicate that a 10–15 min roast at 177°C is the most desirable processing technique (37).

Chemical and physical analysis of hexane-extracted sunflower meal indicates that discoloration due to the oxidation of chlorogenic acid is a problem. An attractive cream color, relatively bland flavor, and excellent stability are possible if the processing conditions do not induce chlorogenic acid oxidation. Unfortunately, the conventional methods of making protein isolates promote the oxidation of chlorogenic acid. Studies have shown that organic solvents produce good extraction of polyphenols from sunflower seed and meal (42). The physicochemical properties of proteins from such extracted meals indicate no significant differences in amino acid content and only slight changes in nitrogen solubility due to protein denaturation. Sunflower flour and protein isolates have excellent emulsion and whipping properties and thus have great potential as functional agents and protein supplements in human food products, provided the polyphenols are removed or care is taken to prevent their oxidation (36).

*Sunflower Hulls.* **Chemical composition of sunflower hulls.** The hull, a by-product of oil extraction, comprises 22–28% of the total weight of oilseed sunflower and may be removed before or immediately following oil extraction or may remain in the meal. Sunflower hulls contain: 4% crude protein; 5% lipid material, including wax, hydrocarbons, fatty acids, sterols, and triterpenic alcohols; 50% carbohydrates, principally cellulose and lignin; 26% reducing sugars, of which the majority is xylose; and 2% ash (35). The high fiber content and low protein and energy content of sunflower hulls reduce their nutritional value.

**Use in animal feeds.** Hulls can be used in ruminant feeds when finely ground and mixed with other ingredients. When adequate energy is provided, sunflower hull pellets may be used as a portion of the roughage component in ruminant rations due to their high content of cellulose and lignin. They are used to add bulk to concentrated rations and to absorb liquids such as molasses. Sunflower hulls are sold to feed manufacturers and livestock feeders at prices comparable to those of other ingredients (38).



**Hulls as a source of fuel.** The utilization of sunflower hulls as a source of fuel has been studied. The heat value of hulls alone is 19.2 MJ/kg, whereas the heat value of hulls and meal combined is 23.6 MJ/kg. The higher heat value suggests that the combination of hulls and meal makes a better fuel (43). In many countries, the burning of sunflower hulls offers an alternative to higher priced fuels. The resulting ash has a high percentage of potassium and can be used as a fertilizer (34). Hulls have been pressed into cylinders with wood waste and sold as fire logs (35).

It has been reported that because of their high content of reducing sugars, it is possible to produce furfural and ethyl alcohol from sunflower hulls (40). Sunflower hulls also represent a source of lignocellulosic material for acid hydrolysis and fermentation. As a lignocellulosic waste material, sunflower hulls can be hydrolyzed with acid to yield material suitable for the production of single-cell protein (44). Purple-hulled sunflowers contain anthocyanin, which may be useful as a natural red food colorant. North Dakota State University has extracted, quantified, and scaled up processing techniques to extract the pigment. Economic analysis suggests that the processing of these unique hulls may be economically justifiable (45).

*Sunflower Stalks.* Finely chopped and dried stalks could be used as deburring and polishing abrasives in the metal manufacturing industry and are a replacement for peat moss in plant starter mix. It has been reported that stalks are easily processed and decolorized by existing pulping and fiber processing techniques. The processed material can be made into acoustical tile. This material weighs less than 60% of standard acoustical tile and has better sound absorbency and strength (46). Sunflower heads and stalks also represent a potential source of low-methoxyl pectin for use in low-sugar jams and jellies (47, 48).

#### 1.4. Safflower

Safflower is a minor oilseed crop limited in production by environmental constraints and by the plant's spiny nature. Unless the seed is well dehulled, the oilcake resulting from oil extraction will have a high fiber content. Uncorticated oil cake has a protein content of 20–22% and an end use as manure. In contrast, removal of the hull improves the protein content to 40%, making it acceptable as cattle feed despite low lysine levels. Leftover hulls and husks are added to cattle feed or are used to manufacture cellulose, insulation, and abrasives (5, 49).

#### 1.5. Cottonseed

Gossypol is a yellow-green polyphenolic pigment contained in discrete bodies in cotton leaves, stems, roots, and seeds. This form of gossypol is readily extractable with 70% aqueous acetone. The glands are ruptured during processing and the released pigment structures are highly reactive with other cottonseed components such as protein. The gossypol binds to the biologically available lysine, effectively reducing the concentration available to an animal. Gossypol also causes toxic effects in monogastric animals, including humans. An additional complication related to the presence of gossypol is the production of dark-colored pigments

in the oil and meal that cannot be removed by conventional refining and bleaching operations (50, 51).

The adverse physiological effects of free gossypol on monogastric animals may be counteracted by making free gossypol a bound form during processing, either by precooking, followed by pressure and shearing in a screw-exPELLER, or by binding to ferrous salts (50, 51). A polar solvent, such as aqueous acetone, acetone-hexane-water, or hexane-acetic acid, may then be used to extract residual free gossypol from the meal (51). The presence of bound gossypol reduces the protein efficiency ratio, presumably by reducing the availability of lysine. Care is required to prevent thermal damage to protein, which would further decrease the nutritive value of the meal (51).

Utilization of glandless cottonseed strains is an alternative to the extensive seed treatment necessary to lower the gossypol content. The goal of plant selection programs has been to minimize the total gossypol concentration in the raw material (50). The Hopi Moencopi variety (*Gossypium hirsutum* var. *punctatum*) was used in the late 1940s by McMichael to produce plants with almost complete elimination of pigment glands from leaves and bolls. His findings stimulated an exploration of commercial-type cotton crosses with glandless lines when the results were finally reported in 1959 (52). Presently, the glandless strains that have been developed are not widely produced due to concerns related to the influence of these gossypol-free characteristics on the yield and the quality of the commercially more valuable cotton fiber. Studies indicate that this concern is unfounded. An additional concern that has been addressed is that gossypol and related terpenoids are natural insecticides, such that the use of glandless cotton may encourage insect preference for the glandless cotton. It has been shown that insects do not prefer either strain (52).

Despite the presence of gossypol, interest in the cottonseed cake has developed as a result of its high content of protein, the valuable component in cottonseed by-products (51). A number of commercial products from defatted cottonseed have been extensively used in the past. Proflo flour was produced from 1939 to 1975, and contained 55–60% protein and 4.5% fat (52). This product was a nonallergenic dietary protein source contributing functionalities such as emulsification, antioxidation, and water absorption to bakery-type products. Commercial production was suspended due to the limited market, but it is still produced for nonfood industrial purposes (52). Incaprina, or INCAP Vegetable Mixture 9, was produced in the 1950s and 1960s and contained 38% cottonseed flour. It had a content of 0.05% free gossypol that was high enough to warrant supplementing the product with lysine to offset binding losses. Incaprina was vital as a low-cost vegetable protein source in South America (52). In addition to human consumption, the post-oil extraction cake is also used for animal feed and, in the past, as fertilizer.

**Cottonseed Meal.** Cottonseed meal is second only to soybean meal with respect to the quantity produced worldwide. This by-product of oil extraction is used in rations for cattle, sheep, goats, horses, and mules. Neither glandless nor normal cottonseed meal is palatable to young pigs (5).

Broiler poultry feeds often contain cottonseed meal, with the potential to cause depressed weight gain and reduced feed efficiency (53). Cottonseed meal is not

used in layer feeds since the gossypol produces a yellow discoloration of the yolks and whites of the eggs. Ferrous iron is added to most poultry diets containing cottonseed (5). A recent study of low-gossypol cottonseed meal found that it successfully replaced soybean meal in the diet of broiler chickens with no harmful effects (53).

A number of alternative uses for cottonseed meal exist. Adhesive and fiber production have used cottonseed meal as a protein source. Plastics that contain cottonseed meal in equal parts with cottonseed hulls and phenolic resin have excellent flow properties, a short curing cycle, water resistance, and strength (50).

*Cottonseed Hulls.* Hulls are used as roughage in animal feed and as mulch and soil conditioner. Additional uses for cottonseed hulls include fuel, insulation, and a xylose and furfural source. Raffinose derived from cottonseed hulls is used in culture media (50).

## 1.6. Palm

The major producers of oil palm products are located in the equatorial tropics and include Malaysia, Nigeria, Indonesia, China, Zaire, and Cameroon (54). Palm fruit, when pressed, yields approximately 43% of crude palm oil and 57% of press cake, which consists of 35% pericarp (fiber) and 65% nuts. Palm nuts consists of 83% shells and 17% kernels, which, when pressed, yield approximately 50% of each of palm kernel oil and palm kernel cake (55).

*Palm Kernel Cake.* Palm kernel cake (PKC) protein is of average quality, which, at a level of 19%, is the lowest of the commercial oil cakes. The positive characteristics of PKC are a valuable calcium to phosphorous ratio, a 48% carbohydrate level, a 5% oil content, and a 13% fiber content (54).

The gritty texture of PKC limits its use in feed for monogastric animals. It is used in poultry diets at an optimum level of 15% in broiler and 20% in layer diets and provides 1500 kcal/kg of metabolizable energy (56). Pig diets may contain PKC if blood meal is added as a supplement. Pigs will consume 20–30% of PKC in their rations if it is introduced gradually to young animals, which otherwise find it distasteful. The use of PKC results in a firmer pork (57).

Ninety-five percent of the 430,000 tons of PKC produced annually in Malaysia is exported to Europe. European farmers use a ration containing 7–10% of PKC for dairy cattle (58). The high fiber of PKC is necessary for dairy cattle to prevent metabolic and digestive problems. Each adult animal requires 2–3 kg each day. The level of fiber found in PKC prevents deficiency problems in lactating cows and may increase the fat content of the milk (57).

*Palm Fiber.* Palm press fiber or pericarp fiber includes not only palm press fiber from the oil extraction process but also empty fruit bunches and kernel shells. Its high fiber and lignin content, comparable to wood, and low palatability limit its use in animal feed (55). It exhibits very slow digestion in the bovine rumen such that processing to increase its nutrient content is required prior to use (59). Supplementation with molasses, urea, and vitamins allows palm press fiber to be

used as a fiber source. Urea produces an alkaline effect on the fiber and adds nitrogen to the feed.

The predominant use of palm press fiber is as biomass fuel for oil mill plants. The palm press fiber and kernel shells are burned to produce steam for generation of electricity. The potential heat energy of palm fiber is 4420 MJ/kg and of the shell 4848 MJ/kg. One ton of shells and fiber used for fuel produces 578 kg of steam. For example, Malaysia required 413 million kWh of electricity in 1985 to process 20.65 million tons of fresh fruit bunches. The industry, therefore, by burning palm shells and fiber, saved 140 million liters of diesel fuel that would have been required to produce this amount of electricity. This source of energy has saved the palm oil industry in a monetary sense and is a convenient disposal method for fiber and shell wastes (60). The ash from the burning of this solid waste does not contain sufficient nutrients to be used as a fertilizer, and dumping creates an airborne hazard and pollutant (58). It has been incorporated in concrete as a replacement for cement with a slight increase in the setting time but within American and British standards (58).

Palm shells are composed of 20% free carbon and are suitable for production of charcoal or activated charcoal. Empty bunch waste can also be used as a field mulch (55).

## 1.7. Coconut

Coconut palms have the greatest economic value and distribution of all the palms and are considered the most useful of all plants, after grasses. Coconut products service both local and international markets (61).

*Copra Cake.* Copra cake is a by-product of oil extraction from coconut. The dehusked coconut is split and the meat is scraped from the kernel cup and dried. The oil is extracted via expeller or solvent processes from the dried coconut meat copra. This product is available throughout the year, making it a cheap local source of animal feed. The cake is ground to meal for use in feed for poultry, cattle, sheep, and swine. Copra cake can be used as a substitute for fish meal in swine feed but may cause constipation. Germany has been an importer of the majority of the cake produced in the Philippines (61).

There are problems associated with the use of copra cake in feed. As the amount of cake in the feed increases, its palatability decreases. Copra meal tends to be less digestible than fresh coconut meal. Despite a protein content of greater than 20%, the addition of methionine and lysine improves growth and feed utilization. The method of oil extraction does not appear to influence the quality of meal produced. Neither expeller-extracted nor solvent-extracted copra meal, at levels of 10–14% in poultry diets, caused any difference in egg production, mortality, or efficiency of feed conversion (61).

*Coconut Flour.* Coconut flour is produced from the shredded kernel, dried in a continuous countercurrent drier, and subsequently extracted with solvent to remove the residual oil. The white meal produced contains 25% protein and 65% carbohydrate, as well as various minerals and vitamins (62). This coconut product

is used as a high-protein additive to enrich other flours such as wheat, rice, and corn flour. Coconut flour has properties compatible with those of wheat flour in the preparation of bread, biscuits, and other food products (62). The protein quality of coconut flour may be very high (lysine, 19%; cystine, 8–9%; histidine, 5–6%; methionine, 3–9%), making it desirable for use in baby food and convalescent food drinks. Its content of essential amino acids may be reduced, however, if excessive heat is generated during mechanical oil extraction. The flour also possesses a relatively high crude fiber content (62).

*Coconut Shell.* The coconut shell comprises 27% of a dehusked nut by weight. This by-product of oil production has many local uses. Coconut shell has a composition similar to that of hardwoods but has a slightly higher lignin content and a lower cellulose content (61). In southern India and Sri Lanka, the shells are used directly as fuel in villages and small holdings and by local industries such as laundries, bakeries, and iron foundries.

Charcoal is manufactured using a simple process. The air surrounding burning shells is limited, encouraging slow carbonization rather than ash production. This process takes place in a kiln over a 3-day period with careful consideration given to the balance of conditions and time. The final product represents 30% of the original weight of the shells (61). Charcoal is a preferred fuel as it produces no waste material when burned. Hot embers emit infrared wavelengths that are valuable in the cooking of foods such as fish, meat, or tubers. Coconut shell produces heat energy at a level of 23 MJ/kg whereas shell charcoal produces 30 MJ/kg. Shell charcoal is also used to manufacture calcium carbide and the carbon electrodes of electric batteries. Both shell and charcoal generate producer gas. Reactors utilizing this product are sold for refrigeration, water pumping, and ground and marine vehicle operation (63).

Destructive distillation of the shell produces some interesting substances. Upon exposure to very high heat in the absence of air, the shell forms products from all three phases (gaseous, liquid, and solid) as noncondensable gases, pyroligneous liquor, settled tar, and retort charcoal will be generated. The pyroligneous liquor, a dark red and odorous liquid, yields acetic acid, methanol (locally called “wood naphtha”), and a variety of other products. The liquor may be used as boiler fuel, and noncondensable gases may be compressed in cylinders for use as domestic cooking gas (62).

Coconut shell is the source of two other products, coconut shell flour and activated charcoal. Powdered coconut shell is used in the plastics industry as a compound filler for synthetic resin glues. It is also used as a filler and extender of phenolic molding powders that give a smooth and lustrous finish to molded articles, thereby improving their resistance to moisture and heat. Activated charcoal is an adsorbent for toxic agents. It has been used in gas masks, but can also be used to remove odors and industrial stench. As well, this by-product is a contact catalyst used to facilitate some industrial chemical reactions (61).

*Coconut Husk.* The husk represents 35% of the intact, mature coconut by weight. A number of products are derived from this by-product of oil production. Particles of husks may be consolidated with little or no adhesive (64). Pith is

chipped with the husk material to produce the best bonding characteristics. The resulting self-bonding chips can be formed into boards of varying densities that are strong, durable, water repellent, and fire retardant. These boards are used as low-cost construction products such as roofing panels. Boards with a density of less than  $400 \text{ kg/m}^3$  are used for thermal insulation whereas medium-density boards of  $500\text{--}900 \text{ kg/m}^3$  are used in construction and furniture (64). A combination of 25% finely ground coconut husk and 75% andesitic sand by volume has been used as a potting medium in nurseries (65).

*Coir.* Coir is a valuable and versatile fiber derived from the coconut husk. The best quality coir is produced from green coconut, which is more difficult to harvest and has a lower copra yield than more mature coconut. The amount of copra and the quantity of coir produced are inversely related (61). Husks must be retted to manufacture coir. This process involves holding the husks under water, away from air, with mud and leaves for a period of a few months to a year. Fermentation is accomplished by short rod bacteria such as *Pseudomonas*, *Rerobacter*, and *Bacillus*. The microbial process is a polyphenolic degradation in which the pectic substances are decomposed. Slow moving and slightly saline water in a natural source speeds the process and produces a better quality fiber (66).

## 1.8. Groundnut

Groundnut is comparable in nutritional value to more expensive animal-derived foods. Protein from this oilseed has the highest quality of the vegetable proteins, equivalent to casein. The oilseed cake from commercial-grade groundnuts is used for animal feed, whereas solvent-extracted edible-grade groundnut cake is milled into flour (67). The digestibility of groundnut meal is high when it is well dehulled. Groundnut meal is lower in available lysine than soybean meal but contains a greater quantity of sulfur amino acids (5). Peanut flour has an amino acid content comparable to that of raw and roasted peanuts, indicating that moderate heat treatment does not alter the amino acid composition (67).

A new process for peanut flour production has been developed that requires treatment of the seed with both heat and moisture prior to oil extraction. The flour end product is white and bland, contains 65% protein, and is devoid of peanut flavor. This allows the addition of this product to a variety of food products without disturbing color, flavor, or texture. A high-quality flour has been used in the treatment of hemophiliac patients (67).

Protein produced from solvent-extracted meal has been used as a thickening agent in soups, baby foods, high-protein foods, institutional meals, and meat products. Groundnut proteins have also been used to manufacture a soft, wool-like, cream-colored fiber, adhesive products such as plywood glue and wettable glue, and for paper coating.

Fermentation of peanut protein with microorganisms increases the level of some essential amino acids. *Rhizopus oligosporus* is widely used to produce a tempehlike product (68). A similar product, oncom or ontjom, is produced in Indonesia using the fermenting agent *Neurospora sitophila* (51). The difference

between the two products is the red color of oncom, derived from the red hyphae of *N. sitophila*.

The seed coat of peanut is a commercial source of tannins and thiamin and sees limited use in feeds, primarily as a bulking agent in reduced-calorie pet foods. The presence of the seedcoat in meal may lead to a decrease in the availability of lysine (5). This apparent waste product is also used as mulch, fuel, litter for poultry houses, and in the production of a high-grade activated charcoal.

The hull of the peanut is low in crude protein and exhibits low digestibility. This has limited the utilization of this by-product, although use as a source of roughage for ruminants has been reported (69). Physical and chemical treatments of the hulls have been applied to promote digestibility, with little success (69,70). Hulls are generally considered waste products.

### 1.9. Olive

Olive oil is produced in warm-temperature and subtropical regions where the olive tree grows well. The fruit develops maximum oil content during the mid-November to February or March period in the Mediterranean basin and from May to June in the Southern Hemisphere. Ninety percent of the olives grown is used to produce oil (71).

Initial extraction of oil from oil fruit produces a cake of fruit skin, pulp, and kernel known as olive pomace or orujos. The value of this primary by-product of oil extraction depends on its oil and water contents, which are, in turn, determined by the method of oil extraction employed and the operating conditions. Pressure extraction yields a residue containing 4–5% oil, whereas classical presses leave 8–12% oil in the pomace (72). Pomace flours are used as animal feed due to their high content of protein, which is also of high quality.

Secondary oil extraction from the pomace produces olive pomace oil containing large amounts of free fatty acids and is considered an inferior oil compared to virgin olive oil. Pomace must be dried in long revolving horizontal cylinders through which hot air is passed before the oil can be extracted (73). The exhausted olive pomace is called kernel wood or orujillo. This product has few uses due to its low protein content and its high content of woody and cellulosic materials (73). Kernel wood is predominantly used as fuel for operation of the processing plant. Ash from kernel wood is used as fertilizer because of its potassium, phosphorus, and calcium content. The low value of this by-product negatively influences the overall value of olive products, contributing to the high price for olive oil (71).

### 1.10. Sesame

Sesame has declined in international trade due to a market preference for other oilseeds that are cheaper and easier to produce, such as groundnut. World trade tends to be in whole seed with only a small amount moving as oil and cake. There is great value in dehulling if the product is to be used as a foodstuff, as removal of the hull lowers the oxalic and phytic acid levels in the meal. The presence of these

components may decrease the bioavailability of calcium, magnesium, zinc, and, perhaps, iron. The protein content, acceptability, and enzymatic digestibility of the meal will also increase (74). There are problems associated with dehulling, however. It is difficult to remove the hull from sesame, and there will be an associated loss of minerals. Whole press cake has a bitter taste due to the presence of the testa and is best used for manure and soil conditioning (51, 75).

Cake or meal from oil extraction contains 40–50% protein when processed in a screw press and 56–60% protein after solvent extraction. Sesame products have a pleasant flavor and contain high levels of methionine and cysteine. The flour produced from sesame meal has a high nutritive value compared to other oilseed flours (75).

Sesame has specific uses in confectionery products such as halva, sesame seed cake, candies, and as a garnish on bread and rolls. Microatomized protein food for feeding unweaned babies represents another use for sesame and other oilseed flours when enriched in vitamins and minerals (75).

### 1.11. Linseed/Flax

Linseed is grown predominately for its quick drying oil and for the fiber from its stalks. Whole seed is often shipped without processing at the location of harvest (76). Oil cake contains approximately 30% protein and is used in feed for sheep, horses, and dairy and beef cattle. A high mucilage content endows many positive properties to the meal. Linseed meal is comparable to soybean meal in composition but energy and protein digestibility are lower than for most other oilseeds. Linseed meal endows “bloom” and mellowness to the hide, which is most valued in the preparation of animals for shows. These qualities can be attributed to residual oil in the cake, slight laxative effects, and appetite stimulation (5). Care must be taken when linseed meal is used as feed for poultry. It contains a vitamin B<sub>6</sub> antagonist, *N*-( $\gamma$ -L-glutamyl)-amino-D-proline, such that supplemental vitamin B<sub>6</sub> must be used to prevent any detrimental effects from occurring. Low levels of lysine and methionine must be balanced using supplements before linseed meal can be used for swine feed. In addition, the possible presence of hydrocyanic acid should be monitored since it varies in concentration according to growing conditions. The cake has seen limited use as a soil conditioner (77).

## 2. BY-PRODUCTS OF OIL REFINING

### 2.1. Lecithin

Lecithin, an edible by-product of oil processing possessing a variety of useful functionalities, is primarily a mixture of phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and phosphatidic acid and contains minor quantities of other water-soluble or hydratable components such as glycolipids and oligosaccharides (78). The degumming of oil with



water or a degumming agent such as citric acid, phosphoric acid, oxalic acid, acetic anhydride, or maleic anhydride yields a lecithin sludge and a degummed oil. Continuous agitated thin-film evaporation removes the approximately 50% water content of the lecithin sludge, resulting in a highly viscous semiliquid or powdered product (79).

Soybean is the predominant vegetable source of lecithin due to its availability and outstanding functional characteristics. Lecithin products from rapeseed and sunflower are increasing in market share. Glandless cottonseed and corn are also potential commercial sources (79–82). The composition of rapeseed lecithin is very similar to that of soybean but is considered to be of lower quality with respect to color, flavor, taste, and general appearance. This product has been predominantly used as a dust control agent in rapeseed meal and is added to livestock and poultry feeds (79). The development of canola or low-erucic-rapeseed varieties and specialized refining processes have increased the use of rapeseed lecithin. Glanded cottonseed contains more phospholipids than any other oilseed with the exception of soybean. Solvent extraction methods, however, cause toxic gossypol to bind to the phospholipids. The resulting lecithin is dark brown in color, limiting its use in food products (81).

*Purification and Fractionation of Crude Lecithin.* Purification consists of the removal of nonlecithin components such as carbohydrates, proteins, and other contaminants. Vegetable lecithin is more difficult to purify compared to other sources of lecithin because of its higher viscosity. The crude product obtained during degumming is contaminated with fines derived from seed material, hulls, and other seed impurities. Regulatory standards require that these contaminants be removed because of the high levels of iron and heavy metals in the impurities, which interfere with the oxidative stability of the products to which lecithin is added. Methods used for purification include filtration, which can be performed on the crude oil, lecithin, or miscella, partitioning between organic solvent and water or salt solution, dialysis, and adsorption on cellulose or Sephadex columns (80, 81, 83).

Fractionation takes advantage of the varying solubilities of different phospholipids in different organic solvents. Many options are available for large scale production of marketable lecithin. The de-oiling process is one fractionation system in which the separation of neutral and polar lipids is based on the insolubility of polar lipids in acetone; more than 60% of lecithin consists of acetone-insoluble substances. Oily lecithin is combined with excess acetone and mixed vigorously, thereby solubilizing triglycerides in the acetone. This process is repeated, after which the polar lipid material is dried and sold as a light yellow powder or in the form of granules (79). De-oiling on a smaller scale can be accomplished using adsorption of lecithin, in a hexane solution, on a silica column. Also, a new technology has been developed for treating the lipid mixture with a supercritical fluid. For example, carbon dioxide at a temperature of 40°C and a pressure of 300 bars has a solubility similar to that of liquid acetone. The gas may be recovered and reused. Oxidation of the lecithin does not occur since oxygen is displaced by carbon dioxide. As well, the absence of solvent residues in either the oil or the lecithin eliminates flammability and environmental concerns. This process, however, has

a number of drawbacks, and its commercial feasibility has not been established (79).

Crude fractions can be obtained by solvent fractionation treatments utilizing lower alcohols, such as ethanol, or alcohol–water mixtures. The product is a soluble fraction rich in phosphatidylcholine, whereas phosphatidic acid and phosphatidylinositol predominate in the insoluble fraction. The shift in the ratio of phosphatidylcholine to phosphatidylethanolamine improves the emulsification and antispattering capabilities of the soluble fraction. The products of this process can be used as they are or can be further purified with adsorbents. The soluble fraction is an excellent oil-in-water emulsifier and is predominately used in margarine. The acidic phospholipids of the insoluble fraction are used in water-in-oil systems. The chocolate manufacturing industry uses this fraction to increase the viscosity of chocolate masses, thereby reducing the requirement for cocoa butter (83, 84).

It is not possible to obtain pure phospholipids from lecithin by solvent fractionation. Chromatographic adsorption processes are capable of separating this complex mixture but cannot be used, in practice, to generate large quantities of the pure phospholipids. Chromatographic adsorption methods include aluminum oxide with ethanol or chloroform/methanol, silica gel with a variety of solvent systems, and a diethylaminoethyl–cellulose system. A high price is demanded for purified phospholipids (83).

*Functionality and Utilization of Lecithin.* There exist a number of functional groups in lecithin that can be modified. Phospholipid products derived by hydrolysis, hydroxylation, and acetylation are the most likely to be used commercially. Hydrolysis can be performed with phospholipase A, acid, or alkali. The resulting lecithin contains 56% or more of acetone-insoluble material. Products of hydrolysis have improved hydrophilic and emulsifying properties. Hydrolyzed lecithins are highly viscous or pasty fluids and tend to be light brown to brown in color (85). The products resulting from hydroxylation of lecithin have improved oil-in-water emulsifying properties and improved water dispersibility. Acetylation of phosphatidylethanolamine creates improved fluid and emulsifying properties, as well as improved water dispersion. Modification of the polar phosphoric acid ester or the glyceride moiety of phospholipids is legally restricted in the food industry (78).

The wide range of functionalities of lecithin has been applied in a variety of industries, including pharmaceuticals, cosmetics, and food. Lecithin components have both lipophilic and hydrophilic groups that respond to changes in pH and differences in ionic strength. These charged surfactants stabilize emulsions of water and oil. The selection of the appropriate raw material and the use of techniques such as fractionation, modification, and compounding can be combined to generate the phospholipid characteristics best suited to a desired application. Different phospholipid and fatty acid combinations influence solubility, emulsifying quality, the type of emulsion (whether oil–water or water–oil), instantizing properties, dietetic value, and sensitivity to oxidation (86).

Lecithin acts as a lubricant and release agent between solids by coating the surface of the solids. A reduction of the surface tension and particle attraction occurs when lecithin coats solids in a solid–liquid mixture, which allows for stable

dispersion and suspension of the solid in the liquid. Lecithin also reduces surface tension and enhances emulsification in immiscible liquid mixtures (87).

Lecithin makes up 0.5–1.0% of the composition of many cosmetic products. The surfactant properties are most valued for the “skin feel” that is produced. Longer wearing cosmetics evolve from pigments and particulates coated in lecithin since they have smoother surfaces, increased adhesion to the skin, and improved color stability. Due to film adhesion, the presence of lecithin reduces the oily or greasy feeling of many products and reduces the transfer of cosmetics to clothing. The emulsification properties, ease of spreading, and wetting ability of lecithin are also utilized in cosmetic products (88).

Lecithin may become a useful component of magnetic recording equipment due to its ability to act as a surfactant, thereby facilitating dispersion of magnetic particles on pigment surfaces. The magnetic and physical properties of the recording tape are enhanced as a result. For lecithin to be active in this capacity, it must function in a variety of solvents including methylethylketone, tetrahydrofuran, cyclohexane, and toluene. Another valuable property of lecithin is its ability to adsorb to the surface of a variety of pigments such as iron oxide, chromium dioxide, iron metal, and barium hexafurite (89).

Industrial coatings, paints, and inks utilize lecithin because of its pigment dispersal characteristics. Lecithin binds to pigment surfaces allowing wetting of the pigment by the vehicle in which it is being dispersed. Lecithins with different functionalities can function in both oil-based and water-based formulations (87).

The food industry relies on lecithin in bakery, beverage, and confectionery product development. The lecithin functionalities of emulsification, release, mixing and blending, and instantizing, many of which were discussed above, are put to use in many aspects of food production (86). Lecithin is used in baking as a dough conditioner for cookie, cake, and doughnut mixes with many positive effects including improved handling, a drier and more elastic dough, improved pan release, more uniform color, texture and grain, and decreased mixing time. Lecithin acts as a dispersor, aiding the mixing of unlike ingredients such as fat and flour or sugar. The activity of lecithin as a surfaceactive agent has been shown to retard the rate of staling in yeast-leavened products. Lecithin plays a valuable role in the instantizing of beverage and food mixes by promoting the incorporation of powders into aqueous environments. Dry edible powders such as cake mixes, nutritional supplements, and milk powders can be quickly integrated into liquids such as milk or water with the aid of lecithin (90).

The confectionery industry utilizes the emulsification, antistick, and viscosity properties of lecithin and benefits from the concurrent effects of shelf-life extension, texture improvement, and decreased production costs (83). A product such as caramel will not blend correctly in the absence of lecithin. Uniform dispersion of fat, aided by lecithin, will decrease stickiness and provide tenderness for ease of cutting. The natural antioxidant properties of lecithin slow the decay of any product in which it is incorporated. Viscosity is very important in the chocolate industry where shape is often a requirement for consumer acceptability. High concentrations of butter, such as cocoa butter, impart high viscosity, which in turn makes

production more efficient. Alternatively, lecithin may be used to provide a portion of the viscosity requirement and to eliminate greasiness in the finished product (84).

## 2.2. Refining By-Product Lipid (Soapstock)

Refining by-product lipid, commonly referred to as RBL, results from the refining of crude oil, where continuous mixing of crude oil with a dilute sodium hydroxide solution produces a by-product consisting of free fatty acids, hydrolyzed phosphatides, and unsaponifiable materials (91). Free fatty acids are the valued component in RBL, the composition of which varies with the oil source, oilseed condition at crushing, the oil removal method used, the solvent employed, the extent of extraction, and the refining conditions. Larger quantities of RBL are produced as the oil becomes more refined. The concentrations of free fatty acids, gums, and impurities, and the efficiency of refining, influence the amount of RBL formed. Refining by-product lipid tends to be the item of lowest value produced in oil refining (92). Acidulation of RBL using sulfuric acid stabilizes it and reduces its weight for shipment. Debris such as phosphatides, proteins, and mucilaginous substances are present in varying quantities depending on the quality of degumming and refining and may cause emulsification to occur and prevent effective acidulation (91). The acidulation of RBL is the greatest wastewater producer in the refining system. Disposal of the effluent or acid water requires expensive treatment measures to comply with environmental regulations (91). The Daniels Fertilizer Company (Shrewsbury, Mass.) views this acid water as a potential resource. The use of nutrient chemicals in the refining, acidulation, and neutralization steps produces an acid water suitable for use as a liquid fertilizer. Vegetable oils are refined with caustic potash (KOH) instead of caustic soda (NaOH), and acidulation with sulfuric acid is followed by neutralization with ammonia rather than NaOH. This is an innovative method that closes the loop of agriculture processing (93). Animal feed is the dominant sink for RBL. Refining by-product lipid can be returned to meal to increase its weight and its fat content. This has been done to a level of 0.9% for cottonseed meal and 0.4% for soybean meal (91). Soybean RBL provides 6694 kcal/kg of digestible energy and 6599 kcal/kg of metabolizable energy to pigs (94). Refining by-product lipid not only increases caloric content but also provides essential fatty acids and increases food utilization. Refining by-product lipid may also be added to feed for purposes of dust control, appearance, ease of handling, and improved pelletability (95).

Raw and acidulated RBL are combined in different ratios with animal tallow to produce soaps of varying characteristics. Palm oil and coconut oil are the dominant fatty acid sources for soap manufacturing. Coconut oil and tallow are complementary in fatty acid composition such that in combination they provide the ingredients of toilet soap (96). There has been speculation regarding the use of safflower and sunflower RBL in this capacity if alterations were made to processing methods. Cottonseed and soybean RBLs are available in large quantities but the cost of upgrading these to the quality necessary for use in toilet soap inhibits their use.

Refining by-product lipid has been considered as a growth medium for microorganisms. It appears to provide a satisfactory supply of nutrients for growth, due

in part to its high sodium content and its suitable trace element composition. Despite its residual oil content and high pH, many species of microorganisms will grow on this substrate (97).

### 2.3. Spent Bleaching Earth

Spent bleaching clay from oil refining contains substantial absorbed oil (20–40% w/w). This product is both a problem and a potential source of recoverable oil. The problems of spent clay are well known (98). Fat-containing clays are prone to spontaneous combustion when in contact with air, particularly if the bleaching earth contains highly unsaturated oils. Spent clay also represents an environmental concern, both as a fire hazard and as a threat to ground waters through fat-containing runoff when discarded in landfills. In addition, there is an economic loss of oil in the clay.

Technical solutions to the recovery of oil from spent clay can be categorized into four areas:

1. *Steam Treatment.* This method, whereby steam is blown through the cake, is commonly practiced in refineries to reduce the oil content of the cake to approximately 20% (99).
2. *Aqueous Extraction.* In its simplest form, this procedure involves pumping 95°C water through the cake for approximately 30 min to reduce its oil content. The oil must then be separated from the water (98). A procedure employing extraction with sodium carbonate has been described (98, 99) where spent clay was mixed with an aqueous 5% sodium carbonate solution. The slurry was heated to 95°C and stirred slowly for 30 min. Although the procedure is simple, hot carbonated water sometimes failed to displace the fat. The quality of the resultant oil was low and disposal of residual slurry [containing clay, water, and salt (NaCl)] was difficult. Suggested methods of slurry disposal include inclusion in cement manufacture or blending into sandy soils to improve soil structure. Boiling the spent clay in a water suspension containing 1.5–2.5% concentrated sodium hydroxide as a surface-active agent has also been described (100). This procedure yielded a dark-colored oil that could only be used for technical purposes. After extraction, the residual slurry was centrifuged and the liquid effluent appropriately treated. The solid material was used as a landfill material or as a replacement for earth or sand for the covering of refuse dumps (100).
3. *Solvent Extraction.* Spent clay oil recovery by solvent extraction with hexane may be accomplished after filtration, either directly from the cake in the filter or after the cake has been removed from the filter (99). Any exposure of the cake to air prior to extraction will cause rapid deterioration of the fatty material. Depending on the intended use of the oil, perchloroethylene or methyl chloride may be effectively employed as solvents (98).

4. *Pressure Extraction.* The use of pressure (5–30 bars) in combination with water and sodium hydroxide has been shown to produce acid oils from spent clay that can be easily separated by decanting (98).

Other options for recovery of oil from spent clay include mixing the spent clay filter cake with milled oilseeds *en route* to solvent extraction. This procedure is used in some refineries having associated crushing and refining plants and is convenient if the fire hazard of the spent clay can be overcome and the level of addition is small enough to not significantly alter the mineral content of the meal (99).

Evaluations of spent bleaching clay as a feed supplement indicate that, for poultry diets, inclusion rates of up to 7.5% spent clay in diets produced no deleterious effects on feed intake, growth rate, or feed efficiency (101). These results suggest that spent clay could be added to poultry feed at 0.5–2.0% which is similar to the amount of bentonite clay currently used as a pellet binder in poultry diets. The metabolizable energy (ME) of spent clay was determined to be 2870 kcal/kg (dry matter basis) but would vary with oil content. Other studies have also demonstrated the feeding value of spent clay (102).

Spent clay and its associated disposal problems represent a concern for all refineries. Additional research may yield new and more valuable uses for this by-product material.

## 2.4. Deodorizer Distillates

Deodorizer distillate is the material collected from the steam distillation of vegetable oils, as it occurs in the process of deodorization or physical refining (103). The material has a sludgelike appearance and consistency so is often referred to as scum or scum oil.

The use and value of deodorizer distillate is dependent on its composition. Deodorizer distillate is a complex mixture of tocopherols, sterols, esters of sterols, mixed fatty glycerides, hydrocarbons, and other materials contained in a substantial amount of fatty acids (104). If the material is high in unsaponifiable components, the tocopherols can be used in the manufacture of natural source vitamin E and sterols for drug manufacture. The quality of the distillate is dependent on the feedstock oil composition, processing equipment, and operating conditions.

Comparisons of the tocopherol and sterol contents from various oils indicate that some oils have appreciably higher contents of specific tocopherols and sterols (94). For example, sunflower is high in  $\alpha$ -tocopherol, whereas soybean is higher in  $\gamma$ -tocopherol. As deodorization strips tocopherols and sterols from the oil, different feedstock oils yield different concentrations and types of tocopherols.

Direct contact cooling of the deodorizer discharge vapor with a stream of circulating distillate is the most common method of condensing distillate vapor. Although designs of distillate recovery towers and their position in the deodorization system vary, the purpose remains the cooling of the vapor sufficiently to condense most of the distillates (105). The operation of the deodorizer has a direct impact on distillate composition and quality. In general, higher deodorization temperatures, longer

exposure times, and lower vapor pressures will increase the yield of distillate and decrease the tocopherol and sterol content remaining in the oil (103). Distillate is frequently collected and sold, representing value to the refinery. The demand and value is based on the total tocopherol content, as it relates directly to the economics of vitamin E production. The value of distillate varies and has been as high as \$1.45/kg (\$0.65/lb) (103). End users of the distillate use a series of chemical and physical treatments such as saponification, esterification, and molecular distillation to separate tocopherols and sterols (106). Research has been conducted on the use of supercritical fluid extraction to separate and concentrate tocopherols and sterols from the sludge (107).

With the increasing interest in natural source antioxidants, such as tocopherols, and the use of both stigmasterol and  $\beta$ -sitosterol as raw materials for the production of progesterone and esterone by the pharmaceutical industry, it is likely that deodorizer distillates will continue to be an important by-product for some vegetable oil processors.

## 2.5. Spent Catalyst

Catalysts are required in the hydrogenation of fats and oils. The most commonly used catalyst is finely divided nickel having a large surface area and used in a flaked or granular form. The nickel component and clay support each make up 50% of the catalyst in a surrounding hydrogenated fat medium. Spent catalyst is removed from the hydrogenated oil using filtration methods such as plate and frame or pressure leaf-type filter presses. The recovered catalyst product contains nickel, clay materials, and residual fat or oil, as well as any filtration media used to aid in filtration of the physically fine spent catalyst. The heat content of spent catalyst ranges from 4500 to 8700 kcal/kg (108).

The use of spent nickel catalyst in its posthydrogenation form is limited. The only available example of its use is by the M.A. Hanna Company (Cleveland, Ohio). This company mines and smelts nickel, producing a ferro-nickel product of primary use in the production of stainless steels. To conserve its nickel supplies, Hanna modified its system for feeding the production line to allow mixing of spent nickel catalyst with the nickel containing ores at up to 10% of the total nickel feed (108).

Landfills have been the most popular depositories of spent nickel catalyst. Environmental concerns regarding the impact of nickel and conservation efforts to preserve nickel supplies have stimulated recycling and reclamation of the nickel component. Solvent extraction of organic material from the nickel is most effective with polar solvents such as isopropanol and methyl ethyl ketone (108).

Incineration is another reclamation technique in use. The multiple-hearth furnace can operate under designed conditions for years with low maintenance and low energy consumption (108). The fluid-bed incinerator is also very effective (108). Tests have shown that all organic compounds are decomposed and most carbon residue oxidized. The ash is of high nickel value being predominately

composed of nickel oxide and silica. The energy input of these reclamation processing methods is fractional compared to the total heat content of spent catalyst. Generation of thermal energy through these processes has the potential for electricity generation as well.

Spent nickel catalyst is a product with limited potential for further utilization. In the future, however, the entire composition of the spent catalyst, including the organic fraction, may be found to have value beyond heat generation (108).

## REFERENCES

1. 2003 *Soya & Oilseed Bluebook*, Soyatech, Inc., Bar Harbor, ME, 2003.
2. W. R. Fehr in G. Robbelen, R. K. Downey, and A. Ashri, eds., *Oilcrops of the World: Their Breeding and Utilization*, McGraw-Hill, New York, 1989, pp. 283–300.
3. T. L. Mounts, W. J. Wolf, and W. H. Martinez in J. R. Wilcox, ed., *Soybeans: Improvement, Production and Uses*, American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Inc., Madison, Wis., 1987, pp. 849, 854–856.
4. K. J. Smith in A. R. Baldwin, ed., *Proceedings of the World Conference on Emerging Technologies in the Fats and Oils Industry*, American Oil Chemists' Society, Champaign, Ill., 1998.
5. J. M. Bell in Ref. 2, pp. 192–207.
6. R. T. Lovell in L. McCann, ed., *Soybean Utilization Alternatives*, University of Minnesota, St. Paul, Minn., 1988, pp. 235–245.
7. T. Klopfenstein and F. Owen in Ref. 6, pp. 227–234.
8. E. F. Sipos in Ref. 6, pp. 57–93.
9. A. E. Sloan, *Food Tech*, **48**, 89 (1994).
10. K. E. Beery in T. H. Applewhite, ed., *Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, American Oil Chemists' Society, Champaign, Ill., 1989, pp. 62–65.
11. D. W. Johnson and S. Kikuchi in Ref. 10, pp. 66–77.
12. A. Visser and A. Thomas, *Food Rev. Internat*, **3**, 1 (1987).
13. W. J. Wolf in I. A. Wolff, ed., *Handbook of Processing and Utilization in Agriculture*, Vol. II; Part 2, CRC Press, Boca Raton, Fla., 1983.
14. J. A. Klavons, R. D. Bennett, and S. H. Vannier, *J. Food Sci.* **57**, 945 (1992).
15. L. A. Johnson, D. J. Meyers, and D. J. Burden, *INFORM* **3**, 282 (1992).
16. B. Fitch Haumann, *INFORM* **4**, 1324 (1993).
17. L. A. Johnson, D. J. Meyers, and D. J. Burden, *INFORM* **3**, 429 (1992).
18. A. Gennadios and C. L. Weller, *Cereal Foods World* **36**, 1004 (1991).
19. A. Gennadios, A.H. Brandenburg, J. W. Park, C. L. Weller, and R. F. Testin, *Ind. Crops Prod.* **2**, 189 (1994).
20. M. D. Pickard in D. Hickling, ed., *Canola Meal Feed Industry Guide*, Canola Council of Canada, Winnipeg, MB, 1993, pp. 4–5.
21. C. G. Youngs and L. R. Wetter in *Rapeseed Meal for Livestock and Poultry*, Rapeseed Association of Canada, Winnipeg, Manitoba, Publ. No. 3, 1969, pp. 2–3.



22. J. D. Jones, *J. Am. Oil Chem. Soc.* **56**, 716 (1979).
23. J. M. Bell, *J. Anim. Sci.* **58**, 996 (1984).
24. R. Hill, *Nutrition Abstracts and Reviews, Series B. Livestock Feeds and Feeding (UK)* **61**, 139 (1991).
25. D. Hickling, ed., *Canola Meal Feed Industry Guide*, Canola Council of Canada, Winnipeg, MB. 2001.
26. A. B. Pierce in Ref. 20, pp. 12–15.
27. D. Hickling and A. Freig in Ref. 20, pp. 16–20.
28. S. Leeson, J. O. Atteh, and J. D. Summers, *Can. J. Anim. Sci.* **67**, 151 (1987).
29. P. J. McKinnon and D. A. Christensen in Ref. 10, pp. 449–462.
30. Y.-M. Tzeng, L. L. Diosady, and L. J. Rubin, *J. Food Sci.* **55**, 1147, 1156 (1990).
31. F. Sosulski, E. S. Humbert, K. Bui, and J. K. Jones, *J. Food Sci.* **41**, 1349 (1976).
32. D. L. Rinker in M. J. Maher, ed., *Mushroom Science XIII Proceedings of the 13th International Congress on the Science and Cultivation of Edible Fungi*, Vol II, A. A. Balkema, Rotterdam, Netherlands, 1991, pp. 781–789.
33. L. D. Gattinger, Z. Duvnjak, and A. W. Khan, *Appl. Microbiol. Biotechnol.* **33**, 21 (1990).
34. Z. Liebowitz and C. Ruckenstein in Ref. 4, pp. 208–212.
35. D. G. Dorrell in J. F. Carter, ed., *Sunflower Science and Technology*, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Inc., Madison Wis., 1978, pp. 407–440.
36. J. A. Robertson, *Crit. Rev. Food Sci. Nutr.* **6**, 201 (1975).
37. L. D. Talley, J. C. Brummett, and E. E. Burns in *Proc. 4th Int. Sunflower Conf.* (Memphis, Tennessee), National Cottonseed Products Assoc., Memphis, 1970, pp. 110–113.
38. D. H. Kinard, *Feedstuffs*, 26 (Nov. 3, 1975).
39. K. O. Lewis in Ref. 37, pp. 271–272.
40. H. O. Doty in Ref. 35, pp. 457–488.
41. *Oil Crops Outlook (OCS-05a)*, Economic Research Service, USDA, Jan. 13, 2005.
42. G. Sripad and M. S. N. Rao, *J. Agric. Food Chem.* **35**, 962 (1987).
43. P. K. Saxena and L. C. Buchanan, Canadian Society of Agricultural Engineers, Paper No. 82–106 (1982).
44. E. Eklund, A. Hatakka, A. Mustranta, and P. Nybergh, *Eur. J. Appl. Microbiol.* **2**, 143 (1975).
45. D. Wiesenborn and co-workers, *N. D. Farm Res. J.* **49**, 19 (1991).
46. D. MacGregor in Ref. 37, pp. 107–109.
47. K. C. Chang and A. Miyamoto, *J. Food Sci.* **57**, 1435 (1992).
48. A. Miyamoto and K.C. Chang, *J. Food Sci.* **57**, 1439 (1992).
49. P. F. Knowles in Ref. 2, pp. 363–374.
50. J. P. Cherry and H. R. Leffler in R. J. Kohel and C. F. Lewis, eds., *Cotton*, American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Inc., Madison, Wis., 1984, pp. 511–569.
51. J. Redhead, *Utilization of Tropical Foods: Tropical Oilseeds*, FAO Food and Nutrition Paper 47:5, Food and Agriculture Organization, Rome, 1989.

52. E. W. Lusas and G. M. Jividen, *J. Am. Oil Chem. Soc.* **64**, 839 (1987).
53. T. Yo, *Agribiol. Res.* **44**, 357 (1991).
54. D. K. Salunkhe and B. B. Desai, *Postharvest Biotechnology of Oilseeds*, CRC Press, Inc., Baco Raton, Fla., 1986, pp. 147–159.
55. D. A. Okly in *Proceedings of the 1987 International Oil Palm/Palm Oil Conferences: Progress and Prospects. Conference II: Technology*, Palm Oil Research Institute of Malaysia, Kuala Lumpur, Malaysia, 1988, pp. 434–437.
56. S. W. Yeong, *1st Asian-Australian Animal Science Congress*, Serdang, Malaysia, 1980, pp. 217–222.
57. W. L. Siew, *PORIM Tech.* **14**, 111 (1989).
58. J. H. Tay, *Resour. Conservat. Recycl.* **5**, 383 (1991).
59. M. Shibata and A. H. Osman, *Jpn. Agricult. Res. Quart.* **22**, 235 (1988).
60. M. A. Ngan and A. S. H. Ong, Potential Biomass Energy from Palm Oil Industry, PORIM Bulletin No. 14, Research Institute, Malaysia, 1987, pp. 10–15.
61. J. G. Woodroof, *Coconuts*, AVI Publishing Co., Westport, Conn., 1979.
62. T. V. P. Nambiar in N.M. Nayar, ed., *Coconut Research and Development*, Wiley Eastern, New Delhi, 1983, pp. 245–253.
63. J. A. Banzon, *Energy Agricult.* **3**, 337 (1984).
64. J. George in Ref. 62, pp. 284–290.
65. R. Erwiyono and D. H. Goenadi, *Indonesian J. Crop. Sci.* **5**, 25 (1990).
66. J. V. Bhat in Ref. 62, pp. 259–273.
67. pp. 35–56 in Ref. 54.
68. T. N. Bhavanishankar, T. Rafashekar, and V. Sreenivasa Murthy, *Food Microbiol.* **4**, 121 (1987).
69. G. M. Hill and P.R. Utley, *Nutr. Rep. Internat.* **36**, 1363 (1987).
70. M. D. Lindemann, E. T. Kornegay, and R. J. Moore, *J. Anim. Sci.* **62**, 412 (1986).
71. pp. 194–195 in Ref. 54.
72. C. Carola in J. M. Moreno Martinez, ed., *Olive Oil Technology*, Food and Agriculture Organization, Rome, 1985, pp. 78–87.
73. A. K. Kiritsakis, *Olive Oil*, American Oil Chemists Society, Champaign, Ill., 1990, pp. 80–85.
74. L. A. Johnson, T. M. Suleiman, and E. W. Lusas, *J. Am. Oil Chem. Soc.* **56**, 463 (1979).
75. pp. 105–117 in Ref. 54.
76. pp. 171–186 of Ref. 54.
77. R. S. Bhatta and P. Cherdkiatgumchai, *J. Am. Oil Chem. Soc.* **67**, 79 (1990).
78. G. L. Dashiell in Ref. 6, pp. 355–363.
79. M. Schneider in Ref. 4, pp. 160–164.
80. J. P. Cherry, M. S. Gray, and L. A. Jones, *J. Am. Oil Chem. Soc.* **58**, 903 (1981).
81. J. P. Cherry and W. H. Kramer in B. F. Szuhaj, ed., *Lecithins: Sources, Manufacture and Uses*, American Oil Chemists Society, Champaign, Ill., 1989, pp. 16–31.
82. L. Somogyi, “Food Additives,” in *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 12, Wiley, Hoboken, New Jersey, 2005.

83. G. L. Dashiell in D. R. Erickson, ed., *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, American Oil Chemists' Society, Champaign, Ill., 1990, pp. 396–401.
84. R. C. Appl in Ref. 81, pp. 207–212.
85. G. R. List in Ref. 81, pp. 145–161.
86. G. L. Dashiell in Ref. 81, pp. 213–236.
87. E. F. Sipos in Ref. 81, pp. 261–276.
88. C. Baker in Ref. 81, pp. 253–260.
89. M. Chagnon and J. Ferris in Ref. 81, pp. 277–283.
90. E. H. Sander in Ref. 81, pp. 197–206.
91. J. B. Woerfel in Ref. 4, pp. 165–168.
92. D. R. Erickson in Ref. 6, pp. 95–105.
93. R. S. Daniels, Can. Pat. 1,256,449 (1989).
94. E. T. Fialho, L. F. T. Albino, and E. Blume, *Pesquisa Agropecuaria Brasileira* **20**, 1419 (1985).
95. K. Zilch in P. J. Wan, ed., *Introduction to Fats and Oils Technology*, American Oil Chemists' Society, Champaign, Ill., 1991, pp. 251–266.
96. N. O. V. Sonnlag, *J. Am. Oil Chem. Soc.* **58**, 155A (1981).
97. C. W. Hesseltine and S. Koritala, *Proc. Biochem.* **22**, 9 (1987).
98. J. M. Klein in Ref. 4, pp. 169–171.
99. J. T. L. Ong, *J. Am. Oil Chem. Soc.* **60**, 314 (1983).
100. C. Svensson, *J. Am. Oil Chem. Soc.* **53**, 443 (1976).
101. R. Blair, J. Gagnon, R. E. Salmon, and M. D. Pickard, *Poultry Sci.* **65**, 2281 (1986).
102. W. M. M. A. Janssen, P. J. W. van Schagen, A. A. Siegerink, and A. J. N. Bisalsky, *Fette Seifen Anstrichmittel* **88**, 25 (1986).
103. R. L. Winter in Ref. 4, pp. 184–188.
104. S. K. Kim and J. S. Rhee, *Korean J. Food Sci. Technol.* **14**, 174 (1982).
105. A. M. Gavin, *J. Am. Oil Chem. Soc.* **58**, 175 (1981).
106. F. Z. Sheabar and I. Neeman, *Riv. Ital. Sostanze Grasse* **64**, 219 (1987).
107. H. Lee, B. H. Chung, and Y. H. Park, *J. Am. Oil Chem. Soc.* **68**, 571 (1991).
108. F. J. Hennion in Ref. 4, pp. 172–183.

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## *Environmental Impact and Waste Management*

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### **1. INTRODUCTION**

Oilseeds processing, refining and processing of vegetable oils, and further processing into edible products and oleochemicals, produces a variety of waste products. In no other industry is the proper handling of these wastes as dependent on the understanding and control of the processes. This article reviews major processes and facilities, particularly as they relate to waste generation and control. Wastes from a well-run facility are first defined, followed by an analysis of those processes that are the largest potential waste generators. In addition, those factors are reviewed that affect process control as it relates to waste generation, followed by a review of current issues.

A final section addresses the fundamentals of wastewater treatment processes often employed in fats and oils and oilseeds processing. This article addresses air and solid waste aspects of the industries as well, but the primary focus is on wastewater.

The article is directed mainly at conventional caustic refining and related downstream processes with related considerations in physical refining and new

processes. The article also references oleochemicals. For further information on oleochemicals, the reader is referred to *Fatty Acids in Industry*, by Johnson and Fritz (1).

## 2. PROCESS COMPONENTS AND MAJOR WASTEWATER SOURCES

Figure 1 is a conceptual flow diagram that shows the major processes of a typical oilseed processing and refining facility. For the purposes of this presentation, the following processes are considered:

- Milling and extraction
- Caustic refining
- Further processing and handling (bleaching, winterization, hydrogenation)
- Deodorization
- Acidulation
- Tank car washing
- Packaging
- Margarine production
- Salad dressing/mayonnaise production

*Primary Parameters.* Table 1 lists these processes and associated wastes loadings. Separate subtotals are presented with and without salad dressing and mayonnaise, as these processes are often absent and portions of this article do not consider them. See (2) for clarification of terms and test methods. Table 1 excludes wastewater flows without organic contamination such as noncontact cooling tower blowdown and boiler blowdown.

Note that only freon extractables (fats, oils, and grease, FOG) and 5-day biochemical oxygen demand (BOD) are listed. Numerous other parameters are often discussed, monitored, and interpreted, but treatment and control strategies can usually be reduced to these two items. Loadings are presented as average and maximums; however, the variable operations of an oilseeds fats and oils facility are such that a true average is less relevant than an operating range. Maximums are reasonable upper figures, with higher loadings possible in spillage situations.

Control of pH is important in the wastewater stream, but it ultimately becomes a byproduct of controlling FOG and BOD. Chemical oxygen demand (COD) is a useful parameter, and it can usually be predictably estimated at 1.4 to 1.6 times BOD for the model plant discussed in this section. These ratio values can vary based on processes in-place and other unusual circumstances. Measurement of COD is more often a tool to determine whether BOD tests are being correctly conducted and are useful in that results can be obtained in a matter of hours rather than days.

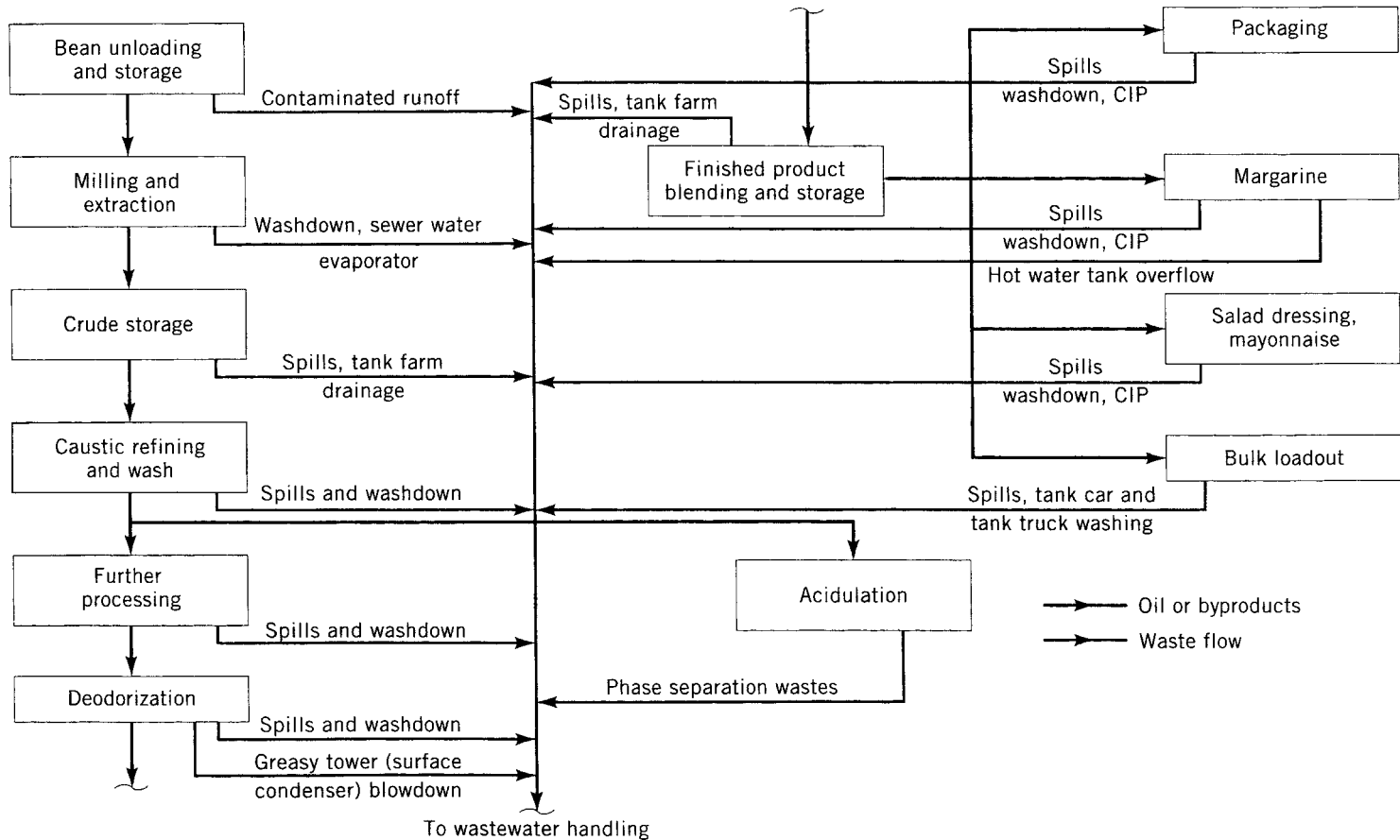


Figure 1. Conceptual flow diagram, oilseed processing.

**TABLE 1. Oilseeds Processes and Wastewater Loads.**

Process	Waste Load <sup>a</sup>				
	Flow (m <sup>3</sup> /day)	BOD (kg/day)		FOG (kg/day)	
	Average	Average	Max.	Average	Max.
Milling and extraction	95	168	272	11	29
Caustic refining	42	100	454	52	181
Further processing	19	68	136	34	68
Deodorization	19	18	45	9	23
Acidulation	72	1451	2268	11	363
Tank car washing	19	113	680	57	113
Packaging	38	113	454	57	227
Subtotal	304	2031	4309	231	1004
Margarine production	265	272	454	136	227
Salad dressing/mayonnaise	189	907	1588	454	771
Total	758	3210	6351	821	2002

<sup>a</sup>Effluent from good in-plant gravity separation of free oils and solids

Many effluent permit limitations also include total suspended solids (TSS). The test procedure for TSS also measures oil and grease that is removed in the filter paper used in the test. Non-oil-suspended solids are normally low in vegetable oil handling wastewaters; therefore, the parameter is not really of significance in most applications. There are some exceptions, such as milling and extraction area wastes along with salad dressing and mayonnaise.

The data in Table 1 are developed around the following criteria:

- Milling and extraction of soybeans: 2800 m<sup>3</sup> bushels per day.
- Caustic refining with single-stage water wash: 27,000 kg/hr nondegummed soybean oil.
- Semicontinuous deodorization with scrub cooler, barometric condenser with atmospheric cooling tower.
- Acidulation of soapstock and washwater with 90% to 95% TFA recovery efficiency.
- Bottling line and/or other extensive liquid oil packaging.
- Margarine, mayonnaise, and salad dressing production and packaging.
- Tank car washing of finished oil cars only; no crude oil cars.

Clearly, operations of different size, and lacking some processes, will have different waste loads. This applies particularly to acidulation as well as mayonnaise and salad dressing processing. In addition, variations in refining process methods will greatly impact wastewater characteristics. This is addressed in more detail in Section 7. The effects of process control and its impact on wastewater loading are outlined in the next section. The values in Table 1 can be adjusted for different processing sizes; however, this adjustment should be done with caution and not

by simply applying a waste load per unit of production. Many processes use equipment that is available only in various increments. Also, smaller or larger sized processes may make other downstream (or upstream) processes inherently uneconomical or imply a fundamentally different process approach. For example, in North America, the economics of onsite soapstock acidulation starts becoming inherently uneconomical at refining capacities less than 12,500 Kg/hr.

These loadings represent a reasonably well-run operation from a process loss control standpoint. Loadings will vary from better or poorer managed facility operations. In addition, the figures in Table 1 assume that all waste streams have been subject to at least a modest degree of gravity separation for removal of large quantities of floatable oils and solids.

A final source of wastewater is contaminated runoff from truck and rail loadout areas, tank farm drainage, and related sources. This runoff can contribute the equivalent of 20 to 40 L/min to total daily average flow during rainy periods and affect peak flows to a much greater extent.

*Other Significant Parameters.* Other parameters of interest include nickel, phosphorus, and sulfates.

Nickel may enter the wastewater stream from cleanup and minor losses in the hydrogenation process areas caused by nickel catalysts. Caustic cleaning of filter screens from the catalyst are a particularly significant source. Often, this problem can be controlled at the source.

Crude oils, particularly soybean oil, contain significant quantities of organic phosphorus in the form of phosphatides. These compounds are removed to a large extent from the oil phase in the refining process. If refinery washwaters and soapstock are acidulated, the phosphorus is then carried into the water phase. Other sources of phosphorus are in an oilseeds operation, but the majority results from the mechanism described above.

Sulfates are present from the use of sulfuric acid in the soapstock acidulation process. Concerns for sulfates are generated from their contribution to dissolved solids as well as their potential to form odor-causing compounds under anaerobic conditions. Sulfate levels in the model plant presented herein would be on the order of 2000 mg/L. Where controlled, regulatory limits are in the range of 200-300 mg/L.

### 3. PROCESS FACTORS AFFECTING WASTEWATER GENERATION AND CHARACTERISTICS

*Soapstock Handling.* The largest factor affecting wastewater loading is handling of refinery soapstock when caustic refining is employed. Four methods are routinely employed:

1. Acidulate soapstock for fatty acid value.
2. Sell as raw soapstock on the open market.



3. Spray on meal as fat additive (if an oilseed extraction operation is present).
4. Partially neutralize and dewater soapstock.

Acidulation is probably the most misunderstood and maligned process in the entire fats and oils industry. The easiest part of an acidulation design is production of acceptable quality acid oil. The difficult challenge is the minimization of the middle phase and creating an oil-free wastewater, which implies that the facility is to be properly designed from the standpoint of reaction kinetics as well as the mechanical aspects of acid/oil mixing, decanting, and middle phase recovery.

Methods 2 and 3 above do not produce any wastes other than from handling losses; however, they are often not the most economical method of dealing with soapstock as the value of the acid oil is not realized.

Method 4 is directed at reducing water content of soapstock sold for acidulation elsewhere. The water phase generated in the decant can have as much or more waste load as that generated from a well-run acidulation plant. Figure 2 shows a relationship developed from one such operation. Note that some gain is realized by a moderate pH reduction; however, in later phases, the operation essentially becomes an inefficient acidulation process.

In summary, the economic advantage of freight cost gained should be weighed against additional waste handling costs.

*Deodorization.* This process should be a minor contributor; however, it can produce significant waste loads if the deodorizer does not have a well-operated scrub cooler. The barometric recycle should have its oil separator/skimmer. In certain instances, an indirect heat exchange system is employed to reduce odor problems

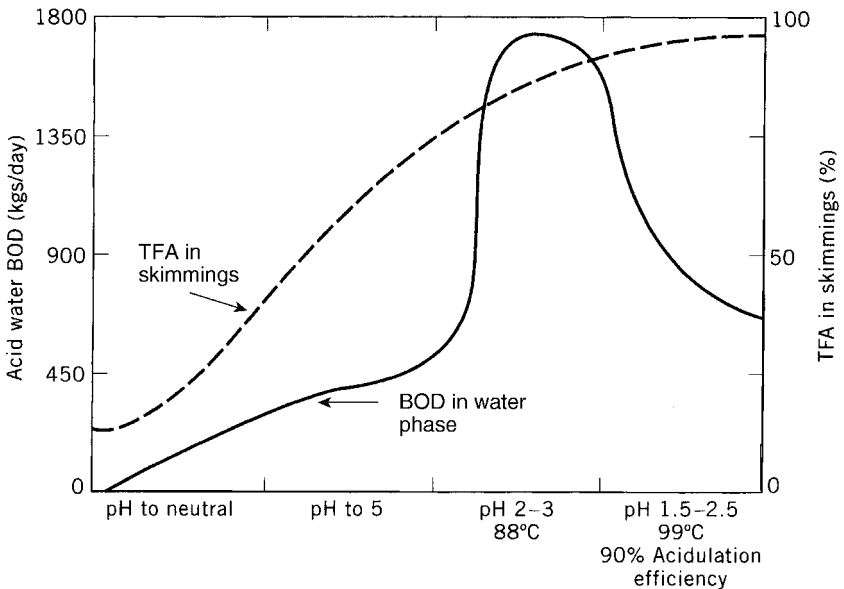


Figure 2. Partial acidulation waste loading.

off the barometric water stream. In any event, the waste load should be no greater than the deodorizer steam load, with about 500 mg/L FOG, the soluble oil fraction in the water phase at steady state recycle. Several processors are using polymer addition to enhance this. Most deodorgic systems in North America now rely on surface condensers rather than on atmospheric “greasy” cooling towers. These surface condensers normally employ a 45–70-L/min caustic wash to keep the condenser surface clear, which results in an overall wastewater load contribution in the same range as that from the blowdown of a contact barometric cooling tower system with a scrub cooler.

As a final note regarding deodorizers, these units experience a maintenance boil-out with sodium hydroxide and citric acid about once a year. This is an extremely high waste load and must be stored for regulated release into the process sewer system. If appropriate, this wastewater can be released on a regulated basis into wastewater neutralization, (assuming low pH wastewater) and some benefit is gained for the waste sodium hydroxide.

*Refining.* The water wash centrifuge stage is, at times, susceptible to oil break-over because of back pressure control. Many plants have a method to monitor or control this other than visual. These breakover conditions can represent substantial quantities of free neutral oil. A secondary gravity separator should be used in-line on the wash water stream to recycle the floatable refined oil directly to the crude oil stream, rather than allowing it to downgrade as acidulated material.

*Cleanup, Handling Losses.* There is no end to the potential to create, and eliminate, waste load water flow through in-house control, which is a matter of employee training and attitude and how management views the importance of training to achieve results. This is an extremely important area; however, it cannot be addressed effectively in detail here. Several references are included (3,4).

There are physical aspects to consider. For example, vacuum pump seal water can make up a large percentage of total water flow if not recycled or otherwise minimized.

To properly address this issue, a plant-wide study of oil loss and wastewater generation points should be conducted and evaluated. From there, a cost-effectiveness analysis of potential operational and physical remedial measures can be conducted. Very few plants exist where such a study cannot produce plenty of opportunities for loss reduction and money savings.

## 4. AIR EMISSIONS, SOURCES, AND CONTROLS

### 4.1. Sources

Major sources of air emissions considered for this presentation include the following:

1. Process dust particulates
2. Solvent from extraction and meal processing

- 3. Odors from meal cooling and oil deodorization
- 4. Boiler emissions

Table 2 summarizes loadings and/or limitations in these areas.

**TABLE 2. Major Air Emission Sources.**

Source	Loss
Hexane at extraction	0.10–0.30 kg/100 kg crush
Odor in meal dryer and deodorizer	—
Coal-fired boilers, particulates	14–23 g/M BTU input
Particulate emissions	—

An excellent overview of the subject in general is “Air Pollution Issues Associated with Soybean Process Facilities” (3).

1. **PROCESS PARTICULATE MATTER**—This arises from a variety of sources throughout the handling and processing of oil seeds and byproduct meal. Elevator losses as a whole usually average 2% to 3%. This loss is a combination of moisture reduction, seed spillage, and dust loss/emissions. The dust portion of the loss is a smaller absolute number; however, it can create substantial challenges when measured as particulate air emissions. Additionally, a portion of these losses can manifest as water-born contaminants in plant site rainfall runoff.
2. **SOLVENT EXTRACTION**—The facilities are regulated as major sources of solvent emissions. The industry controls itself to a certain degree because of the cost of the solvent—normally “commercial” hexane. The U.S. Federal Clean Air Act provides a basis for controlling solvent emissions as volatile organic compounds and, because a major constituent is typically n-hexane, as a hazardous air pollutant.

The Federal Clean Air Act requires the U.S. Environmental Protection Agency to establish hazardous air pollutant emission criteria per unit of production. These standards have been promulgated and range from 0.2 to 0.25 kg for 100 kg of oilseeds processed, for specific categories of extraction and oilseed type. Attainment of this figure requires a properly designed vent and cooling water system with minimal interruptions to the operations.

3. **ODORS FROM MEAL DRYER AND OIL DEODORIZATION**—Odor is a qualitative parameter from the standpoint of both measurement and control. Motivation for abatement usually arises out of complaints of citizens residing nearby a facility. Sources of odor can be meal driers and refined oil deodorization. Acidulation has been an odor producer in some isolated instances; however, is most often because of basic poor design and operation usually coincident with batch processing in open-topped kettles.

The other two processes also can be inherent producers of odors. Meal drying odors vary according to the seed processed and the equipment design.

In deodorization, oil is subject to high temperatures to drive-off volatile fractions to strip odor-causing compounds from the oil. The control of these compounds has become an integral part of the process.

4. **BOILERS**—Steam generation is an integral component of oilseed processing facilities, sometimes coupled with the generation of electricity. Several processors operate coal-fired boilers as a hedge against fluctuating natural gas and fuel oil costs. Particularly in the Midwestern United States, tax incentives expanded intrastate use of coal. More recent increased air emission concerns for coal combustion have reversed some of this. Coal-fired boilers for steam and electrical generation are common in other areas of the world at oilseed plants.

Concerns with boiler emissions now include  $\text{NO}_x$  and CO irrespective of the fuel. For coal, the pollutants of concern include not only these constituents, but also ash particulate emissions and associated visual plumes,  $\text{SO}_2$ , Hg and other trace metals, and HCl.

Certain oilseed processors use byproducts of production as alternative fuels, which is particularly true in sunflower and peanut processing where hulls are burned as boiler fuel. This creates additional combustion challenges, but reduces solid waste.

## 4.2. Controls

1. **PARTICULATE MATTER** —This is generated primarily because of mechanical handling of grain and meal solids. Good design and maintenance of transfer and handling facilities is essential for dust minimization and control. Speed spillage and resulting dust emissions are minimized by the use of enclosed and interlocked conveying systems with flow monitors. Also, as it relates to internal elevator dust formation, for which control and cleanup is essential to prevent fires and explosions, facility designs minimizing internal conveying equipment are common.

Current industry trends are toward the installation of enclosed conveyors and the construction of enclosed areas with negative pressure aspiration for oilseed unloading and meal load-out. Highly efficient dust collection systems and control equipment (cyclones and bag houses) are used throughout the processes to control both fugitive and point source emissions.

2. **SOLVENT**—Adequate control of the extraction solvent loss is primarily a result of sound extraction, distillation, vent and cooling water component and system design, operation, and maintenance. Computer monitoring and control systems can enable improved operation, which results in enhanced solvent control.
3. **ODOR**—As noted in Section 2 facilities to control odor have become essentially a part of the process.

Deodorization of fats and oils is a necessary process for removing disagreeable flavor and odor components that are naturally present or created

during processing. The deodorization of oils produces off-gases that historically have produced the greatest challenge to the industry for odor control.

Three steps eliminate these odor problems. The first is a distillate recovery system that recovers most of the fatty acid as a byproduct and many of the associated odors in the deodorizer vapor discharge. The second is a closed-loop cooling system that keeps the fat-laden hot well water out of the cooling tower. The third is a vapor scrubbing or oxidation system to eliminate volatile organic compounds that are not removed in the previous two steps.

A typical distillate recovery system consists of a scrub cooler located at some point in the deodorizer vacuum system. The deodorizer vapor effluent is stripped of about 95% of the condensable organic material by direct contact in the cooler. The circulating distillate is then cooled to remove the heat of condensation before being returned to the stripping tower. Several equipment companies offer design/equipment packages for this.

The remaining deodorizer vapors and the stripping steam are then condensed in either barometric condensers or shell-and-tube condensers. When barometric condensers are used, the condensate and cooling water from the direct contact condensers and the air discharge of the last ejector is discharged to a hot well. From the hot well, the cooling water then passes to a cooling tower before recirculation to the condensers. This system is the source of characteristic oilseed processing odor of oil process facilities.

This system normally uses a condenser discharge directly to a hot well and then to a cooling tower, often by gravity. The cooling tower overflow and blow down is discharged to a wastewater treatment facility as part of the plant oily wastewater handling needs.

If odors from the cooling tower required further control, the cooling water from the hot well is pumped through plate heat exchangers, which allow for indirect cooling. However, because of fouling problems, an extra heat exchanger must be provided to enable off-line heat exchanger cleaning. Either steam or water and detergent are used for the cleaning. One benefit of using steam is that the fatty material is more easily reclaimed. The closed-loop system is more expensive to install (more equipment) and has higher energy costs for pumping water and motive steam in the vacuum system.

When non-contact cooling water is used with shell and tube condensers, the deodorizer distillate not recovered in the scrubbing system will not build up and foul the cooling tower but is confined to the hot well. The insides of the tubes of the condensers require continuous scrubbing with caustic water to eliminate fouling, which also enters the hot well. The floating distillate is skimmed from the hot well into a recovery tank, whereas a portion of the high pH water is recirculated for cleaning the tubes and the excess water overflows to the wastewater treatment facility.

4. **BOILERS**—The control of combustion products varies with the fuel combusted and boiler design. For natural gas and fuel oil combustion, the control mechanism for  $\text{NO}_x$  and CO emissions is typically a burner system that

recirculates combustion gasses to the burner to control the combustion temperature. These burners are also available as a retrofit for existing boilers; however, a reduction in steaming capacity may result.

Most coal-fired boilers are equipped with baghouse collections for particulate control. Where  $\text{NO}_x$  and  $\text{SO}_2$  control is an issue, fluid bed boiler design can normally enable fire-box control because of lower combustion temperatures and the use of a limestone bed. Another alternative for  $\text{SO}_2$  reduction is the combustion of low sulfur coal. Trace metal and HCl emissions are regulated as hazardous air pollutants. Their concentrations in coal can vary significantly within a coal vein and from vein to vein. Control strategies include switching coals or cooling the boiler exhaust gasses, injecting a sorbent, and collecting the resulting particulate with a baghouse collector.

## 5. SOLID AND HAZARDOUS WASTES, SOURCES, AND CONTROLS

### 5.1. Sources

Generation and handling of solid and hazardous waste has not been a challenge in this industry on the same scale with wastewater or air emissions. However, several significant sources exist. These sources are discussed below, and potential control technologies are presented in the previous section. Table 3 summarizes generation rates.

*Refinery Bleach.* Various bleaching clays purify and remove color bodies from refined oils. These wastes contain 5% to 35% oil. The characteristics of these materials are such that they will combust spontaneously, which has created problems for disposal in sanitary landfills, as well as at factory locations in some instances, but this condition can be overcome by several methods. The model refinery presented in the previous section will generate about 2500 kg of waste earth per day, of which 570 kg is oil.

The material is not normally considered hazardous, other than spontaneous combustion; however, some landfills are reluctant to accept this material because of combustion and/or oil content. The spontaneous combustion is caused by excess heat generated from oxidation of organics. Methods to prevent contamination

**TABLE 3. Major Solid Waste Sources in Oilseeds Processing.**

Source	Loss
Meal and grain solids	1–10 kg/100 m <sup>3</sup>
Bleaching earth	2500 kg/day
Waste nickel catalyst	100 kg/day

include addition of antioxidants or water sprays to the waste earth. The addition of lime is also reported to prevent the spontaneous combustion.

*Spent Hydrogenation Nickel Catalyst.* Nickel catalyst is used in the hydrogenation of oil. Depending on the processor, catalyst may be recycled many times; therefore, waste generation rates differ. However, the model refinery will generate about 100 kg of spent catalyst per day.

This material has been either recycled or disposed of in sanitary landfills (or onsite). The recycling for nickel recovery has been spotty and not well organized until now in the United States and North America.

Although nickel has not yet been designated as hazardous by the EPA. Several states have classified nickel as hazardous and it is anticipated that it will soon be universally treated in that manner. As discussed above, a more organized infrastructure to recycle these materials is developing. Active facilities for spent nickel catalyst recycle are known to exist at this time in the United States, Mexico, and western Europe.

## 5.2. Controls

*Bleaching Earth.* The quantity of earth used is primarily a function of crude oil quality and press efficiency. In both cases, there is already a high degree of problem recognition because of the process and economic implications. Therefore, there will generally not be substantial opportunities to reduce waste earth through process control simply for environmental purposes. Certain brands and types of filter media are better for various crude mixtures and presses; however, this is also dictated by process conditions out of necessity. This subject is addressed in more detail elsewhere in other articles.

There is a tendency to use excess clays to ensure final product quality, and proper dosage should be maintained. Also, physical refining uses greater quantities of waste earth. To some extent, physical refining essentially trades wastewater problems for solid waste.

*Spent Catalyst.* Opportunities may exist to reduce the volume of waste catalyst by reusing the catalyst through several hydrogenation batches; however, experts disagree on the final benefit of this. The measurement of this can be made by measuring hydrogenation efficiency versus catalyst disposal costs. Other catalysts have been explored; however, their costs and effectiveness are less desirable. These catalysts have environmental problems of their own because they are heavy metal-based.

*Grain and Meal Solids.* In this area, the improvements in solid waste can be made through loss control. Transport of these materials is highly mechanical, involving conveyors, elevators, pneumatic systems, and related mechanisms. Losses tend to occur at transfer points along the conveyance system as well as at loading and unloading points (barge, railcar, and truck). In addition, building dust collectors, cyclones, and similar air emission control devices are a constant potential source of grain and meal solids losses because of plugging and overflow. Of importance is that many points are located on building roofs; therefore, the losses may go

unnoticed and undetected. This situation is receiving better because of regulations on control of pollution in stormwater.

A program of good maintenance and repair is essential to minimizing these losses. Again, physical measures can be installed on a cost-effective basis, which include loadback bins at loss points to allow material to be recovered as product rather than as waste. Also, closed chutes and other conveyance devices, particularly at discharge points into railcars, will add greatly to loss control.

These wastes are not considered hazardous and can be disposed of in a sanitary landfill. It is extremely important that these grain solids be kept out of the process and storm sewers as they will exert a substantial wastewater loading.

## 6. CURRENT ISSUES

### 6.1. Wastewater

Selected wastewater issues in oilseeds processing that have application to numerous processors in the United States and elsewhere are discussed here.

1. Increasingly stringent FOG limits in discharges to publicly owned municipal sanitary sewage works.
2. Increasing stringent BOD and TSS limits to POTW discharges of 250 mg/L or less.
3. Increased emphasis on phosphorus control in discharges.
4. Increased emphasis on “unconventional” pollutants/toxins such as heavy metals and chlorinated organics. This category is more relevant to oleochemicals.
5. Increased emphasis on water quality in stormwater discharges.
6. Water costs and availability.

The oil and grease discharge limitation issue has been argued tirelessly by the industry and other groups in the United States to the EPA, states, and municipalities for many years. Although it has been shown that these animal and vegetable oils and greases are readily biodegradable and should not be regulated as stringently as petroleum oils, many municipalities are reverting back to an FOG limit. This might be the more conventional 100 mg/l, or a range of 150–200 mg/l. This results from a combination of real and perceived problems at POTW facilities, all of which can be avoided, at a considerable expense to industry, by more stringent controls on pretreated discharges to POTWs. This subject has been and will continue to be extremely important (5,6).

The BOD and TSS limits are also tightening in many cities, which often is in response to increased regulatory pressure on the municipality to bring their POTW discharges into compliance. Under certain conditions, these more stringent limits are mandated by enforcement directives toward the municipalities. Compliance with such limits by the oilseed processors virtually demands a biological pretreatment facility.



Phosphorus in high levels is associated primarily with acidulation; however, it can be found in varying levels in several other waste streams. Concerns for phosphorus are centered primarily around its impact on algal production in receiving streams, particularly at impounded lakes. Other than acidulation, much can be done to control phosphorus at the source. A major difficulty in treating for phosphorus in this application is that it is an organic form and not readily precipitated using lime or alum. If the wastes are biologically treated, the resulting effluent will contain predominantly inorganic phosphorus and can be readily precipitated.

The issues of nonconventional pollutants is an evolving subject and related primarily to oleochemicals. Parameters involved include various chlorinated organics, heavy metals, and phenols. Each must be addressed on its merits. Solutions involve a variety of pretreatment techniques and occasionally result in a source control solution such as a product being discontinued.

Portable water supply and wastewater treatment costs, coupled with water availability concerns, are causing the industry to look at internal and end-of-pipe recycle options. These projects could not have been justified and/or the technology was not readily available 10 years ago. This situation is addressed further in the next section.

## 6.2. Solid Wastes

*Salable Oils.* The issue of recovering suitable oils for resale or reuse from gravity separation of wastewaters and other secondary recovery units is a major problem, and it will only get worse. More locations recover oils and greases to sell into the feed fat industry, which will drive prices down and quality requirements up.

*Sludge Disposal.* Waste sludges are generated from several areas in oilseeds wastewater treatment, including lime neutralization and dissolved air flotation phase separators, and biological waste.

These materials are becoming much more difficult and costly to properly dispose of. Three options have been used, depending on site-specific economics and other constraints.

1. Divert sludge to meal dryer for introduction to meal stream.
2. Land apply sludge to agricultural row crops.
3. Dewater sludges, and dispose in an appropriate sanitary landfill.
4. Process sludge off-site through contract dewatering or other means. A limited number of municipalities offer this as a service to their industrial discharge customers. Also, several private waste processors offer this service in North America.

On the upper east coast in the United States, the population density and land costs have increased to the point where the market for animal feed has virtually disappeared and no other outlet is available for such recovered materials.

### 6.3. Air Emissions

*Hexane.* Control of hexane emissions is a major issue and will continue to be so for several years. In the United States, hexane is classified as an “air toxic” compound. Air toxic compounds are, for the purposes of this article, a list of potentially toxic compounds emitted primarily to the atmosphere in quantities of 909 Kg per year or greater, from stationary (industrial) emission sources. As a result, total hexane emissions for all extraction plants will need to meet the same standards as the best 12% in the industry are now achieving. As a minimum, this will require a well-run mineral oil absorber and excellent control of other loss points throughout the system.

Toxicity analysis at the plant boundary line will be required for all plants. As a minimum, increased stack height for the meal dryer will be required in most instances.

Permitting requirements under the U.S. Federal Title V for air emissions will essentially encompass most stand-alone refineries because of the volume of hexane brought in as part of the crude oil and removed (“emitted”) during oil processing.

*Particulate Emissions and Stormwater Runoff.* Increased emphasis is being placed on control of particulate emissions, with “pollution” taxes being imposed on a selective plant-by-plant basis. More importantly, particulate emissions ultimately end up on the plant site grounds. In the United States, a program of enforcement of excess contamination in stormwater is now underway. Organic particulates along with fats and oils that reach storm drains are the most significant contaminants. Stormwater discharge limitations have yet to be set; however, it is clear that no reasonably attainable goals can be met without also controlling particulate emissions.

## 7. WASTEWATER TREATMENT PROCESSES AND TECHNOLOGIES

This section briefly describes the various treatment systems encountered in fats and oils and oilseed applications along with their capabilities relative to the parameters of interest. The processes listed are generally consistent with the order in which they might appear in a treatment facility. Table 4 presents the potential effluent quality for oil and grease for various physical–chemical processes.

### 7.1. Gravity Oil Separation

This process can be considered as in-plant process control or as a wastewater treatment process. In either case, it is essential that adequate gravity separation be provided at every fats and oils handling facility. The author felt compelled to address this subject in some detail as numerous poorly conceived gravity separation facilities have been observed.

**TABLE 4. Influent—Wastewater Comparison (Flow Diagram/Figure 4, Reference Point 1).**

Parameter	Conventional Refining, w/Acidulation	Conventional refining, w/o acidulation	Activated Silica Refining	Organic Acid Refining
Flow, gpd	100,000	100,000	85,000	55,000
Cubic meters per day	378	378	321	207
BOD, lbs/day	3,400	1,200	700	512
Kg/day	1554	544	318	232
COD, lbs/day	5,700	2,000	1,165	850
Kg/day	2,590	907	530	386
Oil and grease, lbs/day	290	290	290	290
Kg/day	132	132	132	132
pH	2.5–4.0	6–8	6–8	6–8

Where is the best place to locate gravity separation facilities in an oilseeds operation? The answer is in as many places as possible and as close to the source as possible. This is not always practical or efficient. The main considerations are listed below.

1. Prevent cross-contamination of a higher quality recoverable product with a lower quality material whenever possible; i.e., do not combine packaging area drainage with deodorizer cooling water blowdown.
2. Place gravity separators wherever wastewater gravity flow ends and pumping begins. If any kind of a wet pit is involved in the pumping, the oil will float to the surface despite whatever attempts are made to keep it in suspension. Therefore, one should take advantage of this and construct a gravity separation section as part of, or adjacent to, the wet pit.
3. All waste streams must receive oil separation of some sort before any further treatment. Every oilseeds processing and oil processing plant requires facilities for capturing of floatable fats and oils resulting from both day-to-day losses and larger spill situations.

Gravity separators need to be designed both to contain a defined oil spill size, as well as for managing routine process wastewater flows. Gravity oil separators for these applications should be designed for rise rate loadings to accommodate peak flows, if practical. Experience dictates rise rates in the range of 10 to 20 L/min per square meter of effective separator surface area.

The rise rate concept is applicable to the design of continuous flow separation devices. Many designers use retention time for separator sizing, which is acceptable if desirable separator geometry dimensions such as length to width ratios are maintained. The concept of retention time may be applied directly for design of batch settling vessels.

Skimming of oil and grease from the surface of separation vessels can be performed on a manual or continuous basis. The viscosity of many oils used in vegetable oil processing at ambient temperature is such that surface contact-skimming devices (belts, ropes) are not particularly effective. Chain and scrapper type skimmers are effective, but not physically adaptable to every skimming situation. Shop fabricated, manually operated decant devices have been effective.

A portion of the flow to a separator often originates in rainfall from oil-contaminated runoff areas, which may result in flow rates too high to practically handle at desirable rise rates. This result often dictates a need to handle process waste flows and contaminated runoff in separate systems.

## 7.2. Equalization

The variance in process conditions, rainfall contributions, and related considerations make equalization facilities for stabilization of waste flow and strength essential.

The volume of equalization required will depend on the variation of incoming flows and strength over a reasonable time duration. Experience indicates that the volume of equalization required for flow and strength for the typical oilseeds operation defined in the previous section will be about the same. The only wastewater stream not requiring equalization is the effluent from the milling and extraction operation. This stream is composed primarily of the sewer water evaporator steam condensate and is usually without much variation.

The volume of equalization required can be determined with a volumetric influent hydrograph approach. The required volume can be determined graphically to the level of accuracy required. A check of influent and resulting effluent strength variation can be made to confirm the suitability of the volumetric design for a specific application. Figure 3 is an idealized sample hydrograph.

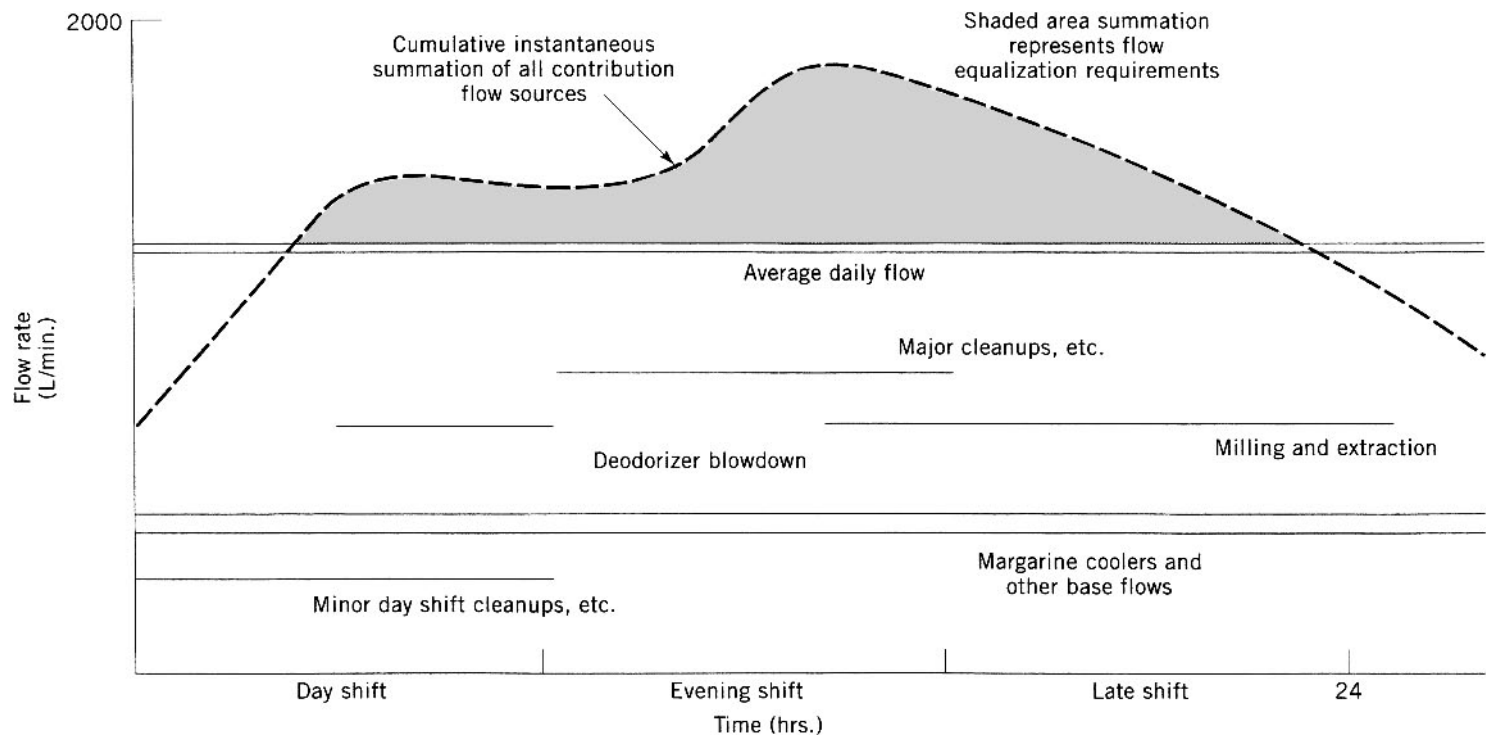
In general, the equalization surge volume required will vary between 30% and 50% of the daily average flow, but this can vary from each application.

## 7.3. Dissolved Air Flotation

Dissolved air flotation (DAF) is a technique that uses minute air bubbles to enhance the flotation of oils and suspended materials not removable in reasonably sized gravity separators. The bubbles are formed by pressurizing all or part of the waste flow and introducing air to a pressurization cell. Subsequent depressurization allows the bubbles to form, according to Henry's law.

This process is enhanced by the addition of polymers, alum, or other flocculants. This chemical addition provides for coagulation and flocculation of colloidal oils and solids into larger particles that can be easily removed.

In fats and oils applications, this technique has been shown to be effective in removing FOG and associated insoluble BOD; however, significant quantities of waste sludges are generated. Table 4 shows the removal rates that can be expected for various processes discussed in this presentation.



**Figure 3.** Wastewater influent hydrograph.

The selection of a chemical flocculent must consider pH conditions as well as the removals desired. In the application of DAF in a fully integrated oilseeds plant, alum, and polymers are effective. An alternative approach is to use sodium hydroxide, the most commonly used refinery waste neutralization agent, to bring pH up to a range of about 5.0, and then lime can be a final pH adjustment chemical. The resulting insoluble calcium sludge will perform the same function as alum at less cost. The lime sludge may settle better than it floats; however, and flotation should be evaluated against settling.

As will be described in the next subsection, the use of chemical flotation in this application may be less desirable considering other processes. DAF has been applied effectively as a trimmer process following other treatment facilities for FOG removal requiring a 100-mg/L FOG limit, which can sometimes be accomplished using food grade-approved polymers. In that event, the recovered materials can sometimes be sold as a low-grade feed fat rather than disposed of as a waste sludge.

#### 7.4. Filtration

In-line filtration using diatomaceous earth is an effective device for reducing FOG and associated insoluble BOD to very low levels. It will not remove soluble BOD components because of acidulation wastes.

Filtration has not found extensive use in fats and oils wastewater treatment application; however, it provides an effective treatment process, particularly as an alternative to DAF as a trimmer device. Another advantage of filtration is its familiarity to refinery personnel as it is used in other in-plant processes. The principal disadvantage of filtration is that it also produces another waste sludge to be disposed of.

#### 7.5. Hydrolysis

An alternative approach used successfully at several fats and oils handling facilities employs a low-pH, high-temperature hydrolysis effect to recover oils and grease.

If the plant acidulates soapstock and/or washwater, the resulting acidulation waste will contain sufficient residual heat and acidity to affect the entire waste stream pH and temperature, which obviously assumes an alkali refining process rather than a physical refinery. This process will produce an acidulation effect on residual oils. These oils can then be readily removed in a gravity separation process.

The process can be used at plants that do not acidulate soapstock; however, site-specific studies should be conducted to determine pollutant removals to be expected. In the overall scheme of things, acidulation of washwater as part of the treatment process will greatly improve effectiveness.

This process offers several advantages.

1. Process control is relatively easy. If the waste stream pH stays at 3.5 or below and a temperature of 37.7°C (100°F) or higher and soapstock and/or

washwater is acidulated, relatively constant effluent results can be obtained virtually independent of the raw influent waste characteristics (high FOG and BOD levels). The exception to this process is carryover of excess middle phase from an inefficiently operated acidulation system. This material will not gravity phase separate and will actually inhibit the process effectiveness for other plant waste streams. Other sources of protein, such as extraction and milling meal fine carryover into the crude oil, will also have an adverse effect of this (and other) oil separator processes.

Under proper conditions, the effluent BOD strength in the separator underflow will be equal to the soluble BOD load from acidulation plus the additional contribution from the residual oil and grease in the separator effluent.

2. The material recovered in the separator will contain a relatively low moisture and high total fatty acids (TFA), thus, the material can usually be sold for feed fat.
3. If the facilities can be constructed near acidulation, existing acid handling, steam, and recovered oil storage tanks can be used for both purposes. The primary disadvantage is the need to utilize materials of construction that will withstand the high-temperature and low-pH conditions. A wide variety of tankage, process controls, and related equipment have been used in designing these treatment facilities, which is dependent on refinery size and processes, client preferences, existing equipment to be used, and other related factors.

Neutralization of the separator effluent is usually accomplished with sodium hydroxide; however, lime or ammonia are occasionally used. A two-stage system using well-mixed tankage of suitable size is recommended. The neutralization invariably occurs on the steep part of the titration curve in these applications. Any inappropriate design, such as an excessively remote pH controllers, will result in a nonfunctional system. As a result, it is highly recommended that an individual experienced in designing pH control systems reviews the final design.

### 7.6. Other Processes

Comments are made here relative to processes which have had only marginal success in oilseed processing.

Various membrane technologies have been applied to these waste streams for pretreatment removal of fats and oils. Although pollutant removals are good in some applications, membrane life and regeneration have presented problems. As a result, operating costs for periodic membrane replacement are high. This technology has advanced considerably in recent years and several system suppliers are working on vegetable oil waste applications. The membranes will have future use in biological treatment, as noted below.

At least one attempt has been made to use activated carbon to remove residual organics. The nature of the soluble components in the acidulation stream (the primary source of solubles) is such that these organic compounds cannot be effectively removed by activated carbon.

Several processors attempted to use a mixed media (sand, anthracite) filter to remove residual oils. This technology was adopted from petroleum waste applications; however, it did not function well in oilseeds applications, because of an inability to backwash these units caused by the highly viscous nature of these oils at ambient temperature.

Attempts have been made to apply inclined plate and other packaged media separator systems. These have not functioned well in vegetable oil applications, because of the viscous oil coating over the separator media.

## 7.7. Pretreatment Applications and Refining Processes

From an applications standpoint, the emphasis is placed on assembling the various processes noted above in an effective manner and realistically predicting the anticipated influent and effluent for various refining processes. Figure 4 is a simplified flow sheet showing an integrated physical/chemical pretreatment scheme that has effectively been used at a number of oilseed and oil refining operations. The sizing, materials of construction, and related considerations are a function of the refining process and related factors.

Tables 4 and 5 reflect anticipated process wastewater influent and effluent for the model refinery noted in the second section. These values reflect data collected and analyzed by the author from over fifty facilities in North America and elsewhere. The values reflect average conditions and values, with reasonably good operations control and facility maintenance. Note that each table has a reference point within the process, as shown on Figure 4.

## 7.8. Biological Treatment

Wastes from oilseeds operations have been successfully treated with biological treatment in any number of circumstances. Traditionally, pretreated effluent has been further treated in combination with domestic wastes in publicly owned pretreatment works (POTWs). Also, several biological systems are in place at oilseeds plants either as pretreatment or for direct discharge.

Figure 5 is a diagram that shows relative rates of biodegradation for a variety of oils. Clearly, it can be seen that oils of animal and vegetable nature are highly biodegradable and compatible with municipal domestic wastes.

Good gravity separation of floatable oils is mandatory before further treatment in a biological facility. Additional oil removal pretreatment may be required for oil value, prevention of sewer line blockage problems or merely to reduce the size of the biological facilities.

Various biological processes have been used with good results, including extended aeration/activated sludge, sequential batch reactors, and biofilm reactors. Anaerobic treatment has not been effective because of high sulfate levels.

Water availability, in conjunction with water and sewer charges, is causing the industry to consider installation of facilities that will allow for a much greater end-of-pipe recycle of process wastewaters. Several refiners are considering the



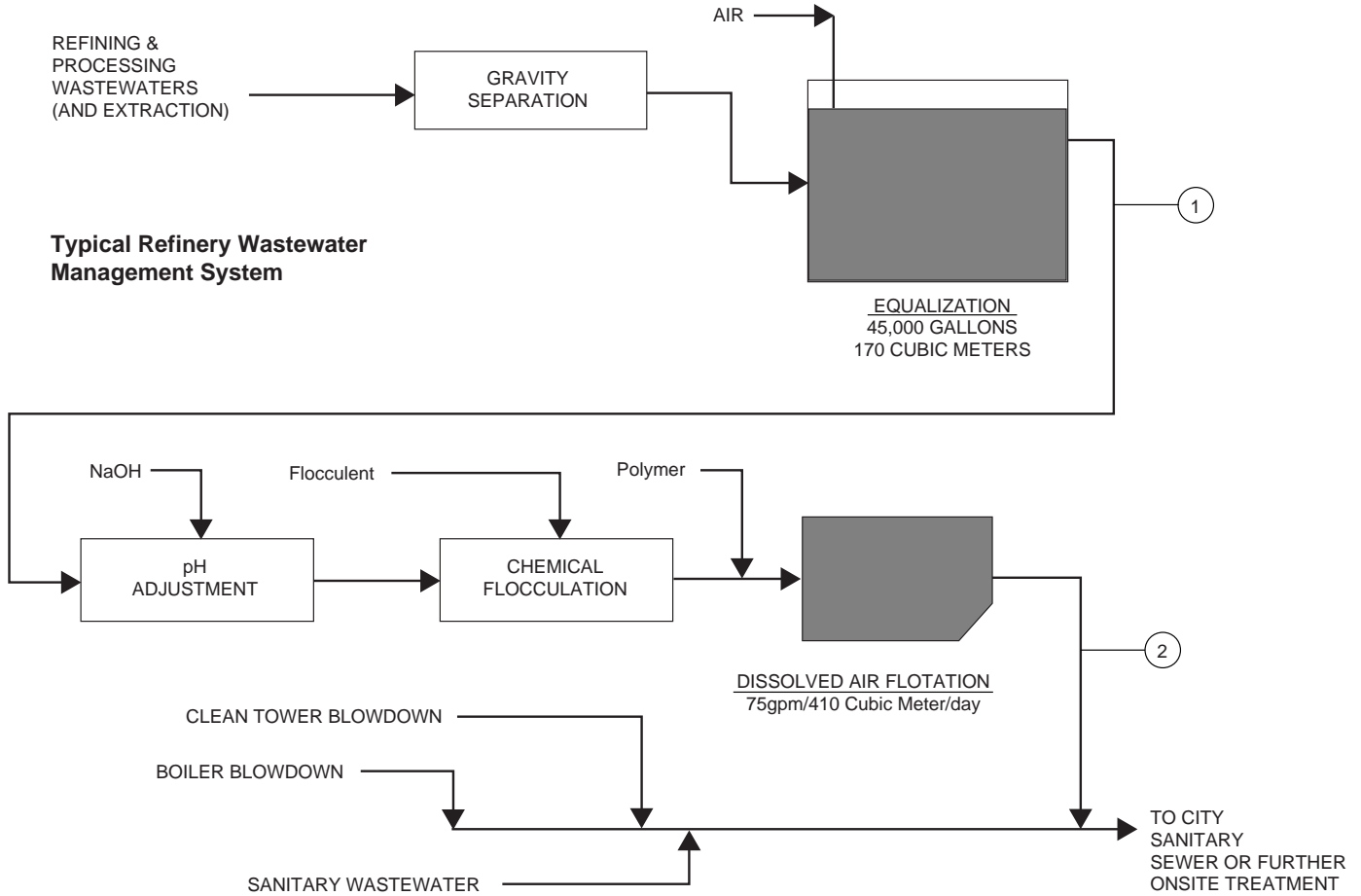
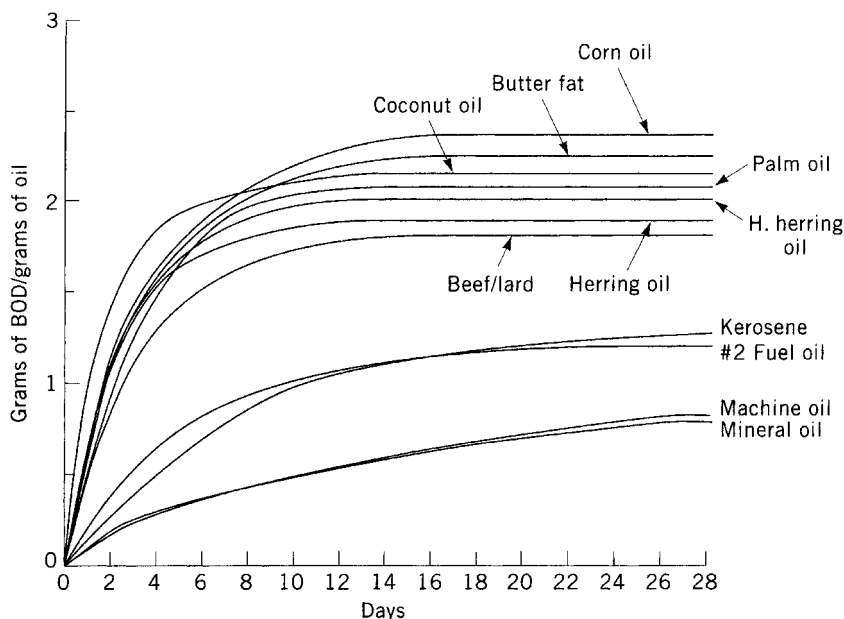


Figure 4. Oil processing wastewater treatment flow diagram.

**TABLE 5. Effluent—Wastewater Comparison (Flow Diagram/Figure 4, Reference Point 2).**

Parameter	Conventional Refining, w/Acidulation	Conventional refining, w/o acidulation	Activated Silica Refining	Organic Acid Refining
Flow, gpd	100,000	100,000	85,000	55,000
Cubic meters per day	378	378	321	207
BOD, mg/l	3,427	360	210	125
lbs/day	2,900	300	150	57
Kg/day	1,318	136	68	26
COD, mg/l	5,711	600	350	206
lbs/day	4,833	500	250	95
Kg/day	2,196	227	114	43
Oil and grease, mg/l	20–50	20–50	20–50	20–50
lbs/day	25	25	25	25
Kg/day	11	11	11	11
pH	6–9	6–9	6–9	6–9

installation of a membrane reactor biological wastewater system (MBR). This technology was developed for use in the municipal wastewater field, and is now well proven and reliable in that application. The MBR technology utilizes one of several membrane pressurization equipment systems to filter biological effluent. The filtered water has very low suspended solids and can be used for cooling tower make-up. The MBR process eliminates the need for a conventional biological clarifier and allows the bioreactor to operate at much higher biological solids level.

**Figure 5. Biological uptake rates.**

## REFERENCES

1. E. Fritz and R. W. Johnson, *Fatty Acids in Industry*, 1st ed., Marcel Dekker, New York, 1989.
2. M. A. H. Fransen, *Standard Methods for Examination of Water and Wastewater*, American Public Health Association, 1992.
3. American Oil Chemists' Society. "Air pollution issues associated with soybean processing." *Short Course Proceedings*, 1984.
4. Metcalf and Eddy, *Wastewater Engineering Treatment/Disposal/Reuse*, 2nd ed., McGraw Hill, New York.
5. M. J. Boyer and K. Krassowski, *Environment Pollution Prevention*, seminar, Technical University of Lodz. Bielsko-Bialn Branch, Poland, 1993.
6. "Regulatory oil and grease discharges to municipal wastewater treatment facilities," *Georgia Water and Pollution Control Association Annual Meeting Journal*, 1980.

# *BAILEY'S INDUSTRIAL OIL AND FAT PRODUCTS*

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**Fereidoon Shahidi**

*Memorial University of Newfoundland*

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# 1

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## *A Primer on Oils Processing Technology*

**Dan Anderson**

### **1. INTRODUCTION**

In the early days of oilseed production, functions were often far removed, and actions taken by one operation were done for optimization of its own performance with little consideration on impacts made on subsequent processes. For example, the elevator dryer operator, in order to get the maximum grain throughput during busy harvest periods, might dry grain at an excessively hot temperature without considering the impact on the oil quality. The degumming operator may set the centrifuge to take advantage of trading rule limits without regard of the downstream impact on the refining operation.

Within the last few years, the emphasis has changed from stand-alone operations toward the integrated manufacturing facility, producing a more complete range of value-added products from the raw seed to the dinner table. During this transition, operations have become more dependent on each other, as the individual functions involved must now consider the impact of their actions on the total process. At the same time, the scope of knowledge each operation must have of other functions has expanded, and it is important that at least a basic understanding of the “big picture” be available to the decision maker. The purpose of this Chapter is to provide an overview of the typical processes and interrelations associated with a total integrated facility. It is also hoped this basic overview will prove to be beneficial

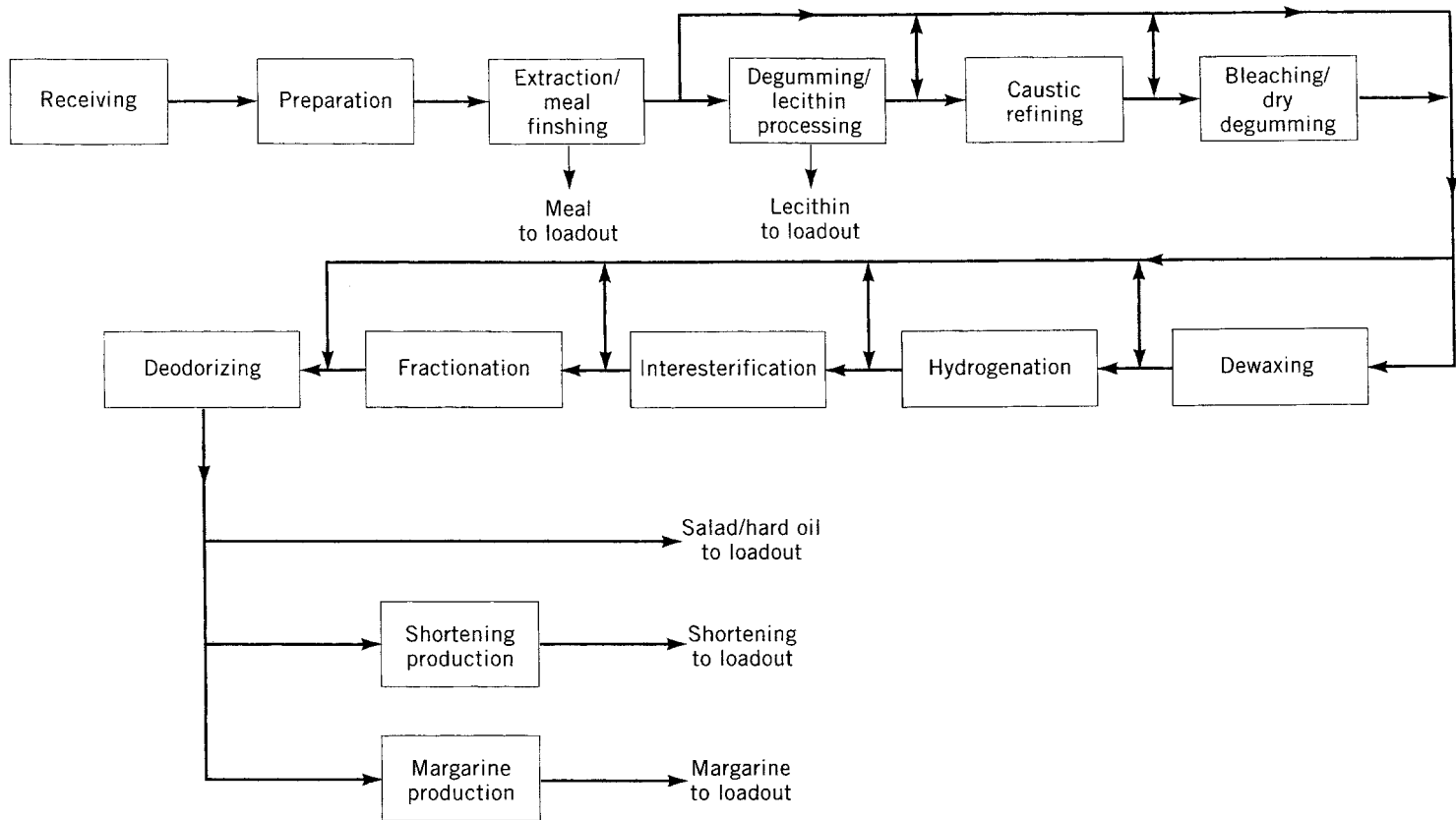
to those new in the industry. It must be stressed that this Chapter attempts to touch in a limited number of pages subjects that are the basis for volumes of books and lifetimes of knowledge. It must also be emphasized that this Chapter does not expand the many viable options of each type of process, nor does it intend to provide details for proper operation of each of the unit operations. For a more detailed discussion, the reader is encouraged to refer to the many fine chapters within this series and other publications dealing in much greater detail with each of the individual functions of the integrated facility.

It is useful to consider the modern manufacturing operation as a set of unit operations and develop a block diagram representing the facility. Figure 1 illustrates the processes involved with the subsequent sections of this Chapter reviewing these unit operations.

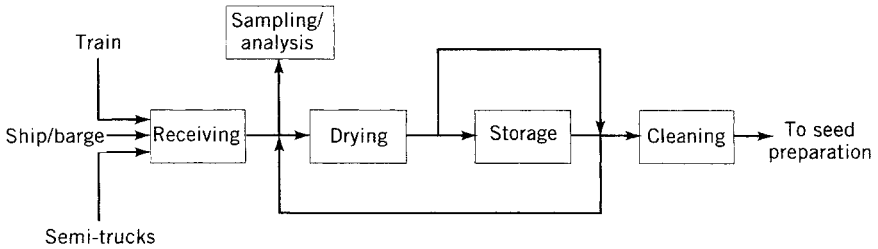
## 2. STORAGE

The typical operations associated with *storage* include receiving, sampling, drying, storage, and cleaning prior to processing. Figure 2 illustrates the common functions of this operation. There are many variants; for example, some processors may clean the grain both before and after the drying operations. In any case, the basic operations are designed to accomplish the same task, which is to provide a safe haven for the grain and deliver it at the proper time and condition to the processing facility.

As the product is received fresh from the farmer's field, the grain will contain foreign material, consisting of naturally occurring sticks and pods, metal and rock accumulated during handling, and contamination from weed seeds and other grains. The elevator manager will sample the grain and make adjustments in the price paid based on the moisture, splits, heat damage, and other factors. Typically grain receipts are segregated based on these quality factors, with the moisture content being one of the prime factors for separation. For proper storage and subsequent processing, the contaminants must be removed and the grain must be dried prior to storage. As grain freshly harvested may have a moisture content of up to 20% (although many farm operations are equipped with dryers), the grain must generally be dried to around 13% moisture for safe extended storage. High moisture damage typically results in reduced oil content, decreased protein, and increased color and refining loss of the extracted oil (1). A precursor to this damage is often indicated by a rising grain temperature, and many storage facilities are equipped with a series of temperature cables embedded in the grain with indicating and recording equipment located in the manager's office. If left unchecked, the grain will spontaneously heat and become damaged and under extreme conditions, a serious fire may develop. It is routine practice to monitor the temperature of the grain daily and, if heating is occurring, immediately process the grain. If this is not possible, or the degree of heating is not severe, the elevator manager may simply "rotate" the grain by moving it from one location to another.



**Figure 1.** Integrated processing facility.



**Figure 2.** Receiving and storage.

Drying is usually accomplished in a vertical column, direct fired unit, although steam, and even solar energy have been used as a heat source for certain installations. Naturally, the hotter the drying temperature, the faster the drying operation will be. However, drying temperatures must be closely monitored as excessive temperatures will damage the seed. It has been found that temperatures in excess of  $63^{\circ}\text{C}$  will significantly increase the color of both the meal and the oil, denature the protein, increase the nonhydratable phosphatide levels in the crude oil, and result in greater potential for grain dryer fires (1).

Storage facilities come in many sizes and varieties, and storage methods are often limited to the imagination of and the assumption of risk by the elevator manager. It is noteworthy that the design is always concerned with protection against the elements, and, despite the design, the condition of the material put into storage always determines the success of the storage program. While on-farm storage is generally limited to aerated steel or stave silo tanks, storage at the local elevator and the processing plant may take several forms. Complexes are situated to serve rail, road, ship, or barge traffic as the locality requires, and the functionality is determined by the success of movement of products into, out of, and within the complex. A popular storage method is traditional concrete or steel storage tanks, with typical sizes of 100,000–750,000 bushels per tank. Structures of this type are normally very visible in many small towns throughout North America. Muskogee houses, which are large warehouse bulk storage facilities, have long been popular for cottonseed and other seeds that are difficult to handle. Undercover “tents” and inflatable warehouses with capacities in excess of 2 million bushels are often used, and even caves with huge capacities are sometimes employed. In small towns, it is common during harvest to see parking lots filled with grain, with the elevator’s manager and stockholders praying for the rain to hold off until sufficient storage becomes available in more permanent facilities.

In some crushing operations producing a high-protein meal, it is common to dry the grain from its 13% moisture storage conditions to 10.5% processing conditions. This is necessary to shrink the meat away from the hull and to remove excess moisture that would end up in the extracted meal. After process drying, it is desirable to temper the grain for an additional 4–10 days prior to processing to allow moisture to migrate evenly throughout the grain. Even when the storage conditions have been at low moisture, it remains common practice to pass the grain through the



dryer to help shrink the hull from the meat, allowing the subsequent dehulling step to be performed more effectively. This additional preprocessing step does increase operating costs, not only because of the energy spent to heat the grain, but also because this represents one additional unit operating with associated depreciation, operating, and handling losses. There are new technologies available for dehulling integrated in the preparation process that largely eliminate the need for a process dryer.

Cleaning methods vary greatly depending on the seed received, but typically consist of a magnet designed to remove tramp metal, a scalper designed to remove large and heavy materials, and a sizing screener designed to remove fine and oversized materials. Aspiration may also be employed to assist in removal of light foreign material. While contaminants removed by the magnet and scalper are normally discharged as waste, contaminants separated in the cleaner may be ground and reintroduced into the meal stream, or may be used as fillers in feed rations. In addition to cleaning, cottonseed is often delinted prior to preparation, with the lint fibers removed by a series of saw cutters prior to processing.

### 3. PREPARATION

The function of the preparation process is to properly prepare the seeds for extraction of the oil, either by solvent or mechanical methods and, if applicable, remove the hulls and other materials from the seed kernel or meat. While a particular seed may contain from 20 to 50% oil, the oil is tightly bound within the cell and mechanical action must be taken to either forcefully remove the oil or to make the oil more accessible to subsequent solvent extraction. The unit operations typically involved are illustrated in Figure 3, and usually involve scaling, cleaning, cracking, conditioning (or cooking), and flaking. Depending on the process and the oilseed in question, process drying, and dehulling (or decorticating) may be employed, as may be expanders and collet dryer/coolers. After the preparation process, the prepared flakes or collets are delivered to the extraction operation.

Once arriving in the preparation facility, the seed is usually scaled through a weighing device or other control means. The scale is often used to check the physical inventory of seed against the production accumulated, with the difference reconciled as shrinkage. After weighing the seed, it is then delivered to the cleaning process, which generally follows the same path as that described in the receiving operation. After cleaning, cottonseed and sunflower seeds may be dehulled by impacting the seed, breaking and separating the hulls. The hulls may be used as a solid fuel source for boiler operations or for animal feed supplement. The traditional process continues with the cracking rolls, which are a set of two- or three-high corrugated rolls turning at relatively high speeds that break the grain into several pieces. For the soybean processor producing high-protein meal, the cracking breaks the bond between the meats and the hulls, and in the traditional process the hulls are then removed by aspiration. After dehulling, the meats are delivered to

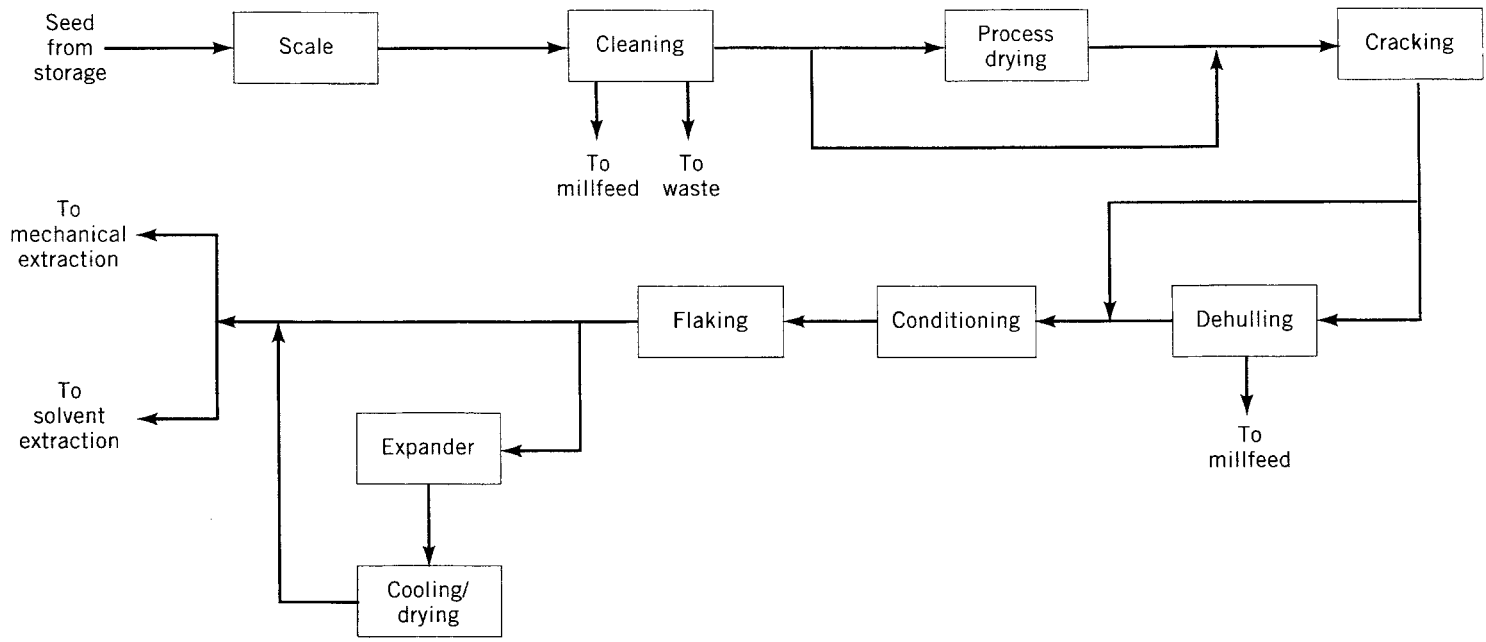


Figure 3. Preparation for extraction of oil.

the conditioner (or cooker) where heat is gently applied to make the cracks soft and pliable for the subsequent flaking operation described later in this section.

There have been several novel approaches applied to preparation in the past few years. One concept that has been widely accepted, especially for soybean processing, is hot dehulling. After traditional cleaning, the seed may be delivered directly to the crackers or may enter the hot dehulling operation. As mentioned before, use of this technology generally eliminates the process drying step traditionally identified with the storage function. The basic principle shared by the three commonly used hot dehulling systems is to dry the bean from storage moisture to process moisture, dehull the seed while still hot, and deliver the conditioned cracks to the flakers without allowing the seed to subsequently cool. This not only saves the energy of one heating step, as much of the air is recycled reducing the energy required for the integrated facility, but reduces the fines generated compared with the traditional system where the grain is cracked cold. One system that has gained wide acceptance is the Escher-Wyss system, which uses a fluid-bed dryer-heater to perform the drying process. After the dryer-heater the grain is discharged to specially designed high-speed cracking rolls, where the seed is cracked while still hot, and then delivered to special high-shear impactors to separate the meats and hulls. The product is then delivered to aspirators, where the hulls are removed, and then to the conditioner, which allows the meats to cool slightly and to temper prior to flaking. Another process that has gained acceptance is the Buhler hot dehulling system, which uses a conditioning column with steam-heated elements to slowly bring the beans to 65°C. The beans are then subjected to a short treatment in a fluid-bed *popper* where the hull–meat bond is broken. The beans are then broken in half by impact splitters, the hulls removed in an aspirator, and the splits further cracked and sent to the flaking rolls. The Crown hot dehulling system uses a similar conditioning column followed by a *jet dryer* to crisp the soybean hull and free it from the meat. A proprietary Hulloosenator then splits the bean and rolls the hulls free where they are aspirated from the meats. The splits are then cracked to the final size for flaking and sent to the Crown Cascade Conditioner for additional aspiration with temperature and moisture adjustment. In addition to the obvious energy savings, these types of systems are reported to reduce residual oil content, improve extractability, and reduce refining loss (2). In all cases, the comments on drying temperatures presented during discussion of storage drying are valid with hot dehulling systems.

While having received the greatest attention, cottonseed, sunflower, and soybeans are not the only oilseeds suitable for dehulling operations. There is research underway to produce a dehulled canola seed. Removing the hulls will increase the protein content and reduce the fiber content of the meal making the product more attractive for feed formulation. A variety of methods have been tested, with encouraging results, although no commercial system has yet been installed.

After conditioning, the meats are generally passed to the flaking rolls where the cell wall is distorted, making the oil more accessible. The rolls being relatively large (70 × 157 cm or larger) are held together with hydraulic or mechanical pressure, squeeze the meats into flakes of approximately 0.30 mm thickness. For grains

with lower oil content such as soybeans, the flakes are typically delivered directly to the solvent extraction plant. For oilseeds with higher oil concentrations, such as sunflower or canola, or installations where solvent extraction is not employed, the flakes are typically sent to mechanical pressing equipment.

A number of processes have been applied to enhance oil extractability and to improve conditions for consistent physical refining. One of the greatest problems associated with physical refining of high-phosphorous oils (such as soybean or corn) is that nonhydratable phosphatides generally cannot be removed without extensive bleaching clays and acid treatments. Because of variances in the crop year, growing conditions, and seed varieties, consistency in the oil is a major factor affecting successful application of physical refining. It is postulated that the presence of an enzyme during conditions associated with certain storage conditions and the subsequent extraction process causes water-hydratable phosphatides to become nonhydratable. Activity of this enzyme is directly impacted by the seed and growing conditions. Lurgi's Alcon process is said to inactivate this enzyme immediately after the flaking step, and provide an oil consistently acceptable for physical refining (3). This process is said to also reduce hexane carryover, although the characteristics of the meal are somewhat different from that obtained from a conventional process. Lecithin produced from degumming the oil is also affected.

Another process that has gained much popularity, especially for deep-bed extraction installations, is expanders. These devices, which operate much the same as mechanical presses but with less pressure, substantially increase the bulk density of the flakes prior to extraction. This allows the processor to increase the throughput of the plant with minimal capital investment, as the oilseed extractability and gravimetric throughput is greatly enhanced. It was originally postulated that the emphasis on thin flaking may be reduced with expanders with one process designer even proposing elimination of flakers, simply grinding the grain prior to the expander. While elimination of the energy, maintenance, and capital investment associated with flaking is certainly attractive, tests to date indicate that thin flaking is still necessary to produce an acceptable residual flake fat.

The use of expanders does have an impact on the quality of the oil that the integrated processor should be aware of. An interesting phenomena of the expander is that while phosphorous levels in the extracted crude oil are normally increased over traditional flake extraction (typically as much as 200 ppm as P in soybean oil), nonhydratable levels in the degummed oil are normally lower. It is postulated that partial inactivation of the lipase enzyme (blamed for conversion of nonhydratable phosphatides) occurs in the expander and, while the crude oil has a higher neutral oil loss, the quality of the degummed oil is higher. In fact, one processor reported that despite all efforts to make a soybean oil physical refining plant function, the oil was of substandard quality until the plant installed expanders. Once installed, the plant could consistently produce a quality physical refined oil (4).

Expanders do have an impact on lecithin production as well, not only in terms of higher quantity but also with respect to the quality. Expanders continue to gain acceptance, and several variations have been introduced for high oil content applications. For oilseeds containing in excess of 30% oil and for instances when the

material cannot be extracted directly, at least one manufacturer has installed drainage cages on the discharge end of an expander, eliminating the need for a traditional mechanical press for certain applications (5). Another manufacturer has designed a set of extruder parts that can be added to its existing prepress. With this equipment, the product can be pressed and extruded within the same unit with reports of a press capacity increase of approximately 20% (6). Many processors take advantage of the increased extractability and percolation rate of collets and send full-fat collets directly to the extraction plant. This not only reduces operation and maintenance costs but also reduces the need for separate oil setting and filtration systems.

The collets from the expander are hot and moist and are often cooled and dried prior to entering the extraction process. The dryers, which may be equipped with steam heating, typically remove about 2% moisture (most of that added by live steam in the expander) and reduce temperature by about 40°C. The dryers also allow time for the collets to approach a more uniform moisture and temperature. Some plants report an increased tendency for the miscella in the second effect evaporator to foam or to coat or plug the evaporator tubes. It is thought that this may be related to a lack of drying and a nonuniformity of the collet such that the center is hot and moist, causing water-related problems in extraction.

#### 4. MECHANICAL EXTRACTION

As the electrical classification of a mechanical extraction operation is generally the same as that in the preparation area, many processors locate the pressing operation in the same building as the preparation process. In the pressing plant, the seed is subjected to extreme heat and pressure with oil mechanically forced from the oil cell. A typical pressing operation is shown in Figure 4, and involves cooking, pressing, cake cooling and finishing, and oil filtration.

Like most processes, the operation and configuration of almost every plant is different, and while the following description refers to a generic pressing operation, the reader is reminded that there are many variances in system design. In this generic operation, pressed meats from the flakers enter a vertical stacked agitated cooker where heat is applied and protein is denatured. After the cooker, the hot meats enter a mechanical press, where roughly 60% of the available oil (or nearly 90% for full press operations) is removed by application of intensive mechanical pressure. The cake passes through the end plate, where through the application of the high friction, it has been cooked, compressed, and often quite hard, while the extracted oil exits through drainage bars in the press. After the pressing operation, the cake is normally broken and cooled, with the prepressed cake usually sent to the solvent extraction plant for final oil removal. Where economics do not permit solvent extraction, a higher degree of oil removal is performed in the press plant (known as a full press operation), with the cake used directly as animal feed. As the material is subjected to great heat during the operation, naturally occurring urease activity is inactivated and protein is denatured, making the product suitable for feed purposes.

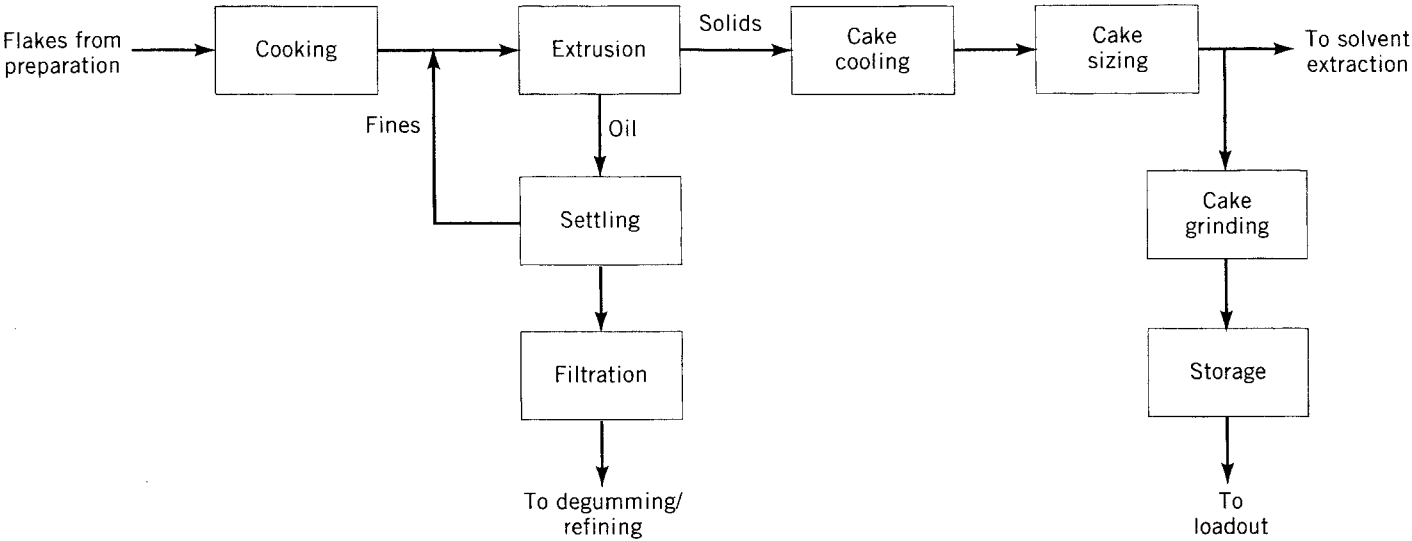


Figure 4. Mechanical extraction/meal finishing.

Oil from a mechanical pressing operation usually contains a high concentration of meal fines, which are removed in a screening tank followed by a pressure leaf or plate and frame filter, prior to delivering the crude oil to the refining process. The quality of this oil may be higher than that obtained from solvent extraction, as less oil-soluble impurities (such as phosphatides, etc.) are removed. In fact, oil from some pressing operations (such as olive or evening primrose) is suitable for direct consumption without additional processing. Removed fines are collected and typically recycled back to the press inlet.

A great number of variations may be used in the pressing operation. As indicated earlier, expanders may be used either before the press or, in some instances, after the press to agglomerate fines and provide consistency to the solvent extraction operation. Material entering the cooker may be flaked, as indicated above, or may be rolled seed with flaking operations taking place on the cake after the press.

## 5. SOLVENT EXTRACTION

After leaving the preparation process, the flakes (or collets) are delivered to the solvent extraction operation. As this process typically uses a flammable solvent (and is classified as a hazardous flammable environment), the operation is usually somewhat removed from other facilities, and access to the controlled area is restricted. Figure 5 illustrates the typical unit operations associated with solvent extraction, which include extraction, solvent distillation, and liquid-phase recovery. Upon discharge from the extractor, solid-phase extracted material is desolventized, toasted, dried, and cooled prior to meal finishing.

In the extractor, which is a countercurrent flow device, the solid material moves in an opposite direction of solvent–oil miscella with an increasing oil concentration. As the material to be extracted enters the unit, it is contacted with miscella at nearly full oil concentration. After this first wash, the miscella, containing around 25–30% oil, leaves the extractor for solvent distillation and recovery. After passing through the various washing stages, finally being contacted with fresh solvent and allowed to drain for a brief period, the extracted material, commonly known as white flakes, is removed from the extractor and is conveyed to the desolventizing process.

Several types of extractors are in existence today, with one of the most discerning differences being that of a deep- or shallow-bed philosophy. The rotary, or deep-bed extractor, operates largely in a semicontinuous fashion with a number of individual baskets with flake depths of 2–3 m being quite common. The baskets are initially filled with the flakes, which are supported on a drainage screen, allowing the miscella to pass but retaining the solidphase material. The screens can be either rotating or stationary, depending on the configuration of the extractor, as can the baskets and washing manifolds. As the rotary extractor is constantly moving at a slow speed, an empty basket is present at the filling mechanism just as the previous basket is filled. The operator adjusts the speed of the extractor to keep the baskets as full as possible, with typical total retention time in the extractor of 30–45 min. After final drainage, the screened basket bottom either drops, or in the case of the stationary

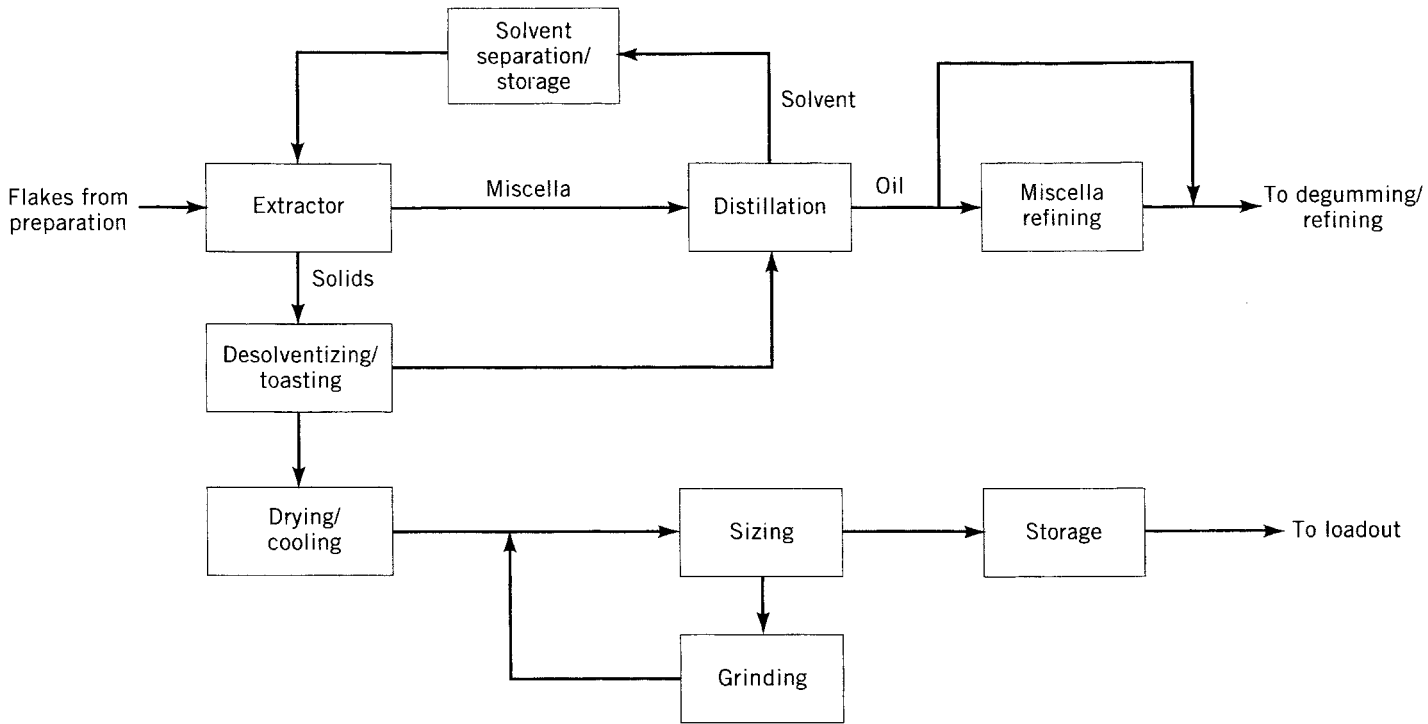


Figure 5. Solvent extracting/meal finishing.



basket bottom design, has an opening strategically placed to allow the solid phase to discharge. As the extracted material drops, the screen is closed, and the cycle begins anew. Extracted material is kept in a discharge hopper prior to conveying to the desolventizing equipment. In this manner, a continuous flow is provided to downstream equipment while the plug of material in the hopper helps prevent the possibility of desolventizer-toaster (DT) sparge steam from entering the extractor.

Shallow-bed extractors, with bed depths generally less than 1 m, provide similar drainage screens in the form of slotted bars. The oil-bearing material is continuously conveyed through zones in the extractor where the countercurrent washing of miscella occurs. At the discharge end of the extractor the white flake material is dropped into a hopper and is conveyed to the desolventizing system. Shallow-bed extractors have gained significant popularity in recent years and, as they lend themselves very well to automated control, are well suited for modern programmable logic control (PLC)-based operations.

Temperature control is extremely important during the extraction process. As extractibility is enhanced with high temperatures, the operator generally desires to keep temperatures as high as possible without flashing the solvent or creating excess pressure in the extractor. However, phospholipase enzyme activity affects the oil quality at these elevated temperature conditions, partially causing an increase in nonhydratable phosphatides. While this trade-off is generally not a problem, when processing field-damaged seed it may be necessary to reduce extraction temperature, sacrificing residual fats somewhat, in order to produce an oil that can be acceptably processed without undue refining losses. As mentioned earlier, several processes have been developed to help inactivate enzyme activity prior to extraction.

While hexane is widely accepted as the most effective solvent used today, there are concerns about its flammability, exposure, and environmental impacts. Research has focused on various alternative solvents in the hopes of finding one with acceptable performance while providing greater safety. Alternative solvents that have received some attention include isopropyl alcohol, supercritical carbon dioxide, and other fluids. However, no economical alternative to *n*-hexane has been accepted at this point, and the best available control technology emphasizes containment and limiting fugitive hexane emissions.

The extracted white flake material, containing around 25–35% residual solvent, is conveyed from the extractor to the DT. In the traditional system; steam is used to countercurrently flash the volatile solvent from the solid phase. The vapor phase then passes through the distillation system on its way to condensing and collection. The desolventized meal contains a high amount of urease activity (measured as a pH rise) that is detrimental for certain animal feeding purposes. Under conditions of heat, moisture, and retention time this enzyme is inactivated, and these variables provide the basis of control for the DT operation. After the desolventizing section, the solid-phase material passes through a series of steam-jacketed heating trays that provide the environment necessary for toasting.

The DT is one of the largest single energy users in the solvent extraction operation. The desolventizing process is also responsible for the bulk of solvent separation and recovery. As much of the DT steam is sparged in direct contact with

the meal, with the condensate not recoverable, much attention has been focused on the efficiencies and economies of this unit operation. New technology has been developed to minimize the amount of sparge steam and provide greater countercurrent contacting of the vapors with the meal. One extremely popular concept is the Schumacher DT, which provides true counterflow contact of the vapor and solid streams. Rather than perform desolventizing exclusively in the top section of the DT, the counterflow design allows maximum effective vapor contact with the material throughout the unit. Recent modifications to the counterflow DT have included addition of pre-desolventizing steam-heated surfaces prior to the DT inlet as an inexpensive way to improve the dry heat-sparge steam ratio.

After the traditional DT, the meal may contain in excess of 18% moisture and may be 100°C or hotter. As trading rules and practical handling conditions do not allow for these types of conditions, the meal must be dried and cooled prior to storage and loadout. Traditional drying methods have utilized a slowly rotating steam-tubed kilnlike dryer or other device with forced aeration to reduce the moisture to proper storage levels. After drying the meal still contains excessive heat, which is removed by another rotary kiln where cooling takes place either through indirect contact with cooling water or by exposure to large amounts of air. Not only is this equipment energy and maintenance intensive but introduces a number of environmental problems with particulate emissions even with high-efficiency cyclones. Wet scrubbing equipment has been introduced on some airstreams to collect much of the fugitive emissions, but the resulting high biological oxygen demand (BOD) loads in the scrubber effluent must be dealt with in an environmentally acceptable manner. Quite often this effluent is introduced into the plant meal or mill feed stream for moisture correction prior to loadout.

Using the same concept as the counterflow DT, the integrated dryer-cooler (DC) reduces energy consumption and combines functions into a single vertical stacked unit. Toasted hot meal enters the dryer section, where hot air is passed counterflow through the meal bed, removing excess moisture. The meal then passes through the cooler section, where the temperature is reduced by a countercurrent fresh airflow. The DT and DC functions can be further integrated in a single vessel (DTDC), not only reducing space and initial capital requirements but energy and operating costs as well.

Of obvious concern in any DT application is the potential for overtoasting the meal and adversely affecting the water solubility of the protein, often expressed in terms of the protein dispersion index, or PDI. For certain edible flour and other applications demanding a high PDI, the DTDC process may be replaced with a flash desolventizing system. These systems, which typically use superheated solvent to evaporate volatiles present in the freshly extracted white flakes, maintain the high PDI while desolventizing the feedstock.

After cooling, the meal is screened to separate large particles from those of acceptable size. This screener is typically either a multilayered inclined reciprocating bed screener or based around a continuously brushed cylindrical screen. Large meal particles (or balls) are ground in a hammer mill and resifted prior to being blended back into the meal stream. At this point, it is common to add mill feed,

the mixture of hulls and other materials removed earlier in the process and a flowability agent, such as calcium carbonate prior to storage. Meal storage can be provided by placement either in concrete or steel tanks and silos or, for longer storage period, a flat storage facility. It is important that meal placed in storage be low in moisture and well cooled. It is common practice to limit silo storage to 2–3 days, but longer storage periods have proven acceptable with occasional meal transfer, or “turning.” As the meal is loaded into customer transport, it is common to add water for moisture adjustment. For certain markets, the meal is passed through a pellet mill directly after the meal dryer. This not only increases the bulk density of the material, allowing for lower shipping costs, but also eliminates the grinding and sizing operations. Belt dryers are normally installed after the pellet mills to cool the product for storage and shipping.

Upon exiting the extractor, the miscella is passed through a series of distillation equipment to separate the oil and recover the solvent. The process usually involves a series of falling film evaporators and stills with the miscella on the tube side and vapors on the shell side. The first effect evaporator, using steam and solvent vapors liberated from the DT, concentrate the miscella from around 28% up to 80% or higher. The liberated vapors are condensed, sent to the work tank for water–solvent separation with the solvent reintroduced into the extractor. The miscella then passes to the second-stage evaporator, typically operating at atmospheric or vacuum conditions, where the miscella concentration is increased up to 95–98% oil. Finally, the miscella enters the oil stripper or still, which, operating at 50 mm Hg abs or less, removes most of the remaining volatiles. The oil typically leaves the extraction plant at this stage with moisture and volatiles (M&V) less than 0.15% or passes through a second oil dryer or “superstripper” to obtain a very low M&V oil. The oil is then cooled and sent to storage. It is extremely important to minimize moisture levels in the final oil, as phosphatides have a great affinity for residual moisture and will separate as gums from the crude oil in the presence of moisture. In fact, one processor considered introducing water in the oil leaving the processing facility and using its long-term storage tanks essentially as batch degumming vessels, decanting the top phase as degummed oil. In practice, this plan was rejected, as the heavy phase gums can be quite difficult to remove from transport vessels and storage tanks, and excessive deposition may result in rejection of an oil shipment. Moisture retained in crude oil can also increase the free fatty acids (FFA), and corresponding neutral oil loss, under certain storage conditions.

Miscella refining of some oils, especially cottonseed, has gained great popularity in recent years. In this process, caustic is added to the half miscella between the first and second effect evaporator, and the mix is subjected to centrifugation separation. The refined light-phase miscella continues on to the distillation system while the heavy-phase soapstock is blended back in the meal stream in the DT. Although there is a legitimate concern about locating high-speed centrifugal equipment in a hazardous environment, there are substantial benefits to be considered. A major advantage, especially with cottonseed, is that color bodies are removed before they are “set” in the oil by exposure to high-temperature distillation equipment. Not only is the oil of excellent quality, but refining losses are considerably lower

than the traditional methods. Other advantages include elimination of the water washing system, and a convenient disposal means for the soapstock. While the miscella refining typically requires a half miscella concentration of about 60% oil, with a resulting higher hydraulic load through the centrifuge, the difference in specific gravity between the two phases is substantial, allowing very high capacity through the centrifuge. Additional miscella processing has been practiced, including miscella bleaching and fractionation.

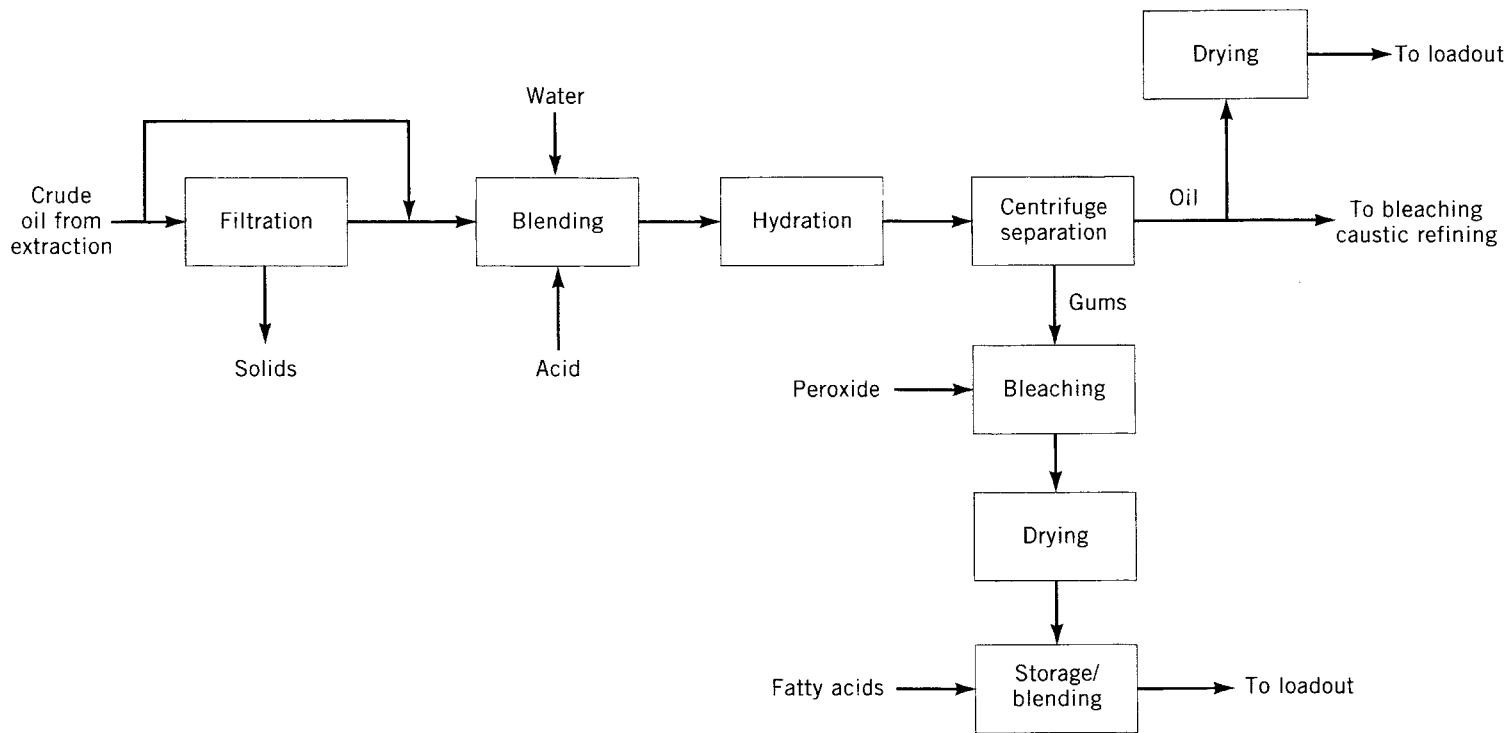
## 6. DEGUMMING, LECITHIN PROCESSING, AND PHYSICAL REFINING PRETREATMENT

A solvent extraction free, caustic refining free process for producing refined soybean oil has been described (7). Oils high in phosphorus, such as soybean, corn, and sunflower, may be degummed prior to refining. Degumming may be considered the first step in the refining process, especially for processors with an integral gums disposal option, and is designed to remove the phosphatides that interfere with subsequent processing. The degumming process is not a mandatory process, as the phosphatides can generally be effectively removed in subsequent processes. In fact, some processors prefer to refine crude oil than crude-degummed oil as a better “break” is obtained in some older style centrifuges (8). This may be especially applicable for solid bowl machines where an additional amount of “bowl flush” water is injected with the feedstock to facilitate removal of the heavy-phase material. Addition of this bowl flush not only increases potential refining losses but represents an additional hydraulic load on the plant acidulation system. With the advent of self-cleaning centrifuges, this bowl flush is not as critical, and coupled with increasing environmental concerns associated with soapstock utilization, the preference for crude over degummed oils may shift.

The fact remains that today the primary reasons for degumming are to either provide a crude-degummed oil suitable for storage or long transit, to prepare oil for physical refining, or to produce lecithin. Processing degummed oil does provide a side benefit for the stand-alone refiner lacking a lecithin disposal option. Phospholipids are an excellent emulsifier (which explains much of the demand for lecithin) and, when discharged as soapstock, introduce problems for oil–water separation in the acidulation process. Processors who experience problems meeting water effluent limitation without extensive waste treatment costs may be forced to specify degummed feedstock for this reason alone.

Traditional water degumming is effective only for water-hydratable phosphatides, those having a greater affinity for a water phase existence than remaining in the oil phase. However, significant amounts of nonhydratable phosphatides (NHP) exist that cannot be effectively removed without special treatments. The presence of significant quantities of NHP usually indicates a poor-quality oil, for soybean oil from fresh good-quality beans about 90% of the phosphatides are normally hydratable. However, when seed is severely damaged, the hydratable phosphatide may be reduced by as much as 50% over time (9).

Figure 6 illustrates a typical degumming operation integrated with lecithin processing. If lecithin is produced for edible purposes, the crude oil is first filtered to



**Figure 6.** Degumming and lecithin processing.

remove meal fines and other insoluble impurities. For straight degumming purposes (without lecithin production), the fines from a well-maintained facility are generally not a major problem for centrifugal removal. The oil continues to hydration, where a rationed amount of softened water is added to the oil, intimately mixed, and then introduced in a gently agitated hydration tank. During this retention period, the gums agglomerate and begin to separate from the oil phase. The mixture is then gently delivered to the centrifuges, with the light-phase oil passed through the moisture dryer, cooled, and finally delivered to storage.

When not used as lecithin, the heavy-phase gums can be reintroduced into the meal stream through the DT, adding energy to the meal and assisting with dust control. When sold as non-food-grade lecithin, the gums are simply dried from 35 to 50% moisture concentration and used either as a plastic, high-AI (acetone-insoluble) product, or blended with either degummed oil or distilled fatty acids for consumption as a fluid product. Gums used for food-grade lecithin may require color correction and after separation in the centrifuge are generally single bleached (using hydrogen peroxide) or double bleached (using benzoyl peroxide) in an agitated retention vessel. Bleaching agents can also be introduced in the oil hydration tank. It should be noted there have been concerns expressed about decomposition products associated with these bleaching agents, especially benzoyl peroxide, which prohibit their use for certain markets. After reaction with the bleaching agent, the phosphatides are introduced into the gums dryer. Divalent metals can also be introduced at this point to improve flowability. The dryer, which generally consists of a vertical or horizontal wiped film evaporator operating at less than 50 mm Hg abs, uses carefully controlled steam jacket heating to evaporate the moisture in the hydrated gums. After leaving the dryer, the lecithin is slightly cooled, and depending on the quality of the product, may be sold as plastic product (AI > 65%) or blended with degummed or refined oil, and sold as a fluid product. Oil or distilled fatty acids may be added to reduce the viscosity and increase the lecithin acid value. Special attention must be paid to the design of the lecithin storage and handling system as the products are very viscous and potentially hard to handle. Storage tanks and lines are generally jacketed with low-pressure steam or hot water to ensure flowability without overheating and darkening of the product. Lecithin is usually sold in drums, pails, or truckload lots.

Production of a clear and sparkling lecithin product is highly vulnerable to effects caused by upstream conditions. Field- or storage-damaged beans can result in a cloudy, dark lecithin that may be unacceptable for use as edible product. The quality is also highly affected by grain drying, conditioning, extraction, and oil distillation temperatures and exposure times. Lecithin periodically seems to develop a cloudy haze, which is extremely difficult to remove using traditional methods. It is postulated that this haze is a form of sugar, starch, or other material extracted from the oilseed. Lecithin from plants using expanders seem to experience this phenomenon more frequently, possibly related to greater extractability of collets.

Lecithin may also be deoiled and sold as a granulated product high in phosphatyl choline. This process typically involves an acetone extraction, leaving a granulated product for packaging.

When the production of lecithin is an objective of degumming, maximum removal of phosphatides from the crude oil becomes a primary concern. It is interesting to note that a significant economic benefit is provided by producing lecithin, not only because of manufacture of an added-value product but also because of its impact on the meal. In a protein control meal situation, approximately 1.25 kg of mill feed can be added for every kilogram of gums diverted from the meal stream. This is due to the protein content of mill feed and may provide as much of the justification of a lecithin production operation as the increased value of the lecithin itself. The effect of producing lecithin is negative, however, in a fiber control situation. When lecithin is not produced as part of a degumming operation, then a controlling factor usually becomes the maximum level of phosphorus permitted in the degummed product. Generally the processor has little trouble removing sufficient gums to meet the trading rules limits, as most of the gums are water hydratable. There are times when simple water degumming is not sufficient as there are, as indicated earlier, several factors that affect the amount of nonhydratable phosphatides in the crude oil. In addition, physical refining continues to gain popularity, and the degumming system is called upon to produce a lower phosphatide oil than normally possible with a traditional water hydration system. These factors have resulted in considerable new approaches for more complete phosphatide removal in the degumming operation. Lecithin recovered from solvent extracted soybean oil had different phospholipid class compositions from those produced by mechanical pressing (10).

Several acid treatment and other processes have gained acceptance in producing a lower phosphorous degummed oil. These processes, including Unilever's super degumming, the TOP process, total degumming, and others, focus on the fact that calcium, magnesium, and iron salts of phosphatidic acid have a greater affinity for the oil phase than the water phase and as such must be removed from the oil by a special process. Pretreatment of oil with phosphoric acid, citric acid, or another agent with proper temperature, time, and agitation conditions, followed by a water hydration as described above, is generally effective in removing phosphatide containing components. Silica absorption processes are also effective in precipitating these phosphatides. Use of these pretreatment systems can produce a degummed oil that when properly bleached will have phosphorous levels less than 3 ppm, although these processes are generally not as flexible or "forgiving" as a traditional alkali refining process. Oil of this quality is generally acceptable feedstock for physical refining, which is discussed later in this Chapter.

As acid degumming has proven to be cost effective, many variations have been developed to improve the consistency of its results. One method, especially developed for canola, suggests introduction of a small amount of dilute caustic after acid pretreatment immediately prior to the degumming centrifuge. The system is said to produce an oil of equal quality to caustic refined oil without the problems of soap generation and requirement for subsequent water washing (5). While super-degumming systems do have advantages both in terms of capital and operating cost reductions, the impact on the gums must be considered. The lecithin resulting from an acid pretreatment system is usually darkened in color and generally considered unsuitable for edible purposes. Wet gums from an acid-treated degumming

system should be neutralized before introduction into the meal stream. Finally, as many degumming and lecithin processing installations are located in the extraction plant, there is concern about the safety of storing the acid (and peroxides for lecithin) in an environment containing flammable solvents.

While the concept of wet degumming usually involves the processes described above, there is an alternate process called dry degumming (as pretreatment for physical refining) for lower phosphatide oils, such as coconut. This process is usually integrated into the bleaching operation and involves introduction of acid, usually phosphoric, with either a brief retention high temperature, high shear retention, or a longer retention lower temperature, less vigorous agitation system. The acid and precipitated phosphatides are removed in the subsequent bleaching operation.

While the continuous degumming operation has become the standard for most operations, some older and specialty processors practice batch degumming. This operation, which is frequently combined in a single vessel performing sequential degumming, neutralization, and bleaching, involves hydration, settling, and decanting the degummed oil from the gums. While initial capital costs of batch operations may be less than a continuous process, the operating costs of labor, chemicals, and neutral oil loss make batch processing unfeasible for all but the smallest operations.

Degumming is an integral part of the physical refining operation, which will see continued growth in response to environmental pressures. While the basics of the degumming process have been largely unchanged for several years, new membrane separation systems have been gaining recent attention (11). Past problems of plugging may be solved by new filter media, miscella filtration, or evolution of self-cleaning designs.

## 7. CAUSTIC REFINING

*Refining* is the term liberally applied to the processes designed to neutralize free fatty acids present in the oil by introduction of an alkali and centrifugal separation of the heavy-phase insoluble material. Refining is also associated with removal of phospholipids, color bodies, and other soluble and insoluble impurities. The term refining can be applied to physical and chemical operations, as they both are designed to perform much of the same tasks. As physical refining operations are generally incorporated into degumming, bleaching, and deodorizing systems (also known as steam refining systems), descriptions of these processes are integrated into discussions of these areas. Miscella refining shares many similarities with chemical refining but has specific considerations as described in the extraction section of this Chapter. The focus of this section will be on the chemical, or alkali, refining process.

Caustic refining in particular does not only effectively perform the separation functions described above but is considered “more forgiving” in operation than alternate physical methods. If the degumming operation has been less than perfect (or is not used), alkali refining will remove the bulk of the phosphatides. If a high amount of metals, particularly calcium and magnesium, are present, these can be



removed in the chemical neutralization process. Caustic refining is also less sensitive to the type of feedstock presented, as a system designed for one oil will generally produce satisfactory results with other oils. Caustic refining does have its substantial downfalls, however, which have led to development of the alternate processes, such as physical or steam refining.

If degumming is not included, caustic neutralization is the traditional first step for edible oil processing. It is important to remove the impurities at this stage of processing as the oil when heated in subsequent steps may turn dark, smoke and foam, or become cloudy from the precipitation of solids. Figure 7 provides a block diagram description of the operations associated with the typical continuous long mix refining process. As the crude or crude degummed oil is delivered from the extraction plant or crude oil storage facility, the oil is usually measured, cooled, and injected with a rationed amount of phosphoric or other acid. The acid is used to facilitate removal of nonhydratable phosphatides, and the mixture is generally gently agitated in a day tank for 8 h or more before further processing. The processor should minimize the use of excessive acid treat, as it is rather aggressive on piping and equipment, and must be neutralized with alkali. It would be desirable to accurately measure the amount of nonhydratable phosphorous in a given batch of oil and adjust the acid treat accordingly, which may be possible with in-line DCP/ICP analysis equipment. However, the operator generally assumes a phosphorous level based on historical operating data and provides an excess over theoretical to cover any variances. After determination of FFA levels, the oil is pumped through temperature correction exchangers and is injected with a rationed amount of temperature-controlled dilute alkali solution, normally caustic soda. Preparation of the dilute neutralizing solution may be accomplished by preparing a batch of heavy caustic and softened water to a known specific gravity and temperature (16–24 Bé is common for most seed oils). The solution may also be prepared using a series of mass-flow equipment measuring precise flow and densities of solutions. This method is very attractive when coupled with ICP and computer control systems. When determining the treat amount, the operator must consider the pretreatment acid affects the FFA titration test, and must be adjusted for accordingly. The total treat is based on the theoretical amount of alkali to neutralize the FFA plus an excess to remove other impurities. The minimum amount of excess alkali must be used to perform the job while minimizing the saponification of neutral oil. The oil–alkali solution is thoroughly agitated to assure intimate contact normally using an in-line high-shear mixer or static agitation devices. The flow of both caustic and oil must be carefully controlled at this point as variations in flow can give variations in the mixture densities in the centrifuge, seriously affecting the separation efficiency (12). Both the oil and caustic should be cooled to less than 38°C.

While the saponification reaction between the caustic and FFA is nearly instantaneous, the mixture requires some time for the excess caustic and water to hydrate the phospholipids and to react with color pigments. A notable exception is the refining of certain lauric oils, where, if not physically refined, the oil and caustic are mixed immediately prior to entering the centrifuge. For other oils, sufficient mixing with mechanical agitation must be provided during this period, but this agitation

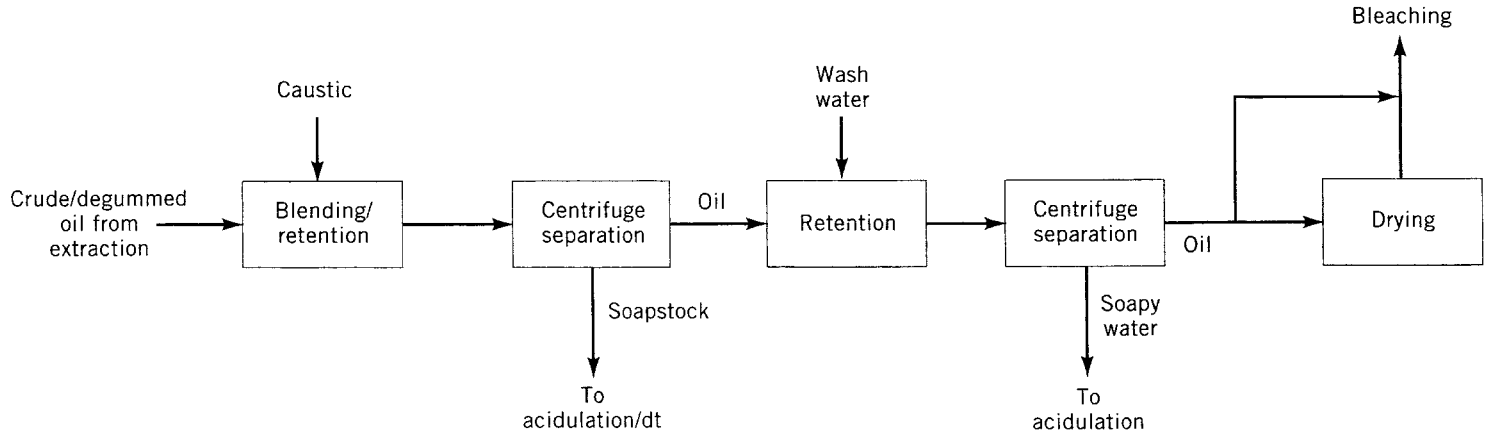


Figure 7. Caustic refining.

must not be sufficiently turbulent to create stable emulsions that will not separate in the centrifuges. The proper retention period is usually provided by zoned mixers that are generally multiple units provided in series for maximum flexibility in processing. For example, while soybean oil may require 5 min or longer for the reaction to be completed, less retention is beneficial for corn oil processing (13). After leaving the retention mixers, the mixture is heated to reduce viscosity and to provide a more definite separation of the soap and the oil. While the optimum separation temperature must be determined on an individual plant basis, practical experience suggests that this heating duty is not a good application for an oil-oil interchanger. While the energy balance between this service and the hot-water-washed oil certainly looks favorable, it has been observed that separation efficiency may suffer if the oil is not subjected to the high-temperature gradient provided by steam. After the heater, the mixture enters the primary refining centrifuge. Centrifuges are generally of the paring disk, hermetic or semihermetic type, where changes of the zone of separation occur as the light-phase discharge back pressure is adjusted. Increasing the back pressure reduces the soap content in the oil phase but allows more neutral oil to exist with the soapstock, while decreasing the back pressure has the opposite effect. The centrifuge operator generally sets the proper back pressure by viewing a slight turbidity in the light-phase discharge sight glass or tests for separation efficiency of the refined oil with a table-top separator. While dramatic zone changes can be made by back pressure control in a hermetic machine, more substantial changes in the separation zone may be made by changing the centripetal pump in the pressure-type centrifuge. Neutral oil in soapstock should be 18% or less on a dry basis, while refined oil soaps in excess of 500 ppm indicate back pressure should be increased. If soaps cannot be effectively reduced through back pressure control, the centrifuge probably requires cleaning.

From the primary, the heavy-phase soapstock enters the acidulation system or, in some instances, is introduced back into the meal stream. Care must be taken with reuse of this material, as the high pH should be neutralized prior to livestock feeding, and the high moisture and fat concentration will affect feed formulation. The light-phase refined oil discharged from the centrifuge is generally heated, mixed with 10–15% hot water and the mixture subjected to an intimate mixing. To maximize the adsorption of soaps, the oil-water mixture should pass through another retention zone mixer, again with sufficient but gentle agitation to avoid emulsification. In some facilities, a water-wash holding tank is provided for this retention period. This tank is not only designed to provide proper mixing and residence time but allows a “wide spot in the road” to avoid affecting the line when the primary centrifuge “shoots” or otherwise disrupts product flow. Phosphoric acid can be added in the washwater to reduce the residual soap in the refined oil, and to provide a better split between the oil and aqueous phase. While common in double-wash systems, it is thought that at least a portion of the water from a single-wash system can be reused, not only reducing hydraulic loading on the waste treatment plant but also maximizing thermal efficiency. At least one processor uses a portion of this soapy water for bowl flush water. For optimum soap removal, the oil-water mixture is heated before entering the waterwash centrifuge. This

centrifuge generally reduces the residual soap by a factor of 10:1, with soap concentration in the oil typically less than 50 ppm. After the washing machine, the oil may be sent to the vacuum dryer, where the residual 0.5% moisture is removed. With some designs, an oil dryer may not be provided, as residual moisture enhances absorption efficiency of the certain bleaching agents. Special care must be taken when storing this wet oil, although most of the natural preservatives are still present at this point of processing. At a minimum, the oil is cooled prior to storage, and some installations may begin nitrogen blanketing at this point.

As indicated earlier, the long mix system requires a certain residence time to allow the caustic to react with certain components in the oil. This system, which has been the standard in the United States for years, is especially well suited for removal of gossypol from cottonseed oil and phosphatides from soybean oil. This system is quite common in large refineries with few stock changes per day. In Europe and developing countries, the move toward larger, continuous plants took longer, and the short mix system was developed to provide greater flexibility and stock change ability. In this process, the zone mixers are generally not used, pretreatment agents are introduced into hot oil, and the hot oil and caustic are intimately blended in a high-shear mixer and immediately introduced into the primary centrifuge. The advantage of longer retention times during the reaction between oil and caustic are becoming more apparent to many seed oil processors. The long mix system is therefore gaining ground with many traditional short mix processors, especially those working with soybean and canola oil (5). It is also common practice in some of these facilities to provide a double water washing, with the water phase discharge of the second centrifuge used for makeup water for the first washing machine. While this does represent a potential savings in water consumption, this practice has generally not been adapted in the United States, as the soaps from the primary centrifuge tend to be lower and may be more easily reduced than with the short mix operation (5).

Modern refining usually involves the continuous operation with centrifugal separation as described above. Refining originally evolved from batch operations, which are still used in some small and specialty operations, and involve much of the same process techniques with separation of the heavy phase performed by settling and draining in the vessel. One serious problem, especially with nondegummed oils, is the creation of the stable emulsion layer. It is common to add a brine and other solutions as part of the batch process to help break this emulsion, and a double (or triple) washing is also common. As one may expect, batch refining is highly labor intensive, introduces significant environmental problems, requires several hours to process a batch, and incurs significant losses. For these reasons even small specialty processors are encouraged to consider continuous refining operations.

An interesting trend in alkali refining is adaptation of acid refining operations. One design optimized for canola introduces acid into the oil through a zone mixer immediately prior to caustic addition and a second zone mixer. This system is said to significantly reduce the refining loss and improve the color. Another interesting trend is close integration of refining with the bleaching operation. As indicated earlier, it is now common practice to eliminate the refined oil dryer, leaving the

residual moisture in the oil to enhance bleaching. A further development is the elimination of the water wash step, using instead hydrated silica or other materials to adsorb the soaps and residual phosphorus during bleaching. This topic is covered more fully in the bleaching section of this Chapter.

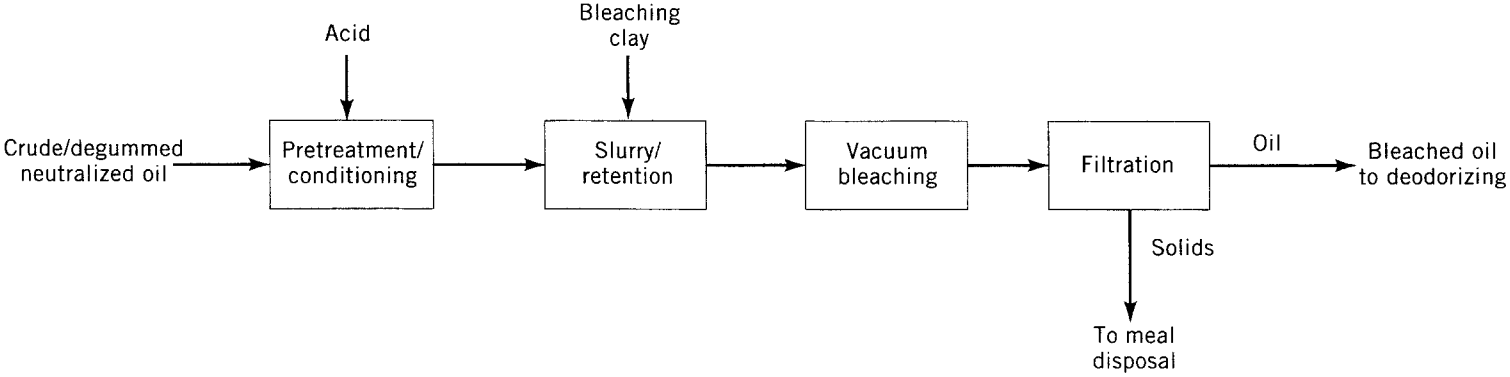
Chemical refining is responsible for a great amount of processing loss (and resulting environmental problems) associated with oilseed processing. For that reason, a great deal of emphasis has been placed on automation and loss monitoring of the process streams. On-line instrumentation to determine FFA, phosphorus, and other process parameters is becoming increasingly feasible. DCP/ICP instrumentation, which although expensive, may prove to be justifiable with improved process control. To date, most control efforts have focused on loss monitoring, measuring the flows of crude, refined, and washed oil, and controlling the corresponding acid, caustic, and water flow addition rates with mass flow devices. Once acceptable losses have been established, the operator is notified if upset conditions are encountered.

While physical refining will continue to receive attention, alkali refining is likely to continue to be the preferred choice where soapstock disposal issues can be resolved economically. Unlike some physical refining systems, alkali refining allows the processor to properly prepare almost any type (and any condition) of oil to produce a quality product. Alkali refining introduces its special set of maintenance, environmental, and neutral oil loss issues for which the processor must continually be alert.

## 8. BLEACHING

Bleaching is the term given to describe the adsorptive cleansing process associated with edible oil refining. This process, as shown in Figure 8, may involve acid pretreatment, introduction of and a retention period with a bleaching agent, and removal of the clay and absorbed materials. While the mechanical process is on the surface rather simple, the importance of the bleaching operation commands significant attention.

Bleaching plants have evolved from open batch systems to continuous processing operations with alternating filters used for clay removal. In practice, oil to be bleached may be pretreated with acid (known as the dry degumming process for crude oils), and after a sufficient residence period, the oil may be dosed with the clay and other agents in a slurry tank. The materials may be introduced in a slip-stream of the oil with the slurry immediately directed back to the main flow of oil or may be introduced in a tank designed to hold the entire flow of oil under nitrogen-blanketed conditions for several minutes. Other materials may also be introduced at this point, such as activated carbon for canola and other oils and filter aids. After leaving the slurry system and prior to entering the bleacher, the oil may be heated to bleaching temperature. Under vacuum conditions, the oil is agitated in the compartmentalized bleacher for several minutes and then delivered to one of two bleaching filters for removal of solid materials. While plate and frame filters may be used for



**Figure 8.** Bleaching/dry degumming.

some applications, the pressure leaf filter is by far the preferred choice for modern installations. The filters are available in either a vertical or horizontal configuration, depending on plant preference, and can be fully automated by using a plant PLC system. During the filtration cycle, as a filter becomes full, as indicated by an increasing pressure differential between the feed and discharge streams, the filter is taken off-line for cleaning while the alternate filter is brought on-line. After removing the residual oil from the filter cake (normally using steam and nitrogen), removing the cake from the filter screens is accomplished by vibrating the screens. A horizontal filter with cake discharge by leaf rotation was introduced several years ago. This design has the advantage of a lower space requirement for bundle withdrawal and has a reported higher cake retention and lower residual oil. However, the drawbacks of higher mechanical complexity and costs may be limiting factors. Regardless of the filter type, the filtered bleached oil passes through a polish filter to remove any traces of solids, and is then cooled prior to storage. As many of the natural antioxidants are removed during bleaching, many processors elect to nitrogen blanket the oil after the bleaching process.

Several modifications to traditional bleaching plant designs have been introduced in recent years. Steam agitation has been used as an alternative to mechanical agitation and bleaching materials have been introduced directly into bleaching vessels without preslurrying the oil in a separate slurry vessel. One interesting concept is to eliminate the traditional retention bleacher, and provide retention time for the clay-oil slurry through a series of specially designed pipes. After the retention period a flash vessel is used to drive off moisture in the oil prior to filtration. Another recent development is introduction of a new filter screen, which is said to have a longer life and eliminate the need for diatomaceous earth precoating (5).

While the degumming operation is designed to remove phosphatides and the caustic refinery converts the soluble free fatty acids to insoluble soaps, adsorptive bleaching provides the processor the last practical opportunity to remove remaining impurities to acceptable levels. While the traditional control for bleaching has been color reduction, the modern processor does not simply use the Lovibond scale to determine the effectiveness of the bleaching operation. While color correction is an important consideration, modern bleaching operations provide even greater benefits.

In addition to modifications to equipment and designs, development of adsorbents and modification of processes to optimize the adsorption has received considerable attention. As indicated earlier, color reduction was the traditional indication of the effectiveness of the bleaching operation. The major color pigments in edible oil are chlorophyll (green) and carotenoids (orange) and while chlorophyll must be reduced in the bleaching process, carotenoids can be reduced in later processes. For high-chlorophyll seeds, such as canola, bleaching clay dosing is quite heavy and is sometimes augmented by the addition of activated carbon or other agents. Carotenoid elimination typically occurs in hydrogenation and deodorizing, sometimes called the *heat bleach effect*. While color correction is certainly important, bleaching provides the last opportunity to remove remaining phospholipids. Not only must phosphatides be minimized for proper deodorizing, but these also affect

hydrogenation selectivity and activity at levels down to 4 ppm (14). Residual soaps created in caustic neutralization should also be removed by the bleaching adsorption process. Not only can these soaps cause polymerization in the deodorizer, affecting not only operation but quality of the oil, but they can also interfere with hydrogenation (15). Products of oxidation, both primary (peroxide value) and especially secondary (anisidine value), should also be removed during bleaching to provide a suitable shelf life oil. Finally, trace metals, especially iron and copper, should also be removed in the bleaching process. While citric acid chelation in the deodorizing process will help inactivate the catalytic oxidative tendency of these metals, it is more desirable to remove them as early as possible in the process. Clearly, while color is an important indicator of bleaching effectiveness (and is easy to check), residual soaps, phosphorus, peroxide, and anisidine values are also some key quality indicators associated with the bleaching process.

As an enhancement to bleaching efficiency, silica gel is commonly added to remove soaps and phospholipids before exposing the oil to bleaching clay. In practice, the clay normally slurried continuously with the oil is applied on the filter leaves immediately after precoating. The silica gel is continuously added in the body feed slurry system and is especially effective when contacting the oil at atmospheric conditions with residual moisture from the water washing step. As indicated earlier, many processors do not use the refinery dryer, leaving the residual wash-water moisture in the oil to enhance this effect. For dry degumming systems, water is sometimes added with the pretreatment acid to increase the crude oil moisture content. After reaction with the silica, the moisture is removed in the vacuum bleacher prior to exposure to the bleaching clay, which has been preloaded on the filters. An interesting alternative to acid and alkali refining has been developed by W.R. Grace & Co., using its silica gel product. As this material has a higher ability to adsorb soap and phospholipids than traditional bleaching clays, it has been used to eliminate the water washing step altogether. This not only reduces the capital costs associated with refinery equipment but significantly eliminates hydraulic load on the plant waste treatment operation. As an increased amount of silica is required for this adsorption service, any savings must be balanced against the silica cost and those associated with the increased cake oil loss, solid waste disposal, and reduced filter cycle time. However, most processors find this economic balance positive, and more activity in this field can be expected.

An unfortunate consideration associated with bleaching is the generation and subsequent disposal of the spent cake. Not only does the residual oil in the cake represent a loss to the processor, but spent cake is prone to spontaneous combustion under certain conditions when exposed to air. For that reason, spent cake may be classified as a hazardous material, making its environmentally responsible disposal difficult. The traditional landfill option may be restricted, not only because of this classification but also because of the limited space available at many locations. Spent cake can be added to meal in some cases, but this practice is frowned upon, especially when processing multiple types of oil. While deoiling the cake does help reduce the risk of combustion (and work is underway to reuse some of this material), most emphasis will be focused on alternate uses for the spent clay. Some

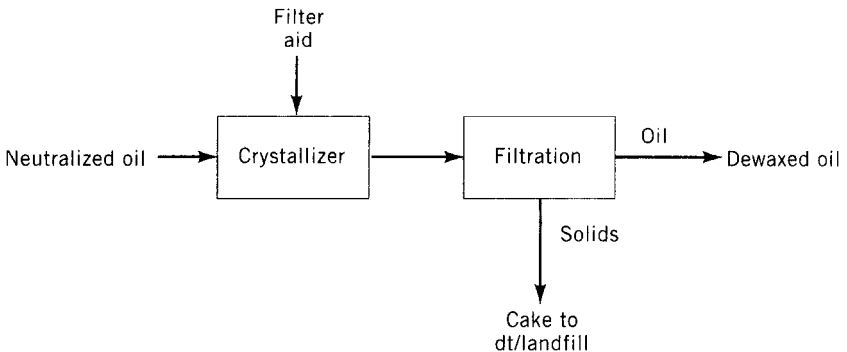


concepts being explored include use as asphalt additive, replacement for plastic parts in refractories, re-refining mineral oils, road foundation, soil stabilizers, and lightweight aggregate applications (16).

## 9. DEWAXING

Dewaxing refers to the removal of high-melting-point “waxes” extracted from certain oilseeds, such as corn, sunflower, and canola. While the wax usually does not negatively affect the functionality of the products, the presence of wax affects the appearance of the product. As products sold as salad oils are often packaged in clear bottles, such as PVC or PET, a haze, and possibly a wax deposition, may form over time when exposed to conditions of the modern supermarket. If refrigerated, this effect will become more pronounced and the end user, not understanding melting point characteristics, will assume the oil is of inferior quality. Consequently, dewaxing has become a process tool in the integrated refinery where waxes are removed by a chilling, settling, and separation process.

Figure 9a depicts a traditional dewaxing process that continues to be used for oils with wax content less than 500 ppm. The refined oil is cooled, and a proportioned amount of filter aid is introduced into the chilled oil. The mixture then enters a gently agitated holding tank (using either mechanical or chilled compressed air agitation), where the wax crystals begin to form. After a certain temperature-controlled residence period, the oil may be transferred to a second maturing tank that may or may not be agitated. Another type of dewaxing system that has proven effective is to simply and quickly cool and filter the oil without use of crystallization tanks. With either system, the solution is then gently transferred to a pressure leaf filter, where the waxy cake is removed. To facilitate filtration, and reduce the viscosity of the solution, the oil may be heated slightly in a laminar flow heater prior to introduction into the filter. When the filter is full, the waxy cake is discharged either to a landfill or, in the case of the integrated facility, transferred to the meal stream. For certain applications, the waxy cake can be slurried with hot



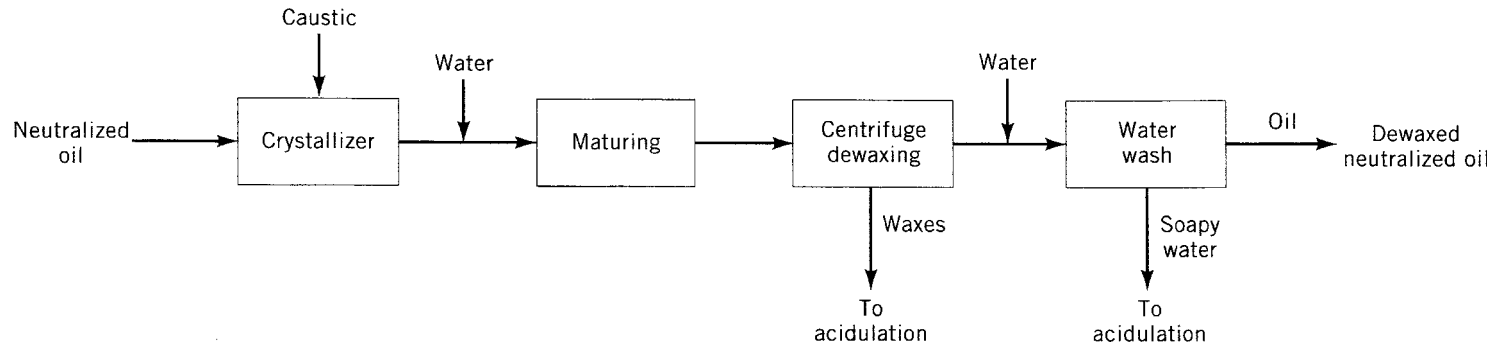
**Figure 9.** (a) Filter dewaxing.

soybean or other oil, and the filter aid removed in a small pressure leaf filter. The oil-wax mixture may be used as hard oil basestock, while the filter aid may be reused for less critical filtration services.

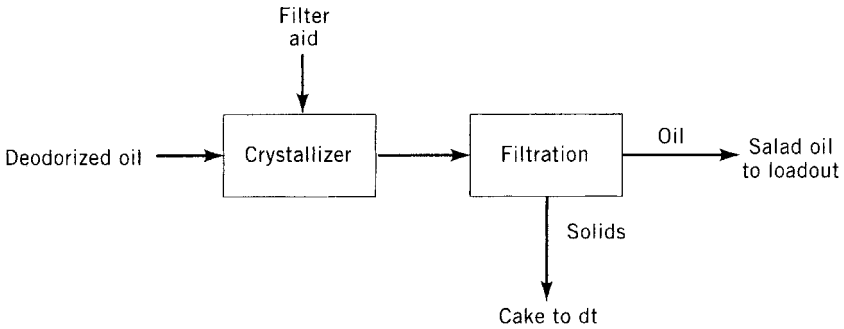
Several years ago, an alternate process was developed using centrifuges to process high-wax-content oils. The process is similar in purpose to the traditional filtration process, but as shown in Figure 9b, it has some significant differences. In this process, the alkali-neutralized oil is chilled and may be treated with additional low-concentration caustic added after the primary centrifuge, increasing the soap content. The slurry is introduced into the first slowly agitated crystallizing tank where the wax agglomerates around the soap particles. After a residence period, the mixture flows to a second maturing tank, with a small amount of chilled water introduced as it enters the tank. After a certain period, the oil may be gently pumped through a laminar flow shell and tube heater, increasing the oil temperature prior to centrifugal separation. The centrifuge removes the bulk of the waxes, and delivers the oil to the hot-water washing equipment as described in the refining section. If capital is limited, a variant of the system is to perform both the dewaxing and water washing step in a single machine. Because of the high losses involved with cold centrifuging, this process has proven to be the least cost-effective approach for all but the smallest processors. Another variant is a combined cold neutralization and dewaxing centrifuging after degumming. In this system the oil is chilled before the caustic is introduced. The reaction is done at a low temperature with very long retention times, but uses little (if any) caustic excess. Excellent quality results have been reported, but losses are relatively high, the capacity of the centrifuge is greatly reduced, and the soapstock is difficult to split (5).

While centrifuge dewaxing is excellent for high-wax oils, this method does not always ensure sufficient material removal for a cloud-free oil. Residual wax content from centrifuge dewaxing systems may be generally around 50 ppm, which may or may not provide a clear oil. One frustration experienced with high-wax-content sunflower oil is that changes in the oil clarity are not necessarily indicated by the wax content or cold test results alone. It has been noted that at times, oil from centrifuge dewaxing will be clear and brilliant while at other times oil with a similar residual wax content will cloud at room temperatures. It has also been noted that despite passing a 24-h cold test, oil may develop a haze after a few days of storage at ambient temperatures in a clear bottle. To help reduce wax content, one variant of the system described above is to perform cold-water washing after the dewaxing centrifuge. While this does reduce the wax levels by another 10–20 ppm over standard centrifuge dewaxing, the cold washing process reduces washing machine capacity by about one half, oil losses are higher, and even then there are periods when the oil clouds. It has been observed, however, that with very low wax contents oils (around 10 ppm) almost always remain clear.

One method used to assure clear oil is to add a polish filtration step after the centrifuge dewaxing operation, as shown in Figure 9c. With this system, a slip stream of oil is cooled and analyzed through a highly sensitive turbidity meter immediately after the deodorizer. When a haze is detected, the flow of oil is diverted through a chiller, filter aid added, and the mixture sent to a crystallizing tank. After



**Figure 9. (b) Centrifugal dewaxing.**



**Figure 9.** (c) Polish filtration.

a proper maturing period, the oil passes through a pressure leaf filter, as described in the traditional system. When the deodorized oil clears, as indicated by the sample passing through the turbidity meter, the oil is again diverted to storage, bypassing the polish filter until a haze again develops.

There is no doubt that polish filtration adds additional cost and processing loss to the operation. One variant of this system is to place the polish filter directly after the dewaxing centrifuge and avoid multiple cooling/heating steps. While this is efficient from a thermal (and initial capital investment) standpoint, the residual moisture in the oil may interfere with material deposited on the cake. To address this concern, a special precoating procedure has been developed that may help alleviate this problem. A second concern dictating the location of the polish filter is that it is postulated that the haze may not be truly a wax but may be a combination of products of polymerization and other materials created during the bleaching and deodorizing process. Polish filtration as the last processing step for salad oil productions appears to be the best use of all resources. The design of systems used after the deodorizer is very critical as any contaminants introduced cannot be removed without redeodorizing the product.

Certain types of equipment may be best suited for dewaxing service. While plate and frame exchangers may be considered for oil cooling, the deposition of wax occurs very rapidly when this oil is exposed to cold surfaces. The processor will find a single unit fouls rather quickly, and having a second cooler readily available will facilitate operations. Scraped surface heat exchangers are excellent for this duty but are considerably more expensive. While self-cleaning centrifuges are generally preferred for refining service, an argument can be made for using solid bowl machines in dewaxing service. While vertical pressure leaf filters can and have been successfully used for dewaxing service, most processors prefer to use horizontal design filters. The waxy cake is difficult to remove and at times must be scraped from the filter leaves. Some facilities are equipped with membrane-type filter leaves, which squeeze residual oil from the waxy cake before discharge, improving yield but demanding higher capital costs.

One of the most important factors affecting the operation is the wax level in the crude oil. Many processors are fortunate to dehull the seed prior to oil extraction,

and as over 90% of the wax occurs on the seed (17), the problem is reduced at the source. For those extracting the nondehulled seed, it is not uncommon to experience wax levels in excess of 3000 ppm in the solvent-extracted crude oil. Unfortunately, the trend is toward increased wax levels, as the hulls from high-oil-yield identity preserved hybrid seeds have significantly higher wax content than less developed seed species. Process control is very important to a successful dewaxing operation, especially those installations using centrifuge separation. In order to provide effective crystal growth, phosphatides must be fully removed from the oil before entering the crystallizer vessels. Phosphorus interferes with crystal growth (in fact a variant of lecithin is sold to inhibit haze formation), and additional pressure is placed on the degumming operation to remove both hydratable and nonhydratable phosphatides. Soap and moisture levels must be carefully controlled, as must temperatures, retention times, and flow rates.

## 10. HYDROGENATION

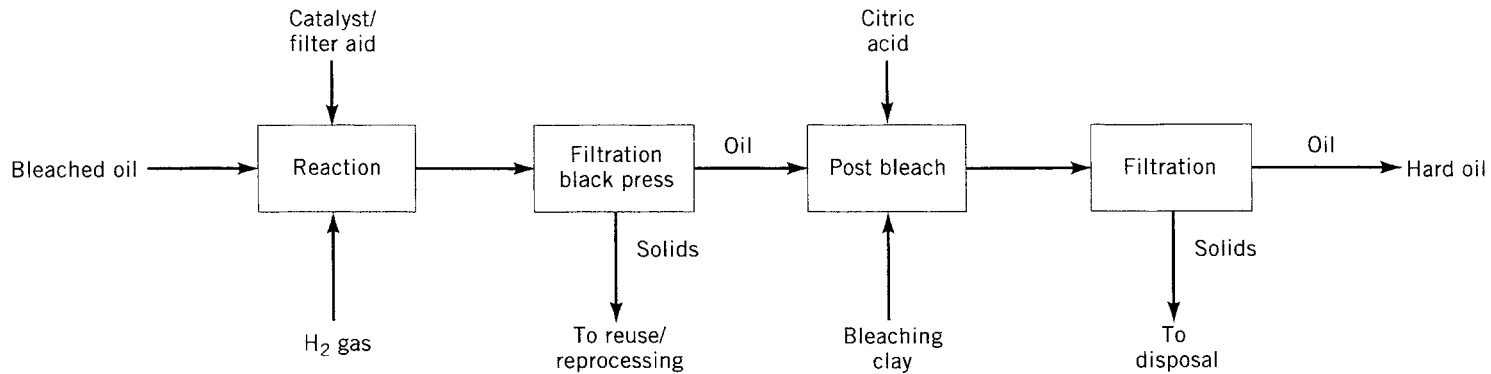
While the processes discussed so far are mainly concerned with filtration, purification, and removal of soluble and insoluble impurities in the oil, hydrogenation involves an actual transformation of characteristics and properties of the oil through chemical reaction in the presence of a catalyst. Hydrogenation allows the processor to tailor the fat system for very specific applications and functions and is one of the key functions of a value-added operation. Hydrogenation was developed to allow vegetable oils as margarine and vanaspati products to substitute for butters and ghees, and this market provides a huge outlet for much of the edible oils produced today. Hydrogenation is generally performed for one of two specific purposes. The first is to provide taste and smell stability and to enhance the shelf life for unsaturated products. The second is to change the functional characteristics of the naturally occurring fats to those required for a specific application. Hydrogenation allows the baker and others to utilize margarines and shortenings while retaining the functionality of products prepared with traditional lards and butters.

Simply stated, hydrogenation is designed to saturate (to the degree desired) double bonds in the fatty ester of the triglyceride molecule. Hydrogenation (and to a certain extent conditions found in deodorizing) also promotes isomerization of the *cis* orientation to the *trans* position. While the fatty ester may contain from 12 to 22 carbon atoms and have a range of 0–3 double bonds, the most prevalent fatty ester of seed oils has a chain length of 18 and has a maximum of 3 double bonds in any given chain. The common names of these esters, based on the number of double bonds, are linolenic (3 double bonds), linoleic (2 double bonds), oleic (1 double bond), and stearic (fully saturated). The number of double bonds greatly affects the fat properties, as does the orientation of the isomer. For example, the melting point of a linolenic ester will be  $-13^{\circ}\text{C}$  while the stearic ester will melt at  $70^{\circ}\text{C}$ . A *cis* isomer oleic may have a  $16^{\circ}\text{C}$  mp while the *trans* isomer oleic may have a  $44^{\circ}\text{C}$  melting point (18). Clearly, these wide swings in properties are of great interest to the modern food processor.

Figure 10 depicts a typical hydrogenation system in use today. This flow-sheet shows a dead-end reactor, as it is known, which traditionally has been an industry standard. In operation, bleached oil is pumped from a holding tank with known characteristics, including phosphorus, soap, and initial iodine value (IV), to the pre-heating equipment. As the oil must be hot (typically 140°C or higher) before a reaction commences, heating may be performed either externally or internally. In the system illustrated, the feed oil is preheated with reacted hot oil to be filtered and then is introduced into the reactor or a fill tank for final steam heating. During final heating, the head space of the reactor is evacuated, removing not only any moisture present in the system but also eliminating any gasses that may interfere with the reaction. After the oil has been evacuated, and is at initial reaction temperature, catalyst slurry is introduced into the reactor. Based on the desired properties of the batch, the operator normally consults a “recipe book” to determine the reaction temperatures and pressures, and calculates the required amount of catalyst and hydrogen gas (usually estimated at 1 standard cubic meter per metric ton per IV drop). The required values are normally entered into the corresponding measuring and controlling equipment. After a brief period for agitation of the catalyst, hydrogen is introduced into the reactor.

During reaction, the automation equipment monitors the amount of gas delivered and controls the pressure of reaction. Many U.S. processors control based on head-space pressure, while many European facilities control based on hydrogen line pressure. While pressures at these locations are usually not the same, especially during the initial reactive period, consistency is the key, and the plant recipe book is written according to the practices of the facility. Only the hydrogen that has been dissolved in the oil is available for reaction, which is an extremely important consideration in both the reactor and agitator design. As the reaction is exothermic, steam is normally turned off when the gas is introduced, and after an allowable temperature rise, cooling water is circulated through the reactor coils to maintain reaction temperature. The reaction continues, with hydrogen gas delivered to maintain the desired reaction pressure. Depending on the purity of the hydrogen gas, inert gases accumulated in the reactor headspace may slow the reaction, and it is common practice to vent a small amount of these (under 1%) to the atmosphere.

When most of the gas has been delivered, the operator may sample the oil and measure the degree of saturation, typically using a refractive index. Upon verification that the refractometer (correlating to the iodine value, a measure of degree of unsaturation) shows the reaction is completed, the operator may vent the headspace gas to the atmosphere or to another reactor and start evacuation of the vessel. It is important that the headspace hydrogen gas be totally eliminated at this point, to avoid the risk of a fire or other serious situation. An alternate to this venting procedure involves maintaining a positive pressure on the reactor at all times to avoid the possibility of oxygen being introduced into the reactor. After securing the reactor, the oil may be cooled and held for filtering, or may be discharged into a *drop tank* where the feedstock for the next batch is preheated, as described earlier. After this partial cooling, the oil typically passes through an oil-oil economizer to bring the hard oil to filtration temperature, prior to entering the catalyst filter, or



**Figure 10.** Hydrogenation.

*black press*. As the oil is highly reactive at this point and temperature control is very important, special care is taken not to expose the oil to oxygen. After the catalyst filter, the oil typically has a residual nickel content, which if used directly may impart a gray-green appearance to the otherwise white hard fat. To remove the remaining colloidal nickel, the oil may be slurried with a mixture of filter aid and bleaching clay, agitated in an evacuated *postbleacher* and then filtered through a final polishing filter. The bleaching earth is also used to compensate for any color degradation that sometimes takes place after hydrogenation in a carbon steel reactor (5). Chelation of the catalyst, using citric acid in place of the bleaching clay, has gained acceptance in recent years, especially considering the additional equipment necessary for the traditional postbleaching system and disposal problems associated with bleaching clays. Some processors feel postbleaching can be minimized if catalyst is not being reused. Many processors do reuse catalyst, although prediction of resulting physical and chemical parameters of the hydrogenated product can become more difficult. Hydrogen gas can be provided from a variety of sources. For small or infrequent use operations, the processor may purchase commercial hydrogen gas, stored on site using cryogenic storage or tube trailers. Larger operations may elect to generate their own hydrogen from steam reforming natural gas, propane, or similar fuels. Hydrogen can also be generated from electrolytic cracking operations and is generally viable in remote locations without commercial hydrogen service.

Several options exist in hydrogenation system design. As mentioned earlier, the *dead-end* reactor recirculates the hydrogen gas accumulated in the head-space above the oil by a gas entrainment vortex blade of the turbine agitator. For large operations, a heat recovery system is often incorporated to utilize the exothermic heat of reaction, using this heat for washwater/heating, heat tracing, or other imaginative purposes. The gas recirculation-type converter, used extensively at one time, operates by circulating large volumes of gas through the oil by means of external compressors. This arrangement was popular when hydrogen gas contained considerable quantities of inert gases; a small amount of gas could be vented continuously, and the circulated gas provided the agitation (19). Another design popular in Europe is the Buss reactor, which has no mechanical agitator, but provides agitation by pumping oil through a headspace eductor. These systems provide fast reaction times and boast high mass transfer rates, but do not provide the flexibility of the dead-end reactor. Another concept is the helical impeller autoclave, which is similar to the dead-end reactor but uses a specially designed agitation and gas entrainment system. Continuous hydrogenation systems continue to be topics of discussion for plants processing significant quantities of homogeneous product, and it is expected that installations of this type will become more common.

As indicated earlier, the operator has several variables under his control to selectively tailor the products. There are two types of selectivity the processor is most concerned with, these being preferential and orientation selectivity. Preferential selectivity refers to the double-bond position the hydrogen atom attaches itself to during the saturation process. The study of orientation selectivity is centered on the fact that during contact with the catalyst, some of the double bonds may be twisted

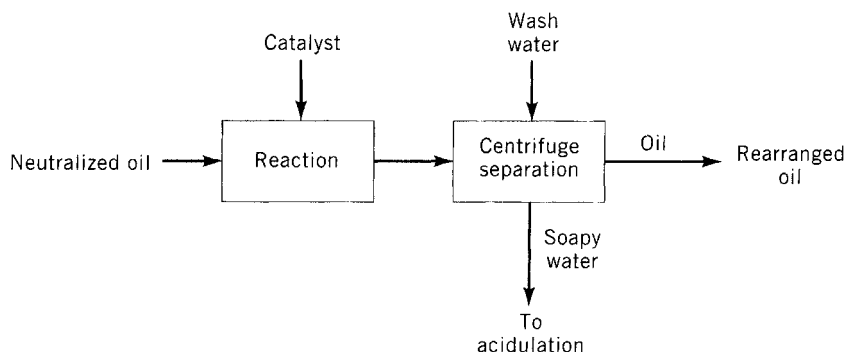


to the higher-melting-point trans form. Pressure, temperature, agitation, and catalyst type and concentration are the most effective tools the processor has to affect selectivity. As this topic is beyond the scope of this Chapter, the reader is referred to the many texts written about these subjects listed in the general references at the end of this Chapter.

One cannot review hydrogenation without touching on the health aspects of saturated and modified fats. For several years, the prevailing medical advice was to limit saturated fats. As a result of this advice, the fast-food and other industries have moved from traditional tallow frying to use of canola, soybean, and other oils. Even timeless favorites have come under fire as of late. The Center for Science in the Public Interest recently attacked coconut oil, traditionally used to give movie theater popcorn its distinctive taste and aroma. As a result of this negative press, many major theater chains are switching to canola and other oils (20). There have been studies suggesting a link between trans isomers and health problems, which further confuses the issue and the ultimate consumer. As a result, some have warned that consuming partially hydrogenated products containing trans isomers was no better (and possibly worse) than eating saturated fat products. The market responded with an immediate drop in margarine sales over a 12-week period while butter volume increased (21). While some of these studies have been questioned as artificially high both in trans isomer content of the products used and in the quantity of products consumed (as the total amount of trans isomer content in one study was three to four times greater than the estimated dietary intake) (22), consumers clearly reacted to the news negatively. In response to the market pressures, some companies are developing products with lower or no trans fatty acids. One of these spreads is a canola oil with soybean-based flavor compounds to provide a buttery flavor. Other approaches have been to use high-oleic oils or specialty tailor-made oils to provide better functionality. High-oleic oils provide the stability normally achieved through partial hydrogenation and are viewed as a promising replacement to hydrogenated products. High-oleic canola blended with canola high in stearic acid has been used in margarine and confectionery products. A dwarf sunflower with high-oleic-acid content has been introduced, as has a low-linolenic soybean variety. While the health debate continues, hydrogenation continues to be an important part of the integrated facility.

## 11. INTERESTERIFICATION

Interesterification is one of the lesser practiced unit operations of the integrated refinery. Generally, the transesterification practiced in the edible oil refinery, known as rearranging (random or directed), modifies the characteristics of the fat without chemically modifying the individual fatty acid composition. The process of interesterification may be applied to a variety of interchange reactions of fats with other components. The various types of reactions are (1) alcoholysis (with monohydric alcohols to produce methyl esters or with polyhydric alcohols to form monoacylglycerols), (2) acidolysis (acid interchange), or (3) transesterification (rearrangement



**Figure 11.** *Intesterification.*

of fats). This process has great potential as the ramifications of natural versus modified fats continue to be debated.

Figure 11 depicts a typical interesterification process. This random interesterification uses a reactor quite similar in design (if not identical) to the hydrogenation dead-end reactor. Oil is heated after being neutralized and dried to around 90–120°C and is blended in the reactor with a catalyst, such as sodium methoxide (0.2–0.3%) or an alkali metal (0.1–0.2%). During reaction, the reactants become orange-brown in color (the first quality check of reaction process). Once the color develops, the reaction is normally completed within 30 min, where an equilibrium exists of the random distribution of fatty acids on the glycerol molecule. When completed, water is introduced to stop the reaction, vacuum is released, and the oil discharged to a holding tank. As a soap residue is normally present in the oil, the mixture may be washed and centrifuged from the oil. The oil is dried and bleached and deodorized in the normal manner.

Continuous interesterification processes exist but to date none have been commercialized. Interesterification is generally performed in small batches by specialty processors. An alternate method of increasing interest is directed interesterification using enzymes. The process is generally applied to palm-oil-based materials such as cocoa butter substitutes and to coconut oils. There is some concern about the effectiveness of interesterification with physical refined oils as low levels of FFA must be present or the reaction will not proceed as planned (5). Although not a hazardous process, interesterification is often included in the hydrogenation section of the refinery because of the similarity of the reactors.

As the debate concerning health effects of saturated products and that of trans isomers generated during hydrogenation continues, interesterification may offer a viable alternative to the refiner. Outside the United States interesterification is used to produce hardened fats without trans isomers. These products are available in Canada and continental Europe. This technology has been available for quite some time, as a patent on the product was granted to Unilever in 1961 (22). The ability to tailor the melting point and functional crystallization characteristics without

changing the fatty acid composition makes interesterification a process with interesting potential. A comprehensive review on hydrogenation can be found in Ref. 23.

## 12. FRACTIONATION

Fractionation, or winterizing as it is sometimes called, is a practice gaining importance today. The phenomenon of winterizing was originally observed when storage of refined cottonseed oil during cold weather caused deposition of high-melting-point triglycerides leaving clear liquid oil on the top. The later was decanted, deodorized, bottled, and marketed as a “winter salad oil” (24). While the commercialized dry fractionation plant design is only some 30 years old, it is estimated that around 10% of the world’s production passes through a fractionation plant (25).

Today, edible triglycerides are fractionated for one of the following reasons: (1) to remove waxes and other nontriglycerides (2) to remove naturally occurring high-melting-point triglycerides, or (3) to remove high-melting-point materials formed during hydrogenation (26). Fractionation usually refers to the dry process, although a wet or solvent process is also utilized. The wet fractionation process, which is usually applied to cottonseed products, uses hexane or acetone, and allows separation of the various components in the miscella stage. However, the costs incurred by solvent recovery, the hazardous nature of the operation, and the process losses make the wet process less frequently used than dry fractionation.

Fractionation is widely practiced with palm oil, especially with the large amount of palm olein exported from Southeast Asia, but is also used with tallow, lard, and other fats. Fractionation of partially hydrogenated soybean oil (PHSBO), canola, and other brush hydrogenated products is gaining importance as well. A typical fractionation basestock may be a PHSBO with a feed IV of 100, fractionated into a high stability liquid frying oil with the stearine used for margarine basestock. Refined, bleached, and deodorized palm (RBD) with a starting 50–55 IV is commonly fractionated into an 56 IV olein and 48 IV stearine.

A typical dry fractionation process is shown in Figure 12. The process involves chilling the fat at a prescribed rate allowing the stearine to crystallize and then

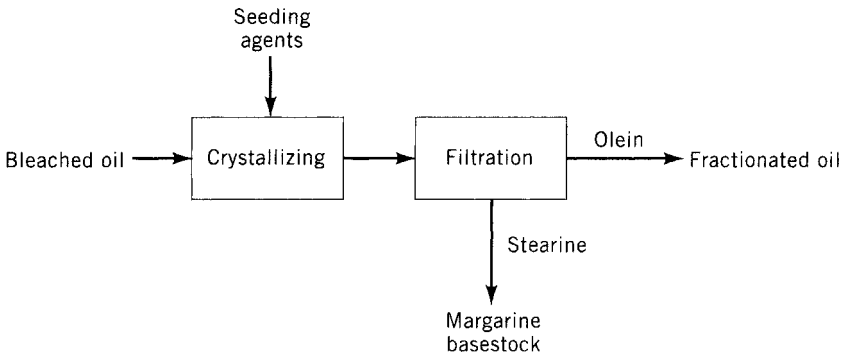


Figure 12. Fractionation.

separating the two phases by filtration. One of the key factors determining the success of the fractionation process is the efficiency of the separation. Consequently, both the crystallizing and subsequent filtration require careful control. The crystallization cycle usually starts with the operator selecting one of several recipes programmed into the plant PLC, depending on the feedstock and desired products. The process then continues by loading a cell (or crystallizer) with a prescribed quantity of dry (and for seed oils, hydrogenated) feedstock. The oil is normally loaded sufficiently hot to melt any components that could interfere with the crystal development process, and to melt any crystallized fat present in the system from a previous batch. After a brief holding period, the system controller then starts the flow of chilled propylene glycol (or water) through the crystallizing cooling coils and jacket. In some designs, a direct expansion refrigeration system is provided, eliminating the glycol system so that the crystals form and grow properly; the temperature differential between the oil and coolant is carefully controlled during this initial cooling period. During this cycle, agitation is introduced by either very slow rotation of mechanical agitators or by bubbling cold air through the cell. After a certain period, the temperature differential is changed for the crystal maturing phase, and the agitation may be reduced or stopped.

For certain products, stearine seeds may be introduced during the cycle to provide a stable base for crystal growth. Agitation during the crystallizing process is necessary but also potentially detrimental to the process. Without sufficient agitation, the heat transfer rate becomes very low and the coil surfaces build up with crystallized fat. Too much agitation breaks up the crystals and imparts mechanical work into the mixture. A balanced agitation has been shown to be necessary for proper crystal formation, as an olein–stearine mixture slowly cooled without agitation produces a stiff and nonpourable mass. Photomicrographs showed that large-sized crystals were formed having long thin tentacles that trapped liquid oil within the crystal structure (26).

After the proper crystallizing period, the stearine fraction is separated from the olein by one of several filtration methods. From the crystallizer cells, the oil mixture is either gently pumped or is transferred by gravity or slight pressurization of the cells to the filter. The olein fraction passes through the filter media while the solid stearine fraction is trapped. The stearine is then dropped into a receiver where it is melted and pumped to a holding tank. The olein may be blended with a crystal inhibitor, such as phosphatides, polyglycerol esters of mixed fatty acids, or other commercial inhibitors, to increase the time of the cold test. The cold test involves chilling and holding oil at or near freezing temperature for a certain period of time.

Fractionation is designed to separate the various fractions based on differences in crystallizing temperatures. Often, the products of a single fractionation are not sufficiently fine tuned to produce some of the higher value specialty fats. Hence, the concept of double fractionation was introduced, with one of the phases (usually the olein) refractionated. This process is used quite frequently with palm oil. The products of the second fractionation are a “super” olein and a stearine used as tropical margarine base or a confectionery base.

While the designs of commercially available crystallizer cells have some variances, all are intended to cool and agitate the oil as a batch more or less in the same manner. There are, however, several types of filters in use based on the products and style of operation. These basic designs include plate and frame filters, continuous vacuum systems, membrane systems, and pressure leaf designs.

At one time, plate and frame filters were standard equipment for fractionation service, especially for cottonseed applications. It is generally necessary to introduce the oil–stearine mixture into the filter by gravity during the first hour of operation and then apply low air pressure to the tank forcing the mixture through the filter. As these designs require location within a refrigerated room, are generally high in labor requirements, and have slow filtration rates, they have largely been replaced by other designs. A modern variant of the traditional plate and frame design that is gaining acceptance is a filter press equipped with membrane plates. When the crystals are rather small and hard, a membrane system provides an excellent olein–stearine yield. The membrane filter is similar to a traditional plate and frame press, except that each chamber is equipped with one or more flexible membranes, such as rubber or polypropylene. The operation of this system involves a filtration step where olein passes through with the stearine retained in the chamber. Next, a squeezing takes place where compressed air is applied behind the membranes to expel the trapped olein, leaving the hard stearine cake. Finally, the chambers are opened and the cake discharged into the melting tank. Yields from membrane filters are usually quite high, and the entire process has been automated to reduce the labor requirements. However, membrane filters tend to be rather capital intensive and require replacement of the limited life membranes.

A very popular filtration system is the continuous vacuum filter. When the stearine crystals are durable and less sensitive to deformation during filtering (such as cake from PHSBO or palm), this filter provides an excellent separation. The introduction of this technology represented a major advance as filtration rates were increased several magnitudes over the rates observed with plate and frame systems. Operation of the vacuum filter is well suited for large-capacity operations as oil from the crystallizer cell is gently and continuously pumped to the filter receiver. A slowly rotating drum, partially submerged in the oil, uses vacuum to draw the oil through the filter of the compartmentalized drum. The air–olein mixture is separated and the filtrate discharged to a holding tank. The stearine cake collects on the rotating drum and is dried as the drum turns. As the drum rotates to the discharge section, the cake is removed from the filter cloth by either a knife blade, string discharge, or a blast of air, and drops into the stearine receiver for melting and subsequent pumping to storage. The vacuum filter is often hood shrouded for hygienic and temperature control purposes.

A popular variant of the rotating drum vacuum filter has been widely applied especially for the palm oil industry. The Florentine vacuum filter uses a horizontal stainless steel perforated belt with vacuum applied under the belt. This design includes a recycle from the first filter section, which is said to allow the first layer of stearine to act as a continuous precoat (25).

Another type of filter in use today is a horizontal (or vertical) pressure leaf filter with a special cake squeezing device. Between each pair of hollow filter leaves is an impermeable flexible diaphragm that can, when inflated, apply pressure on one side of each adjacent leaf. As the cake forms, the pressure on the leaf side is less than on the chamber side. The diaphragms move toward the leaf, compressing the olein from the stearine cake.

Dry fractionation applications have enjoyed substantial growth in the past years, and with growing emphasis on value-added specialty fats, the processor finds fractionation a most valuable tool. It is expected that rapid growth fractionation capacity will occur as processors search for additional revenue enhancement opportunities.

### 13. DEODORIZATION AND PHYSICAL REFINING

Typically the last step in the edible oil refinery is deodorization, and this process is included in almost every refining operation, regardless of the other unit operations selected. While in the past limited natural fats could be utilized as edible products (such as butter and animal fats), most oils used today come from sources having natural components that if not removed impart objectionable flavors and tastes. These oils contain not only flavor bodies that must be eliminated to produce a palatable product but other unit operations, such as hydrogenation, impart a negative flavor and color that must be removed in the deodorizer. Shelf life and color are also important considerations upon which the deodorizer has a direct impact.

Deodorizing was developed to remove the relatively volatile odoriferous compounds from the host triglyceride. From the early efforts to boil the volatiles at atmospheric conditions and high temperatures, the modern practice of applying heat under low absolute pressure for a particular period of time has developed. As there is a substantial difference between the vapor pressure of the oil and the volatile materials affecting the flavor, color, and stability, deodorizing can consist of any method to evaporate these substances without damaging the oil.

*Physical refining* refers to the process where the free fatty acids in a crude or degummed oil are removed by evaporation rather than being neutralized and removed as soap in an alkaline refining process. In design and operation, the deodorizer is very much like the physical refiner, with the major exception being the higher load of fatty acid removed in physical refining. While some deodorizing designs can use carbon steel for certain noncontacted parts (such as the outer shell for a double-shell deodorizer), the high level of fatty acids (and subsequent corrosion if left unchecked) generally demands that a physical refining system be made from stainless steel or other material not affected by contact with fatty acids. A physical refining operation should also have sufficient heating capacity to compensate for the cooling produced during fatty acid evaporation, which is normally a negligible figure for alkali-neutralized feedstocks.

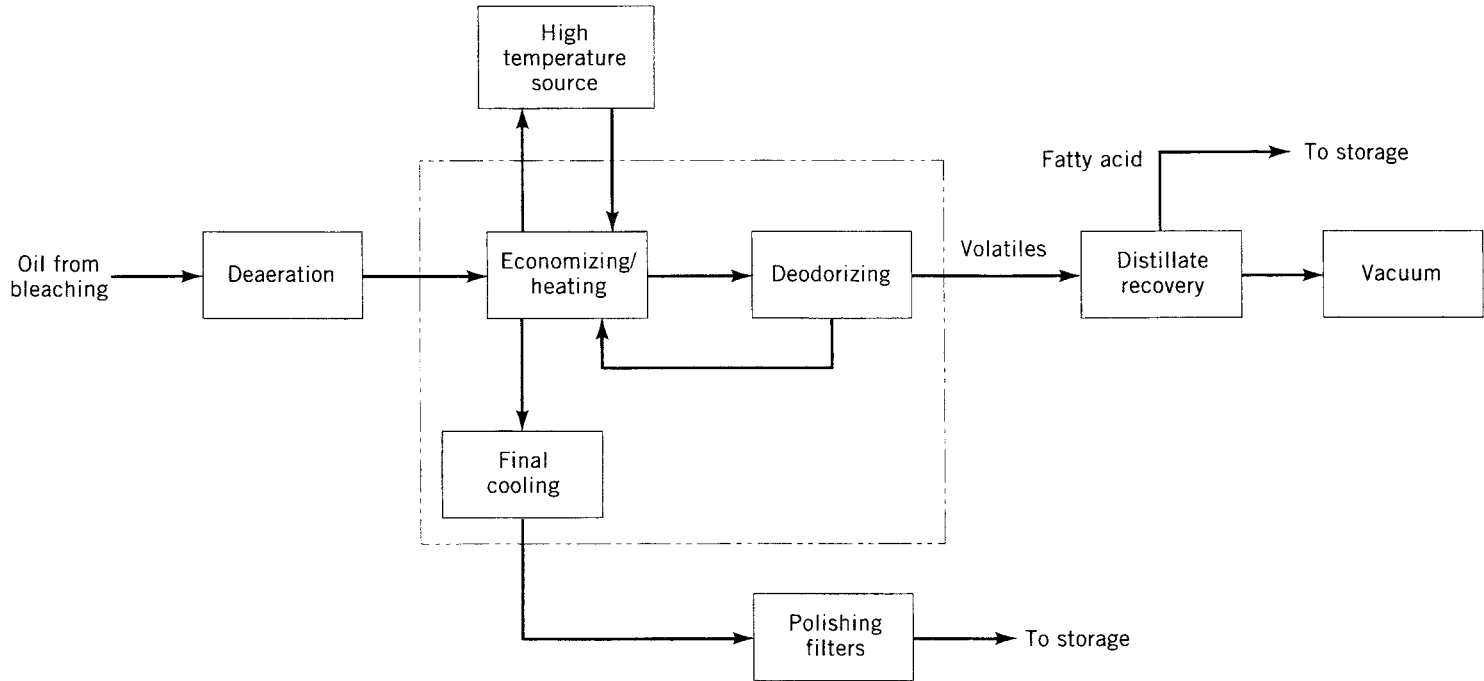
Deodorization is a mass transfer purification process, where the oil is exposed to surface conditions forcing the volatiles into the vapor state. Deodorizing conditions

normally involve exposing a thin film of oil to a carrier gas at an elevated temperature and low pressure. A stripping gas (normally steam) is usually introduced to agitate the oil, ensuring that all the oil is subjected to surface conditions, and to carry the volatiles from the deodorizer to the vapor recovery system. One of the key factors in successful deodorization is to provide thin-film mass transfer for each particle of oil. Each of the deodorizing concepts described attempts to provide some type of rapid agitation to accomplish this duty. Heat bleaching is also normally provided in most deodorizing designs, which refers to the process occurring at these conditions where the carotenoid pigments and other coloring compounds either break down or are evaporated. Effective deodorization requires this exposure and agitation to be performed for a certain period of time, not only to ensure equilibrium conditions but also to provide sufficient time for heat bleaching.

Figure 13a depicts the unit functions in a typical deodorizing system in use today. From the bleaching plant (or physical refining pretreatment process) the oil (normally at 60–90°C) is first deaerated. This step is extremely vital prior to heating the oil to temperatures over 100°C, as most oils, especially those derived from seed sources, will deposit products of polymerization when oil containing oxygen is exposed to heating surfaces. The deaerator may be a separate vessel or in the case of certain designs, an integral part of the deodorizer itself. After the deaerator, the oil normally passes through an oil–oil interchanger, being preheated by freshly deodorized product. The hot oil is then heated to deodorizing temperature. These heat exchangers can either be external to the deodorizer or these heating (and cooling) functions can be performed under the same vacuum conditions as the deodorizer itself. After being brought to temperature, the oil is vigorously agitated in the deodorizing vessel for a certain period of time until the bulk of the volatiles are removed and heat bleaching is accomplished. The oil is then cooled, normally first passing through a heat recovery economizer as described above, and then through final cooling. During cooling, a small amount of chelating agent, such as citric acid, may be introduced into the oil, as may an antioxidant. The volatiles removed during the deodorizing process are condensed and usually recovered in a fatty material direct condenser, known as a vapor scrubber. The balance of the volatile gases, including the stripping steam and other more volatile compounds, are condensed in the vacuum system.

While the basic principles behind any deodorizer are essentially the same, there are several types of systems used to perform these functions. General classifications include the batch, continuous, and semicontinuous operations.

The *batch process*, which was the first type of deodorizer to be developed, generally uses a single vessel to accomplish the functions desired. These units, which are generally suitable for total daily output of under 60 metric tons per 24 h, consist of relatively tall vertical cylindrical vessels designed for substantial level rise during the steam agitation process. A batch of incoming fresh oil is normally slowly heated under vacuum conditions to deodorizing temperature, at which time sparging steam is introduced through a sparging pipe, or through a gas-lift pump. After a suitable agitation period (which is normally several hours) the oil is cooled, under vacuum conditions, and is discharged to storage. While heating and cooling are

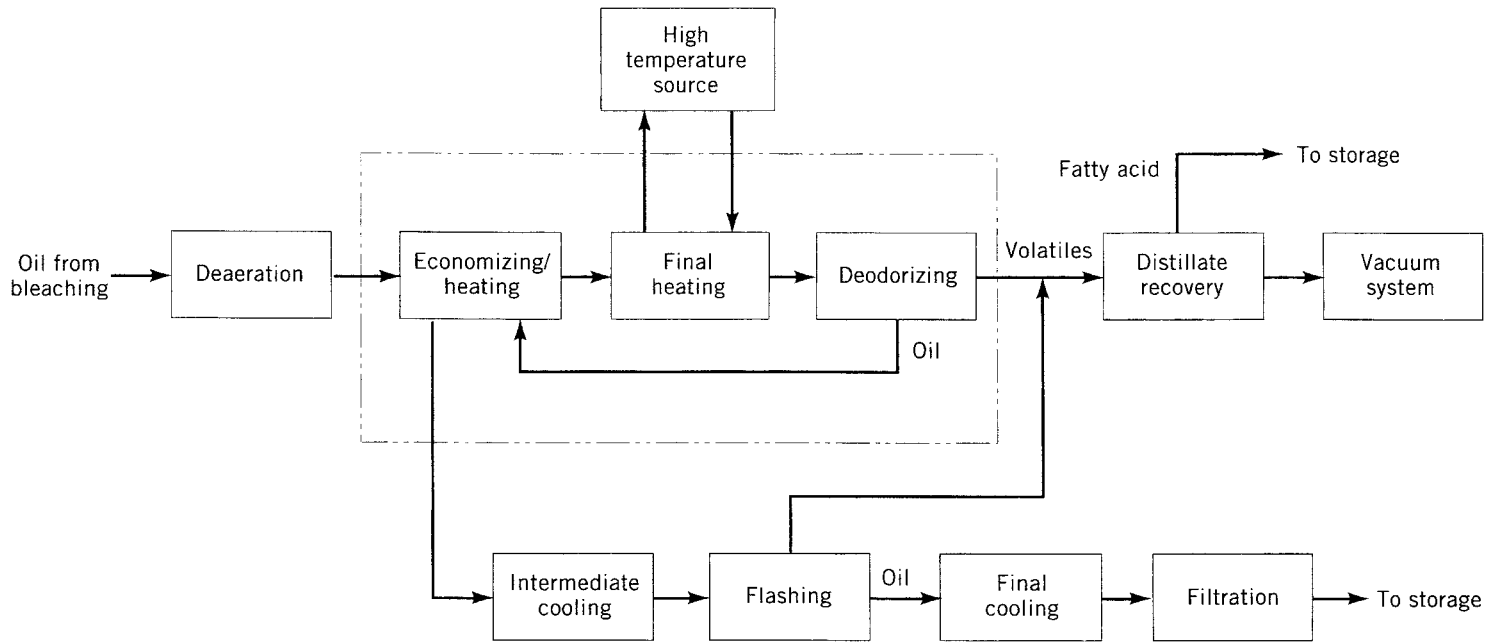


**Figure 13.** (a) Semicontinuous deodorizer.



normally performed in the vessel utilizing internal coils, forced circulation through external heat exchangers allows for a quicker cycle and possibly lower overall utility costs. Because of the relatively small batches involved, the long times between filling and emptying, and the economies of scale, heat recovery is normally not incorporated in batch deodorizing designs.

The *continuous deodorizer*, illustrated in Figure 13b, performs the same basic functions but is designed for a larger operation requiring few changes of stocks per day. The continuous deodorizer allows for low utility consumption, reduced labor costs, and high heat recovery and is generally the best choice for the modern integrated refiner who is sufficiently flexible in scheduling production runs. There are several options for continuous deodorizer design today, but the following description follows a typical installation found in a modern integrated facility. The bleached oil enters the deaerator where, operating at 50 mm Hg absolute or less, oxygen and some moisture are removed prior to heating to deodorizing temperature. The deaerator is normally fitted with a demister to eliminate oil carryover to the vacuum system. After the deaerator, the oil passes through the economizer and then the final oil heater. The final oil heater is normally designed to heat the incoming oil to final temperature using high-pressure steam or thermal fluid. The oil then enters the deodorizer, which is normally a single-shell vertical unit (or a horizontal vessel) designed for operation of 3 torr (or less) at the vapor outlet. The vessel has several deodorizing compartments fitted with special steam distribution devices to promote intimate contact between the oil and steam. These devices can be gas-lift pumps (also called mammoth pumps) or sparge rings. The retention time in each compartment varies between 10 and 30 min, depending on the design. As the oil passes through a series of baffles installed in the trays, it overflows through a overflow pipe or weir to the next lower tray. After a sufficient deodorizing period, the oil is ready for cooling. In order to achieve the high heat recovery typical for continuous deodorizers the oil is often brought outside the vessel and is cooled in an oil-to-oil economizer, as described above. To provide additional cooling or to compensate for a lack of cooling media when changing feedstock, an intermediate cooler is also often provided. After cooling, a small amount of citric acid (around 200 ppm) is introduced to chelate any trace metals present in the oil. Certain oils, such as palm and lauric oils, can safely be delivered to storage at this point, while other oils, such as soybean, may develop flavor and stability problems if cooled to storage temperatures under pressure conditions. It is theorized that the development of substances detrimental to flavor occur very rapidly at temperatures present in the deodorizer, and without constant removal of these volatile components, the oil quality is affected. If cooling is accomplished entirely under vacuum, these volatiles are removed almost as fast as they are formed. To remove these volatiles formed under pressure conditions while the oil is being cooled, the oil is often reexposed to low-pressure conditions in a flash vessel or a lower section of the deodorizer after external heat exchange. Finally, the oil is passed through a tubular or polyester bag filtration system to remove any particulate material that may have been picked up in previous operations.



**Figure 13.** (b) Continuous deodorizer.

Continuous deodorizers can be supplied with many options tailored to the specific application. For example, while maximum heat recovery is usually accomplished using external heat exchangers, the continuous vessel can be designed to perform all heating and cooling functions internally. Orientation of the piping, vessels, and heat exchangers can be arranged to minimize product contamination during stock change. A variety of heat recovery options have been developed, including a design generating steam to assist driving the vacuum system.

As mentioned earlier, there are several configurations of continuous deodorizers in addition to the traditional vertical tray unit described above. One type uses a multisectioned tray (box) contained in a horizontal cylindrical vessel. Heat transfer baffles are used as flow baffles, with agitating steam injected between the baffles. Another system is based on a series of vertically stacked horizontal single-shell cylindrical vessels, with agitation steam injected by multiple pipe distributors running the length of the vessel. Another type of continuous deodorizer takes maximum use of thin-film processing, using structured packing to maximize the steam-oil contact. This system provides excellent fatty acid stripping with a minimum of steam, but because of the relatively short retention times involved, it does not provide a great deal of heat bleaching unless a retention vessel is provided as part of the system.

The *semicontinuous deodorizer*, Figure 13a, again follows much of the same processes but is specifically designed for an operation demanding frequent stock changes. Like the continuous deodorizer, many variants are in operation today, some utilizing multiple deodorizing sections, a greater number of heat recovery options, etc. The process described is for a typical unit found in operation today. Semicontinuous deodorizers, developed in the late 1940s, were specifically designed to process an individual batch of oil in a fixed-capacity tray, allowing the operator to follow one batch of oil with a different feedstock with minimum lost production time and contamination. The term *semicontinuous* stems from the fact that although each batch is treated individually (with heating, deodorizing, and cooling functions performed in a specific tray of the vessel), the flow to and away from the deodorizing vessel is generally continuous. As significant temperature swings occur during the cycle, and heat exchange ability is limited by physical constraints of the available surface area and operating conditions, overall heat recovery in the semicontinuous deodorizer is typically around 50–60%. The semicontinuous design requires considerable control to manage the drop valves and stock change system, and its installed cost is typically greater than a similarly sized continuous design.

In operation, properly pretreated oil flows continuously to the measuring tank. This tank, which usually also performs the deaeration function, accumulates oil at a constant rate. When signaled by the plant control system, a drop valve opens for 1–2 min to admit a quantity of oil into the top heating tray before closing. In this first of a typical five-tray configuration, the oil is preheated using a device commonly known as a thermosyphon. This process uses the temperature differential between the cold incoming feedstock and the high temperature of deodorized oil in a lower holding section using steam or thermal fluid as the heat transfer medium. This medium is naturally circulated by alternately evaporating in the holding

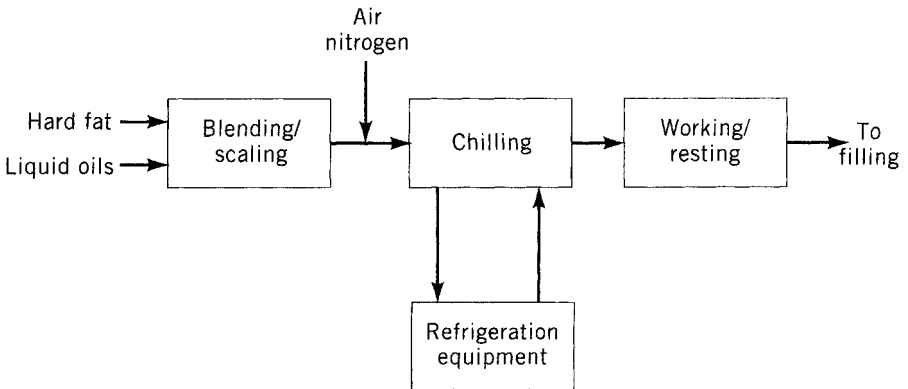
section while condensing in this preheating section or is pumped for a single-phase fluid. After the proper period, the preheat tray drop valve opens briefly to discharge the oil to the final heating tray. This tray, which is also packed tightly with coils, uses high-pressure steam or thermal fluid to bring the oil to proper deodorizing temperature. After a suitable period, the drop valve on this tray opens, and the oil drains into the deodorizing tray. Now at deodorizing temperature, the oil is subjected to high agitation through either a single or series of mammoth pumps, as described in the continuous deodorizing section. When the deodorizing period is over, the drop valve opens, and the oil flows to the holding tray. This tray, which forms the lower section of the thermosyphon heat transfer system described above, allows the oil to partially cool and is a main source of heat recovery in the semi-continuous system. After a suitable period, the oil is discharged to the final cooling tray, where cooling water (or other cooling fluid) brings the oil to (or close to) final storage temperature. Finally, the oil enters the holding section, where it is pumped continuously by the discharge pump through final polish filtration on its way to storage. An external cooler may also be supplied to reduce the oil temperature prior to storage.

Traditionally, the deodorizer was considered the last step in the refining process. For a custom blend shipment, basestocks were blended and after being deodorized were loaded directly on a customer's vessel. Even with a homogeneous oil product, deodorizing was performed almost immediately prior to loadout to provide the freshest oil possible. With an increased use of stainless steel piping and equipment, and judicious use of inert gas purging, several commercial operations have found it acceptable to hold deodorized product for a longer period of time, and in fact perform blending of deodorized products without redeodorizing the load. As customer requirements are quite variable, especially for higher value custom fat blends, and the concern of scheduling and potential contamination is significant, this practice allows a longer run of product through a plant system, resulting in smoother operation without the need for frequent changeovers. This practice also eliminates much of the previous justification for a stock change deodorizer.

While the deodorizer generally represents the final step in edible oil processing, and can make a good product from less than perfect feedstock, the deodorizer cannot forgive the sins made in earlier processes. While the primary products of oxidation are removed (as indicated by the peroxide value), severely damaged oil may contain secondary products of oxidation (measured as anisidine value). Phosphatides must be at very low levels prior to entering the deodorizing system. Soaps must also be completely eliminated and the oil must be handled and stored to minimize exposure to oxygen.

## 14. SHORTENING AND MARGARINE MANUFACTURING

*Shortenings* are edible fats that have undergone special processing to yield products with certain physical characteristics. They are suitable in a wide variety of applications but are primarily used in cooking and baking of foods. Shortenings act to



**Figure 14.** Flow process for shortening.

“shorten” or tenderize foods, particularly baked goods, by their ability to interpose films of fat through the food in such a manner that the protein and carbohydrate components do not cook to a hardened mass. In cakes and icings, shortenings also make possible the incorporation of tiny air bubbles that greatly assist in the attainment of fine, uniform, and stable structures. Liquid oils do not possess this ability.

The simplest type of shortening consists of a fully hydrogenated fat (stearine) blended with a liquid vegetable oil. These products are plastic at room temperature and are marketed as “all-purpose” shortenings, generally suitable for general pan frying and less critical baking operations in the home. Other products have been developed (which may include emulsifiers and other ingredients) that have greater creaming qualities and exhibit greater resistance to oxidation.

Figure 14 illustrates a typical shortening manufacturing operation. Configurations of equipment vary widely based on the local conditions, and this discussion is designed to provide but one example of a typical process. The basestocks and ingredients are weighed through either a series of scale tanks or mass-flow meters and are introduced into a feed tank. The mixture is thoroughly blended and introduced into the first scraped surface heat exchangers (SSHE), commonly referred to as the A unit. The mixture is super-cooled as a fluid, but has the crystal nuclei established for fine crystal ( $\beta'$ ) growth. The product then passes through a worker unit equipped with a number of projecting fingers on a rotating shaft, commonly known as the B unit. In this unit the product is plasticized, and the crystallization process continues under controlled mechanical conditions. After passing through an extrusion (or homogenizing) valve, the product is packaged into either a bulk container or smaller consumer quantity package. After packaging, the filled containers are usually moved to a temperature-controlled tempering room for 36–48 h prior to shipment. Some installations have included an additional controlled cooling step prior to packaging to reduce the need for the tempering period.

While the process is fairly straightforward, a properly produced shortening requires judicious control over the chilling, plasticizing, and tempering operations to make it into a satisfactory product. Control of the SSHE equipment, which is

normally cooled by direct expansion of ammonia liquid, involves monitoring both the product and refrigerant temperatures. Plastic fats at room temperatures may contain between 10 and 30% solid crystal fraction dispersed in the liquid oil, depending on the formulation and the oils used. The most important factors governing the product are the number, size, and structure of the crystal solids dispersed in the liquid oil. A slowly cooled fat crystallizes in relatively few and large granular clusters. Such a fat exhibits less plasticity and is not as firm as is a rapidly chilled fat where many small and well-dispersed crystal solids are developed. A fat with large crystals has a “gritty” feel in the mouth and can best be demonstrated by heating and subsequent cooling of a commercial shortening product. While the properly prepared product will appear homogeneous and have an agreeable mouthfeel, an uncontrolled heating and subsequent cooling operation will produce a fat with large crystals, in which the liquid and solid portions may separate. Because crystals of the solid fat fraction are polymorphic (existing in more than one crystalline form), quick solidification and subsequent plasticizing functions require careful control to ensure functional products with stable crystalline structures. The conditions of supercooling can be established to take advantage of crystal reorientation, or tempering, which is allowed to take place during storage. Proper crystal reorientation will yield a higher melting product and a more stable crystal pattern.

In addition to the operations discussed, it is also common practice to introduce 8–12% nitrogen or air into the chilled product. The effect of incorporating gas into the shortening is to improve its textural appearance and color, stabilize it by making it more homogenous, and improve its creaming properties by contributing gas to expand dough and batter for subsequent baking operations.

While shortening is primarily a fat product, *margarine* is generally defined as a plastic fat food product containing 80% (U.S. minimum) edible fat with the balance of about 20% consisting of an aqueous phase (usually a specially cultured skim milk) and including salt, vitamins, food coloring, and other additives. In addition, a host of spreads containing greater aqueous and lesser oil phases have been developed and widely accepted by the consumer.

Unlike household shortening, consumer margarine is generally consumed as a spread on bread as well as for home cooking and baking. Margarines must therefore melt readily and completely in the mouth and impart “buttery” characteristics to products containing them. Any residue that fails to melt at body temperature may not be noticeable in a shortening (in fact, such a residue may be desirable to give shortening high-temperature firmness), but in margarine it imparts an undesirable “pasty” sensation in the mouth. An ideal margarine should be plastic and spread freely on soft, fresh bread. At refrigerator temperatures, however, it should be sufficiently firm to permit forming into the desired package for marketing. Margarine also differs from shortening insofar as flavor is concerned. Shortenings are invariably produced to be completely bland whereas margarine is modeled after butter. It is essential that the basestocks for margarine production are properly processed and well deodorized.

A typical process flow for margarine production is shown in Figure 15. While several types of margarine are produced today, and each has its own variant of the

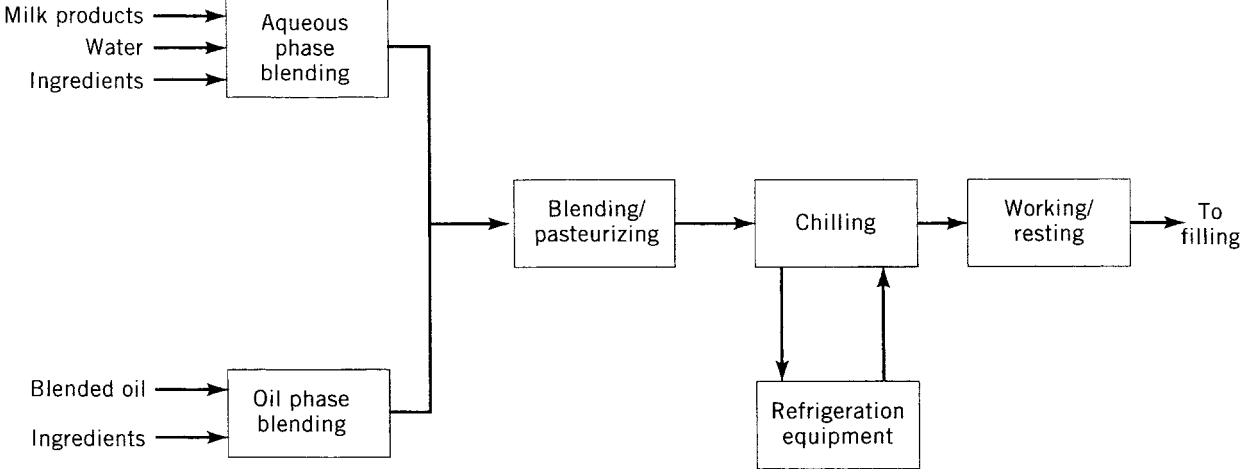


Figure 15. Flow process for margarine.

processing stages followed, the basic process is similar with most configurations. The oil phase (and oil-soluble components) are weighed through a series of scale tanks or in-line batch metering equipment. Meanwhile, potable water is blended with milk products and other aqueous-phase ingredients, with inventory of all components carefully measured and recorded. As the aqueous phase provides an excellent growth media for harmful bacteria, it must be very carefully prepared and must be sterilized. To ensure proper blending, aqueous-phase tanks are usually equipped with hot-water jackets and high-efficiency turbine agitators. After thorough mixing, the aqueous phase is carefully blended with the oil phase, and the mixture is heated to pasteurizing temperature either in a batch pasteurization process or in an inline sterilization process. The mixture is then cooled, often using a regenerative heat exchanger and, when ready, is pumped to the scraped surface heat exchangers. Pressures associated with margarine manufacturing can be quite high, with some puff pastry products requiring upward of 50–55 bars pressure at the inlet of the SSHE.

As mentioned earlier, there are several variants of margarine in production today. Table margarine, a traditional 80% fat product, typically passes through one set of coolers, then through an intermediate worker/resting unit, and through a final SSHE prior to packaging as wrapped bricks. Tub-filled margarine may follow a similar path, except it may not pass through the intermediate worker unit, but rather passes through a worker unit after the final SSHE, staying somewhat less plastic until after filling. An example of cake margarine (or biscuit fat) production involves passing the product through a SSHE, then an intermediate worker, then a second SSHE, and finally through worker/resting units prior to packaging in cartons. Puff pastry margarine may be subjected to as many as three cooling and subsequent intermediate working steps prior to final cooling. After final cooling, puff pastry product may pass through a “resting tube,” which provides a controlled amount of retention time and mechanical working prior to packaging (27). Several other variants exist, and it is common for individual facilities to develop their own hybrid process after observing the impact of the various operations on the product (and often changing) market. The modern production facility is suitably flexible to change the configuration depending on local conditions. Like shortening manufacturing, control of the SSHE equipment, which is normally cooled by direct expansion of ammonia gas, involves monitoring both the product and refrigerant temperatures.

While the basic process of margarine manufacturing is fairly straightforward, much of the secret for success lies with the formulations and processing conditions established to develop the functionality demanded for the specific application. Since plasticity and spreadability are two of the most important physical properties of margarine, the ratio of crystal solids to oil over a given temperature range should be used to determine the exact quantities of the various fats and oils to be blended together. Milk, or milk powder, may be favored for margarine productions as it imparts the desired flavor and aroma to the product. Milk also aids in the production and stabilization of the emulsion and provides certain nonfat ingredients that give the characteristic aroma akin to butter. It is generally necessary to add special emulsifying agents to margarine in order to achieve the physical properties associated with this product. Improperly emulsified or inadequately stabilized margarine



will tend to “leak” or exude the aqueous phase while in storage. Emulsifiers are also used to prevent the aqueous and oil phases from separating. Lecithin is a natural emulsifying agent added with mono- and diglycerides as well as common emulsifiers added to margarine formulations. Salt, preservatives, vitamins A and D, antioxidants, food color, and flavoring are also included in typical formulations.

Of all sectors of the edible oil processing plant, the margarine/shortening facility usually receives the most attention, both because of hygienic considerations and customer interest. While the crushing plant, refinery, and other operations are the subject of many tours, it is the margarine/shortening manufacturing function with which the nontechnical customer base can best identify. It is not uncommon, therefore, to find this facility the cleanest and most up to date of all the integrated plant facilities, as the image portrayed in this operation sets the overall impression formed of the organization.

## 15. ACIDULATION

Throughout the process, waste streams have been generated by many of the unit operations that have been reviewed. Most of these waste streams are a mixture of oil and oil-soluble products along with aqueous-phase materials. While a few processors possess the ability to blend these products directly into the extracted meal, many do not have this as an option, find that either the quantity or quality of these materials is incompatible with product formulations, or find the oil products are not compatible with the meal products. Few producers can afford the transportation costs involved to sell these waste streams commonly known as raw soapstock. As the oil and water phase has a very high FOG (fats, oil, grease) concentration, high TSS (total suspended solids), BOD, and COD (chemical oxygen demand) loads, and includes a potentially valuable glycerine and fatty acid source, most processors include acidulation as part of the integrated facility.

Acidulation is one of the least desirable processes in the integrated facility. Not only is the process rather difficult to perform effectively, but it generally represents a cost without significant return. Figure 16 depicts a typical acidulation system based on gravity separation. The separations can be performed in either a continuous or batch operation. In operation, soapstock, discharge streams from tank farm collection systems, and other waste streams enter a equalization or holding tank. The facility may or may not add additional caustic at this point to saponify the

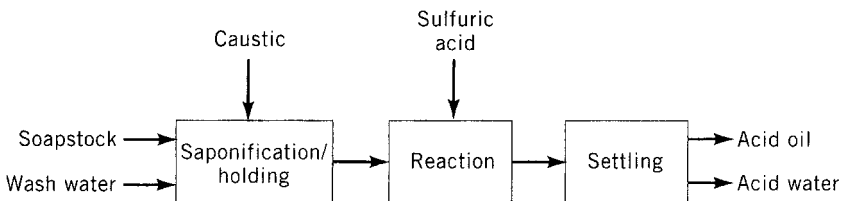


Figure 16. Acidulation.

fat. Washwaters, fat separator skimmings, and other sources may also be added at this point, or may be introduced into one of the later settling tanks. The mixture is heated and is treated with sulfuric or other acid, at a controlled pH of around 2–2.2 and, in the continuous system, enters an acid-resistant reactor. Batch operations use an acid treatment followed by a boiling period. After reacting with the acid, the mixture then enters a series of holding/settling tanks, where (in an ideal world) the oil and aqueous phases split. A small amount of aeration may be introduced in the tanks, and a small amount of washing water may also be sprayed on the oil surface. Acid oil skimmed from the top surface may be water washed, boiled, and settled, and either is sufficiently dry to sell as is or may pass through an evaporative heat exchanger to remove excess moisture. The product is typically sold as a feed ingredient energy source but may also be used as a feedstock for soaps, fatty acids, distillation, and purification and other industrial applications.

Acid water passing as underflow through the system is normally neutralized with lime or other alkaline material prior to leaving the acidulation system. As the pH has been significantly neutralized by the properly functioning acidulation system, some facilities blessed with adequate municipal waste treatment plants can discharge the water to these facilities. Often, however, the residual BOD, COD, TSS, and other contaminants exceed the municipal discharge standard, and a substantial penalty may be incurred. For these processors, and for others lacking a municipal option, the water continues to the waste treatment plant for further processing. Effluent from the extraction operation may be introduced at this point as well. Discussion of these systems is beyond the scope of this chapter, but some of these facilities can become rather elaborate in design and operating cost to meet the effluent standards.

One problem with acidulation in the integrated facility concerns the emulsion tendencies of certain products, especially phosphatides removed as gums. As indicated earlier, if the gums are not removed from the soapstock stream, but are allowed to enter the acidulation system, a third phase may be evident in the settling tanks. This phase is extremely difficult to split into separate oil and aqueous phases and may require several passes through the system until the emulsion is broken.

Several alternative acidulation systems have been developed. Various configurations of the acidulation vessel and settling tanks have been adapted, including a fairly simple design said to be effective for degummed soybean oil (28). Centrifuge separation may prove to be an attractive alternative if accurate pH control can be provided. Separation of raw soapstock by evaporation of the water phase has also been proposed, which is said to perform as well as commercial feed fats (29). Other processes are under development and improvements to the separation technology will be welcomed by the integrated processor.

## 16. SUMMARY

The industry has evolved over the past decades from small stand-alone operations to larger, integrated food processing facilities. Both suppliers and processors have

recognized that integrating operations at a common source allows the operations to enjoy greater economies of scale, utilize common service functions, and provide a greater range of services for their customers. As the trend toward integration continues, the knowledge base of the individual operation must include the impact of its actions and decisions on other operations of the integrated facility.

## REFERENCES

1. L. W. Shoemaker, Effects of Soybean Handling and Storage on Product Quality in *Soybean Extraction and Oil Processing*, presented at Food Protein Research & Development Center, Texas A&M University, 1987, p. 15–13.
2. Sulzer Escher Wyss Ravenburg, "Processing of Oilseeds using Fluid Bed Technology," VF 2, 3 (1986).
3. G. Penk and American Soybean Association, in Ref. 1, p. 32–5.
4. O. Balloni, Soybean Oil Physical Refining in Brazil, presented at the First America Congress on Oils and Fats Processings, Guaruja, Brazil, November 1989.
5. K. F. Carlson and J. D. Scott. *Inform* 2(12), 134 (1191).
6. R. P. Pavlik, *Latest Developments of French Process Equipment*, presented at AOCs meeting, 1992, Toronto, Canada.
7. J. E. Tysinger, J. F. Richmond, and R. B. Dawson, *U.S. Pat. Appl.* 2004017185 (2004).
8. D. R. Erickson, in Ref. 1, p. 60A–2.
9. J. B. Rossell and J. L. R. Pritchard, *Analysis of Oilseeds, Fats and Fatty Foods*, Elsevier Applied Science, London, 1991, p. 488–449.
10. Y. Z. Wu and T. Wang, *J. Amer. Oil Chem. Soc.* **80**, 127–132 (2003).
11. C. Pagliero et al., *J. Amer. Oil Chem. Soc.* **78**, 793–796, (2001).
12. T. L. Mounts and P. P. Khym, in *Handbook of Soy Oil Processing and Utilization*, ASA and AOCs, Champaign, Ill., 1980, p. 91.
13. W. E. Farr, in Ref. 1, 1987, p. 67–10.
14. D. V. Okonek, *Hydrogenation of Clay Treated Soybean Oil: Effect of Phosphoroas*, presented at the 1986 AOCs annual meeting, Honolulu.
15. O. L. Brekke in Ref. 11, p. 108.
16. R. Fahn and W. Zschau, *Possibilities for the Use of Spent Bleaching Earth*, presented at the AOCs 75th Annual Meeting, 1984.
17. W. H. Morrison, *JAOCs* **59**(12), 519 (1982).
18. D. V. Okonek and G. M. Lengen, in Ref. 1, p. 76A, 3.
19. T. L. Mounts in Ref. 11, p. 138.
20. R. Fukushima, *The Hull Truth*, St. Paul Pioneer Press, St. Paul, Minn., 1994.
21. *Wall Street Journal*, June 24, 1993.
22. *Inform* **5**(4), 358 (1994).
23. M. Kellens, in W. Hamm and R. J. Hamilton, eds., *Edible Oil Processing*, Sheffield Academic Press, Sheffield, UK, 2000.
24. G. M. Neumunz, *JAOCs* **55**, 397 (1978).
25. A Tirtiaux, *Dry Multiple Fractionation*, presented at the IFSC meeting, 1992, Denmark.

26. G. R. List and T. L. Mounts, in Ref. 11, pp. 193–199.
27. Crown Chemtech Limited, Chemetator sales brochure, 1993.
28. F. M. Bloomberg and T. W. Hutchins, U. S. Pat. 3,425,938 (1969).
29. J. B. Woerfel, *JAOCs* **3**, 188 (198).

## GENERAL REFERENCES

- D. R. Erickson, and co-workers, *Handbook of Oil Processing and Utilization*, American Soybean Association and AOCS, St. Louis, 1985.
- L. R. Watkins, E. W. Lusas, and S. S. Kaseoghu, eds., *Soybean Extraction and Oil Processing Manual*, Food Protein Research and Development Centers, Texas, 1987.
- E. Turkulov, D. J. Dimic, Karlovic and V. Vaksa, *JAOCs* **63**(10), 1360 (1986).
- H. B. W. Patterson, *Handling and Storage of Oilseeds, Oils, Fats and Meal*, Elsevier Applied Science, London, 1989.
- G. Anderson, *Oilseed Extraction and Meal Processing*, presented at the AOCS World Conference in Singapore.
- G. D. Brueske, *New Desolventizing and Toasting Techniques*, presented at the International AOCS Convention in Cannes, France, 1988.
- P. J. Wan and W. Farr, eds., *Introduction to Fats and Oils*, AOCS Press, Champaign, Ill., 2000.
- B. F. Szuhaj and G. R. List, eds., *Lecithins*, American Oil Chemists' Society, Champaign, Ill., 1985.
- M. J. Boyer, *Fundamental Practice and Current Wastewater Issues in Fats and Oils and Oleochemical Processing*, presented at the Environmental Short Course, Chicago, Illinois, May 1991.
- R. F. Wilson et al., *Lipid Biotechnology*, Marcel Dekker, New York, 2002.
- H. B. W. Patterson, *Hydrogenation of Fats and Oils*, Elsevier Applied Science, London, 1983.
- Ainelo, and co-workers, *JAOCs* **64**(9), 1341 (1987).
- Solvent Extraction Plants*, NFPA 36, 1993 edition.
- K. P. Eickhoff and K. Klein. *Winterzing of Vegetable Oils in Conjunction with Alkali Refining*, presented at the AOCS 79th Annual Meeting, May 3–6, 1989, Ohio.
- A. S. A., "Hydrogenation of Soy Oil," proceedings of the Third A.S.A. symposium of soybean processing.
- Y. Basiron and co-workers, *Progress, Prospects and Challenges Towards the 21st Century*, proceedings from the PORIM International Palm Oil Conference, Sept. 9–14, 1991.
- T. H. Applewhite, ed., *Proceedings from the World Conference on Oleochemicals into the 21st Century*, AOCS, Champaign, Ill., 1991.
- A. R. Baldwin, ed., *Proceedings from the World Conference on Emerging Technologies in the Fats and Oils Industry*, AOCS, Champaign, Ill., 1986.
- N. T. Dunford and H. B. Dunford, *Nutritionally Enhanced Edible Oil Processing*, AOCS Press, Champaign, Ill., 2004.

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## *Oil Extraction*

Timothy G. Kemper

### 1. EVOLUTION OF OIL EXTRACTION

#### 1.1. Hand Press Extraction

Documented oil extraction dates back to 1650 B.C. when ripened olives were pressed by hand in Egypt using wooden pestles and stone mortars. The extracted olive oil was filtered through goat hair filters and used as a lubricant. Sesame, linseed, and castor oils were recovered in Egypt by hand pressing as far back as 259 B.C. (1).

#### 1.2. Early Mechanized Extraction

By 184 B.C., the Romans developed more sophisticated technology such as edge-runner mills and screw and wedge presses. These technologies combined leverage and the use of animal power to aid in the milling and extraction of the oil. From Roman times until the eighteenth century, similar technology was used for oil extraction (1).

In the eighteenth century, wind and water power largely replaced animal power to assist in oil extraction. Large wind-driven stamper mills became popular in Europe. The wind turned a vane outside the oil mill, and the rotational energy was transmitted into the mill via shafts and gears, eventually rotating a horizontal cam shaft. The horizontal cam shaft had vertical stamper shafts connected to it. The initial stamper shafts were used as mortars to beat the oilseeds into a pulp inside a wooden pestle. The pulp was then transferred into filter bags woven from horse hair

and placed vertically between opposing wedges. Additional stampers pounded the wedges together, squeezing the oil through the filter bags, where it could be collected. Hundreds of such oil mills sprung up across Europe (2).

### 1.3. Hydraulic Press Extraction

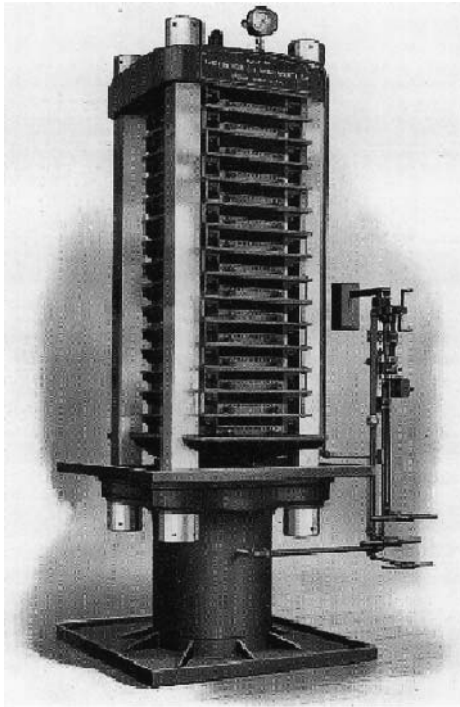
In 1795, J. Bramah of England invented the hydraulic press for oil extraction (1). Oilseeds were milled, cooked, and wrapped in filter cloths woven from horse-hair. The oilseeds wrapped in filter cloths were manually loaded into perforated, horizontal boxes below the head block and above the ram of the press. The boxes were pressed together using upward hydraulic pressure on the ram. The oil was pressed out through the filter cloths surrounding the oilseeds. The filter cloths and spent cake were manually removed from the hydraulic press. The residual oil in spent cake was approximately 10%.

In 1801, the first cottonseed oil mill was constructed in the United States using hydraulic presses (1). By the 1870s, American technology in hydraulic pressing had outpaced European technology. Large hydraulic presses with up to 16 press boxes and up to 400 tons of force were being used. In 1874, Rose, Down, and Thompson (2) of Hull, England, began marketing the American-designed hydraulic presses with the advantage of preforming the cakes to increase productivity. Facilities using this joint technology were commonly referred to as Anglo-American oil mills. In the late 1800s, German companies were producing hydraulic cage presses, with rams pressing the oilseeds inside of vertical slotted barrels that did not require filter cloths. By the end of the nineteenth century, hydraulic press oil mills were the standard technology for oil extraction.

In 1900, Alfred French founded the French Oil Mill Machinery Company in Piqua, Ohio, for the purpose of advancing hydraulic press technology. He was awarded a patent (3) for the automatic cake-trimming machine for automating the sizing of the cakes prior to pressing. He also developed and patented the “change valve” in 1905, which allowed the hydraulic press to change pressures near the end of the pressing cycle to squeeze additional oil. The continuous stacked cooker was patented in 1907 along with an innovative cake former. Two-pass pressing was another of French’s developments, taking final residual oil in cake below 5%. French hydraulic presses became the industry standard in the United States in the 1920s (3). Figure 1 indicates a typical French hydraulic press of that era.

Hydraulic press oil mills remained in use as late as the 1950s before the last of them were replaced with continuous screw presses and continuous solvent extraction plants, both of which required far less labor and could process at much higher rates.

The olive oil industry is the only oilseed industry still using hydraulic presses today. This is possible because of the price premium paid for natural olive oil, processed without the use of heat or chemicals.

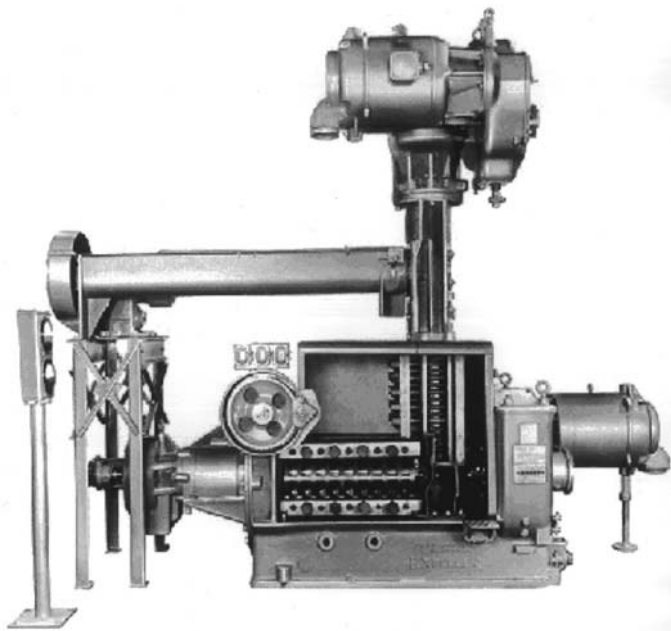


*Figure 1. Hydraulic press. Courtesy of French Oil Mill Machinery Company.*

#### **1.4. Screw Press Extraction**

In 1900, Valerius D. Anderson invented the mechanical screw press in Cleveland, Ohio. He was awarded a U.S. patent for the apparatus (4). The mechanical screw press was a radical departure and significant technological advancement over the hydraulic presses being used at the time. The mechanical screw press used a vertical feeder and a horizontal screw with increasing body diameter to impart pressure on the oleaginous material as it proceeded along the length of the screw. The barrel surrounding the screw was slotted along its length, allowing the increasing internal pressure to first expel air and then expel the oil through the barrel. The expelled oil was collected in a trough under the screw, and the de-oiled cake was discharged at the end of the screw. The primary advantage of the mechanical screw press was that it allowed continuous oil extraction and could process large quantities of oleaginous materials with minimal labor. Figure 2 illustrates an early Anderson Expeller<sup>®</sup> (trademark for Anderson mechanical screw press).

In the early 1900s, a number of European companies developed variations of the V. D. Anderson design concepts and began manufacturing mechanical screw presses. In the 1930s, after the U.S. patent had expired, other American firms did the same. These machines were able to replace large numbers of hydraulic presses.



**Figure 2.** *Expeller. Courtesy of Anderson International.*

In 1951, the V.D. Anderson Company was again the pioneer in mechanical screw pressing, being first to patent the process of using a mechanical screw press to continuously prepress oleaginous materials ahead of continuous solvent extraction plants. The company was awarded U.S. Patent 2,551,254 (5).

In the past 100 years, the primary improvement in mechanical screw press design has been developing materials of construction that extend wearing part life. Screw and barrel parts that once lasted three months before requiring replacement may now last up to two years. Additionally, mechanical screw presses have been built to much larger scale, going from initial capacities of 5 tons per day up to present-day capacities of over 100 tons per day for full pressing and over 800 tons per day for prepressing applications.

### **1.5. Solvent Extraction**

In 1855, Deiss of Marseilles, France, was first to employ solvent extraction (1). He used carbon disulfide to dissolve olive oil retained in spent olive cakes. This technology used batch solvent extraction, where the material was held in a common kettle for both the extraction process as well as the subsequent meal desolventizing process. Deiss obtained a patent for batch solvent extraction of olive oil in 1856 (1). Small batch solvent extraction plants were installed in France and Italy, and by 1870, small batch solvent extraction facilities had spread across Europe. Larger scale solvent extraction plants were supplied by Rose, Downs, and Thompson (2) of Hull, England, starting in 1898.



In the early 1920s, with the availability of petroleum-based solvents, German inventor Hildebrandt invented the continuous countercurrent immersion extractor and German inventor Bollman invented the continuous two-stage percolation extractor. The first commercial scale continuous solvent extraction plants were installed in Germany in the late 1920s, with the Hansa-Muhle facility in Hamburg using Hildebrandt extractors being the largest (6). Between 1934 and 1937, the Hansa-Muhle Company of Germany supplied the first continuous solvent extraction plants to oilseed processors in the United States, using Hildebrandt-type extractors. Leading German solvent extraction technology was set back due to World War II.

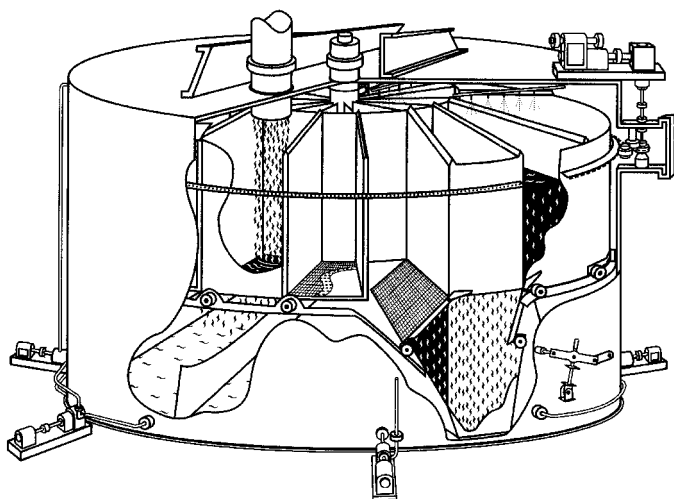
The American company, French Oil Mill Machinery Company of Piqua, Ohio, developed continuous solvent extraction plants starting in 1939, using a multistage Bollman-type percolation extractor (3). Soon thereafter, the Belgian Company, Extraction De Smet, started manufacturing continuous multistage belt-type extractors in 1945. V.D. Anderson of Cleveland, Ohio, joined the solvent extraction equipment supply market in 1948 with continuous Hildebrandt-type immersion extractors (5).

A small-scale chain conveyor type of extractor was developed in the 1940s at Iowa State University with the intent of using trichloroethylene solvent to extract soybeans. Crown Iron Works of Minneapolis, Minnesota, licensed the technology and extractor design, and supplied several continuous solvent extraction plants in 1951 using the new extractor and trichloroethylene solvent (6). The meal from these plants proved detrimental to animals, so the plants were either closed or converted to petroleum-based solvents (7). The chain-type extractor apparatus continued on and is the basis of the modern Crown Iron Works Model III extractor used today.

In the first continuous solvent extraction plants in Germany, the meal was desolventized in Schnecken's desolventizers. The Schnecken's desolventizer design consisted of a series of steam-jacketed conveyors, stacked one above the other, which used indirect heat to desolventize the meal. This was the prevalent meal desolventizer design used by all equipment suppliers through the 1940s.

In 1939, Kruse and Soldner (8) of Central Soya Company patented a process of mixing water into desolventized soybean meal and drying it in a vertical stacked cooker to improve meal quality. In 1940, Hayward (9) of Archer Daniels Midland described a similar process, referred to as "toasting" the meal. In 1948, Hutchins of French Oil Mill Machinery Company and Kruse of Central Soya Company worked together to develop an apparatus and process for desolventizing soybean meal and toasting soybean meal in the same vessel. The apparatus was a vertical stacked vessel. Live steam was introduced primarily in the top tray of the vessel to desolventize the meal and increase its moisture. The high moisture meal was then dried using indirect steam conducted through the trays lower in the same vessel. This new apparatus was known as a combination desolventizer-toaster, commonly abbreviated as "DT." Hutchins was awarded an apparatus patent, and Kruse was awarded a process patent for the DT (10, 11). This new desolventizing technology was considered state-of-the-art until the 1980s.

The Blaw Knox Company of Buffalo, New York, invented a rotary-type continuous solvent extractor in 1958 (12). The rotary-type extractor held the material in



**Figure 3.** Rotocele extractor. Courtesy of Kvearner Davy.

cells that rotated in a horizontal plane around a vertical axis, and it included four countercurrent extraction stages. The Blaw Knox trademark for this apparatus was the Rotocele<sup>®</sup> Extractor. The Rotocele Extractor was licensed to equipment suppliers around the world and quickly became very popular. Figure 3 illustrates a Rotocele Extractor. A similar apparatus, named the Stationary Basket Extractor, was invented by French Oil Mill Machinery Company in 1962 (3).

As solvent extraction plants were scaled up in size in the 1960s, the drying trays of the DTs were often not scaled up proportionately. The result was higher meal moistures exiting the DT, requiring a subsequent downstream meal drying step. Meal drying was generally accomplished using a rotary steam-tube dryer. In 1972, Schumacher of Germany invented the vertical stacked meal dryer-cooler, often abbreviated as “DC.” This apparatus used heated air to dry the meal and cool air to cool the meal. As the DC was similar in construction to a DT, Schumacher also developed the concept of stacking a desolventizer-toaster above a dryer-cooler to form a combination desolventizer-toaster-dryer-cooler, commonly abbreviated as a “DTDC.”

Desolventizing technology was further advanced in the late 1970s by Schumacher. The original Schumacher DT was a single, deep material depth tray with live steam injected from below. Steam injection from below allowed countercurrent stripping of solvent, and the deep material depth allowed adequate time for toasting. Steam distribution through the deep material bed was uneven, and the original Schumacher DT was difficult to operate. By 1980, Schumacher developed an improved version of the Schumacher DT, which included predesolventizing trays at the top of the vessel, along with perforated countercurrent trays below that supported thinner layers of meal and allowed the steam entering the bottom of the vessel to pass through the meal more evenly. Schumacher patented the hollow stay-bolt type of perforated countercurrent tray (13) used in the improved Schumacher DT in

1981, and he licensed this technology to equipment suppliers around the world. The Schumacher DT was often supplied above a DC to form a Schumacher DTDC.

In 1985, Mason of French Oil Mill Machinery Company developed a variation to the Schumacher DTDC design. The Mason design used a hollow stay-pipe type of perforated countercurrent tray design with clusters of perforations through a single plate at the top of the pipes. The DC also included the option of steam dryer trays prior to the air dryer trays, with the water vapor from the steam dryer trays being returned to the solvent extraction process for heat recovery. Another variation of the Schumacher DT was developed in 1997 and patented in 1999 by Kemper of the French Oil Mill Machinery Company and the Farmer of Bunge Corporation, which used a perforated countercurrent tray composed of a combination of indirect heating surface and slotted screen elements for more even distribution of countercurrent stripping steam (14).

The latest extractor to be developed for mainstream oleaginous material extraction applications is a rotary-type extractor with fixed slotted floor and bevel gear drive trademarked as the Reflex<sup>®</sup> Extractor. This extractor was developed by Kemper et al. (15) of the French Oil Mill Machinery Company in 1995 and patented in 1997. The Reflex Extractor is an improvement on the Rotocel Extractor. One of these units installed at the Louis Dreyfus solvent extraction plant in Argentina has processed in excess of 9000 metric tons of soybeans per day, making it the world's highest capacity extractor.

## 2. SEED PREPARATION

Upon receipt at the processing plant, oleaginous materials require varying degrees of seed preparation prior to the oil extraction process. Seed cleaning, seed drying, size reduction, hull removal, heating/drying, flaking, and extruding are all potential unit processes involved in seed preparation.

### 2.1. Seed Cleaning

Good quality oleaginous materials entering a processing plant will contain up to 2% foreign material. Foreign material is generally removed twice, once prior to storage and again as the oleaginous material enters the continuous process. The foreign material to be removed may consist of a combination of weed seeds, sticks, pods, dust, soil, sand, stones, and tramp metal.

Tramp metal is generally the first foreign material to be removed to protect all downstream equipment from damage. Tramp metal is separated using magnetic force to pull the iron-based materials from the oleaginous materials. Magnetic devices commonly used are plate magnets, drum magnets, and magnets suspended over a conveyor. Plate magnets allow the oleaginous materials to pour across their surface and rely on the metallic material sticking to the magnetic surface. Plate magnets are relatively inexpensive but require stopping the process occasionally to clean the magnetic surface and suffer from high wear rates. Drum magnets

rely on a fixed magnet inside a quadrant of a rotating drum. The metal debris sticks to the rotating drum, allows the oleaginous materials stream to fall away, and then the metallic objects fall off the drum later in the rotation. Drum magnets have the advantage of being continuous and self-cleaning, but suffer from high wear rates. The last type of magnet is a magnet suspended over a conveyor. This system generally consists of a pair of electromagnets suspended over the oleaginous materials passing in a thin stream over a belt conveyor. The metallic debris is pulled upward onto the surface of the electromagnets. This type of system has the advantage of no wear and, due to redundancy, allows cleaning during operation. There are numerous suppliers of various devices to remove tramp metal.

Sticks and pods are typically larger than oleaginous materials. In addition, sticks, pods, and dust are typically lighter than oleaginous materials. Therefore, the method of removing this foreign material from the oleaginous materials stream is typically a combination of coarse screening followed by aspiration. This combination of processes is commonly referred to as scalping. Companies such as Buhler of Switzerland, Carter-Day of the United States, and Kice Metal Products of the United States are well known for supplying scalping equipment.

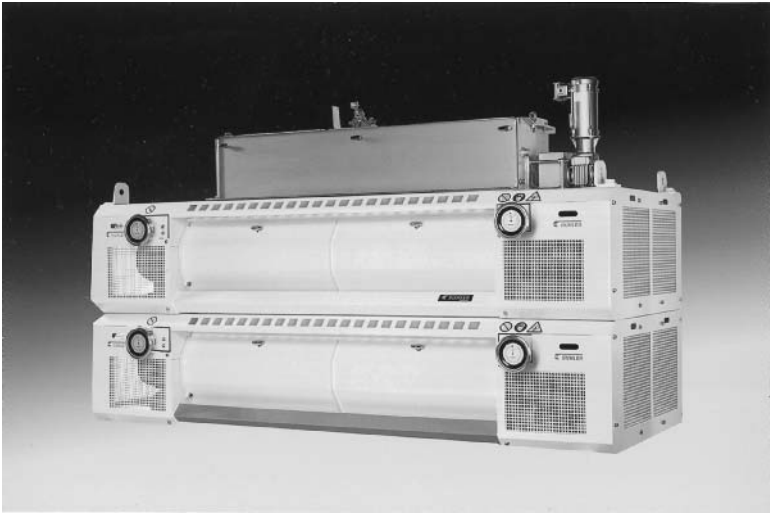
Weed seeds, sand, and soil are typically smaller in physical size than the oleaginous materials. These foreign materials can be removed by fine screening. The hole sizes in the screen are selected slightly smaller than the oleaginous materials, allowing the soil, sand, and weed seeds to sift through. Buhler of Switzerland and Rotex of the United States are popular suppliers of such screening equipment.

Particularly with ground nuts (peanuts), there may be a significant quantity of stones approximately the same size as the ground nuts. These stones are not removed by scalping or by fine screening. The stones are heavier than the ground nuts and need to be removed by gravity separation. Gravity separators manufactured for stone removal are commonly referred to as destoners. Buhler of Switzerland and Triple-S Dynamics of the United States are suppliers noted for manufacturing destoners.

## 2.2. Seed Drying

The moisture of oleaginous materials often needs to be reduced to minimize degradation in storage and to enhance the effectiveness of downstream unit operations. For example, soybeans are often received at 13% moisture and need to be dried to 10% moisture to facilitate hull removal.

Oleaginous materials are generally dried in large, vertical, open-flame grain dryers. These dryers have multiple columns of oleaginous materials that slowly migrate downward in a plugged flow. The oleaginous materials are dried in the upper portion of the column and cooled in the lower portion of the column. Cool air is pulled laterally through the lower portion of the column to cool the oleaginous materials and warm the air. The warm air is further heated with open flame and pushed laterally through the upper portion of the column to heat and dry the oleaginous materials. Many regional manufactures of cereal grain dryers are used to supply this equipment.



**Figure 4.** Cracking mill. Courtesy of Buhler.

### 2.3. Size Reduction

Most oleaginous materials require size reduction prior to further processing. Exceptions are canola, rapeseed, and corn germ, which are already sufficiently small in size. For most oleaginous materials, they need to be broken into pieces 2 to 3 mm across to enhance the downstream unit processes of hull removal, heating/drying, and flaking.

Soybeans, ground nut (peanuts), and copra are typically reduced in size using an apparatus known as a cracking mill. These mills generally consist of two sets of cylindrical corrugated rolls in series. The rolls operate at differential speeds to assist in breaking the oleaginous materials apart. The first set of rolls often has a coarser corrugation, and the second set of rolls has a finer corrugation to reduce the particle size in two steps. Modern cracking mills can process up to 1000 tons per day of oleaginous materials each. Figure 4 illustrates a modern, high-capacity cracking mill. Buhler of Switzerland and CPM Roskamp of the United States are the primary providers of cracking mills to the vegetable oil industry.

Sunflower seed and delinted cottonseed have very course hulls on the exterior of the seed, requiring more intense breaking. Devices for breaking these seeds into smaller pieces are generally supplied by Buhler of Switzerland and Carver of the United States.

### 2.4. Hull Removal

Soybeans, sunflower seeds, and delinted cottonseed have a course outer seed coat known as a hull. The hull fraction is high in fiber content and low in oil and protein

content. Soybeans, sunflower seeds, and delinted cottonseed often have a large portion of their hull fraction removed so that the finished meal fraction will be higher in protein by weight, and therefore higher in value.

The process of removing the seed coat of soybeans is commonly referred to as dehulling, and the process of removing the seed coat from sunflower seeds and delinted cottonseed is commonly referred to as decortication. In both dehulling and decortication, the process has two distinct stages. In the first stage, aspiration is used to remove the lighter hull fraction from the heavier meats fraction. A certain amount of small meats particles are also aspirated away with the hulls stream. In the second stage of dehulling, the fine meats are separated from the hulls through various means of hull agitation and screening. The effectiveness of a dehulling system is often measured by the residual fiber content (from hulls) in the meal fraction, and the residual oil content (from meats) in the hull fraction. For soybeans, the industry standards are less than 3.5% fiber content remaining in the meal fraction and less than 1.5% oil content remaining in the hull fraction.

In soybean dehulling, the aspiration can take place with the soybean at elevated temperature, or at ambient temperature. Dehulling at elevated temperature is referred to as hot dehulling, and dehulling at ambient temperature is referred to as cold dehulling. Dehulling at elevated temperature has the advantage of fewer fine meats particles being aspirated away with the hull fraction in the first stage of dehulling and, therefore, requires less separation of meats from the hull fraction in the second stage of dehulling.

Dehulling systems are most commonly supplied by Buhler of Switzerland, Crown Iron Works of the United States, De Smet of Belgium, Kice of the United States, and Rotex of the United States. Decortication systems are most commonly supplied by Buhler of Switzerland and Carver of the United States.

## 2.5. Heating/Drying

With the exception of cold pressing, all oil extraction processes require that the oleaginous materials be heated and sometimes further dried before oil extraction.

In order to enhance the downstream unit operation of flaking, oleaginous materials are typically heated in the range of 60–75°C temperature. By heating and softening the oleaginous materials, it enables the oleaginous material to stretch and flatten in the flaking operation with a minimum of fragmented particles being created.

For oleaginous materials being prepressed prior to solvent extraction, they are typically heated in the range of 90–110°C temperature to decrease the viscosity of the oil and allow the prepress to expel the oil and make a good quality cake. This heating is often in two stages, initial heating to approximately 65°C prior to flaking and then final heating to approximately 100°C prior to entering the prepress.

Oleaginous materials that are full pressed are often heated to temperatures of 110–150°C temperature, and dried to as low as 3% moisture. The high temperature decreases the viscosity of the oil, making it easier to expel. The high degree of drying largely ruptures the cellular structure of the oleaginous material as the internal

moisture vaporizes and expands. The low final moisture maximizes friction within the full press to maximize internal pressure. These functions all improve de-oiling and allow residual oil in cake to be minimized.

Another aspect of heating soybeans in particular is the impact on the phospholipase enzyme. The phospholipase enzyme is activated at approximately 55°C and remains activated up to approximately 100°C. In this temperature range, and with sufficient exposed surface area and time, the phospholipase enzyme modifies a portion of the phosphatides in the oil fraction by splitting off the non-fatty acid moiety (16). The resultant calcium and magnesium salts of phosphatidic acids that are formed tend to be more oil-soluble than water-soluble, thereby converting phosphatides from a hydratable form to a nonhydratable form (16). This has a resultant impact on the quantities of acid, caustic and silica needed to reduce the phosphorus content of the soybean oil in the downstream degumming and refining unit operations.

There are several types of apparatus used for heating/drying oleaginous materials. The rotary steam-tube conditioner, stacked-tray cooker, plugged flow conditioner, and hot air conditioner are most popular.

Rotary steam-tube conditioners are horizontal, cylindrical, shell, and tube heat exchangers that rotate on large rollers mounted under the shell. High-pressure steam is used on the tube side as the heating medium, and the oleaginous materials fill the lower half of the shell side, between the tubes. The rotating vessel is sloped downhill, with the oleaginous material particles entering at the center of the higher end of the vessel, and exiting at the center of the lower end of the vessel. Inside the unit, the oleaginous materials are mixed and heated by conductive heat transfer with the steam-filled tubes. The distribution of residence time of the oleaginous materials within the rotary steam-tube conditioner is very consistent. Typical residence times for oleaginous material applications are in the range of 10 to 30 minutes. Single units process as much as 5000 tons per day. The disadvantages of the rotary steam-tube conditioner are that it requires significant floor space, and it is difficult to maintain the seals at the product inlet and outlet. There are a number of regional suppliers of rotary steam-tube conditioners that supply the industry.

The stacked-tray cooker is a vertical, cylindrical vessel with a multitude of horizontal trays. The oleaginous materials enter at the top and are supported by the tray. The material is mixed above each tray and conveyed downward from tray to tray by agitating sweeps anchored to a center rotating shaft. The heat for increasing particle temperature and evaporating moisture is conducted into the oleaginous materials from the upper surface of the trays, filled with high-pressure steam. The stacked-tray cooker has the advantage of limited floor space. However, the stacked-tray cooker has the disadvantage of a wide distribution of residence time among particles, and it is limited to about 2000 tons per day capacity in a single vessel. Major suppliers of stacked-tray conditioners are Crown Iron Works of the United States, De Smet Group of Belgium, and Krupp Elastomertechnik of Germany.

Plugged flow conditioners are vertical, square columns with a multitude of horizontal oval-shaped tubes inside the column. The oleaginous materials have a

plugged flow and totally surround the steam-heated tubes. The oleaginous materials discharge the conditioner by variable-speed live bottom screws. The advantages of the plug flow conditioner are that they require no electrical mixing power, they have very tight distribution of residence time among particles, and they require little floor space. Plugged flow conditioners are supplied by Buhler of Switzerland and Crown Iron Works of the United States.

In soybean plants using hot dehulling, the cracked soybeans are shocked by hot air to aid in the separation of the hull fraction from the meats fraction. The hot air conditioner uses either a hot air fluidized bed to heat and convey or hot air spouting to heat and a belt to convey. The hot air is partially recirculated for heat recovery. Hot air conditioners for soybean plants are provided by Buhler of Switzerland, Crown Iron Works of the United States, and Escher Wyss of Germany.

## 2.6. Flaking

Most oleaginous materials are flaked prior to solvent extraction. Flaking distorts the cellular structure of the oleaginous material and reduces the distance that solvent needs to penetrate to reach the oil in the oleaginous material cells. The apparatus used for flaking is commonly referred to as a flaking mill. The flaking mill has two large diameter rolls in parallel, turning in opposing direction at approximately 250 to 300 r.p.m., and forced together by hydraulic cylinders. The softened oleaginous materials are uniformly fed into the nip of the two rolls. As the oleaginous materials are pulled through, they are stretched and flattened. Typical flakes are in the range of 0.3 to 0.4 mm thick and 8 to 18 mm in diameter.

Good solvent extraction performance is highly dependent on good flaking performance. It is critical that no particles pass through the flaking mill without being flaked, and it is critical that flake thickness is kept uniform across the entire width of each flaking mill. To accomplish good flaking mill performance, the flaking mills must be fed very uniformly at all times, and the roll surfaces must be kept parallel. Common practice is to grind the surfaces of the rolls on an as-required basis to maintain the parallel condition. It is also important to maintain good top-down aspiration of the flaking mill to remove dust from the feeding apparatus, and to remove surface moisture from the flakes.

Modern flaking mills are manufactured in capacities of 300 to 500 tons per day. Figure 5 illustrates a modern, high-capacity flaking mill. Buhler of Switzerland and CPM Roskamp of the United States are the major suppliers of flaking mills to the vegetable oil industry.

## 2.7. Extruding

A wet extruder can be used to enhance the performance of solvent extraction on soybeans and cottonseed, and a dry extruder can be used to enhance the performance of a full press.

A wet extruder is a high-strength cut-flight screw conveyor equipped with steam injection along the housing and a restricted orifice discharge. These units are





**Figure 5.** Flaking mill. Courtesy of CPM ROSKAMP.

commonly referred to as Expanders<sup>®</sup> (Anderson International trademark). The oleaginous flakes are heated in the range of 90–110°C temperature using a combination of electrically generated internal friction and live steam heat. The pressure developed prior to the restricted orifice discharge increases the product density. As the high-temperature, high-moisture product exits the restricted orifice, the pressure drop allows the product to abruptly flash off moisture. The result is a hot, fragile, pellet-like product, with greater bulk density than the incoming flakes, greater porosity than the incoming flakes, and significant cell rupture. To enable good solvent extraction performance, the extrudate must be dried to 10% moisture and cooled to 60°C temperature to firm up the soft extrudate and prevent evaporative cooling and recondensing of moisture enroute to solvent extraction.

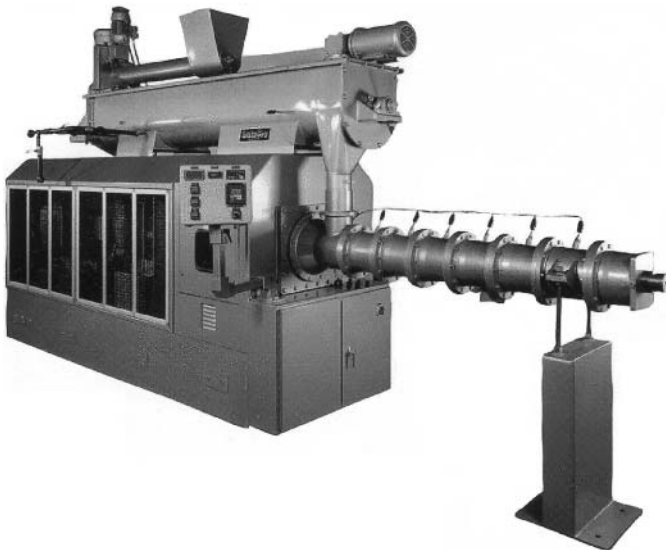
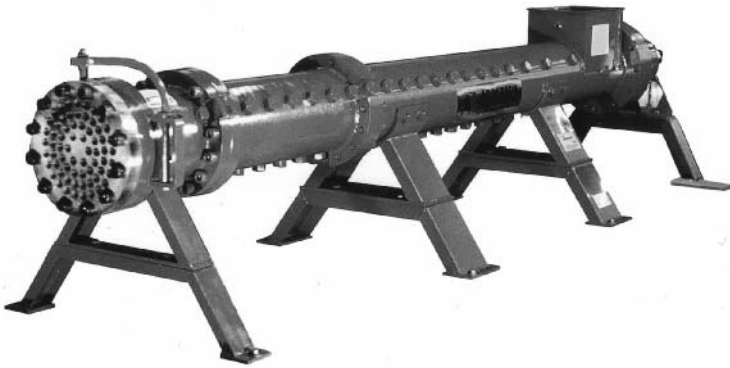
Wet extruders are used extensively prior to solvent extraction in cottonseed processing. Cottonseed flakes with 30% oil content are very fragile and have poor miscella flux rates within the extractor, causing inconsistent results and requiring very long extraction times. By extruding the flakes, consistently good results can be achieved with moderate extractor sizing.

Wet extruders are also used ahead of a number of soybean solvent extraction plants. In plants with undersized extractors, the energy used by the extruder can be compensated by additional oil yield from the extractor. Steam consumption is reduced in the desolventizer toaster. Additionally, the wet extruder in a soybean plant increases temperature sufficiently to stop the phospholipase enzyme from converting additional hydratable phosphatides into nonhydratable phosphatides. This reduces acid, caustic, and silica consumption in the downstream refinery. However, in soybean plants with adequately sized extractors where potential oil yield

improvements are minimal, the additional operating costs of wet extruders are difficult to justify.

Modern wet extruders can process up to 1500 tons per day of flakes per unit. They are equipped with variable orifice apertures to aid cleaning after power stoppages. Figure 6a illustrates a modern, high-capacity Expander. Anderson International of the United States and Technal of Brazil are the two major suppliers of wet extruders used in the vegetable oil industry.

Dry extruders are occasionally used ahead of full presses. Dry extruders use electrical power to generate internal friction to heat the product as high as 150°C temperature. When the extruded product exits the dry extruder, it is liquid-like in consistency with thorough cell rupture. When this product is fed to a full press, the



**Figure 6.** (a) Expander. Courtesy of Anderson International. (b) Dry-type extruder. Courtesy of Insta-Pro International.

press throughput can be increased significantly. Because of the short residence time of less than 30 seconds in the dry extruder (17), the protein solubility of the meal produced is higher than with traditional processing, whereas trypsin inhibitors are sufficiently deactivated. The resultant high-protein solubility meal is best suited for poultry and swine consumption.

The principle advantage of dry extruder preparation is that no expensive stacked-tray cookers or steam boiler are required, and the total capital investment for facilities under 100 tons per day in size is significantly less than for traditional full-press or solvent extraction processes. Therefore, this means of preparation is commonly applied in very small-scale plants commonly referred to as “mini-mills.” The primary disadvantage of dry extruder preparation is that the meal produced is higher in fat content than many poultry producers desire, and lower in bypass protein than many cattle producers desire. Thus, meal produced from a full-press mill using dry extrusion preparation generally obtains a lower price than meal produced from a traditional full-press operation.

Dry extruders available today are limited in capacity, typically processing less than 50 tons per day each. Figure 6b illustrates a typical dry-type extruder. Major suppliers of dry extruders are Anderson International of the United States and Insta-Pro International of the United States.

### **3. MECHANICAL EXTRACTION**

#### **3.1. Palm Fruit Extraction**

Palm fruit is different than most oleaginous materials and requires a unique preparation consisting of sterilization, stripping, and digestion. Sterilization is a heating process under pressure to stop the development of lipolytic enzymes in palm fruit bunches. Stripping is the mechanical process used to separate the stalks and leaves from the palm fruits. Digestion is a process of heating the palm fruits to approximately 90–100°C temperature for 20 minutes prior to pressing (18).

Mechanical extraction of the palm oil from the digested palm fruit is a delicate process. The screw press must squeeze as much oil as possible from the fruit without breaking the palm kernels. Palm-kernel oil will mix with the palm fruit oil if the kernels are crushed. International trading rules allow a maximum of 5% palm-kernel oil in palm oil.

The presses used to extract the palm oil are low-pressure screw presses. A low-pressure screw press is a mechanical device that uses a horizontal screw with increasing body diameter to impart pressure on the palm fruit as it proceeds along the length of the screw. Some designs use parallel twin-screw technology. The barrel surrounding the screw(s) is perforated along its length, allowing the increasing internal pressure to first expel air and then expel the oil through the barrel. The expelled oil is collected in a trough under the screw(s), and the de-oiled material discharges at the end of the screw. Low-pressure screw presses can reduce residual oil in palm fruit fibers to 5% while keeping palm-kernel breakage rates below 15% (18).

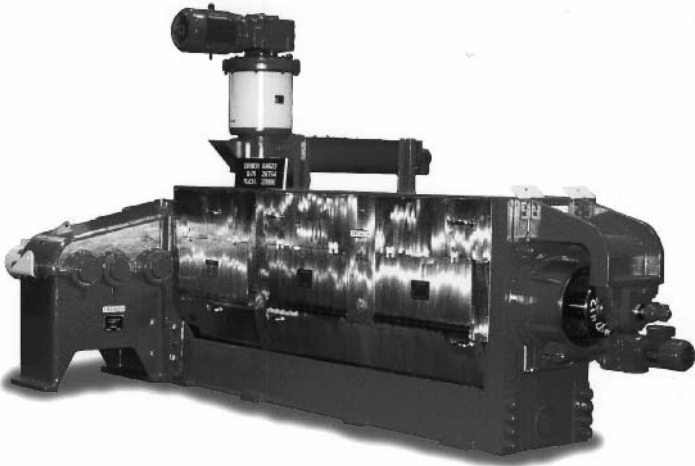
The palm oil exiting the low-pressure screw press has approximately 66% oil, 24% moisture, and 10% solids (18). The solid particles are typically separated from the oil using the traditional method of pumping the oil into a tank with approximately 2 hours of residence time to allow the heavier solid particles to settle and be continuously dredged from the base of the tank. After gravity separation, the oil is then pumped through a liquid cyclone to remove residual solids. Solid particles separated from the clean oil stream are saturated with oil and recycled back into the process. After the palm oil is cleaned of solid particles, it is heated and pumped through a vacuum oil-dryer to remove moisture.

### 3.2. Full Press Extraction

Because of comparatively poor oil yields, sole use of mechanical extraction to separate the oil and meal fraction is not as commonly used as solvent extraction. The mechanical extraction process can reduce the oil in meal to 5% to 10% by weight, whereas the solvent extraction process reduces the oil in meal to less than 1% by weight. As the value of the oil fraction is typically two to three times the value of the meal fraction by weight, the loss of yield is very costly. The mechanical extraction process also has comparatively higher energy and maintenance costs per ton of oleaginous materials processed.

There are four primary reasons why the mechanical extraction process is still selectively used. First, the mechanical extraction process can be furnished in very small scale, as low as 10 tons per day. The capital cost for small mechanical extraction facilities is considerably less than small solvent extraction facilities. In remote locations, freight differential can compensate for higher operating costs and lower yields. Second, there is a niche, high-value market for natural oils that have not been in contact with solvents or chemicals, requiring the use of mechanical extraction. Third, mechanical extraction can create a high bypass protein meal for ruminant animals that sells at a price premium over solvent extracted meal. Finally, mechanical extraction is often considered more reliable than solvent extraction when processing difficult materials (copra and palm kernel) in hot, tropical climates.

The apparatus used to mechanically extract the oil fraction from the meal fraction is a full press or Expeller<sup>®</sup> press (Anderson International trademark). A full press is a mechanical device that uses a horizontal screw with increasing body diameter to impart pressure on the oleaginous material as it proceeds along the length of the screw. The barrel surrounding the screw is slotted along its length, allowing the increasing internal pressure to first expel air and then expel the oil through the barrel. The expelled oil is collected in a trough under the screw, and the de-oiled oleaginous material cake discharges at the end of the screw. The full press has one function, to de-oil the oleaginous material as low as possible. The key to full-press performance is to apply maximum pressure to a thin cross section of the oleaginous material to squeeze out as much of the oil as possible. As a result, full presses create tremendous heat. It is common for full presses to use water-cooled shafts and water-cooled or oil-cooled barrels to dissipate the heat to



*Figure 7. Full press. Courtesy of French Oil Mill Machinery Company.*

maintain adequate internal friction and pressure. Most full presses are capable of processing 10 to 100 tons of oleaginous materials per day. Figure 7 illustrates a 100 ton per day, modern full press. Smaller capacity full presses can typically obtain lower residual oil in meal, at the expense of consuming more power and maintenance cost per ton of seed processed. Today there are numerous suppliers of full presses, with Anderson International of the United States, De Smet Rose-downs of the United Kingdom, French Oil Mill Machinery Company of the United States, Insta-Pro International of the United States, and Krupp Elastomertechnik of Germany being the most notable international suppliers.

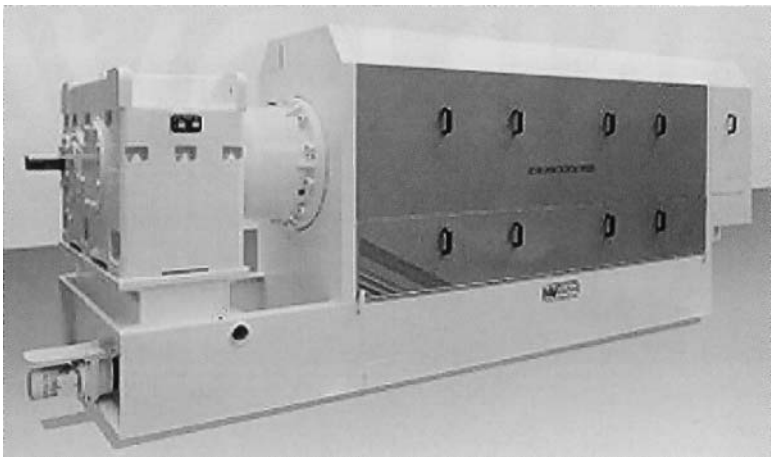
The fraction of oil removed in the full press is laden with fine meal particles, typically in the range of 5–15% by weight. These meal particles are typically separated from the oil using the traditional method of pumping the oil into a tank with 30 to 60 minutes of residence time to allow the heavier meal particles to settle and be continuously dredged from the base of the tank. After gravity separation, the oil is then pumped across a vibratory screener or through a pressure-leaf filter for final meal particle separation. Meal particles separated from the clean oil stream are saturated with oil and recycled back into the process ahead of the full press.

### **3.3. Pre-Press Extraction**

The solvent extraction process relies on the prepared oleaginous material maintaining its structural integrity while in the extractor. Oleaginous materials with greater than 30% oil by weight tend to break down in the extractor after a large portion of the oil is extracted, causing poor final extraction and high solvent retention. A solvent extraction plant operating with an oleaginous material containing approximately 20% oil is in thermodynamic balance. The waste heat from desolventizing

the meal fraction is sufficient to serve as the principle heat source for evaporating the solvent from the oil fraction. If a high oil content material is sent to the solvent extraction plant, the thermodynamic balance is lost and the operational energy required in the solvent extraction process increases significantly. As a result, oleaginous materials with greater than 30% oil content typically have their oil content reduced to 20% prior to solvent extraction. Rapeseed/canola, sunflower, ground nut, wet process corn germ, palm kernel, and copra are common oleaginous materials with an excess of 30% oil content, which require a reduction in oil content prior to solvent extraction.

The apparatus used to reduce the oil content of oleaginous materials prior to solvent extraction is commonly referred to as a prepress. A prepress is a mechanical device that uses a horizontal screw with increasing body diameter to impart pressure on the oleaginous material as it proceeds along the length of the screw. The barrel surrounding the screw is slotted along its length, allowing the increasing internal pressure to first expel air and then expel a portion of the oil through the barrel. The expelled oil is collected in a trough under the screw, and the partially de-oiled oleaginous material cake discharges the end of the screw. The prepress has two important functions, partially de-oiling the oleaginous material to 20% oil content and producing a porous cake with adequate structural integrity to allow high downstream solvent extraction efficiency. The key to prepress performance is to convert electrical energy into pressure rather than heat, by configuring the segmental screw elements to provide sufficient forward conveyance with an optimum combination of intermittent pressurization and mixing along the length of the barrel. Modern prepresses are capable of processing 500 to 1000 tons of oleaginous materials per day. Figure 8 illustrates an 800 ton per day, modern full press. These large machines are typically driven with 200–400-kw motors. Today there are two major suppliers of prepresses, De Smet Rosedowns of the United Kingdom and Krupp Elastomertechnik of Germany.



**Figure 8.** Prepress. Courtesy of De Smet Rosedowns.

The fraction of oil removed in the prepress is laden with fine meal particles, typically in the range of 5–10% by weight. These meal particles are separated from the oil using one of two methods. The traditional method is to pump the oil into a tank with 30 to 60 minutes of residence time to allow the heavier meal particles to settle and be continuously dredged from the base of the tank. After gravity separation, the oil is then pumped through a pressure-leaf filter for final meal particle separation. The alternative method of separating the meal particles from the oil is through use of a high-speed centrifugal decanter. In both methods, meal particles separated from the clean oil stream are saturated with oil. These oil-rich meal particles are either recycled back into the process ahead of the prepress or are mixed with the partially de-oiled cake and sent forward to the solvent extraction process.

## 4. SOLVENT EXTRACTION

### 4.1. Introduction

The overwhelming majority of all vegetable oil is extracted using solvent extraction. The solvent extraction process has the benefit of significantly higher oil yields than mechanical extraction, along with lower unit operating costs. The major drawback of solvent extraction is the high initial capital cost to construct a facility. Solvent extraction facilities constructed today are commonly in the size range of 1000 to 5000 tons per day, costing \$15 million to \$75 million to construct.

The solvent used in the majority of oilseed solvent extraction plants around the world is commercial hexane, a mixture of hydrocarbons generally boiling in the temperature range of 65–69°C. Most commercial hexane available contains approximately 65% normal hexane, with the remaining 35% of the composition consisting of cyclopentane and hexane isomers. As hexane vapor is three times heavier than air and slight amounts of hexane mixed in air can create an explosive mixture, special care must be taken in constructing and operating solvent extraction plants. The National Fire Protection Agency bulletin *NFPA-36 Solvent Extraction Plants* is the recognized guide for safe construction and operation of such facilities.

Because of special safety considerations, the solvent extraction process is constructed in a separate facility from the seed preparation process. The solvent extraction process consists of five closely interrelated unit processes: solvent extraction, meal desolventizing, meal drying and cooling, miscella distillation, and solvent recovery.

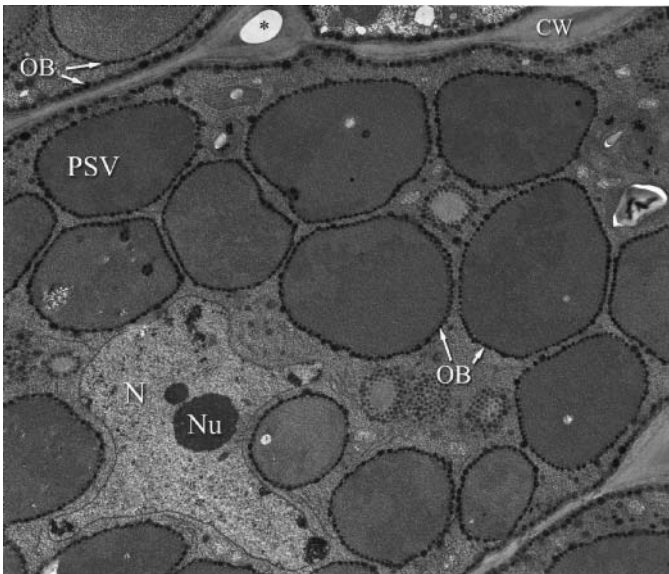
### 4.2. Solvent Extractor

The extractor is the apparatus in the solvent extraction process where the vegetable oil fraction of the oleaginous material is separated from the meal fraction of the oleaginous material by dissolving the oil fraction in a solvent.

The prepared oleaginous material is conveyed from the seed preparation process to the solvent extraction process and enters the solvent extractor. The solvent

extractor conveys the prepared material from its inlet to its exit, providing the prepared material approximately 30 to 120 minutes of residence time. While the material is being conveyed forward, miscella (solvent and oil solution) is washed down through the bed of material to extract out the vegetable oil. Each miscella wash is of a decreasing concentration of vegetable oil. After four to eight miscella washes, the material is washed once more by fresh solvent, ending the extraction process. **Before the material exits the extractor, it is allowed to gravity drain to reduce its solvent retention. The extracted, spent material then falls into the extractor discharge and exits the apparatus. The miscella with the highest concentration of vegetable oil also exits the apparatus to a full miscella tank.**

To understand the extraction process on a macroscale, it is helpful to understand the extraction process on a microscale. Figure 9a indicates the microstructure of a soybean cotyledon parenchyma (meats fraction) cell. This transmission electron micrograph is at 9000:1 magnification and represents a 0.020-mm tall by 0.023-mm wide cross-sectional view of a soybean flake, the approximate size of a single cell. The cell wall (CW), protein storage vacuoles (PSV), oil bodies (OB), nucleus (N), nucleolus (Nu), and intercellular spaces (\*) are all indicated on the micrograph. As clearly seen, the oil within the cell exists as thousands of spherical oil bodies clinging to the inside surface of the cell walls and to the exterior surface of the protein storage vacuoles.



**Figure 9.** (a) Soybean cellular structure. Courtesy of USDA-ARS. Special thanks to Dr. Robert Yaklich and Dr. Charles Murphy at the Soybean Genomics and Improvement Laboratory in Beltsville, MD, for creating this electron transmission micrograph especially for this chapter. (b) Crown extractor. Courtesy of Crown Iron Works. (c) Reflex extractor. Courtesy of De Smet Group. (d) LM extractor. Courtesy of De Smet Group.



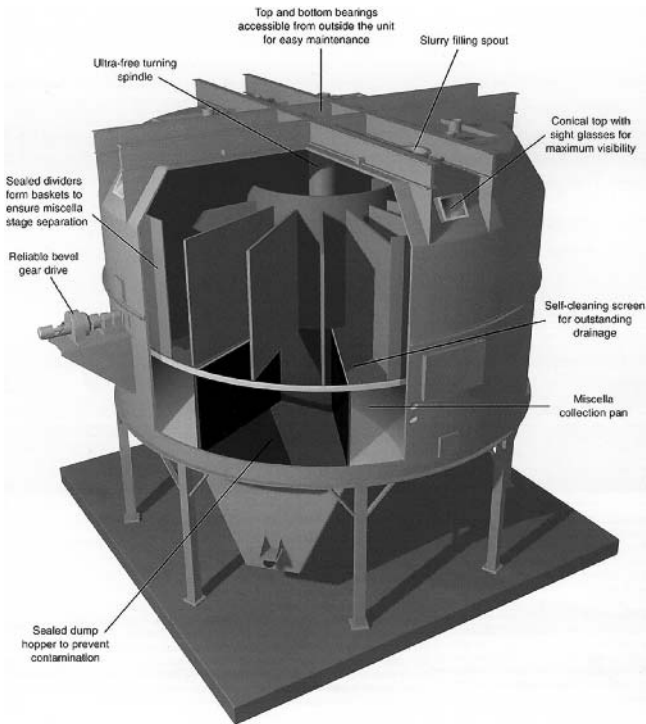
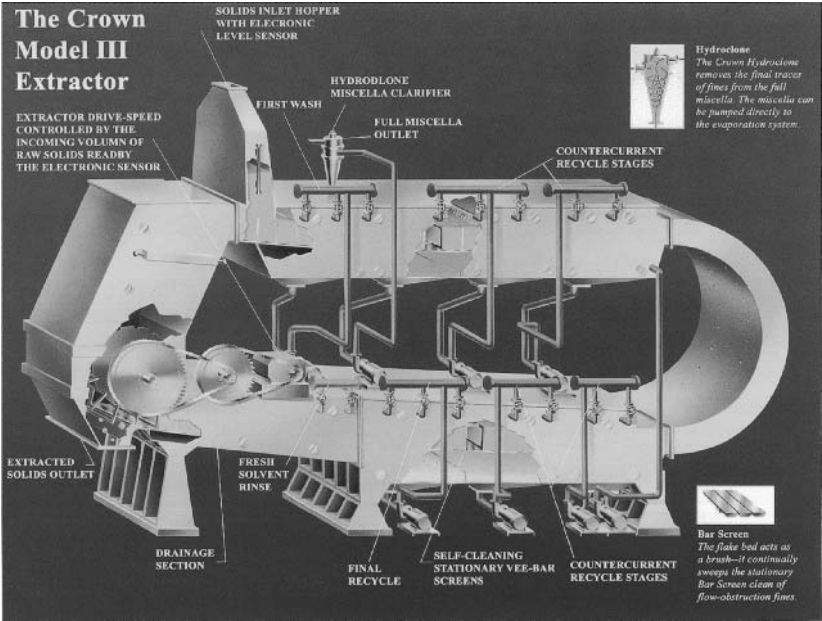
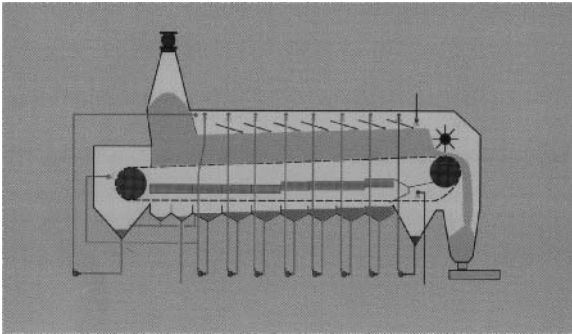


Figure 9 (Continued)



**Figure 9** (Continued)

In the solvent extraction process, the miscella at the surface of the oleaginous material diffuses through the cell walls to the oil bodies located within the cells. The miscella quickly goes into solution with the oil bodies. As miscella continues to enter and go into solution, internal pressure builds within the cell and concentrated miscella diffuses back out of the cell. This concentrated miscella diffuses through the adjacent cell walls and eventually reaches the surface of the particle. Once the more concentrated miscella reaches the bath of miscella outside the oleaginous material, it quickly goes into solution with the miscella, incrementally increasing its concentration. This process continues until the concentration of the miscella inside the cells of the oleaginous material comes into equilibrium with the concentration of miscella outside of the oleaginous material.

All oleaginous materials have a somewhat different cell structure and, therefore, a different time required for the miscella in the cells of the oleaginous material to come into equilibrium with the miscella outside of the oleaginous material. Soybean flakes have a cellular structure (16) that will allow equilibrium to occur within approximately 5 minutes at each extractor wash, whereas sunflower cake requires approximately 9 minutes and rapeseed cake requires approximately 12 minutes.

There are six parameters that affect the performance of the solvent extractor apparatus. These six parameters are outlined below.

**4.2.1. Contact Time** The total time that the oleaginous material spends in the extractor is residence time. Residence time can be subdivided into wash time and drain time. Wash time is the time the oleaginous material spends under the washing nozzles of the extractor, and drain time is the time the oleaginous material spends draining prior to discharge.

Wash time can be further subdivided into contact time and dormant time. Contact time is the time a particle of oleaginous material spends in the washing zone of the extractor where the particle is in contact with miscella. Extraction only takes place during contact time. Dormant time is the time a particle of oleaginous material spends in the washing zone of the extractor where it is not

in contact with miscella. The ratio of contact time to dormant time varies with extractor design.

Extractors with deep material bed depth and small bed surface area are generally operated by immersing the oleaginous materials in miscella. This is accomplished by providing a sufficient miscella flow rate per unit of material bed surface area to ensure that miscella fills all of the voids around the oleaginous material particles as it passes down through the material bed. This type of extractor operation provides a very high ratio of contact time to dormant time in the washing zone.

Extractors with shallow bed depth and large bed surface area are generally operated by percolating the miscella down through the oleaginous materials. With most oleaginous materials, the oleaginous material particles occupy 40% to 50% of the material bed and 50% to 60% of the material bed is composed of void spaces between the particles. In percolation, the oleaginous material particles are surrounded by both solvent vapor and miscella as the miscella rains downward. This type of extractor operation provides a lower ratio of contact time to dormant time in the washing zone.

In comparing two different extractor designs, the contact time can be similar, despite very different configurations:

#### *Deep Bed Extractor Example*

50 min. residence time = 30 min. wash time + 20 min. drain time.

30 min. wash time – 5 min. dormant time = 25 min. contact time.

#### *Shallow Bed Extractor Example*

50 min. residence time = 45 min. wash time + 5 min. drain time.

45 min. wash time – 20 min. dormant time = 25 min. contact time.

Regardless of extractor design, adequate contact time is critical for maximizing extraction efficiency and minimizing the amount of residual oil remaining in the oleaginous material. Increased contact time requires a larger extractor. Even though this represents a higher initial investment, the long-term benefits of surplus contact time are significant and justify oversizing the extractor.

**4.2.2. Particle Thickness** Various oleaginous materials are prepared for extraction using different process steps, but with virtually all oleaginous materials, one process step is flaking. The principle purpose of flaking is to reduce the thickness of the oleaginous material to reduce the distance and number of cell walls that miscella needs to diffuse through to reach the oil bodies. Note that for soybeans, a typical 0.38-mm-thick flake is approximately 20 cells thick. By reducing the particle thickness, the time required for miscella within the cellular structure of the oleaginous material to reach equilibrium with the miscella surrounding the oleaginous material is reduced. By reducing particle thickness, desired results

can be achieved with less contact time. If all other extraction parameters remain constant, reduced particle thickness will allow a smaller extractor to be used.

Reducing the particle thickness represents additional cost. For example, on soybeans, reducing particle thickness from 0.38 mm down to 0.30 mm will increase flaking mill electricity requirements by 1 to 2 kwh/ton of soybeans processed. As this is a significant ongoing operating expense, it is not economically feasible to undersize an extractor and reduce particle thickness. Conversely, by increasing particle thickness, desired results will demand more contact time. An extractor can be oversized to obtain desired results with increased particle thickness, thereby reducing ongoing operating cost.

For all oleaginous materials, the economic balance between the initial cost of the extractor and the ongoing electricity costs required for flaking can be analyzed and the optimum particle thickness can be determined.

**4.2.3. Extractor Temperature** As the temperature of the miscella increases, its rate of diffusivity through the cell walls of the oleaginous material increases. As the prepared oleaginous material enters the extractor at approximately 60°C, and both the oil and meal fractions are heated in excess of 100°C in subsequent process steps, there is no extra energy required for operating the extractor at a warm temperature. As a result, optimizing extraction results requires operating the extractor as warm as possible.

There is an upper limit for the extractor operating temperature. The solvent must remain safely in a liquid state. As the boiling range of commercial hexane is typically 64–69°C at sea level, the maximum possible temperature to prevent boiling is 63°C. Operating on the edge of the boiling range could cause rapid evaporation during an upset condition. Rapid evaporation can cause pressurization of the extractor, leading to excessive solvent loss, a safety hazard. Therefore, most processors operate the extractor at 60°C to provide several degrees of safety margin below the lower end of the solvent boiling range.

If the prepared material temperature is too low, or heat loss in the extractor is too high, then it may not be possible to achieve an extractor temperature of 60°C. Additional contact time will be required to achieve desired results. Insulating the conveying system and extractor to prevent heat loss to enable operation at 60°C is typically less expensive than oversizing the extractor to compensate for low operating temperature.

**4.2.4. Miscella Flux Rate** The miscella flux rate is the maximum volumetric flow rate of miscella that can flow down through the bed of material per unit of material bed surface area. In SI units, it is commonly expressed as m<sup>3</sup>/h per m<sup>2</sup>, and in Imperial units, it is commonly expressed as gpm per ft<sup>2</sup>. By simplification of units, the miscella flux rate can also be expressed as the maximum downward velocity of the miscella through the material bed (in m/min or ft/min). Miscella flux rates for various prepared oleaginous materials vary widely (see Table 1).

The miscella flux rate is determined by the screen below the bed of material. As stated earlier, the material bed is approximately 40% to 50% solids and 50% to 60%

TABLE 1. Miscella Flux Rates

Miscella Flux Rates	m <sup>3</sup> /h/m <sup>2</sup>	gpm/ft <sup>2</sup>	m/min	ft/min
Cottonseed flakes (0.38 mm)	10	4	0.17	0.53
Cottonseed extrudate	30	12	0.50	1.60
Rapeseed cake	20	8	0.33	1.07
Soybean flakes (0.30 mm)	15	6	0.25	0.80
Soybean flakes (0.38 mm)	25	10	0.42	1.33
Soybean extrudate	40	16	0.67	2.13
Sunflower cake (ground)	25	10	0.42	1.33

void space. Therefore, as the miscella is moving downward, it has 50–60% open area. The screen under the material bed has less open area, and therefore, the material interface with the screen creates the greatest restriction to flow. Most deep bed extractor screens have approximately 30% open area, whereas most shallow bed extractor screens have less than 10% open area. In both cases, the screens have less open area than the material bed, and the material interface with the screen provides the greatest flow restriction.

As downward miscella flow reaches the miscella flux rate, the material/screen interface reaches its maximum flow rate and begins restricting the flow of miscella. All void spaces between the oleaginous material particles fill with miscella as the solvent vapors are pushed out of the top of the material bed. Eventually, the entire material bed becomes immersed in miscella with no void spaces. At this point, miscella breaks through the top of the miscella bed and forms a pool. This phenomenon is often referred to as “flooding” the material bed. Once the material bed is flooded, no additional rate of flow will pass down through the material bed.

At each washing stage of the extractor, miscella needs to have an opportunity to wash the material bed, pour through the screen, and then enter the proper miscella collection receptacle underneath the material bed. For a given extractor and prepared oleaginous material, each miscella collection receptacle is carefully calculated to be located a specific distance after its washing nozzle to maintain separation between washing stages.

For example, if there is a deep bed extractor operating on 0.38-mm-thick soybean flakes with a 3.0-m bed depth and a forward velocity of 0.3 m/min, the distance that the miscella collection receptacle needs to follow the washing nozzle can be calculated as follows:

Downward miscella velocity for 0.38-mm-thick soybean flakes = 0.42 m/min.

Time for miscella to pass through material bed = 3.0 m/0.42 m/min = 7.1 min.

Wash nozzle to miscella receptacle distance = 7.1 min\* 0.3 m/min = 2.1 m.

As another example, if there is a shallow bed extractor operating on 0.38-mm-thick soybean flakes with a 1.0-m bed depth and a forward velocity of 1.0m/min,

the distance that the miscella collection receptacle needs to follow the washing nozzle can be calculated as follows:

Downward miscella velocity for 0.38-mm-thick soybean flakes = 0.42 m/min.

Time for miscella to pass through material bed =  $1.0 \text{ m} / 0.42 \text{ m/min} = 2.4 \text{ min}$ .

Wash nozzle to miscella receptacle distance =  $2.4 \text{ min} * 1.0 \text{ m/min} = 2.4 \text{ m}$ .

If the miscella flux rate is significantly reduced for a given prepared oleaginous material, the miscella can partially discharge into a later, undesired miscella collection receptacle. This will cause concentration contamination caused by mixing of the extractor washes and reduce the efficiency of the extractor.

Miscella flux rates can reduce as a result of thinner than normal flakes, surface moisture, or an abundance of fine particles. Flake thickness is a normal operator controlled parameter, and surface moisture and an abundance of fine particles are more difficult to control.

As hexane solvent is not soluble in water, the liquids repel each other. If surface moisture exists on the material in the extractor, the solvent has difficulty penetrating the particle surface. Also, the moisture can collect on the screen at the bottom of the material bed, building a protein layer and narrowing the screen slots, thus further reducing the miscella flux rate. To prevent this from occurring, it is very important to adequately aspirate flakes from flaking mills, cake from screw presses, or extruded pellets from extruders to remove all water vapor created when the material evaporatively cools from preparation temperature to extraction temperature. As a secondary precaution, it is ideal to have an extractor designed such that the oleaginous material moves with respect to the screen surface to constantly keep the screen surface brushed clean so that the impact of surface moisture on miscella flux rate is minimized.

The material bed is approximately 40–50% material particles and 50–60% void space. If there is an abundance of fine material particles, these particles can sift down through the material bed and settle in the void spaces just above the screen. This causes an additional flow restriction, and the miscella flux rate will be reduced. An abundance of fine material coming to the extractor is generally caused by over-drying the material at some point in the seed preparation stage, or by rough handling of the friable material during a seed preparation step. Both should be avoided to ensure uniform material shape to the extractor and uniform miscella flux rates.

**4.2.5. Number of Miscella Stages** In most extraction applications, the prepared material has approximately 20% oil by weight and the goal is to reduce the oil content to approximately 0.5% oil by weight. If an extractor had one miscella stage, then the miscella concentration exiting the extractor (1.15% oil) would be in equilibrium with the miscella concentration remaining in the material cells (1.15% oil). A mass balance for a single-stage extractor can be calculated (see Table 2). An extractor that has only one miscella stage would require 17.2 parts of solvent per 1.0 part of material to be extracted. The energy required to evaporate the solvent in

**TABLE 2. Single-Stage Extractor Mass Balance.**

Prepared Material Entering		Solvent Entering	
Solids:	800 units	Solvent:	17200 units
Oil (20%):	200 units		
Total	<b>1000 units</b>	Total:	<b>17200 units</b>
Spent Material Exiting		Miscella Exiting	
Solids:	800 units	Solvent:	16856 units
Oil (0.5% residual):	4 units	Oil:	196 units
Solvent (30% retention):	344 units		
Total	1148 units	Total:	17052 units
Miscella Concentration		Miscella Concentration	
4 / (4 + 344) = <b>1.15% oil</b>		196 / (196 + 16856) = <b>1.15% oil</b>	

the miscella would be tremendous. As a result, countercurrent, multistage extractors are required.

Through iteration of the mass balance, the minimum number of miscella stages can be calculated for a given solvent to material ratio. For an energy-competitive distillation system, the solvent to material ratio should be maintained below 1 to 1. To achieve a ratio below 1 to 1, the minimum number of stages required can be calculated and determined to be four stages.

A four-stage extractor with sufficient contact time to allow the miscella concentration in the material cells to come into equilibrium with the miscella concentration in the surrounding miscella bath at each miscella stage can be designed to extract to less than 0.5% residual oil using a solvent to material ratio of 1 to 1. The resultant outgoing miscella concentration will be in the range of 27% oil. A four-stage extractor is sufficient in a theoretical sense, but it leaves no contingency for lack of achieving equilibrium at each miscella stage.

The more miscella stages, the greater the theoretical extraction efficiency will be. In practice, however, if too many miscella stages are designed into an extractor, causing the individual stages to have insufficient contact time to reach equilibrium, residual oil will not be further reduced by adding stages. In this case, more miscella stages simply lead to more pumps, and therefore more pumping energy and more potential points for solvent loss. The number of miscella stages is generally determined by the total washing zone time and the number of stages that can theoretically come to equilibrium within the washing zone time. Commercially, most extractors have in the range of five to nine miscella stages.

**4.2.6. Solvent Retention** After the washing zone of the extractor, the extracted material is left to gravity drain. This gravity drain time is generally in the range of 5 to 20 minutes. Extractors designed with shallow material bed depths will generally have a drain time closer to 5 minutes, and extractors designed with deep material

bed depths generally have a drain time closer to 20 minutes. After gravity drainage, the solvent retained with the extracted material will be in the range of 25% (fast-draining extrudate) to 35% (slow-draining flakes).

The “solvent retention” of the drained material is a bit of a misnomer, and it would be more accurately defined as the “weak miscella retention.” The weak miscella retained in the drained material contains approximately 1% oil. In the meal desolventizer, the solvent is evaporated leaving behind the traces of oil, often referred to as the residual oil. In order to minimize the residual oil left in meal, it is important to minimize the amount of weak miscella carried forward to the meal desolventizer.

Adequate extractor drain time is the most economic manner in which to minimize weak miscella retention. Maintaining the desired miscella flux rate is also important. Thus, once again, there is a need to maintain proper flake thickness, minimize surface moisture, and minimize fine material particles in the material bed to minimize weak miscella retention.

Today there are two major suppliers of solvent extractors, Crown Iron Works, headquartered in the United States, and the De Smet Group, headquartered in Belgium. The Crown Model III Extractor is a shallow material bed depth extractor, using a chain conveyor design to convey the material over fixed screens in a loop pattern (see Figure 9b). De Smet supplies two major types of extractors, a Reflex<sup>®</sup> Extractor and an LM<sup>®</sup> Extractor. The De Smet Reflex Extractor is a deep material bed depth extractor, using a cylindrical rotating set of baskets to convey the material over a fixed circular screen (see Figure 9c). The De Smet LM Extractor is provided in both shallow and deep material bed depths, using a belt conveyor made of screens to convey the material along a linear path (see Figure 9d). Extractors of these three types process in the range of 25 tons per day of specialty oilseeds to 10,500 tons per day of soybeans through a single unit.

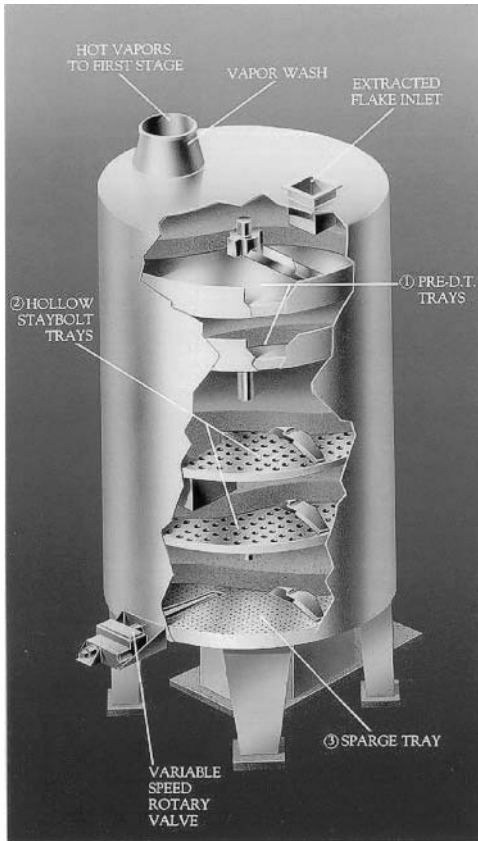
### 4.3. Meal Desolventizer Toaster

After the prepared material has had its oil extracted in the solvent extractor, it is conveyed to the meal desolventizer toaster, commonly referred to as the DT. The material entering the DT is typically at the extractor temperature of 60°C, and it contains 25–35%(w/w) of solvent. The primary purpose of the DT is to remove the solvent from the meal fraction so that the solvent can be recovered.

DTs are vertical, cylindrical vessels with a multitude of horizontal trays. The extracted material enters at the top and is supported by the tray. The material is mixed above each tray, and it is conveyed downward from tray to tray, by agitating sweeps anchored to a central rotating shaft. The heat for increasing meal temperature and evaporating the solvent is supplied by steam, introduced directly and indirectly into the meal via the trays. Figure 10a illustrates a typical Schumacher DT.

The trays of the DT are designed with an upper plate, lower plate, and structural members between designed-to-hold pressurized steam. The DT has three different types of trays: predesolventizing trays, countercurrent trays, and a sparge tray.



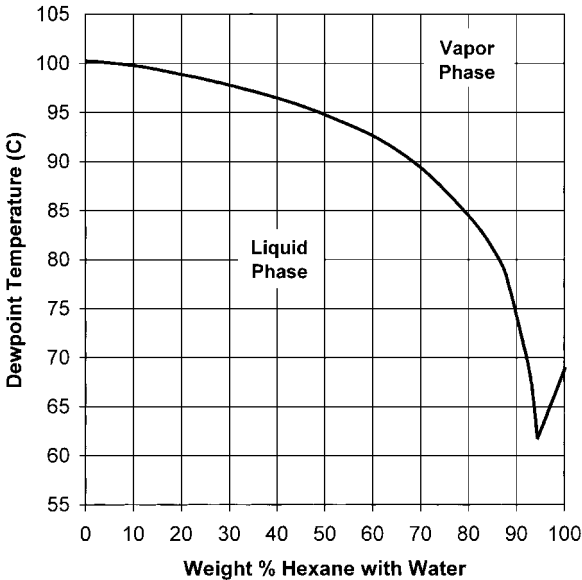


**Figure 10.** (a) Desolventizer toaster. Courtesy of Crown Iron Works. (b) Phase equilibrium.

**4.3.1. Predesolventizing Trays** The predesolventizing trays have the sole purpose of providing conductive heat transfer through their upper surface to the solvent-laden material supported above. The steam is typically held at  $10.5\text{-kg/cm}^2$  pressure within the predesolventizing trays, providing a surface temperature of  $185^\circ\text{C}$ . Steam condenses within the tray, providing its latent heat to maintain the  $185^\circ\text{C}$  tray surface temperature and allow heat to be conducted into the solvent-laden meal layer above. Heat is transferred into the meal at a rate of  $500\text{--}600\text{ kJ/m}^2\text{-C-h}$  with a temperature differential of approximately  $120^\circ\text{C}$ .

A DT may have as many as seven predesolventizing trays, or as few as one. The predesolventizing trays are located in the upper portion of the DT and must allow ascending vapors from below to pass around them to the vapor exit at the top of the DT. Some manufacturers design disk-shaped trays providing space for the ascending vapors to pass between the outside perimeter of the tray and shell wall, and others design donut-shaped trays providing space for ascending vapors to pass

**Phase Equilibrium**  
**hexane & water**  
 (760mm Hg absolute pressure)



**Figure 10** (Continued)

between the inside perimeter of the tray and the central shaft. As an alternative to a large number of predesolventizing trays, the upper section of the DT is often expanded in diameter to enable fewer, larger diameter trays.

**4.3.2. Countercurrent Trays** The countercurrent trays have three purposes, providing conductive heat transfer through their upper surface to heat wet material supported above, providing convective heat transfer through their lower surface to superheat direct steam swirling below, and providing apertures for direct steam to evenly ascend up through the tray and into the meal supported above. The steam is typically held at 10.5-kg/cm<sup>2</sup> pressure within the countercurrent trays, providing a surface temperature of 185°C. Steam condenses within the tray, providing its latent heat to maintain the 185°C tray surface temperature and allow heat to be conducted into the meal layer above, and transferred into the direct steam swirling below. Heat is transferred into the meal above at a rate of 450–550 kJ/m<sup>2</sup>-C-h with a temperature differential of approximately 85°C. Heat is also transferred into the direct steam swirling below at a rate of approximately 100 kJ/m<sup>2</sup>-C-h with a temperature differential of approximately 75°C.

A DT will have from one to four countercurrent trays. The countercurrent trays are located directly under the predesolventizing trays in the center of the DT. The apertures in the trays must allow the direct steam swirling below to pass through the

tray and into the meal supported above. There are three designs of countercurrent trays with differing apertures for allowing the ascending direct steam to pass through. Initial modern DTs (Schumacher, circa 1982) used hollow stay-bolts as apertures and generally had 1% to 2% open area for the vapors to ascend upward. Later designs (Mason, circa 1985) used hollow stay-pipes capped by a perforated plate, with generally 2% to 4% open area for vapors to ascend upward. The latest design (Kemper, circa 1997) uses pie-shaped enclosures capped with stainless steel slotted screens, with approximately 10% open area for the vapors to ascend upward. The choice of countercurrent tray design is generally determined by the amount of open area required to maintain stable operation for the quantity of direct steam passing through.

**4.3.3. Sparge Tray** The sparge tray has the dual purpose of providing a uniform means of introducing direct steam into the meal layer, and providing conductive heat transfer through its upper surface to the wet material supported above. The direct steam introduced through the sparge tray provides approximately 75% of the total heat required for desolventizing and heating the meal in the DT. The sparge tray is typically designed with a plurality of apertures across its entire upper surface to evenly introduce direct steam into the meal. The size and quantity of apertures is calculated based on the anticipated direct steam flow rate to provide a pressure drop of 0.35 to 0.70 kg/cm<sup>2</sup>. The direct steam supply is 10.5-kg/cm<sup>2</sup> pressure saturated steam (185°C), and after passing through a flow control valve, its quality changes to 0.35–0.70-kg/cm<sup>2</sup> pressure superheated steam (150–160°C). Therefore, the upper surface of the sparge tray is maintained at approximately 155°C. Heat is transferred into the wet meal above at a rate of 450–550 kJ/m<sup>2</sup>-C-h with a temperature differential of approximately 50°C. The sparge tray is the last tray in a modern DT.

Solvent-laden meal enters the DT at a temperature of 60°C, and it contains 25% to 35% (w/w) of solvent. The solvent-laden meal is stirred across the surface of the predesolventizing trays by the rotating sweeps. As the heat is transferred into the meal layer by conduction, a shallow layer of 150-to-300-mm meal depth is held above each tray. The solvent-laden meal temperature is increased to approximately 68°C and approximately 10% to 25% of the solvent is evaporated on the predesolventizing trays.

The material exits the predesolventizing trays of the DT and falls onto the top countercurrent tray. This is perhaps the most critical tray of the DT. As most of the heat is transferred into the meal layer by condensation of direct steam, a deep layer of 700-to 1000-mm meal depth is held above the tray. The solvent-laden meal is stirred above the top countercurrent tray by the rotating sweeps. The direct steam passes from below up through apertures in the countercurrent tray. As the direct steam penetrates the upper meal layer, it reaches the solvent-laden meal and condenses, providing direct latent heat to evaporate solvent. The solvent evaporates and exits the meal layer as vapor. The condensation of steam causes the meal exiting the tray to be wet, typically in the range of 17–21% moisture. After the majority of the solvent evaporates, the meal temperature increases by direct and indirect steam

heat, surpassing 100°C before the material exits the tray. The protein solubility of soybean meal is reduced from approximately 90 PDI down to 45 PDI as a result of the elevated moisture and temperature conditions.

After the wet meal exits the top countercurrent tray, it has had over 99% of its solvent removed. On the remaining countercurrent trays and the sparge tray, the meal is held in a 700-to 1000-mm deep layer on each tray to provide residence time for stripping solvent and toasting. The wet meal is stirred above each tray by rotating sweeps. The final desolventizing takes place as the ascending steam passing through the meal slowly strips out final traces of residual solvent down to 0.01–0.05% (w/w). The meal temperature increases from 100°C up to 105–110°C, and the meal moisture decreases approximately 1% before the meal discharges from the sparge tray. The meal color darkens slightly and provides the meal with a toasted color. For soybeans, antinutritional factors such as trypsin inhibitors and urease are reduced on these trays by maintaining the meal moisture and temperature elevated for a period of time. The protein solubility drops approximately 1% PDI for every minute the meal spends in the remaining countercurrent trays and the sparge tray. Ideal feed for monogastric animals (poultry and swine) is high in protein solubility, and ideal feed for ruminant animals (cattle) is low in protein solubility (high in rumen bypass protein). Meal residence time on the remaining countercurrent trays and the sparge tray is dictated by both the degree of solvent recovery required as well as by meal quality parameters.

An important parameter in the energy efficiency of the DT is the exit vapor temperature. The condensing sparge steam provides a plentiful supply of surface moisture, allowing the solvent and water to evaporate as an azeotropic mixture. Figure 10b indicates the solvent/water equilibrium boiling curve. As the chart indicates, a mixture of 94% solvent with 6% water can boil as low as 62°C. Therefore, the lowest possible DT exit vapor temperature would be 62°C. In practice, to maintain low solvent loss and maintain a safety margin, modern DTs are operated with exit vapor temperatures ranging from 66–78°C, with the most typical temperature being 71°C. As the DT vapor temperature increases, the ratio of water vapor to solvent vapor increases. Therefore, to minimize total DT energy, it is very important to maintain a vapor temperature as low as safely possible.

Determining the optimum DT configuration for a given process application is complex. It requires determining all input parameters and calculating the mass and heat balance of both the DT and the follow-on meal dryer cooler (DC). The mass and heat balance of the DC will determine the maximum allowable DT exit moisture that will minimize meal drying energy. This moisture is generally in the range of 18–20%. With the DT exit moisture determined, the amount of direct steam introduced into the meal can be calculated. The DT diameter is generally determined by the direct steam flow rate per unit area. It is important to have a sufficiently high direct steam flow rate per unit area for adequate solvent stripping. The number of countercurrent trays is determined by the residence time needed to balance meal quality with residual solvent objectives. By calculating the total DT heat demand and subtracting the heat supplied by live steam, the total heat supplied by indirect steam can be determined. The total heat supplied by indirect steam less

the heat supplied by countercurrent tray indirect steam will provide the amount of indirect steam heat needed to be supplied by the predesolventizing trays. With this data in hand, the diameter and quantity of predesolventizing trays can be selected. Major manufacturers of DTs use process simulation tools to assist processors in optimizing the DT configuration for a given application.

#### 4.4. Meal Dryer Cooler

After the solvent-laden material is desolventized, it is conveyed to the meal DC. The material entering the DC is typically at the DT exit temperature of 108°C and contains 18–20% moisture. The primary purposes of the DC are to reduce the moisture in the meal to within trading rule limits and to lower the meal temperature prior to storage.

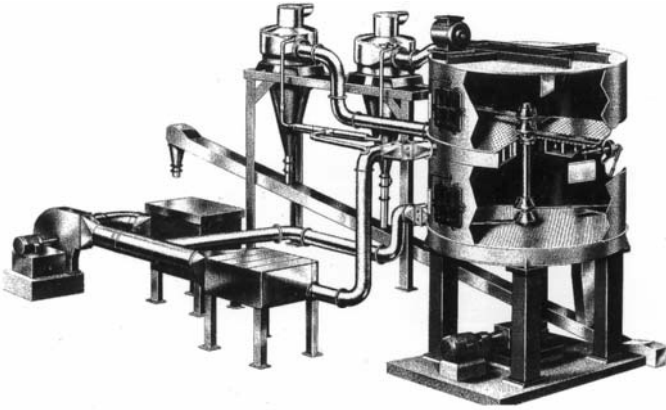
DCs are vertical, cylindrical vessels with a multitude of horizontal trays. The desolventized material enters at the top and is supported by the tray. The material is mixed above each tray and conveyed downward from tray to tray by agitating sweeps anchored to a central rotating shaft. The DC has three different types of trays: steam drying trays, air drying trays, and air cooling trays.

**4.4.1. Steam Drying Trays** The steam drying trays of the DC are designed with an upper plate, lower plate, and structural members between designed-to-hold pressurized steam. The steam-drying trays have the purpose of providing conductive heat transfer through their upper surface to wet meal supported above. The steam is typically held at 10.5-kg/cm<sup>2</sup> pressure within the steam-drying trays, providing a surface temperature of 185°C. Steam condenses within the tray, providing its latent heat to maintain the 185°C tray surface temperature and to allow heat to be conducted into the wet meal layer above. Heat is transferred into the meal at a rate of 450–550 kJ/m<sup>2</sup>-C-h with a temperature differential of approximately 77°C.

A DC may have as many as five steam-drying trays or as few as none. The water vapor evaporated from the meal can be compressed in an ejector and have its heat recovered within the solvent extraction plant.

**4.4.2. Air Drying Trays** The air-drying trays of the DC are designed with an upper plate, lower plate, and structural members between designed-to-hold low-pressure air. The air-drying trays are designed with a plurality of apertures across their entire upper surface to evenly introduce air into the meal. The size and quantity of apertures is calculated based on the design air flow rate to provide a pressure drop of 0.02 to 0.03 kg/cm<sup>2</sup>.

The air supplied to each air dryer tray is first filtered to remove dust and then pressurized using a spark-proof centrifugal blower. The air for the dryer trays is passed through a steam-heated coil between the blower and the entrance to the dryer trays. After the air enters the trays, it flows upward through the meal at a nominal velocity of 14–21 m/minute, partially fluidizing the meal. The meal evaporatively cools, and the released moisture is transferred to the ascending air. The warm, damp air exits the top of the meal layer and then exits the sidewall of the DC to a



**Figure 11.** Meal dryer cooler. Courtesy of De Smet Group.

cyclone collector to remove dust prior to discharge to atmosphere. Figure 11 illustrates a meal DC with two air trays and related air-handling equipment.

The major source of heat for evaporating the moisture in the meal is the high temperature of the meal exiting the DT or the DC steam-drying trays. When the meal drops in temperature from 108°C to 38°C, the heat provided is adequate to reduce the meal moisture by 6.5%. For soybean meal, the trading rule moisture limit is 12.5%; therefore, if the incoming moisture from the DT, or DC steam-drying trays, does not exceed 19.0%, the DC will typically require no additional evaporative heat source to dry the meal. If additional heat is required for evaporating moisture from the meal, the air entering the meal dryer trays can be heated to temperatures up to 150°C prior to entering the air drying trays. The heat source can be recovered flash steam, hot glycol-water solution (oil cooler), or fresh steam.

The air must have adequate capacity to carry out the moisture released from the meal without becoming saturated. Cold air can hold less moisture than warm air, so winter conditions may limit the moisture-carrying capacity of the air. If additional heat is required for increasing the dew point of the incoming air, the air entering the air dryer trays can be heated to temperatures up to 150°C.

The energy required to heat the incoming drying air is largely dictated by the incoming meal moisture to the DC. Adequate predesolventizing heat transfer area in the DT or steam-drying tray heat transfer area in the DC is the key to minimizing meal moisture to the DC air drying trays and therefore minimizing DC heater coil steam consumption.

**4.4.3. Air cooling trays** The air cooling trays of the DC are designed with an upper plate, lower plate, and structural members between designed-to-hold low-pressure air. The air cooling trays are designed with a plurality of apertures across

their entire upper surface to evenly introduce air into the meal. The size and quantity of apertures is calculated based on the design air flow rate to provide a pressure drop of 0.02–0.03 kg/cm<sup>2</sup>.

The air supplied to each air cooler tray is first filtered to remove dust and then pressurized using a spark-proof centrifugal blower. After the cool air enters the trays, it flows upward through the meal at a nominal velocity of 14 to 21 m/minute, partially fluidizing the meal. The meal continues to evaporatively cool and convectively cools. The cool, damp air exits the top of the meal layer and then exits the sidewall of the DC to a cyclone collector to remove dust prior to discharge to atmosphere.

Ambient air is heated approximately 5°C in temperature by the energy of the blower, before it blows into the air cooler trays of the DC. The meal cools down to within approximately 5°C of the air temperature passing through the meal. Therefore, the temperature of the meal exiting the DC is typically cooled down to within 10°C of ambient air.

The dry cool meal is conveyed from the DC to outside the solvent extraction plant for size reduction and then on to meal storage. It is important to properly dry and cool the meal to prevent continued evaporative cooling in storage or transport, which will cause reduced flowability, solidification, and bridging of the meal inside storage and transport vessels.

#### 4.5. Miscella Distillation System

The miscella exiting the extractor contains particles of meal, up to 1% (w/w). Therefore, the first step in miscella distillation is meal particle separation. Meal particles can be separated from the miscella via filtration or centrifugal separation. The large meal particles (+80 mesh) need to be removed to prevent them from settling out in the distillation equipment. The fine meal particles (–80 mesh) are removed from the oil after the solvent extraction plant, typically in the oil degumming process, or oil-refining process. For those plants producing food grade lecithin, the fine meal particles must be completely filtered out prior to degumming.

Some extractor designs include an internal miscella filter (100 mesh) enabling the miscella exiting the extractor to be sufficiently free of meal particles such that it can go directly to distillation. Other extractor designs require external meal particle separation, accomplished by pumping the miscella through a liquid cyclone. The liquid cyclone spins the miscella at high velocity and uses centrifugal force to separate the larger meal particles (over 80 mesh) from the miscella. The larger meal particles, along with 5–10% of the miscella flow, exit the liquid cyclone underflow orifice and return to the extractor. Separation of meal particles by external filtration of the miscella is not recommended because of the safety hazards associated with opening the filter to remove the solvent-laden meal fines.

Once the large meal particles are separated, the clean miscella is stored in a surge tank, generally referred to as a full miscella tank. The full miscella tank has several purposes: It separates the continuous extraction process from the continuous distillation process, it provides miscella storage capacity during a power

outage, and it provides surge capacity so that fluctuations in miscella flow rate from the extractor can be absorbed prior to distillation. The miscella in the full miscella tank is generally 25–30% oil and 70–75% solvent by weight, and at the typical extractor temperature of 60°C.

The miscella is pumped from the full miscella tank to the first of two rising film evaporators. The first-stage evaporator, often referred to as the economizer, uses the waste heat from the DT as its heating source. Miscella enters through tubes at the base of the evaporator, at a temperature of approximately 60°C. As the tube side of the evaporator is held at approximately 300–400-mm Hg absolute pressure, the miscella temperature will drop to approximately 43–48°C temperature upon entry into the tubes. Solvent will begin evaporating and solvent vapor bubbles will rise up through the center of the tubes. Additional DT vapor heat is transferred through the tubes into the miscella, and additional evaporation takes place. When sufficient solvent vapor is formed, the vapor velocity through the center of the tubes will become sufficiently high to drag a thin film of miscella up the inner walls of the tubes, creating high heat transfer rates. At the top of the tubes, the high-velocity solvent vapor and remaining miscella contact an impingement plate to break foam, and they are then centrifugally separated in the evaporator dome. Solvent vapors exit the top of the dome, and concentrated miscella exits the base of the dome. The concentrated miscella exiting the first-stage evaporator is generally 75–85% oil and 15–25% solvent, at a temperature of approximately 48°C.

As the temperature of the miscella exiting the first stage evaporator is low, it is a good heat sink for heat recovery. In various facilities, heat from hot finished oil, heat from steam ejector exhausts, or recovered flash steam is used to preheat the miscella to approximately 75°C in temperature. The preheated, concentrated miscella is then typically heated to 110°C in a steam-heated exchanger prior to entering the second rising film evaporator.

The preheated, concentrated miscella is pumped into the second-stage evaporator, which uses low-pressure steam as its heating source. As the tube side of the evaporator is held at approximately 300–400-mm Hg absolute pressure, the solvent temperature will drop to approximately 43–48°C temperature upon entry into the tubes, providing sufficient heat to immediately begin vigorous evaporation. The vapor velocity through the center of the tubes will be sufficiently high to drag a thin film of miscella up the inner walls of the tubes at relatively high velocity. This is very important to prevent baking phosphatides and fine solid particles in the miscella to the lower, inner surface of the tubes. Additional low pressure steam heat is transferred through the tubes into the miscella and additional evaporation and heating takes place. At the top of the tubes, the solvent vapor and remaining miscella contact an impingement plate to break foam, and they are then centrifugally separated in the evaporator dome. As velocities exiting the tubes are insufficient to break all foam, the dome must be sufficiently large in diameter to allow remaining foam to collapse and not discharge with exiting vapors. Solvent vapors exit the top of the dome, and concentrated miscella exits the base of the dome. The concentrated miscella exiting the second-stage evaporator is generally 95–98% oil and 2–5% solvent, at a temperature of approximately 110°C.



Miscella from the second-stage evaporator is pumped or gravity fed into the oil stripper. The oil stripper is a tall, thin cylindrical vessel that is commonly operated at 150–300-mm Hg absolute pressure. The hot oil passes downward through the vessel across trays. Simple, robust disk-donut trays, or grid-bar trays, are typically used because fouling caused by baking of gums and fine particles onto stripper trays is common. Live steam is introduced into the oil at the top of the vessel to initiate evaporation and again at the base of the vessel to provide countercurrent stripping. The steam and solvent vapors exit the top of the oil stripper through an enlarged diameter dome to prevent entraining oil mist. The oil typically exits the base of the oil stripper with 0.1–0.3% moisture and 0.005–0.020% solvent, at a temperature of approximately 110°C.

The oil leaving the oil stripper, particularly in soybean plants, is often water degummed. In these facilities, the oil temperature is reduced to 70–80°C, and 1–2% soft water is injected and mixed into the oil inline. The oil is then held for approximately 30 to 60 minutes in an agitated tank to allow gums to hydrate. The gums (water, phosphatides, fine meal particles, and some neutral oil) are centrifugally separated from the oil using a high-speed centrifugal separator. The gums are often pumped back into the DT and mixed into the meal fraction. Alternatively, the gums may be dried for food grade lecithin, or feed grade lecithin for animal feed applications. The degummed oil, at a moisture level of approximately 0.5% and a temperature of approximately 70°C, is then heated in a heat exchanger to approximately 110°C.

Whether or not the oil is degummed, it is typically pumped to an oil dryer. The oil dryer is a vertical cylindrical vessel that is commonly operated at 50–80-mm Hg absolute pressure. The hot oil is sprayed downward into the vessel, with or without trays. The solvent and water vapors exit the top of the oil dryer and the oil exits the bottom. The oil typically exits the base of the oil dryer with 0.05–0.10% moisture and 0.002–0.010% solvent, at a temperature of approximately 105°C.

The dried oil must have its temperature reduced from 105°C to 50°C to prevent degradation in storage and transport. The hot oil is commonly cooled in two stages. First, the oil is cooled from 105°C to approximately 70°C temperature in a heat exchanger using concentrated miscella or a water-glycol solution (for preheating DC air) as the cooling medium. Second, the oil is cooled from 70°C to 50°C temperature in a heat exchanger using cooling water as the cooling medium. The cool oil is then pumped to storage.

#### 4.6. Solvent Recovery System

Modern solvent extraction plants recover over 99.9% of the solvent pumped to the extractor. The solvent recovery system includes solvent and water vapor condensation, solvent-water separation, stripping solvent from water and air effluent streams, as well as heating the solvent prior to reuse in the extractor.

The solvent vapors from the first-and second-stage evaporators are typically condensed in a common medium-vacuum condenser. The medium-vacuum condenser is a shell and tube vessel with the vapors typically on the shell side and the cooling

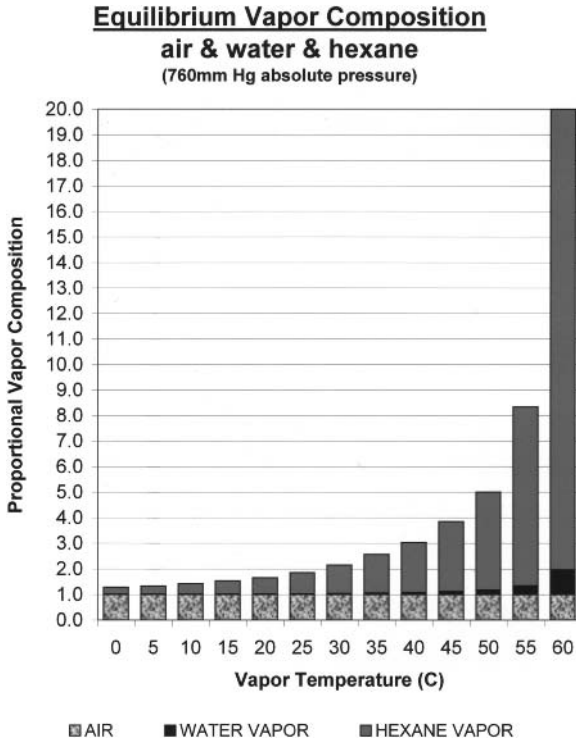
water on the tube side. The noncondensable vapors are removed from the condenser by a steam ejector to maintain the 300–400-mm Hg absolute pressure on the shell side and are typically discharged into the first-stage evaporator for heat recovery.

The water and solvent vapors from the oil stripper and mineral oil stripper are typically condensed in a high vacuum condenser. The high vacuum condenser is a shell and tube vessel with the vapors typically on the shell side and the cooling water on the tube side. The noncondensable vapors are removed from the condenser by a steam ejector to maintain the 150–300-mm Hg absolute pressure on the shell side and are typically discharged into the first-stage evaporator for heat recovery.

The water and solvent vapors are evacuated from the oil dryer by a steam ejector to maintain the 50–80-mm Hg absolute pressure on the oil dryer and are typically discharged into the base of the oil stripper. These vapors along with the ejector motive steam serve as the oil stripper's source of countercurrent stripping steam for heat recovery.

The solvent and water vapors from the DT typically pass through a vapor scrubber to remove meal particles. Two types of vapor scrubbers are commonly used: water-spray scrubbers and centrifugal scrubbers. Water-spray scrubbers use a heavy spray of hot water droplets being sprayed through the vapors in the duct exiting the DT to entrap meal particles. The meal particles and hot water are collected in a tank and recirculated, with a small stream of dirty water being returned to the DT. Centrifugal scrubbers use centrifugal force to separate meal particles from the vapors, allowing the particles to fall out of the bottom of the scrubber directly into the DT. Some centrifugal separators use a solvent wash to keep the walls of the centrifugal scrubber clean.

The clean vapors exiting the DT vapor scrubber, along with the much smaller vapor streams from the steam ejectors and the waste water stripper, are partially condensed in the shell side of the first-stage evaporator. The vapors enter the top of the evaporator shell side at approximately 70°C temperature and quickly condense water vapor until the temperature drops to 62°C, the minimum hexane/water equilibrium temperature. The vapors continue condensing at the mixture of 94% solvent and 6% steam and temperature of 62°C as they progress downward through the shell of the first-stage evaporator. The remaining solvent vapor and water vapor exiting the first stage evaporator shell (approximately 20% to 30% of what entered) are further condensed in either a vapor contactor or a solvent preheater. In a vapor contactor, cool condensate from condensers is pumped and sprayed into the top of a vertical tank. Remaining solvent and water vapors from the first-stage evaporator pass through the cool liquid stream and condense on the droplets, causing the liquid spray temperature to rise. In a solvent preheater, cool fresh solvent en route to the extractor is pumped through the tubes of a shell and tube heat exchanger and the solvent and water vapors from the first-stage evaporator pass through the shell side, condensing on the tubes while warming the fresh solvent passing through the tubes. Whether using a vapor contactor or a solvent preheater after the first-stage evaporator, the remaining DT vapors are reduced to less than 10% of what exited the DT, recovering 90% of the waste heat from the DT. The remaining vapors pass on to the vent condenser.



**Figure 12.** Equilibrium vapor composition.

Approximately  $1 \text{ m}^3$  of air enters the extractor with each  $1 \text{ m}^3$  of material. To maintain the extractor under a slight vacuum, the air must be continuously vented from the extractor. At the extractor temperature of  $60^\circ\text{C}$ , an equilibrium condition will occur where the vent gas exiting the extractor will contain approximately 10 parts of solvent vapor for every one part of air (see Figure 12). The solvent vapor and air exit the extractor and are typically condensed in an extractor condenser. The extractor condenser is a shell and tube vessel with the vapors typically on the shell side and the cooling water on the tube side. The noncondensable vapors exiting the extractor condenser pass on to the vent condenser.

The vent vapor streams from the extractor condenser, vapor contactor or solvent preheater, and atmospheric tanks pass on to the vent condenser. The normal heat load on the vent condenser is low. However, in the case in which miscella is not passing through the first-stage evaporator, or solvent is not passing through the vapor contactor or solvent preheater, the load on the vent condenser can be very high. Therefore, the vent condenser is generally sized for these contingency conditions and has an excess of heat transfer area for normal operating conditions. The vent condenser is a shell and tube vessel with the vapors typically on the shell side and the cooling water on the tube side. The noncondensable vapors exiting the vent condenser pass on to the mineral oil absorption system.

The composition of solvent vapor with the air exiting the vent condenser is a result of an equilibrium condition determined by the vent gas temperature. Figure 12 illustrates the equilibrium vapor compositions at various temperatures. For example, at a vent gas temperature of 30°C, the vent gas composition will be slightly greater than one part solvent for every one part air. However, if the vent gas temperature increases to 40°C, then the vent gas composition will be slightly less than two parts solvent for every one part air. Therefore, to minimize the load on the mineral oil system, it is important to minimize the vent gas temperature exiting the vent condenser. Some facilities, particularly in hot and humid climates, install a refrigerated vent gas cooler after the vent condenser to minimize the solvent vapor load on the mineral oil system.

The vent gas from the process enters the bottom of the mineral oil absorber. The mineral oil absorber is a tall, small-diameter packed column. Cold mineral oil cascades down through the column, absorbing solvent vapor from the vent gas as the vent gas rises up through the packing. When the vent gas exits the mineral absorber, it generally contains less than 10-g solvent per cubed meter of air. The vent gas is pulled from the mineral oil absorber via a spark-proof suction fan and is discharged to the atmosphere through a flame arrestor.

The mineral oil enters the mineral oil absorber at approximately 30°C temperature and contains 0.1–0.4% (w/w) moisture plus solvent. When the mineral oil exits the mineral oil absorber, its temperature rises slightly because of the heat of absorption, and the mineral oil contains 3–5% (w/w) moisture plus solvent. The cool solvent-rich mineral oil is then heated to 65°C by hot/cool mineral oil heat recovery and then further heated to over 100°C using a steam-heated heat exchanger. The hot, solvent-rich mineral oil enters the mineral oil stripper, a packed column vessel similar in construction to the mineral oil absorber. As the mineral oil stripper is typically maintained under 150–300-mm Hg absolute pressure, much of the solvent evaporates as soon as the mineral oil enters the top of the mineral oil stripper. Most of the remaining solvent is removed as the mineral oil cascades down across the packing countercurrent to rising stripping steam. The water and solvent vapor exit the dome of the mineral oil stripper. The hot, solvent-lean mineral oil exits the mineral oil stripper and has its temperature reduced to 65°C by hot/cool mineral oil heat recovery and is then further cooled to 30°C temperature using a heat exchanger with water as the cooling medium. The cool, solvent-lean mineral oil is then recirculated to the top of the mineral oil absorber.

All water and solvent that drain from the various condensers in the solvent extraction plant enter a decanting tank. As the solvent is immiscible with water, the lighter solvent (0.66 specific gravity) floats above the water. The key to the decanting tank performance is to minimize turbulence within the tank to allow sufficient time for the gravity decanting to take place. The elevation of the interface between solvent and water is established by the highest elevation of the water drain pipe. Water, typically containing 0.01% solvent, exits the decanting tank to the waste water stripper. Solvent, typically containing 0.05% water, exits the decanting tank to the solvent work tank.

The waste water stripper is a small tank used to increase the temperature of the waste water to approximately 95°C to evaporate residual solvent prior to discharge to the plant sump. The water exiting the waste water stripper is typically less than 0.001% (w/w) solvent. The hot water exiting the waste water stripper is often interchanged with the cool water entering the waste water stripper for heat recovery.

Solvent enters the work tank from the decanting tank. The work tank is a surge tank for holding solvent prior to the extractor to ensure that there is always ample solvent available to be pumped to the extractor. If a large surge of solvent flows into the work tank, the work tank will automatically overflow to solvent storage. If the level in the work tank becomes low, additional solvent is pumped from solvent storage to the work tank. The temperature of the solvent in the work tank is typically in the range of 52–57°C in plants equipped with a vapor contactor, and 43–49°C in plants without a vapor contactor.

In plants with a vapor contactor, solvent is pumped from the work tank at 55°C temperature to a steam-heated solvent heater to increase its temperature to 60°C prior to entering the extractor. In plants with a solvent preheater rather than a vapor contactor, solvent is pumped from the work tank at 45°C temperature to a solvent preheater where its temperature is increased to 55°C. The solvent is then further heated in a steam-heated solvent heater from 55°C to 60°C in temperature prior to returning to the extractor.

## 5. SUMMARY

The solvent extraction process consists of the unit operations of solvent extraction, meal desolventizing, meal drying and cooling, miscella distillation, and solvent recovery. These unit operations are highly interrelated, primarily because of various heat recovery methods that link the operations together. A process upset in any one of the unit operations will typically cause abnormal operation in the others. The key to efficient solvent extraction operation is process consistency. Consistency of seed preparation, consistency of rate, and consistency of solvent extraction operating parameters are all important factors in operating a safe, environmentally friendly, and cost-effective solvent extraction process for oil extraction.

## REFERENCES

1. H. G. Kirschenbauer, *Fats and Oils*, Reinhold Publishing, New York, 1944, pp. 122–123.
2. Anonymous, in *The Extraction of Oil from Seeds and Nuts*, June 1997.
3. Anonymous, in *Chronology*, **May** (2000).
4. V. D. Anderson U.S. Patent 637,354, 1900.
5. Anonymous. (2002, March 18). Anderson International Corp. Available: <http://www.andersonintl.com/history.html>.

6. P. J. Wan and P. J. Wakelyn, *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*, AOCS Press, Champaign, Illinois, 1997, pp. 14–18.
7. N. Kessler, *Understanding Solvent Extraction of Vegetable Oils*, Volunteers in Technical Assistance, Arlington, Virginia, 1985.
8. N. F. Cruse and W. L. Soldner, U.S Patent 2,260,254, 1941.
9. J. W. Hayward, *Flour and Feed*, 24 (1940).
10. N. F. Kruse, U.S. Patent 2,585,793, 1952.
11. R. P. Hutchins, U.S. Patent 2,695,459, 1954.
12. Karnofsky, U.S. Patent 2,840,459, 1958.
13. H. O. Schumacher, U.S. Patent 4,503,627, 1985.
14. T. G. Kemper and R. D. Farmer, U.S. Patent 5,992,050, 1999.
15. T. G. Kemper, et al., U.S. Patent 5,705,133, 1998.
16. D. R. Erickson, *Practical Handbook of Soybean Processing and Utilization*, AOCS Press, Champaign, Illinois, 1995, pp. 10 and 189.
17. INTSOY. (2003, January 10). University of Illinois at Urbana-Champaign. Available: <http://www.ag.uiuc.edu/~intsoy/extrusn.htm>.
18. W. Hamm and R. J. Hamilton, *Edible Oil Processing*, Sheffield Academic Press Ltd., Sheffield, U.K., 2000, pp. 73–77.

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## *Recovery of Oils and Fats from Oilseeds and Fatty Materials*

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### **1. INTRODUCTION**

Recovery of oils and fats is a specialized branch of technology (1–9). Differences in source materials dictate the use of different recovery techniques. Prior editions of this book covered many methods for obtaining fats from animal materials and oils from oilseeds. This edition retains what is still current about oil and fat recovery and adds what is new.

Good oil and fat recovery procedures produce undamaged products, free from impurities, obtaining as much yield as possible, and producing low-fat residues of high economic value. Animal materials generally release fat easily. Oilseeds usually have the oil encased in minute “cells” that are deeply embedded in fibrous structures. Most oilseeds, therefore, require grinding or flaking to rupture the cells and heat treatment, sometimes cooking and drying, to help release the oil.

Heat treatment also hardens pulverized plant tissue so it can withstand the pressure applied to squeeze out the oil.

The oils and fats discussed in this chapter are all triacylglycerols (TAGs). As such, they could all be referred to as fats. Traditionally, "oil" is used for oilseed TAGs (which are usually liquid at room temperature) and "fat" is used for animal TAGs (which are usually solid at room temperature). However, "oil" is a broader term that also includes nontriacylglycerol materials like petroleum oils and oils of essence. There is no simple word that can be used for all TAGs when something common to all of them is discussed. Therefore "oil" and "fat" will be used in their traditional meanings, but whenever it seems not to cause confusion, "fat" will be chosen over "oil" when discussing properties common to all TAGs.

After efficient "crushing" in a mechanical screw-press, the solid residue will contain 2.5–5% residual fat. Materials high in fat and low in solids produce solid residues containing only a small fraction of the original fat. However, materials low in fat and high in solids produce solid residues (at the same 2.5–5% residuals) that contain as much as 15–20% of the original fat. Solvent-extraction is an attractive procedure for these materials because it takes out more fat. Solvent-extracted meals typically have less than 1% residual fat. The chief disadvantage of solvent extraction is high equipment cost, the impact on the environment, and the use of solvents in contact with the fat and the solid residues. Solvent extraction plants usually are not economically feasible unless the capital equipment cost can be spread across a very large production, several hundred to a few thousand tons per day.

The solids remaining after fat removal are generally rich in protein and find a ready market in animal feeds. Some oilseed solids, especially soybean, go into human foods as flours, concentrates, textured particles, or protein isolates. Some oilseed solids contain toxins or allergens that make them unfit for animal feeds: tung nut and castor bean, for example. Unless treated, these solid residues go into fertilizers. Various processes have been developed to remove or chemically destroy undesirable compounds (10). One process developed at Texas A & M University for UNIDO (11, 12) uses a chemical additive and extrusion to detoxify and deallergenate castor meal making it suitable for animal feed.

Correct preparation is required for efficient crushing and for solvent extraction. Wherever possible, oilseeds should be dehulled before the oil is removed. This will increase the capacity of the processing equipment and avoid loss of oil that would otherwise be absorbed into the hulls. Oilseeds should be flaked, rolled, or ground, and dried to optimum moisture and temperature. Some oilseeds, especially those low in fiber or high in protein, should be cooked at elevated moisture for 15–20 minutes (to harden the protein) and then dried. Cooking and drying also ruptures the oil cells and reduces oil viscosity. This allows the oil to more easily drift from the solids and be extracted by the solvent.

Extrusion converts ground, rolled, flaked, or prepressed oilseeds into porous collets that handle very well in solvent extractors. Extrusion can also pretreat, in



**TABLE 1. Average Yield of Oil from Commercial Processing of Common Oilseeds (Percent Oil from Seed of Normal Moisture Content).<sup>a</sup>**

Oilseed	Yield (%)	Oilseed	Yield (%)
Babassu (kernels)	63	Perilla seed	37
Castor beans	45	Poppyseed	40
Coconut (copra)	63	Rapeseed	35
Corn (germs)	45	Rice bran	14
Cottonseed	18	Safflower seed	28
Flaxseed	34	Sesame seed	47
Hempseed	24	Soybeans	18
Kapok seed	20	Sunflower seed	25
Oiticica (kernels)	60	Tea seed	48
Palm, African (kernels)	45	Tung	35
Palm	40		
Peanuts	35		

<sup>a</sup>Yields are by mechanical expression for all except soybeans, cottonseed, and rice bran, which are by solvent extraction, and refer to whole or undecorticated seeds, unless otherwise stated.

**TABLE 2. Approximate Proportions of Hulls and Kernels in Different Oilseeds and Oil Content of Whole Seed, Kernels, and Hulls.**

Oilseed	Percent Kernel	Percent Hull	Percent Oil in		
			Whole Seed	Kernel	Hull
Usually decorticated					
Oil palm (kernels)	25	75	—	48	—
Babassu	9	91	—	67	—
Cohune	10	90	—	67	—
Tacum	30	70	—	47	—
Murumuru	40	60	—	—	—
Tung	60	40	30	50	—
Oiticica	65	35	38	58	—
Cocoa beans	88	12	50	—	—
Caster beans	70–80	20–30	40–50	—	—
Cottonseed (delinted)	62	38	19	30	1–2
Peanuts	75	25	38	50	0.5–2
Sunflower seed	45–60	40–55	22–36	36–55	1–2
Kapok	60	40	20–25	40	—
Safflower	50	50	28–33	55–65	1.5–2
Soybeans	93	7	18	19	0.6
Usually not decorticated					
Flaxseed	57	43	—	58	22
Perilla seed	68	32	34	—	—
Hemp seed	62	38	31	—	—
Rapeseed	82	18	42	—	—
Mustard seed	80	20	—	—	—

a single step, unprocessed whole seeds, like soybean, for full-pressing. An overview of state-of-the-art equipment is discussed along with the theories by which the equipment work.

The earliest methods of fat removal were probably rendering procedures practiced by primitive societies, similar to the cooking techniques they developed for preparing meats for food. The pressing of oil from olive pulp probably antedated the pressing of oilseeds. Some seeds, however, were processed by the Chinese at a very early date using mechanical presses operated by wedges or levers. The more efficient hydraulically operated mechanical press was not developed until the early nineteenth century. The continuous screw-press was an early twentieth-century development. Solvent extraction on a large scale became widely practiced in the mid-twentieth century.

Early development of crushing and solvent extraction has been reviewed (13, 14). The entire screw-pressing operation has been discussed (14). Average yields from many common oilseeds are summarized in Table 1. Additional data are summarized in Table 2.

## 2. MECHANICAL PRETREATMENT

### 2.1. Mechanical Pretreatment of Animal Materials

Fatty animal materials require comparatively little preparation before the rendering operation. Pure fat tissue, destined for the production of neutral, low-temperature fats, such as oleo stock or neutral lard, is carefully trimmed from the carcass and washed before rendering. Lower grade fatty tissue, such as that used to make prime steam lard, is not always washed, and is less carefully trimmed. The various tissues and parts of animals not used for human consumption are sorted into different classes, partly to avoid mixing better quality materials with those of lower quality, and partly because some parts, such as those containing large bones, require more severe handling than others. The material is then cut or ground into small pieces.

### 2.2. Mechanical Pretreatment of Oilseeds

*Cleaning.* The first processing step for oilseeds is to clean and separate foreign material. Sticks, stems, and leaves are removed through revolving reels. Sand or dirt is removed through vibrating screens. Strategically placed magnets remove tramp iron. Special "stoners" remove stones and mud balls from shelled peanuts. Pneumatic aspirating chambers separate lightweight chaff from the heavier seeds. Oilseeds should be cleaned before storage, but often they are not, because cleaning equipment of high capacity is more costly than what would suffice for continuous, onstream production capacity.

*Dehulling and Separation of Hulls.* Wherever practical, oilseeds should be decorticated before screw-pressing. Hulls usually contain less than 1% oil. Any mixture of hulls with kernels will allow the hulls to absorb oil, thereby reducing

the amount of oil liberated. The presence of hulls will also reduce screw-press capacity. Hulling machines for decorticating medium-sized oilseeds with flexible seed coats, such as cottonseed, peanuts, and sunflower seed, are of two principal types: bar hullers and disc hullers.

The rotating member of a bar huller is a cylinder equipped on its outer surface with a number of slightly projecting, longitudinally placed, sharply ground, square-edged knives, or bars. Opposed to the cylinder, over an area corresponding to about one-third of its surface, is a concave member provided with similar projecting bars. The seeds are fed between the rotating cylinder and the concave member, and the hulls are split as the seeds are caught between the opposed cutting edges. The clearance between the cutting edges may be adjusted for seeds of different size.

The disc huller differs from the bar huller in that the cutting edges consist of grooves cut radially in the surfaces of two opposed and vertically mounted discs, one of which is stationary and the other rotating. The seeds are fed to the center of the discs and are discharged at the discs' periphery by centrifugal force. With either type of huller, the condition of the seed, especially moisture, is critical. Excessively moist seeds are difficult to split cleanly and may clog the huller; excessively dry seeds may disintegrate.

Different seeds vary considerably in the readiness with which they fall out of their hulls. Peanuts, for example, are loose in the hull and separate easily. Cottonseed kernels or "meats" adhere more tightly to the hull; consequently, the hulls are passed through a hull beater to detach small meat particles after the first separation of hulls from the meats. For cottonseed, the following separations are commonly carried out: separation of large meat particles from hulls and uncut seed by screening (15); separation of hulls from uncut seed by an air lift (16); separation of small meat particles from hulls by beating and screening (17); and separation of hull particles from meats by air (18).

Maximum yields are obtained by balancing the degree of hull separation. If an attempt is made to separate hulls from the meats too cleanly, there will be a loss of oil because of meats being carried over with the hulls. If too much hull is left in the kernels, there will also be an undue loss of oil through absorption by the hulls. Kernels and hulls should be separated as quickly as possible, once dehulling is accomplished, to avoid excessive contact between hulls and kernel particles (19).

Cottonseed is usually received at the oil mill encased in short fibers, or *lint*. In order to obtain efficient recovery of oil, cottonseed must be delinted before it is hulled. There are two types of delinting machines: saw delinters and abrasive delinters. Saw delinting machines are similar in principle and appearance to cotton gins. They consist of a revolving assembly of closely spaced circular saws that pick the lint from the seed. The fibers are removed from the saw teeth by a revolving cylindrical brush or by an air blast that suspends the fibers in an airstream, and they are airveyed to the collection equipment. The lint is not ordinarily removed from the seed in a single operation, but is taken off in two or three cuts. Fibers from each successive cut are of shorter length and lower grade than from the preceding cut, as increasing quantities of hull are removed by the saws as they work closer to the kernel to grab the shorter-length fibers.

Although saw-type delinting machines are the industry standard, abrasive delinting machines are also available. Abrasive delinters were originally developed by an American cottonseed processing plant (Buckeye Cotton Oil Co., Louisville, Kentucky) in the 1930s. They were subsequently improved by Buckeye and another American cottonseed processor (Anderson-Clayton, Gilbert, Arizona) and finally by Murray Carver Co. (Wabash, Indiana) (20) in the 1960s. In abrasive delinting, cottonseed is fed into the center of the machine and is forced against an abrasive surface by centrifugal action. As the lint is scraped from the seed, it is drawn off in an airstream. The amount of delinting is controlled by an adjustable discharge damper, which controls the amount of time the seed stays in the machine. Abrasive delinting requires that the first-cut lint be removed by saw-type delinters (20). Abrasive delinting has never met wide acceptance: saw-type delinters are more commonly used.

Historically, the value, or in some years the nonvalue, of linters varies widely. As a result of this, the U.S. Department of Agriculture (USDA) contracted Texas A&M to conduct extensive studies to determine the economic and oil extractability effects of milling cottonseed to various levels of residual linters (21, 22).

Soybeans are usually dehulled before processing. Dehulling is accomplished by first drying and tempering the beans, then cracking the beans on cracking rolls and then separating the hulls from the kernels in two stages:

1. Hulls are screened from the kernels and aspirated from the top deck of a double-deck shaking screen. Any uncracked beans in the kernels are returned to the cracking rolls while the kernels go to the lower deck and pass over a finer mesh screen, at the end of which the hulls are again aspirated.
2. Hulls that have been aspirated contain some kernel particles. The hull stream is, therefore, subjected to an air separation using a gravity table that pulls the light hulls away from the heavier kernels. Depending on the desired degree of separation, a "middling" fraction may also be taken, which is broken down on another gravity table.

A somewhat different system makes use of simultaneous grinding and aspiration to dehull the beans and separate the hulls. In general, the choice of system depends on the processor, who may add some private modifications to the system.

An important part of the dehulling process is to precondition the seed before dehulling. Moisture should be adjusted, usually by drying; and, in the traditional method, the seed should be left to "temper" for a few days. As the moisture equilibrates between the freshly dried surface and the harder-to-reach interior, stresses develop, which help to loosen the hull from the kernel, thereby improving dehuller performance. Drying has normally been done in steam-jacketed vessels, either with the jackets on the walls or with a steam tube assembly revolving within the vessel. However, there are alternative ways of drying.

Florin and Bartesch described fluid-bed drying of soybeans (23). Unlike steam-jacketed vessels that transfer heat from the vessels' wall into the seeds as the seeds tumble through the vessel, a fluid-bed conditioner uses air to suspend and move the seed through the conditioning chamber. If the air is heated, it can transfer heat

directly into the seeds. Working with an Escher Wyss (Ravenberg, Germany) fluid-bed conditioner, Florin and Bartsch observed a more uniform retention time for each seed under heat. This ensured a more uniform final moisture per seed. Adequate heat could be transferred into the air (which contacts the seed) through 20% of the heat transfer area required by a jacketed conditioner. Required residence time could be reduced from 20 min for a jacketed vessel to 6 min for a fluid-bed vessel. Florin and Bartsch achieved good dehulling after fluid-bed conditioning, requiring fewer overall processing steps and using less equipment (23).

A method for dehulling soybeans was developed by Crown Iron Works Co. (Minneapolis, Minnesota). Whole beans are preheated gently and then shocked with high-temperature air in a Crown Jet Dryer to break the bonds holding hulls to kernels. The beans then pass through a device that rolls the loosened hulls free and splits the kernels along naturally occurring debarkations that divide the kernel into two embryonic leaves (cotyledons). Called the Hulloosenator, this device loosens the hulls without producing fines. The split beans are aspirated to remove hulls and then cracked into appropriate size for subsequent conditioning and flaking. Hulls are screened and aspirated to ensure no carryover of meats.

Fetzer described other "hot" dehulling systems developed by Escher Wyss of Germany and Buhler of Switzerland (24). Although hot dehulling systems can save on conveying and energy costs, because of gravity flow and heating the beans only once, more horsepower is required; and, if overdrying occurs, excessive fines may be produced (25).

Small oilseeds, such as flaxseed, perilla, rapeseed, and sesame, are often handled without decortication because of processing difficulties. Although several decortication installations were tried on safflower, they were not very successful (26). Sunflower is usually decorticated; however, the final decision to do so depends on the meal market available to the oil mill and if economical uses for hulls can be found (27, 28).

The various palm kernels, such as oil palm or African palm, babassu, and cohune, are a special class of oilseeds, because they are large and are surrounded by a hard, thick shell. As a result of low-cost labor in the producing regions, the large size of the nuts, and the refractory nature of the shells, these nuts are often cracked and the kernels separated by hand. The entire production of Brazilian babassu kernels, amounting in some seasons to over 72.6 Mg (80,000 short tons), once was deshelled by hand.

African oil palm nuts, which are less thick-shelled than most American palm nuts, are hand cracked to some extent; but in Indonesia and Malaysia they are usually machine cracked. In one type of cracking machine, the nuts are fed to the center of a rotor equipped with curved baffles, along which the nuts are flung out against a heavy steel housing and broken by impact. Another type of machine resembles a hammer mill. The rotor consists of a frame supporting four heavy steel paddles: the nuts are dropped into the path of the paddles and cracked by impact.

After the nuts are cracked, they drop into rotary screens where some separation of kernels and shells is obtained. Some shell fragments, however, cannot be separated by screening. They also are too heavy to be removed by air separation

compared with what is used on cottonseed and peanuts. Two special methods (the "dry" method and the wet "floating" method) are used to separate palm kernels from large shell fragments.

The dry method takes advantage of the fact that the kernels are round and roll easily, whereas pieces of shell are almost flat and do not easily roll on an inclined surface. Dry separators consist of inclined belts moving uphill. When a mixture of kernels and broken shells is fed onto the belt, the kernels roll down and are collected at the lower end. The shell fragments remain on the belt and are carried over the top into a separate bin. Both kernels and shells are then further processed to ensure minimal carryover of kernels in the shell stream or shells in the kernel stream.

The floating method consists of floating the lightweight kernels from the more dense shells in salt or mud baths. A hydrocyclone can also be used to separate kernels and shells from an aqueous slurry. Kernels from these wet methods are free-drained on a vibrating strainer and then sent to a dryer for about 10 h. The dried kernels will average 6–7% moisture and approximately 50% palm kernel oil.

American palm nuts of the *Attalea* family, including the babassu and cohune, are very thick shelled and difficult to decorticate by machinery. The babassu is particularly troublesome because it contains several kernels, each of which is enclosed in a separate cavity within the shell. Whereas splitting oil palm or cohune nuts along a single plane of cleavage will usually free the kernel, similar splitting of a babassu nut may not release any of its four to eight kernels.

Different machines have been devised for cracking American palm nuts. Machines designed for round nuts, like coyol, have either been of centrifugal or hammer mill design, or they use pulsating hammers striking against the nut as it is jammed against a stationary anvil. Some machines designed for cohune or babassu nuts employ chisel-like cutting edges to open the shell. One type of babassu-opening machine has opposed cutting edges that split the nut into several segments resembling orange segments. Other machines for cohune and babassu nuts resemble hammer mills. These machines do a poor job and break the kernel as well as the shells.

With all palm nuts, adequate drying prior to cracking is mandatory to ensure that the kernel will not adhere to the shell. Green or undried kernels fill the shell cavity tightly and adhere very strongly. Nuts are commonly dried in silos where, after being separated from the givers, they remain for about 10 h in a stream of warm air (29). Drying is not only necessary for efficient decortication, it also inhibits enzyme action during shipment. The shelled kernels are also dried to protect the kernels from enzyme action during storage.

*Size Reduction of Oilseeds.* The extraction of oil from oilseeds, either by mechanical pressing or by solvent extraction, proceeds more efficiently if the seed is first flaked or ground. Opinion is divided whether grinding or flaking is more effective in rupturing the oil cells. Flaked oilseeds yield a large fraction of "easily extractable" oil on treatment with solvents and a smaller fraction (usually 10–30% of the total oil) that is difficult to extract (30, 31). The former fraction was presumed to come from ruptured oil cells. It has been shown (32), however, that some seeds (like

soybean) that are cracked rather than rolled, also yield a large fraction of oil that is easily extracted with solvents. Woolrich and Carpenter (33) noted little rupturing of oil cells in flaked cotton-seed. They pointed out that cottonseed oil cells are only 0.025–0.038 mm (0.001–0.0015 in) in diameter, whereas cottonseed flakes are more than six times thicker. In any event, it appears that many cells remain intact after even the most careful grinding or flaking, and the walls of these cells are ruptured only by the action of heat and moisture during cooking. The cells, however, are more readily reached by heat and moisture if the seed particles containing them are small.

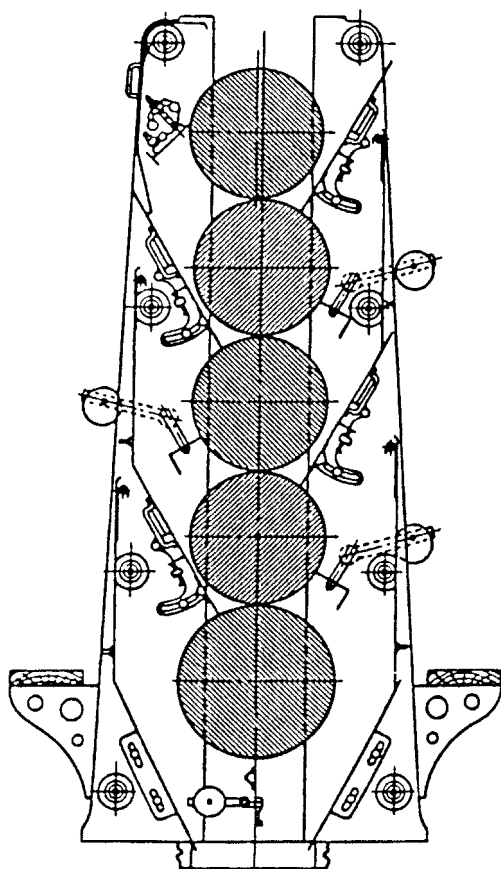
Many oilseeds, after flaking, are processed through steam-heated extruders that combine high-pressure mastication with heat and steam to rupture the cell walls, liquify and liberate the oil, and agglomerate any fine particles that might result from poor flaking. **This converts the oilseed into cylindrical collets of great internal porosity. These collets are larger, heavier, and stronger than flakes. This makes them more easily extracted than flakes. The use of extruders in pretreatment of oilseeds is covered in more detail in Section 3.4.**

Hammer mills, attrition mills, or other grinders are sometimes used for the preliminary size reduction of large oilseeds, such as copra and palm or babassu kernels; but, for the final reduction, it has been almost universal in the United States to use flaking rolls. A roll assembly commonly used for size reduction of cottonseed, flaxseed, and peanuts in the United States consists of five rolls placed one above the other (Figure 1). The seed is introduced by a feeding mechanism between the two top rolls. The seed then passes back and forth between adjoining rolls as it travels from top to bottom of the assembly; hence it is rolled four times. Each roll supports the weight of all the rolls above it, so that the seed particles are subjected to progressively increasing pressure as they pass from one roll to another. Although the lower rolls are smooth-surfaced, the top roll is commonly corrugated to ensure that the seeds will be “nipped” (pulled in) as fast as they are fed.

A typical five-high roll assembly consists of four upper rolls, each 35.6 cm (14 in) in diameter by 121.9 cm (48 in) in width, and a bottom roll, 40.6 cm (16 in) by 121.9 cm (48 in), operating at a peripheral speed of about 192 m/min (630 ft/min). This unit can process 72.6 Mg (80 short tons) of cottonseed or 10,571 dm<sup>3</sup> (300 bushels) of flaxseed in 24 h. However, the actual capacity depends on flake thickness. Detailed data on the capacity and efficiency of cottonseed flaking rolls have been reported by Wamble (34).

Cottonseed meats are usually rolled to a thickness of 0.127–0.254 mm (0.005–0.010 in) for mechanical pressing. For solvent extraction, flake thickness is seldom less than 0.2–0.25 mm (0.008–0.010 in). The repeated passage of the material through the rolls results in considerable breaking of the individual flakes, but this is not disadvantageous for seeds that are to be mechanically pressed. Many oilseeds, especially small seeds such as flaxseed and sesame, are usually flaked in preparation for pressing.

Flaking thin is not as essential for screw-pressing as it is for hydraulic pressing, because heat is generated and seed particles are further crushed by the intense shearing stresses developed in the screw-press. Soybeans for screw-presses are



**Figure 1.** Five-high crushing rolls (Courtesy of The French Oil Mill Machinery Co., Piqua, Ohio).

usually cracked by corrugated cracking rolls into 10–16 mesh particles (passed through a U.S. Series 12 sieve, retained on a U.S. Series 18 sieve) and are then pressed without rolling or further reduction. Schumacher described a particular cracking tooth pitch corrugation that produces a cleaner cut and less fines production when processing low-moisture soybeans for subsequent solvent extraction (35). Palm kernels, copra, and peanuts are handled in screw-presses both with and without flaking.

The rolls used for flaking soybeans or other oilseeds for solvent extraction are different from the five-high crushing rolls just described. As large, thin, and coherent flakes are desired, flaking is commonly carried out by a single passage of the whole or cracked seed through parallel rolls. Therefore, only one pair of rolls is provided. The rolls are mounted side by side and heavy springs or hydraulic rams maintain the pressure of one roll against the other (36). Sometimes the rolls revolve at different speeds to help smear the particles into larger, thinner flakes as they pass through the rolls. As the clearance between these rolls is adjustable,



flakes of uniform thickness are produced. Rolling seed or seed particles into thin flakes disrupts oil cell structure, increases surface area, and facilitates solvent extraction by reducing the distance that solvent and oil must diffuse in and out of the seed during the extraction process.

Karnofsky showed how flake thickness directly affects the percolation extraction rate of soy with hexane (32). He also showed that grinding and screening cottonseed into grits fractions, having comparable diameters with flakes, gave similar extraction rates as the flakes. However, grits do not provide a permeable bed necessary for efficient extraction in commercial extractors. Although thin flakes yield their oil very easily, they can significantly reduce percolation rate or extractor throughput if excessively thin. Therefore, flake thickness must be balanced with percolation rate.

Excessively dry seeds do not flake well; therefore, a higher moisture content is required in oilseeds if they are to be formed into the thin, coherent flakes required for solvent extraction. Conditioners are usually used to add the proper amount of moisture. A conditioner can be either a vertical stacked kettle or a horizontal rotary cylinder. The units are steam jacketed and provision is made so that either steam and or water can be injected as a source of additional moisture (38). For solvent extraction, cracked soybeans are adjusted to a moisture content of 10–11% and flaked while still hot and slightly plastic; that is, while at a temperature of 71–77°C (160–170°F) (36). Flaking hot minimizes fines production and also reduces power consumption (35). In the flaking rolls and transport equipment, the flakes will lose about one percentage point of moisture and enter the extractor at around 57–60°C (135–140°F).

### 3. HEAT PRETREATMENT

The heat treatments given fat-bearing materials may be divided into two categories according to whether the heat treatment itself releases the fat or whether it merely facilitates the subsequent release of fat by mechanical means. The term *rendering* is generally applied to heat treatment designed to remove fat from fatty animal tissues or other materials with a high ratio of fat to solid matter. The heat treatment applied to oilseeds and similar materials is termed *cooking* and serves to facilitate the release of fat in the mechanical press. In rendering or cooking, one object of heat treatment is to coagulate the proteins in the walls of the fat cells and cause the walls to rupture. Heat lowers fat viscosity and makes it easier for the fat to flow away from the solids.

Moisture is present in all materials rendered or cooked and plays an important role in the cooking reactions. Without sufficient moisture, the desired “cook” would not be achieved. Water or steam or both are often added to elevate moisture and to ensure proper denaturing and hardening of protein, rupturing of oil cells, and inactivation of undesirable enzymes, micro-organisms, and antinutritional factors. Water also assists in the displacement of fat from the surfaces of solid materials. Solids have a greater physicochemical affinity for water than for fat.

### 3.1. Rendering of Animal Materials

Fatty animal tissues, free from muscle and bone, usually contain 70–90% fat, the balance being water and connective tissue. Connective tissue is high in protein, hence the dried solids from rendering (tankage, cracklings, stick, etc.), like the solid residue from oilseeds, is marketed as a protein concentrate for animal feeds.

The product of highest fat content (92–95%) obtained in meat-packing establishments is leaf fat from hogs. Fatty tissue from cattle contains 60–80% fat. Back fat and other cutting fats from pork have 80–85% fat. Lard and tallow in significant tonnages are also obtained from bone stock and other low-fat material, which contain 10–15% fat. Whole carcasses of animals (dead stock, etc.) are also rendered for fat recovery. Fish oil is produced by rendering whole small fishes, such as sardines and herring, which contain 10–20% fat.

Rendering methods are tailored to source material and also to the characteristics desired in the finished fat and to the type of rendering equipment available (39–41). There are four general methods of rendering: wet rendering, dry rendering, slurry rendering, and digestive rendering.

*Wet Rendering.* Wet rendering is used to obtain edible fats where color, flavor, and storage qualities are important and the amount of solids in the raw material is small. Wet rendering uses large amounts of water. Separated fat can be removed by skimming, but it usually is removed by centrifuging. There are two methods of wet rendering: low temperature, done at temperatures below the boiling point of water; and high temperature, or steam rendering, done under pressure in closed vessels. Lard produced by steam rendering is known as *prime steam lard*.

An apparatus used in U.S. packing houses is a vertical cylindrical steel autoclave or digester with a cone bottom, designed for a steam pressure of 276–414 kPa (40–60 psi) and a correspondingly high temperature. The vessel is filled with the fatty material plus a small amount of water, and steam is admitted to boil the water and displace air. The vessel is then closed except for a small vent, and the injection of steam is continued until operating temperature and pressure are attained. Time required for digestion varies with temperature and with composition of the charge. The usual digestion time is 4–6 h.

Under the influence of high temperature, the animal tissues disintegrate and release fat. The fat rises to the top of the vessel, leaving a layer of *tankage* (solids) and *stick water* in the bottom. Pressure is then slowly relieved and the fat–water interface is adjusted to coincide with a draw-off cock mounted on the vessel. The fat is drawn off and purified of water and solids by settling or by centrifuging. The fat may also be filtered.

In steam rendering of high-fat stock, 99.5% of the fat is ordinarily recovered. The unrecovered fat consists of a small residue in the tankage plus a small amount that remains in the stick water. The usual packing house “killing” and “cutting” fats yield about 80% and 70% lard, respectively. Dried tankage solids and stick solids contain about 10–12% and 1.5–2% fat, respectively. Both are high in protein: tankage from good stock may contain 70–72% protein; stick solids may contain 90% or more.

Steam rendering is advantageous in that fat can be obtained using simple equipment and the method can be adapted to a variety of materials. There is little tendency for proteins and other substances to disperse into fat in the presence of water. Therefore, steam rendering can be used on materials containing large amounts of nonfatty tissue. Bony material can also be handled by this process because even that material is effectively disintegrated by prolonged treatment with steam under elevated pressure.

Steam rendering is, however, less rapid than other methods and less efficient in heat consumption. A large amount of water must be evaporated to recover the low-fat solid residue. Some hydrolysis of fat into free fatty acids occurs during steam rendering. Free fatty acid level depends on rendering time and temperature, storage temperature, and length of storage before processing (39–41). By careful scheduling of operations, *killing* fats may be rendered when the animals are slaughtered. *Cutting* fats are taken from the carcasses later, after the carcasses have been chilled to 0–3°C (32–36°F).

Antioxidants added before rendering improve the fat stability. Sims and Hilfman (42) studied the stabilization of lard and edible beef fats during pressure steam rendering. Antioxidants tested included butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate and citric acid combinations, and a mixture of BHA and BHT. Best results for a given stabilizer level were obtained with the individual phenolics BHA and BHT. Poorer results were obtained with the mixtures in propylene glycol.

Norris described modifications of continuous wet rendering operations in the previous edition of this book (43). He described several methods, current at that time, that involved disintegration to separate fat from animal tissue, followed by centrifuging to remove sludge from the liquid phase, followed by clarification of the finished fat (44–47). He also described a different operation that involved hammer milling bony material in a stream of cold water from which the fat was subsequently recovered (48, 49).

Since then, the rendering industry, as well as the edible vegetable oil industry in general, have undergone transformation in response to growing pressure to treat effluent water and air streams to minimize impact on local environment (50). This has led to consolidation of many small plants into fewer high-volume plants, more able to afford effluent pollution control equipment. Several equipment manufacturers serve the needs of this industry with integrated rendering systems (both wet and dry systems) that address all the processing requirements.

Atlas Danmark (Ballerup, Denmark) provides a low-temperature, wet rendering system. It features prebreaking; “hashing” to a smaller particle size, more suitable for low-temperature rendering; cooking in a heated, double-screw coagulator; draining of freshly released liquified fat in a strainer screw; followed by pressing in a twin-screw, low-pressure screw-press. The still moist solids from the screw-press are dried, and the liquified fat is clarified in a three-phase decanter.

*Dry Rendering.* Dry rendering is a simpler method of fat extraction. It differs from wet rendering in that the expulsion of fat is accompanied by dehydration of the fat and solid residue, so that both are of low moisture at the end of the operation.

The frying of bacon, to cite a familiar example, is essentially a dry rendering process. Dry rendering can be batch or continuous.

Batch dry rendering is done in horizontal steam-jacketed tanks with a large inlet opening on top and an agitator within. The agitator has paddles attached by arms to a horizontal shaft. After the charge—usually 2,268–4,537 kg (5,000–10,000 lb)—is dried to the desired moisture level, the contents are discharged into a steel box equipped with a perforated liner, and all possible free liquified fat is drained off. The solid residue is pressed, and the fat thus obtained is combined with the free drained fat. After settling, centrifuging, or filtering, the fat is ready for market. The solid residue is ground as a protein supplement for animal and poultry feed (34–41).

Dupps (Germantown, Ohio) provides a continuous dry rendering system involving a continuously discharging, steam-heated, horizontal cooker to dry-render the raw material. Fresh wet material continuously enters one end. Dry-rendered material plus liberated fat continuously discharge from the other end and pass over a drainage screen to free-drain the liberated fat. The solids continue to a screw-press. Fat from the screw-press, combined with fat from the drainage screen, goes to a centrifuge.

An alternative system mixes processed fat with ground raw material to form a slurry, which is heated to release fat, then goes to a twin-screw, low-pressure screw-press to separate an aqueous liquid phase from the still moist solids. The liquid phase goes through an evaporator to dry the fat; the moist solids go through the horizontal cooker, drainage screen, and screw-press as described above.

Stord Bartz (Bergea, Norway) provides a continuous dry rendering system that involves prebreaking, sterilization, cooking, and drying in a horizontal, continuously discharging vessel that is equipped with a rotating, steam-heated rack of discs that agitate and transfer heat into the incoming material. The cooked, dewatered, fatty solids then go to a screw-press for recovery of fat.

*Slurry Rendering.* A slurry rendering process was developed by Charles Greenfield in 1953 (51, 52). The raw material is coarsely ground, mixed with finished fat, and fine-ground to form a pumpable slurry of approximately 68% fat, 10% solids, and 22% moisture. The slurry is pumped through an evaporator under vacuum for moisture removal, then through a centrifuge for fat removal. The solid residue, at approximately 35% fat, is pressed to 10% fat in screw-presses. Greenfield licensed the V.D. Anderson Co. (Cleveland, Ohio) to market the system. It became known as the Anderson C-G system. Most Anderson C-G systems employed double-stage evaporators, some were triple-stage, and small-capacity plants were single-stage.

The advantages of multiple-stage evaporation are reduced steam consumption and improved product quality. Batch or single-stage moisture reduction uses heat from steam to boil off the moisture, and the hot moisture vapors are then condensed and discarded. Multiple-stage evaporators use the hot moisture vapors to boil off more moisture in the other stages. A double-stage evaporator uses the incoming heat twice. Moisture in the last stage absorbs the incoming heat and vaporizes. The vapors then condense against the first stage and release the heat, which causes moisture to evaporate from the product passing through the first stage.

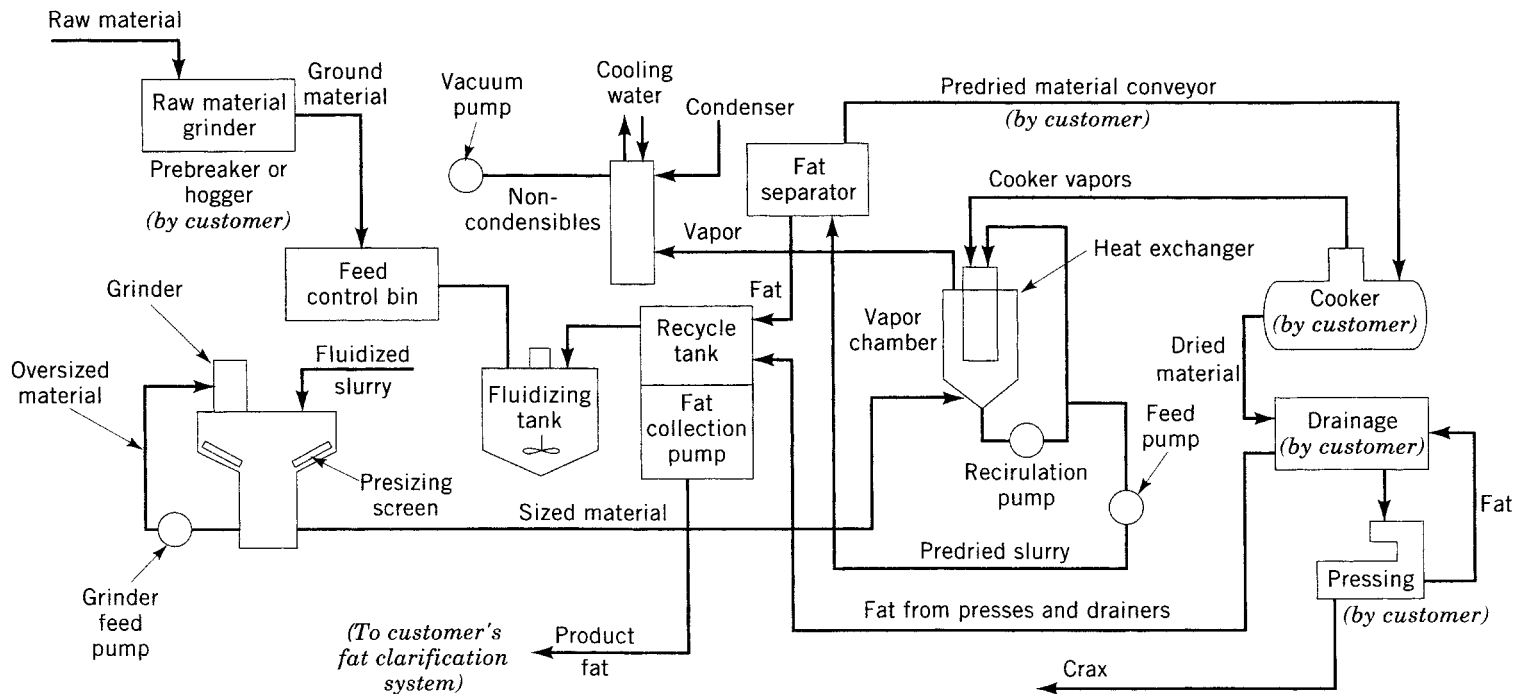
Each stage consists of a heat exchanger and a vapor chamber. Vacuum is drawn across the first-stage vapor chamber. This allows moisture to boil off at low temperature. Vapors released from the last stage condense against the first-stage heat exchanger, providing heat to boil moisture from slurry entering the first-stage vapor chamber. Slurry from the first stage is then pumped through the last-stage heat exchanger, which is heated by steam. That allows moisture to boil off (at a higher temperature) from the last-stage vapor chamber. The driving force controlling evaporation is the difference between the steam temperature on the last-stage heat exchanger (directly proportional to steam pressure) and the vapor temperature from the first-stage vapor chamber (inversely proportional to vacuum). The higher the vacuum or the greater the steam pressure, the greater the driving force.

Greenfield also supplied C-G evaporator systems to handle municipal sewage waste (53) and watery waste streams from other industries (like breweries) independent from his relationship with Anderson Co. In 1981, Anderson adapted the C-G process to connect an evaporator to existing batch cookers (Figure 2). A bank of batch cookers, or a large, continuous, single cooker can be used as the last stage. The hot vapors from the cookers condense against the slurry evaporator serving as the first stage. This allows existing plants to increase capacity and take advantage of multiple-stage evaporation without abandoning existing equipment (54).

TM-1, for Thermomiser Phase One, was envisioned as the first step toward complete conversion to slurry evaporation. Phase two would replace the batch cookers with a steam-heated slurry evaporator to convert the whole process line to a C-G system. A plant in Holland, directed by one of the inventors, employed a double-stage evaporator together with a bank of batch cookers serving as the last stage (55). A similar plant in Detroit, Michigan was placed under computer control (56).

*Digestive Rendering.* Considerable attention has been given to wet rendering of animal fats with the assistance of added chemicals or enzymes that promote the separation of fat by hydrolyzing and dissolving the connective tissue. Deatherage (57, 58) described laboratory and pilot plant experiments on the alkali rendering of lard and beef fats. Best results were obtained when the fat was digested at 85–95°C (185–203°F) for 45 min to 1 h with a 1.75% sodium hydroxide solution. After digestion, the fat was separated from the aqueous liquid (which contained a small amount of undigested solids) by centrifuging. The fat was then washed, first with 2–5% salt solution and then with water. Fat recovery was equivalent to or better than that obtained by steam rendering, and fat quality was better. There was no significant hydrolysis or darkening of the fat, and there was no sign of the typical cooked flavor of steam lard.

The process was best adapted to fresh fat. Stocks in which hydrolysis occurred were difficult to process because of the formation of soap in the aqueous phase. The soap was derived only from already liberated free fatty acids. Under the mild conditions of this digestion process, there was no appreciable saponification of neutral fat. The fat was also alkali refined to produce a final product free of acidity. A typical final product had a free fatty acid content of 0.01% and a Lovibond color of 2 yellow and 0.3 red. This process gave improved yields over conventional wet rendering (59, 60).



**Figure 2.** Flow diagram of slurry evaporator with batch cookers (Courtesy of Anderson International Corp., Cleveland, Ohio).

The use of proteolytic enzymes in rendering is described in several patents. However, an enzyme process does not appear to have been used commercially except perhaps in the recovery of fish fats. The patent of Parfentjev (61) covers the digestion of fish livers with pepsin at low pH and low temperature. The process of Keil (62) for the recovery of lard or other animal fats involves digestion of the fatty stock with a proteolytic enzyme of vegetable origin, for example, 0.005–0.020% papain at a pH of 6.0–7.5, followed by heating to 60–85°C (140–185°F) to separate the fat. Halmbacker (63) patented the use of an enzyme, such as papain or ficin, with a cysteine activator, to decrease digestion time while increasing yield. Other publications deal with treatment of eggs with papain (64), fish rendering (65), and rendering of coconut meats (66). Normally, the use of added enzymes is avoided because of their high cost. A unique use of natural enzymes in the recovery of fat from catfish offal was reported by Freeman (67).

Catfish farming has become a major industry in the southern United States. The fish are processed in large cooperative plants where adequate disposal of wastes has become a serious problem. Usually, catfish offal is sent to poultry rendering plants or pet food manufacturers. However, because of high transportation costs, catfish processors never realize the full value of the waste. Catfish offal contains approximately 10% fat, which Freeman found could be recovered from liquified offal. He liquified offal by adding either 0.5% formic or 0.4% hydrochloric acids, which activated natural enzymes (68). Total liquification of skin and flesh occurred in about 2 h at 55°C (130°F). The silage was then screened to separate bones and centrifuged to recover oil. The deoiled liquid was concentrated to about 50% solids. The concentrate was found to be stable against spoilage for over 4 weeks at 38°C (100°F) and could be used as a direct feed additive or a pet food flavoring.

### 3.2. Cooking of Oilseeds

*General Considerations.* It is universally recognized that oilseeds yield their oil more readily to mechanical expression after cooking, but a complete explanation of why this is so is lacking. The changes caused by cooking are complex and are chemical and physicochemical in nature. Oil droplets in oilseeds are ultramicroscopic and are distributed throughout the seed. One effect of cooking is to cause these minute droplets to coalesce into drops large enough to flow from the seed. An important factor in cooking is heat denaturation of proteins and similar substances. Before the proteins coagulate through denaturation, the oil droplets are virtually in the form of an emulsion. Coagulation causes the emulsion to break, after which there remains only the problem of separating gross droplets of oil from the solid material.

As the surface of the seed particles is highly extended, surface activity figures prominently in the displacement of the oil. Cooking, in turn, has a profound influence on surface activity. The primary objectives of cooking/drying are to coagulate seed proteins (coagulating proteins makes the seed more permeable to the flow of oil), to decrease the oil's affinity for the solid surfaces, and to agglomerate the oil

into larger droplets—all of which permit better yields when the seeds are subsequently pressed or solvent extracted. Important secondary objectives of cooking/drying are to lower seed moisture to a more optimum level for rolling and pressing, to insolubilize phosphatides and other undesirable impurities, to destroy molds and bacteria, to increase oil fluidity, and, for cottonseed, to detoxify gossypol (69).

Control of moisture is important for efficient pressing. Optimum moisture varies from oilseed to oilseed and with methods used for pressing. For cottonseed, as an example, 5–6% moisture was found best for hydraulic pressing, whereas 3% was found best for screw-pressing. Soybeans are ordinarily dried to 2.5–3% moisture before pressing in screw-presses; copra and sesame seed require moisture levels of about 2%.

Moisture control is also important in solvent extraction. Generally, intermediate moistures are needed for efficient extraction. Low moisture usually results in lower efficiency caused by the lower solubility of phosphatides, whereas high moisture can result in swelling of protein with the subsequent reduction of flake porosity and solvent diffusion rate (70). As with pressing, optimum moisture varies with the material being extracted: approximately 7–10% for cottonseed and 8–12% for soybean (70).

*Effect of Heat on Product Quality.* Beyond its influence on oil yields, cooking also influences the quality of both oil and press-cake. Cooking is particularly important in relation to refining loss. Some oil lost in caustic refining of crude oil is neutral oil, which is emulsified in the foots. Certain surface-active agents naturally present in the oilseed favor this emulsification; other agents inhibit it (71). The effectiveness of these two classes of surface-active agents is influenced by cooking. There is little published information on the identity of the surface-active agents in crude oils, but it appears that the substances responsible for high refining losses are phosphatides and related substances. Free gossypol in cottonseed oil was once thought to coincide with cooking conditions that permitted low refining loss (71); however, Wamble and Harris conducted experiments at five different screw-press mills and found no connection between free gossypol in the crude oil and refining loss or refined color of the oil (72).

Reasonable variations in cooking conditions have little effect on oil color or refining loss, but wide variations do. Eaves et al. (73) showed that solvent extraction of raw, tempered, and cooked cottonseed flakes gave different yields of crude oil, but they also showed that the yield of neutral oil was virtually unaffected. The greatest yield of crude oil came from raw flakes. It had the highest percentage of impurities and (by mathematics) the lowest percentage of neutral oil. The lowest yield of crude oil came from cooked flakes, but it had the lowest percentage of impurities and (by mathematics) the highest percentage of neutral oil. The reason the yields varied was that different amounts of impurities were extracted along with the neutral oil (the triglycerides), but the quantity of neutral oil extracted was almost the same in all three cases. In good cooking practice, flaked cottonseed meats are brought to a minimum of 12% moisture and are maintained at that moisture and a temperature of 85–93.3°C (185–200°F) for 15 to 20 min. The cooked meats are then dried for expression of oil (74).



King et al. (75) have studied how cottonseed oil is affected by pH during cooking. They concluded that oil made from meats cooked at low pH is high in gossypol and is subject to color reversion during storage, whereas oil from meats cooked at high pH is low in gossypol, is not subject to color reversion during storage, and has a lower refining loss.

Overcooking of oilseeds may produce abnormally dark-colored oil and cake. Prolonged cooking also impairs the nutritive properties of the cake. With cottonseed, for example, increasing cooking temperature or cooking time will decrease the protein efficiency for chicks and for rats (76). Similarly, soybean meal loses nutritive value as time under heat is increased, but its nutritive value is definitely improved by proper cooking. This is caused by heat inactivation of trypsin inhibitor, goitrogenic factor, anticoagulant factor, diuretic principle, lipoxidase, and other anti-nutritional factors. This has been summarized by Liener (76) and by Cowan (77).

To improve palatability and nutritive value, solvent-extracted soybean flakes intended for animal feeding are toasted before shipment from the extraction plant. This is done by adding moisture and cooking in a conventional cooker after the solvent is removed. With improved desolventizing techniques, desolventizing and toasting are accomplished simultaneously by injecting live steam into the solvent-laden flakes as they leave the extractor (78). Steam condenses on the cooler flakes, thus furnishing heat to boil off solvent while simultaneously adding moisture. Thus, by the time the solvent is removed, there has been sufficient moist-heat treatment to inactivate the heat-labile antinutritional factors mentioned above.

Rapeseed is an important, worldwide-produced oilseed. However, after extraction of its oil, the full potential of its resulting meal has been limited because of the presence of large amounts of toxic, sulfur-containing compounds called glucosinolates. Rhee summarized various cooking methods that use moisture, heat, or added chemicals to detoxify glucosinolates (79).

A prime purpose of cooking cottonseed is to significantly reduce the amount of gossypol extracted with the oil and to reduce the gossypol's toxic effect in extracted meal. Gossypol is a polyphenolic, binaphthyl aldehyde compound that is highly reactive and toxic to nonruminants such as poultry and swine (69). Heat and moisture cause gossypol to complex or bind to carbohydrates, phospholipids, and to the  $\alpha$  and  $\omega$  amino groups of protein and amino acids to form what is called bound gossypol. Bound gossypol was once thought to be totally inactive; however, it is now suspected that some of the bound gossypol can be reacted on in the digestive tract of some animals and can become free gossypol again (80).

Gossypol is associated in the seed with several related compounds. One or more of these may be actually responsible for the bulk of the observed toxicity, as separated whole pigment glands appear to be more toxic than purified gossypol (81). Usually, all toxicity of cottonseed is attributed to free gossypol, but there have been cases of nontoxic meals containing levels of free gossypol above that normally considered toxic (82, 83). Again, this may be caused by other gossypol-like compounds or the method of analysis for gossypol. It is possible that gossypol bound to protein or other seed components may be released by some analytical procedures, thereby giving abnormally high free gossypol figures.

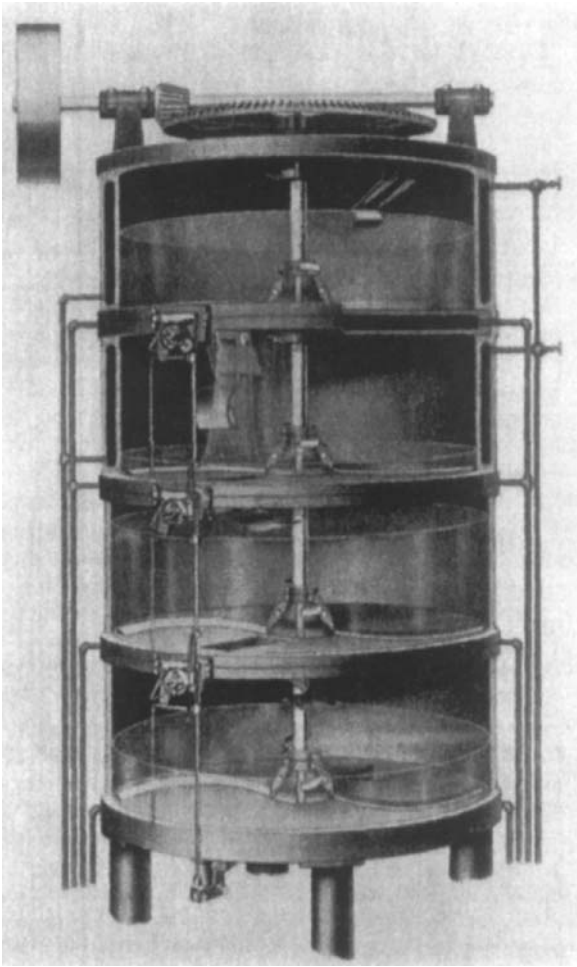
Gossypol is not soluble in hexane or similar nonpolar solvents. However, it is soluble in crude cottonseed oil. If pigment glands are ruptured, and the gossypol is not bound to the protein, some gossypol will be extracted during solvent extraction by a hexane miscella. Essentially all of the gossypol contained in seed can be extracted using certain polar solvents. Aqueous acetone was proposed by Pons and Eaves (84) and a combination solvent composed of acetone-hexane-water (53:44:3) was proposed by Mann et al. for extracting gossypol along with the oil and leaving a gossypol-free meal that need not be denatured to "bind" the gossypol (85). Recently, Hron and co-workers have investigated ethanol-water mixtures (86–90), and Lusas et al. have investigated isopropanol-water mixtures for the extraction of gossypol along with the cottonseed oil (91). The use of alcohol solvents will be discussed in detail in Section 5.4.

### 3.3. Types of Cooking and Drying Equipment

The cooking of oilseeds is carried out either in stack cookers (Figure 3) or horizontal cookers (Figure 4). *Cooking*, as the word is generally used, covers two distinct steps: wet cooking and then drying (to moisture levels of around 3%). Actually, these are two distinct procedures, as Dunning has pointed out (92). Stack cookers cook and dry in the same vessel. Horizontal cookers usually are set up only to cook and are followed by separate horizontal vessels for drying. A stack cooker consists of a series of four to eight closed, superimposed, cylindrical steel kettles, each usually 182.9–335.3 cm (72–132 in) in diameter and 45.7–76.2 cm (1.5–2.5 ft) high. Each kettle is jacketed for steam heating on the bottom (and sometimes on the sides) and is equipped with a sweep-type stirrer mounted close to the bottom and operated by a common shaft extending through the entire series of kettles. There is an automatically operated gate in the bottom of all but the last kettle for discharging material to the kettle below. The bottom kettle feeds into a cake former or a continuous press. The top kettle may be provided with spray jets for the addition of moisture to the material. Each of the lower kettles is provided with an exhaust pipe with natural or forced draft for the removal of moisture. Thus it is possible to control the moisture content of the material at each stack of the cooker.

Flaked meats are delivered at a constant flow rate to the top kettle by means of a conveyor. The meats remain in the top kettle to cook for a predetermined time (controlled by the linkage in the bottom gate) before dropping to the kettle below. The meats then remain in the second kettle before dropping into the third, and so on through the cooker. The gates governing the flow of meats from one kettle to another are opened and closed automatically by a mechanism that is tripped by the meat level in each kettle. The mechanism for each kettle is adjustable. This permits control over residence time in each kettle. The cookers are made in different sizes, with different numbers of stacks, and are supplied by several equipment manufacturers.

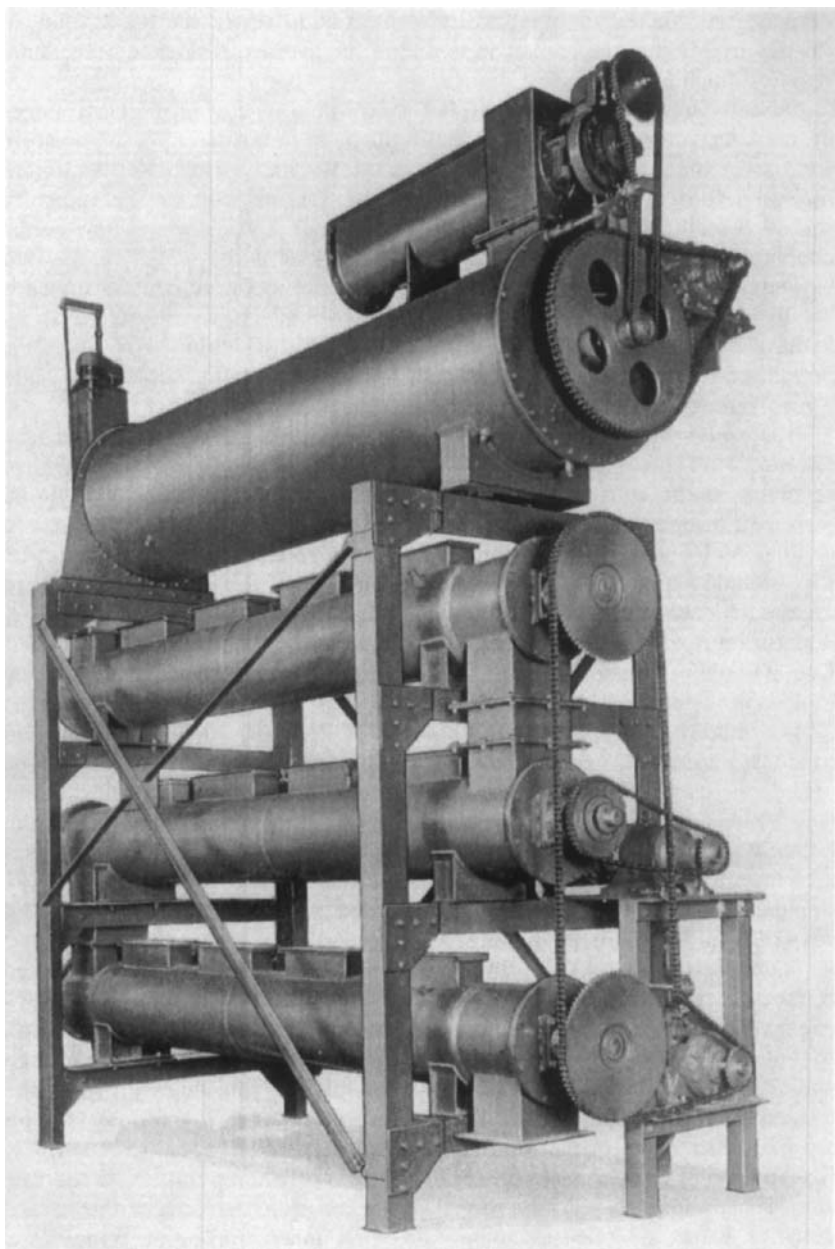
Steam pressure on the top kettle is usually 483–620 kPa (70–90 psi), to quickly heat the incoming material. Lower steam pressure is used on the lower kettles as the material entering the lower kettles is already hot. The important parameter is



**Figure 3.** Interior view of four-high stack cooler (Courtesy of The French Oil Mill Machinery Co., Pigua, Ohio).

temperature. Enough steam pressure is used to maintain an  $87.8^{\circ}\text{C}$  ( $190^{\circ}\text{F}$ ) cook temperature and, on the last kettle, to provide enough temperature to drive the moisture down to around 3%.

Cottonseed meats are usually kept in the stack vessel for 60–120 min and leave at a temperature of  $110\text{--}132.2^{\circ}\text{C}$  ( $230\text{--}270^{\circ}\text{F}$ ). Poor-quality seed is normally not cooked as long as good seed, because oil in poor-quality seed darkens on prolonged cooking. The material first in is not always first out. This has been noted by Alderks (1) and can be demonstrated by adding corn kernels, dyed flakes, or other easily spotted additives to the feed. Some of the material leaves the cooker in less than the average residence time, some remains much longer.



**Figure 4.** Horizontal cooker and dryers (Courtesy of Anderson International Corp., Cleveland, Ohio).

Oilseeds are usually moistened before cooking or during the early stages of cooking, unless they are initially high in moisture. Their moisture content is then reduced in the bottom kettles of the cooker. An initial moisture content of 9–14% is common in the top kettle. The moisture should remain constant in the top two to four kettles where the actual cooking takes place. In the bottom kettles, drying is the objective, assisted by increased temperatures and venting. The final moisture content depends on the material processed and whether cooking is to be followed by hydraulic pressing, screw-pressing, or solvent extraction. For hydraulic pressing 5–6% is used for cottonseed, for screw-pressing, around 3%, and for solvent extraction, 7–10%.

Horizontal vessels are built differently but serve the same function (see Figure 4). The vessels are 3.6 m (12 ft) long, are steam jacketed, and have revolving shafts with spokes and paddles that agitate and move material forward through the vessel. The first vessel is used for cooking and is sealed at inlet and outlet so that a humid atmosphere can blanket the material. Live steam is injected, sometimes mixed with water, to elevate moisture and accelerate cooking. Cooking, among other things, hardens the protein by denaturing it so it can withstand the pressure generated by the screw-press. Usually, holding the oilseed at 87.8°C (190°F) and 10–12% moisture for 15–20 min is sufficient to harden the protein.

The material then passes to the rest of the vessels where it is dried to 2–3% moisture. As a result of boiling point rise, temperature has to be raised to 104.4–115.5°C (220–240°F) or higher to drive the moisture out (the same as for a stack cooker). The hot, freshly dried material (from either type of cooker) should go directly to the screw-press before it has a chance to cool. Horizontal cookers are 60.9 cm, 91.4 cm, or 101.8 cm (24 in., 36 in., or 40 in.) in diameter, depending on capacity. Dryers are 45.7 cm and 91.4 cm (18 in. and 36 in.) in diameter.

There are pros and cons for stacked and horizontal vessels. A stacked cooker/dryer is more compact compared with a bank of horizontal vessels (a cooker followed, e.g., by three or four dryers). Watkins points out<sup>1</sup> an advantage of horizontal cookers in that the raw feed is metered into a large volume of tumbling material that is already hot (93). This quickly brings the raw feed up to temperature. In a stack cooker, the raw material falls on top of a thick layer of material that is slowly agitated beneath the surface. It takes longer for the raw material to come up to temperature. If the raw material contains an enzyme that damages oil quality, reduces the meal's nutritive value, or releases compounds that inactivate catalysts used for hydrogenation, the enzyme can become active and cause damage before temperature becomes high enough to inactivate the enzyme.

This would not happen in a horizontal cooker. There is a possibility, however, that some raw material could migrate quickly toward the discharge end and leave the horizontal cooker before it is fully cooked. If an enzyme is present, and if it is critical to achieve adequate retention time, it is prudent to use two horizontal cooking vessels in series before sending the material to the dryers.

<sup>1</sup>Watkins conducting short courses at Texas A&M University cites (93).

### 3.4. Heat Preparation by Extrusion

Another machine used to pretreat oilseeds is an expander. Expanders are a type of extruder that operate hot and cause the extrudate to puff or expand. This type of expanding/extruder was originally developed in the 1950s to cook and puff animal feeds (94), especially pet food, where a thorough starch cook is required. Later, in the 1960s, these machines found application in the extrusion-cooking of full-fat soy and of binders and adhesives from cereal grains (95, 96), the drying of synthetic rubber (97), and the manufacture of floating and sinking fish feeds (98). The discovery that extrusion/cooking can inactivate enzymes as well as agglomerate fine particles led Baer et al. to develop a process for extrusion of oilseeds, particularly rice bran, ahead of solvent extraction (99). After a slow start extruding rice bran in the mid-sixties, these machines, by the 1970s, began to be used for soybean and other oilseeds.

*Closed-Wall Extrusion.* An expanding/extruder consists of a rapidly rotating wormshaft within a cylindrical barrel. Material enters one end of the barrel and is forced out through a die plate at the discharge end. The wormshaft flighting is interrupted rather than continuous, and stationary pins protrude from the barrel wall to intermesh between the individual flights (Figure 5). This combination of rotating flights and stationary pins imparts a highly turbulent mastication to the material. Live steam is injected to elevate the moisture and supply heat to soften the material.

By the time the material reaches the die plate, it is under high pressure, 1379–4137 kPa (200–600 psi) and has been cooked to some desirable degree based on moisture level and temperature. Oilseeds usually receive a mild cook that converts them into a tacky, elastic-like condition. The injected steam condenses from the vapor state as it is absorbed into the material and releases its heat of vaporization. This sudden release of energy helps to heat the oilseed. Frictional heat generated by the rotating shaft continues the heating.

The oilseed reaches typically 112.8°C (235°F), as read by a thermometer near the die plate, and approximately 10–13% moisture. Internal pressure is 13–40 times greater than atmospheric pressure. At this pressure and temperature, all moisture, even injected steam, is compressed into the liquid phase. On release into atmospheric pressure, some of the moisture flashes to reach equilibria. This vaporizing moisture inflates the collets with internal pores and surface cracks, imparting a porous, sponge-like structure to the collet.

Stronger cooks are required for starchy cereal grains and animal feed formulations containing starch. A stronger cook is achieved at higher moisture and temperature, typically 27% moisture and 137.8°C (280°F). Residence time in an extruder is 10–30 s depending mainly on desired capacity, but the degree of cook can be varied at any capacity by varying moisture and temperature conditions.

The first oilseed application for extrusion was rice bran (99, 100). Really a cereal grain rather than an oilseed, rice seeds have a brown, paper-thin coating called rice bran that surrounds the starchy kernel. Once the hull is removed, the brown kernel is exposed. The bran is removed by polishing process that breaks up the bran into

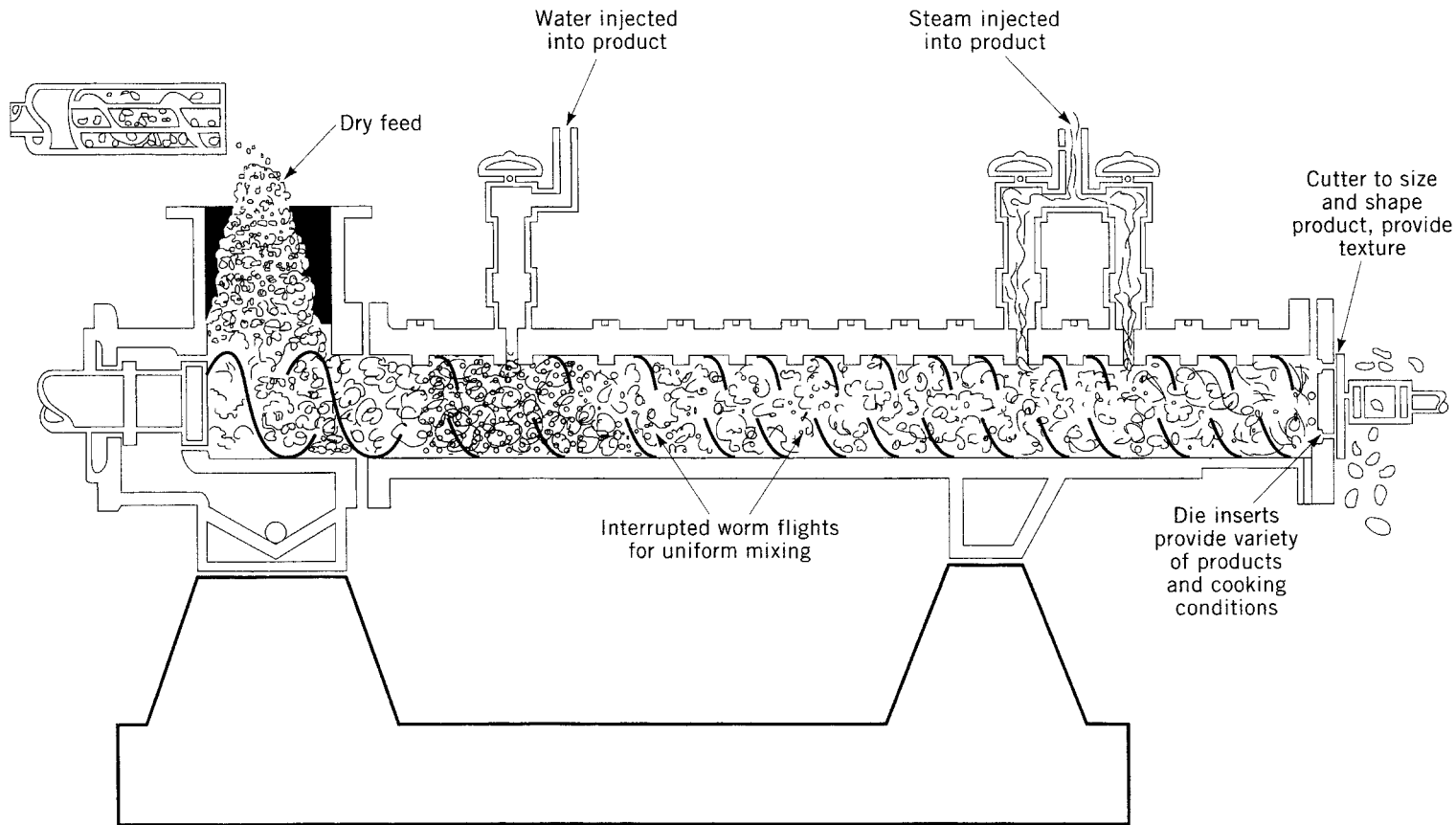


Figure 5. Sectional view of closed-wall expander (Courtesy of Anderson International Corp., Cleveland, Ohio).

finely divided particles and exposes the white kernel. The bran contains 16–22% oil and is very difficult to extract because of the extremely slow flow of solvent through the small particles. The bran, being so finely divided, retains an excessive amount of solvent. Rice bran also contains an enzyme, lipase, that breaks fat into glycerol and free fatty acids. Lipase is activated when the bran is removed from the rice. Once activated, lipase raises free fatty acid level approximately 3–7 percentage points every day. Aside from inactivating lipase, extrusion converts the bran into porous, sponge-like particles, or collets, that permit rapid flow of solvent. The collets drain to a low solvent level before leaving the extractor.

The recent use of extrusion in oilseed preparation is a significant milestone in the history of oilseed solvent extraction. Extrusion can transform flaked soybean and flaked cottonseed into porous collets that process significantly better than flakes in an extractor. Farnsworth et al. described some of the early extrusion research with cottonseed (101). Flakes, if prepared properly, extract well, but if flaking is poor or if the oilseed already contains fines, then extraction suffers. Extrusion can convert poor-quality flakes into easily extracted collets. Even if the flakes are good, extrusion offers great advantages because extruded collets are larger, denser, stronger, and more porous than flakes. These qualities: (1) improve solvent flow because of larger particles, (2) increase extractor capacity because the denser collets occupy less space, allowing almost a doubling of capacity without changing bed depth, and (3) result in better drainage because the collets are porous and strong enough not to crumble as easily as flakes. Watkins and Lusas and co-workers described these effects in a series of articles (102, 103). These effects will be covered in greater detail below when extraction theory is discussed in Section 5.2.

The cooking conditions within an expanding/extruder soften and gelatinize the oilseed's protein, converting the protein into a glue-like condition that binds all the particles together. The gelatinized protein is elastic and allows the collets to inflate with internal pores as some of the superheated water flashes into steam when the collets exit into atmospheric pressure (104).

Some oilseeds are normally prepressed in screw-presses to rupture the oil cells and partially reduce the oil level. The cake from the screw-press is then cooled and either crumbled and flaked or sent directly to a solvent extractor. The hot, tacky press-cake can also be formed into porous collets by extrusion. Enough steam is injected into the expander to raise the moisture 2–4 percentage points. This allows the expander to transform the cake into porous collets. This must be done on stream, before the cake has had a chance to cool and harden. Once cool and hard, protein denatures, and the cake can no longer be transformed into a tacky, inflatable condition.

Prepressed high-oil-containing oilseeds, containing 15–30% residual oil, extrude similarly to flaked soybean (at 18% oil) and flaked cottonseed meats (at 30–33% oil). The main variables that are changed during extrusion are the number and size of dies. Most materials are extruded through 9.5 mm (0.375 in.) to 14.3 mm (0.5625 in.) diameter dies. Although maximum extruder capacity is determined by extruder diameter, operating capacity can be influenced by the number and diameter of the dies.



Land length (the thickness of the metal through which the die opening is cut) helps determine the firmness of the collet (the longer the land, the firmer the collet). Total die area and operating conditions also influence firmness. Collets should be firm enough to survive transport from the expanding/extruder into the extractor. Some breakage, however, is desired. Long, firm collets would allow solvent to flow too fast, and the solvent might channel and not contact the edges of the bed of material. Finely crumbled collets would inhibit solvent flow. Unlike flakes, where the particles are never too large and are frequently too small, collets can be made larger and firmer, or smaller and less firm, by changing the moisture/temperature conditions within the extruder and by changing die configuration. The ideal collet size would be large enough to allow adequate solvent flow, but not so large that solvent cannot spread across the bed of collets. The collets are usually 6.3–12.7 mm (0.25–0.5 in.) diameter and 12.7–50.8 mm (0.5–2 in.) long. Collet firmness and porosity is also influenced by preparation (flaking or heat treatment) prior to extrusion.

*Slotted-Wall Extrusion.* Oilseeds containing more oil than cottonseed meats (30–33%) cannot be efficiently processed through a closed-wall extruder because the liberated oil pools within the extruder and results in oil slugging, which interferes with steady-state operation. Also, the extruded collets cannot reabsorb all the liberated oil. In such situations, extracted meal is sometimes blended with incoming material to dilute the oil level. Although this corrects the problem, the recycling of meal increases the solids passing through the extruder, the solvent extractor, and the meal desolventizer. This increased load on the solids-handling equipment may reduce the total overall daily throughput of the oil mill.

As an alternative answer to the problem, Anderson International (Cleveland, Ohio) developed and patented a new slotted-wall extruder equipped with a drainage cage and internal modifications that can accept high oil materials and produce collets at 20–30% oil along with a separate stream of free oil (105) (Figure 6).



**Figure 6.** Slotted-wall expander (Courtesy of Anderson International Corp., Cleveland, Ohio).

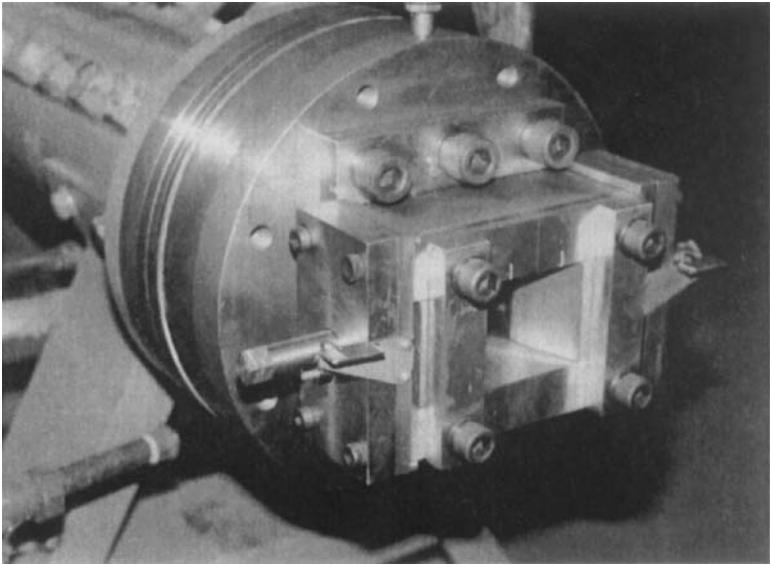
Preliminary field trials with canola were promising (106, 107). Subsequent work with canola and other oilseeds showed that this new machine, the Hivex expander, can process full-fat safflower (at 42% oil), sunflower (at 42–44% oil), peanut (at 45% oil), and some varieties of full-fat canola and rapeseed as well as cottonseed. Typical preparation prior to extrusion of safflower, sunflower, and peanut is to crack to approximately 1.6 mm (0.0625 in.) particles, or flake: heat to approximately 60–71.1°C (140–160°F): and reduce moisture to approximately 8% without denaturing the protein. Canola and rapeseed, being small seeds and low in protein, need to be flaked as thin as possible, 0.15 mm (0.006 in.) if practical. Canola flakes thicker than 0.25–0.3 mm (0.010–0.012 in.) do not extrude very well. If seed protein is denatured too much before extrusion, the canola collets will be loose and crumbly.

Extruders can also be equipped with discharge cone chokes rather than die plates. The cone is a mushroom-shape device that is moved toward a mating socket mounted on the extruder's discharge. A hydraulic cylinder, operated through a positioning lever, moves the cone in and out. Pressure generated by the extruder causes the oilseed to bear against the cone. The hydraulic fluid trapped in the cylinder (when the positioning lever is not in the close or open position) holds the cone in a fixed position.

An alternative method is to control hydraulic pressure so it remains uniform at any desired value and leave the positioning lever in closed position. The cone will now find an equilibria position where the force exerted by the hydraulic cylinder constantly readjusts itself (by maintaining a uniform hydraulic pressure) to balance the force the extruder exerts against the cone. This allows the cone to float, or automatically adjust to, and partially compensate for, pressure upsets in the extruder. The controlled hydraulic pressure can be raised to overcome pressure exerted by the extruder and move the cone to a more closed position. Conversely, the hydraulic pressure can be lowered to allow the cone to retract to a more open position. It should be noted that dies in a die plate cannot adjust themselves to respond to pressure upsets.

Hydraulic cone chokes can be mounted on closed-wall or slotted-wall extruders. Cake flowing across the cone to atmosphere “expands” with internal pores similar to collets from dies. The sheets of cake dropping from the cone break up in transit to the solvent extractor similar to the way collets break up.

*Dry Extrusion.* Another application for extruders is to prepare oilseeds for subsequent screw-pressing. Nelson et al. processed coarsely ground soybean through a high shear, closed-wall extruder without addition of injected steam or water and converted the soybean into a hot, frothy meal of small particle size and almost fluidlike consistency (108). Fluidization was caused by moisture boiling through the freshly liberated oil. Soybean entered the extruder at 10–14% moisture and ambient temperature. High shear extrusion pulverized the particles and ruptured the oil cells. This allowed the oil to spill out of the cells. Frictional heat raised the meal temperature to 135°C (275°F) (108), causing native moisture to flash from 10–14% down to 6–7% as the meal exited the extruder.



**Figure 7.** Adjustable-jaw expander (Courtesy of Anderson International Corp., Cleveland, Ohio).

Extruders, both closed-wall and slotted-wall, can be fitted with adjustable jaw chokes to replace the die plates (Figure 7) and can operate at low moisture to provide high shear rupturing of oil cells. Cracked or uncracked oilseeds, with or without preheating, and at ambient moisture are converted to a foamy, frothy, semi-fluid extrudate at 121–148.9°C (250–300°F) that flashes down to 5–7% moisture as the oilseed enters the screw-press. Shear is generated by a rotating cone point at the end of the shaft and two laterally positioned, nonrotating (but adjustable) jaws through which the extrudate exits. Shear is influenced by the proximity of the cone point to the jaws and by the opening between the jaws.

A 203 mm (8 in.) diameter extruder, powered by a 112-kW (150-hp) motor, can process raw cracked soybean at up to 2722 kg/h (6000 lb/h). The product, having low levels of urease and trypsin inhibitor, is marketable, without any further processing except cooling, as full-fat soymeal. However, the hot product, flashed down to 5–7% moisture, having almost all the oil cells ruptured, is ready to pass immediately to a screw-press. A conventional screw-press, with some modification to wormshaft configuration and rotational speed, can process this material at a three-fold increase in capacity using the same motor and pressing to the same residual oil.

This procedure has been used successfully on soybean and cottonseed. One manufacturer, Anderson International Corp (Cleveland, Ohio), has applied this procedure to copra, increasing traditional copra plant capacity 40% while still maintaining residual oils at 7–8%. Recognizing the current trend favoring “organic” foods, (foods made from products that have never been exposed to chemical fertilizers, pesticides, or solvents during growth and manufacture), Anderson is developing large capacity systems using extrusion followed by full-pressing. These systems will permit a few machines to replace a large-size solvent extractor.

## 4. MECHANICAL EXPRESSION OF OIL

### 4.1. Batch Pressing

Around 1890, oilseeds were pressed in manually loaded batch presses. Workers stacked layers of oilseed, separated by filter cloths and hollow pressing plates, into the press and applied pressure through a manually operated jack screw or a hydraulic cylinder. Oil flowed from the compressed material into the hollow plates and then out through the side walls of the press. After the oil stopped flowing, workers opened the press, placed the deoiled solids in a hopper, and recharged the press with fresh material.

Manually operated presses, using jack screws to apply pressure, have been used for hundreds of years. Hydraulic cylinders made the presses more efficient and less labor intensive. The greater pressure generated by a hydraulic cylinder liberated more oil. In the early 1900s, hydraulic presses dominated the oilseed crushing industry. However, even then they were considered labor intensive. Valerius D. Anderson, as will be discussed in Section 4.2, had already developed a continuous screw-press, but it was 20–30 years before it swept through the animal fat and oilseed crushing industries.

Today, continuous screw-presses dominate the mechanical extraction of fats. The only applications still favoring hydraulic presses are those requiring gentle handling, for example, pressing cocoa butter from cocoa beans where the defatted residue is to be fine-ground to make cocoa powder. The development of continuous screw-presses will be outlined in Section 4.2. The development and technology of hydraulic presses was covered in detail in previous editions of this book (109). This edition briefly summarizes the early development of hydraulic presses and then focuses on modern, continuous cocoa butter hydraulic presses used today.

Batch presses can be divided into two main types: open type, where the oily material is wrapped in press-cloths, and closed type that, omitting press-cloths, confine the material in a metal chamber that holds the oilseed under pressure. Open-type presses can be subdivided into plate presses and box presses. Closed-type presses can also be subdivided into two varieties: cage presses that confine the material in a cylindrical cage with slotted walls and pot presses that confine the material in potlike chambers with perforated bottoms.

*Open-Type Presses.* In plate presses, the fresh material, after proper preparation, was wrapped in press-cloths and placed between the plates. The plates were either corrugated or covered with hair mats to assist drainage and overcome cake creepage. Box presses, in contrast, had special box-like enclosures for the oilseed that simplified the wrapping of the oilseed.

Hydraulically operated open-type presses generally had a 40.6-cm (16-in) cylinder operating at 27,580–31,027 kPa (4000–4500 psi); hence, the pressure on the oilseed was 11,376–12,755 kPa (1650–1850 psi). It is important on batch presses to build up pressure gradually. A typical press cycle is 2 min to charge the press, 6 min to attain maximum pressure, 20 min to drain, and 2 min to remove the solids: total time, 30 min.

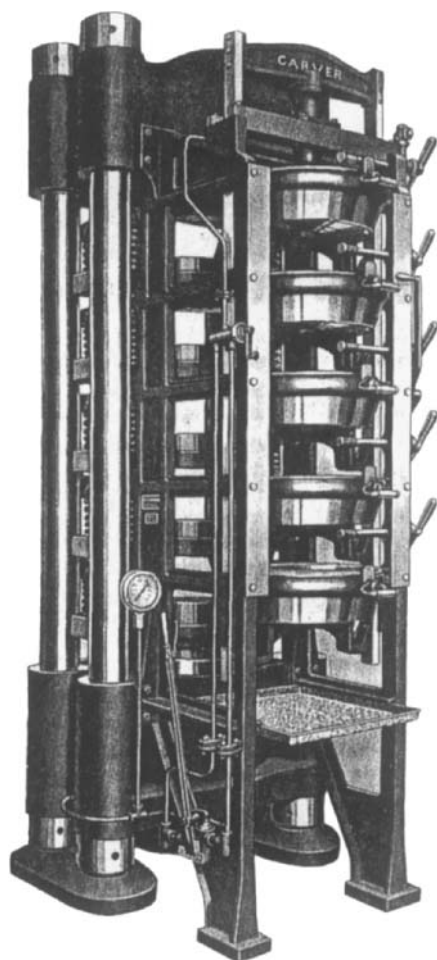
*Closed-Type Presses.* Cage presses enclose the oilseed within a strong perforated steel cage that can stand greater pressure than an open press. Cages were round or square and were assembled of closely spaced steel bars or slotted steel plates arranged within a heavy supporting frame. Oil flowed through channels that increased in size from the interior of the cage outward. This minimized any tendency to clog with solid particles. Oil was squeezed from the oilseed by moving a close-fitting piston into the cage by means of a hydraulically operated ram. The cage could be in a vertical position or a horizontal position. As the oil tended to flow in the compressed cake longitudinally rather than radially, the cage could not be packed solidly with the fresh material but had to be packed with layers of seed separated by drainage plates and press-cloths.

A pot press is a special form of cage press used for extracting cocoa butter or other fats that are solid at room temperature. In this press, the cage is replaced by a series of short, steam-heated cylinder sections, or pots. The walls of the pots are solid; drainage occurs through perforated plates and filter mats in one end of each pot. Pot presses are arranged vertically or horizontally. The advantages of a pot press are that the pots can be heated and the press can handle very soft, nonfibrous material, such as fruit pulp, at high pressure without forcing large quantities of solid material into the oil. Fred S. Carver designed a hydraulically operated batch press in 1915 and later applied it to the pressing of cocoa beans. His company<sup>2</sup> continually improved the cocoa butter press and, by 1983, had captured 75% of the U.S. market and 50% of the world market. Their standard cocoa butter press is a pot press made in many models, the most widely used being the model 5–34 (Figure 8).

In the pressing of cocoa beans, cocoa bean cotyledons, containing about 54% fat, are heated and ground into a slurry of melted cocoa butter and finely ground solids. The pots are aligned in the press to accept feed, and the slurry is pumped into the pots from a feed manifold. The butter flows out of each pot through fine-mesh, metal grids that retain the solids. As the pots fill with solids, more pressure is required to pump the slurry. When the pressure reaches a preselected value (1000–2000 kPa, or several hundred psi), the press stops the flow of fresh slurry and applies pressure from a hydraulic ram to compress the pots against the trapped solids. This squeezes out as much fat as possible. The residual fat level can be as low as 8%. Hydraulic pressures up to 41,370 kPa (6000 psi) are used. When butter stops flowing, the cylinder retracts and the pressed cakes are thrown into a hopper. The press is then closed and the cycle repeated.

Carver's model 5–34 is a vertically arranged, semiautomatic press containing 5 pots of 45.7 cm (18 in.) diameter. Carver's later models were horizontal and automatically ejected the press-cakes. The entire cycle of charging, pumping, pressing, ejecting cake, and recharging could then be automatically controlled. Carver's largest press is their model 12–22, having 12 pots of 55.9 cm (22 in.) diameter.

<sup>2</sup>Fred S. Carver Inc., Wabash, Indiana Carver no longer manufactures or reconditions cocoa butter presses but does service existing presses and provides repair parts. Carver still offers, however, hydraulic presses for food products, chemicals, plastic, and environmental applications.



**Figure 8.** Carver Model 5-34 cocoa butter press (Courtesy of Fred S. Carver Co., Wabash, Indiana).

Another supplier of cocoa butter presses is BV Machinefabriek PM Duyvis (Kong aan de Zaan, Holland). P. M. Duyvis and D. W. Stork founded the company in 1885 as a small workshop to repair vertical cocoa butter presses made by other manufacturers. In 1967, they began adding innovative modifications to the presses they were repairing, and in 1976, they built their own proprietary design cocoa butter press. Today, Duyvis offers a line of cocoa butter presses with 6–14 pots of 425 mm (16.7 in.) diameter. A 580-mm (22.8-in.) hydraulic ram, operating at up to 55,000-kPa (7977-psi) hydraulic pressure, exerts the compression. Their newest and largest press has 450-mm (17.7-in.) pots and a 600-mm (23.6-in.) ram. This permits 25% more capacity.

To press cocoa beans in a Duyvis press, the cocoa bean cotyledons are first pulverized into a fluid-like slurry. The slurry is then heated in an agitated conditioning

tank to the desired temperature. A typical temperature is 100°C (212°F), but the optimum temperature can vary from plant to plant. Slurry viscosity is influenced by temperature, and viscosity is usually brought to a desired uniform value before the slurry is pumped into the press. The slurry then flows into all the pots, and clarified cocoa butter flows out of the pots through fine-mesh grids.

When pumping pressure reaches the maximum value of 2000 kPa (290 psi), pumping stops. A hydraulic ram then exerts pressure against the pots, causing them to move closer to each other, thereby compressing the solids trapped within each pot. When the hydraulic pressure reaches 20,000–25,000 kPa (3000–3700 psi)—which, because of the rams large diameter, equates to almost twice that pressure on the cakes—the residual fat drops to 22–24%. This takes about 6 min from the start of the pumping cycle. Adding nine more minutes, and increasing the hydraulic pressure to 50,000–55,000 kPa (7400–8100 psi), lowers the residual fat to 10–12%. Holding that pressure for ten additional minutes reduces the fat to 8–10%. Final residual, however, depends on the preparation of the cocoa liquor.

Longer press time reduces the cycles per hour, so there is a corresponding decrease in capacity as residual fat is decreased. Starting with 2100 kg/h (4628 lb/h) at 22–24% fat (6-min cycle time), capacity is reduced to 585 kg/h (1289 lb/h) at 8–10% fat (25-min cycle time). The press is then opened, the cakes pushed out into a cake conveyor, and the press closed to start a new cycle. The press cycle can be controlled manually by plant personnel or it can be automatically controlled through a microprocessor. The Duyvis press can process slurries of other oilseeds, peanut, for example, but the major application for this type of press is cocoa butter.

## 4.2. Continuous Screw-Pressing

A screw-press is a mechanical device that accepts a continuous stream of fat-bearing material, compresses it under very high pressure to squeeze out the fat, and then discharges a continuous stream of squeezed solids. Considerable effort was made in the late 1800s to develop a continuous press that would work. The batch-operated hydraulic presses of that era were already considered too labor intensive.

Valerius D. Anderson (Anderson International Corp., Cleveland, Ohio) developed a workable continuous screw-press in 1900. He started working with continuous press designs in 1876 and tested a series of screw conveyor-like devices that had perforated walls and a flapper-type choke at the discharge end. However, every time he closed the choke, product would simply spin with the shaft. His breakthrough discovery (the principle by which today's continuous screw-presses work) was to provide interruptions in the flighting. His first successful press, patented in 1900 (110), used standard conveyor flighting cut into segments and mounted on a central shaft so as to have some unflighted space between the segments. The shaft was placed inside a cylindrical housing with holes drilled throughout to allow fat a means of escape. A spring-loaded conical choke created back pressure against the material. The material still tended to spin with the flighting, but spinning would stop in the areas where there was no flighting (Figure 9). This would allow the shaft to generate enough pressure to push the material against the choking device.

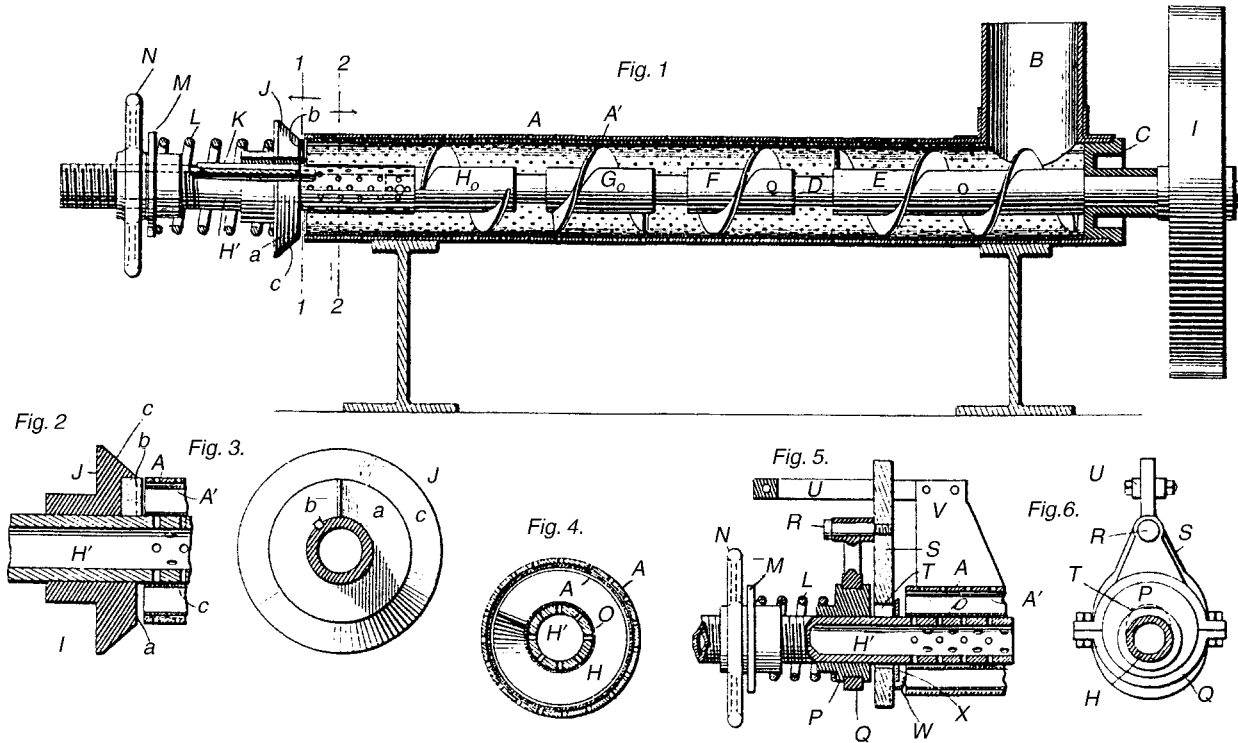


Figure 9. First working oilseed screw-press (Courtesy of Anderson International Corp., Cleveland, Ohio).

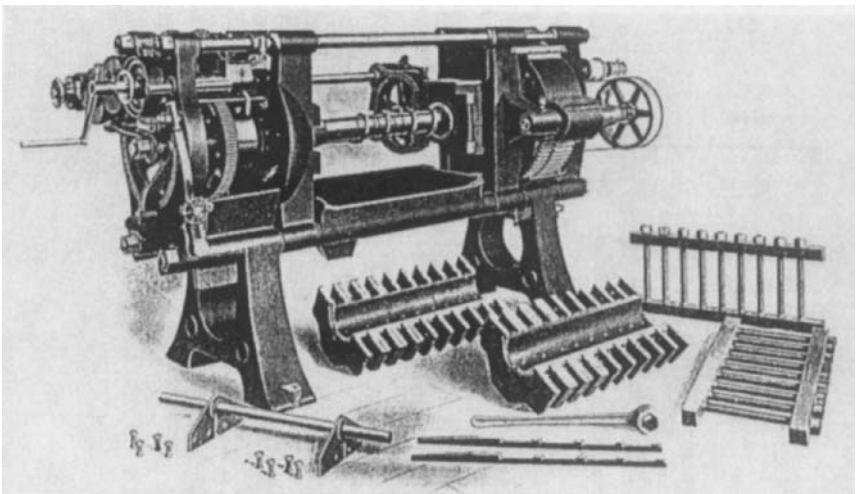


Several years later, Anderson discovered that flat lugs protruding from the barrel wall and intermeshing with the flighting were more effective in preventing material from spinning. The screw-press now generated such high pressure that the housing ruptured. In 1902, Anderson redesigned the screw-press to withstand this pressure. The barrel housing was assembled of heavy steel bars arranged within a cylindrical supporting frame, having thin spacers inserted between the bars to provide longitudinal slots for fat to escape.

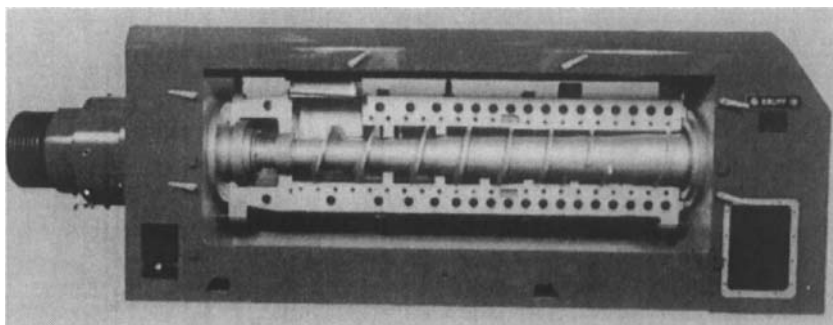
The cylindrical barrel was constructed in two longitudinal halves clamped together with heavy clamping bolts to contain the pressure. Inserted between the barrel halves were two lug bars called “knife bars,” 180° apart, running the entire length of the barrel. Both bars had lugs protruding through the channel and intermeshing with the individual worm flights. The shaft was now made of individual heavy-duty worm segments of narrow channel depth. Figure 10 shows a disassembled view of Anderson’s 1907 screw-press. This basic principle of interrupted flight design has never been changed. All high-pressure screw-presses have the same interrupted flight design with knife bars and the same basic barrel design of barrel bars and spacers within a heavy supporting frame.

In the early 1900s, this new continuous screw-press, being immensely less labor intensive than hydraulic presses, revolutionized the crushing industry. It found ready acceptance in the pressing of animal residues as well as oilseed materials. In 1900, Anderson registered the trade name Expeller (111) for the screw-press. The name is still registered to Anderson.

Anderson then developed a second screw-press, this one designed to generate lower pressure and to operate at higher capacity. It was used to prepress oilseeds and animal scraps and to dewater steeped corn germ and other wet materials. The high-pressure press (The No. 1 Press) had a 15.2-cm (6-in.) diameter barrel,



**Figure 10.** Disassembled view of 1907 screw-press (Courtesy of Anderson International Corp., Cleveland, Ohio).



**Figure 11.** Interior view of Krupp screw-press (Courtesy of Krupp Maschinentechnik., Hamburg, Germany).

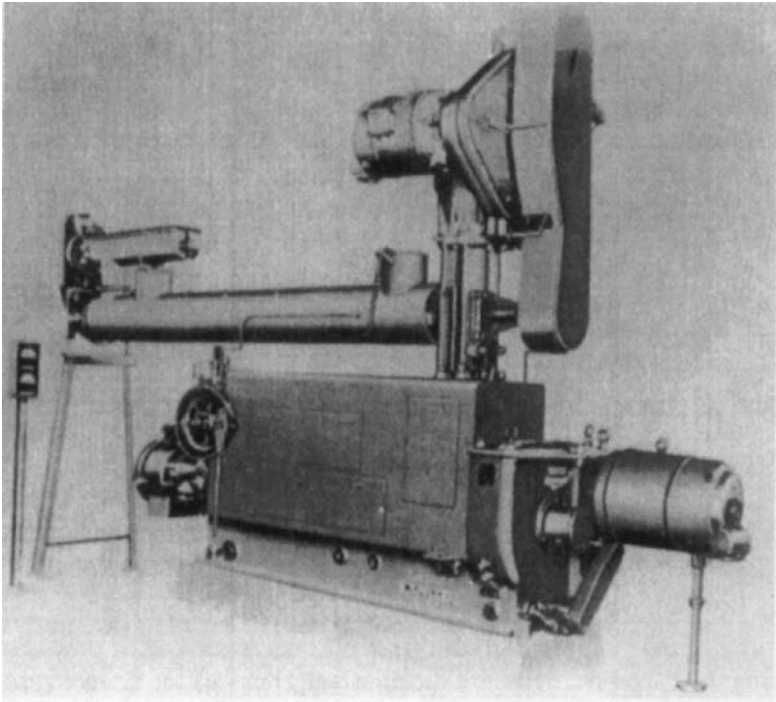
83.8 cm (33 in.) long. The first low-pressure press (No. 2 Press) had a 20.3-cm (8-in.) diameter barrel, 83.8 cm (33 in.) long. It failed to perform properly, so Anderson replaced it in 1907 with the No. 3 Press, having a 30.5-cm (12-in.) diameter barrel, 111.7 cm (44 in.) long.

Around 1910, Anderson licensed Krupp (Hamburg, Germany) to manufacture these screw-presses. Krupp concentrated primarily on the No. 3 Press for prepressing ahead of hydraulic presses. In the United States, Anderson continued improving both the low-pressure and the high-pressure presses. By 1934, when the original patents expired, Anderson was marketing the No. 4 Press for prepress applications and the RB (for roller bearing) Expeller for fullpress applications.

Around 1934, French Oil Mill Machinery Co. (Piqua, Ohio) came out with its screw-press. About the same time, Krupp developed its own screw-press. Today, Anderson International Corp. (Cleveland, Ohio) and French Oil Mill Machinery Co. (Piqua, Ohio) are the leading manufacturers of continuous screw-presses in the United States, and Krupp Hamburg, Germany (Figure 11) and De Smet Rosedown of England (North Humberger, United Kingdom) are the leading European manufacturers.

*Full-pressing.* The most widely used Anderson screw-press for full-pressing is the Super Duo (Figure 12). This press has two shafts, one that prepresses, followed by one that presses out the rest of the fat. The first shaft is positioned vertically above the main (horizontal) shaft and force feeds it. The vertical shaft is often equipped with a drainage cage. It normally turns faster than the horizontal shaft and turns with opposite rotation compared with the horizontal. This helps the oilseed make the transition from the vertical barrel into the horizontal barrel.

Screw-presses by other manufacturers employ different means to facilitate feeding the high-pressure chamber of the press. Some use two-speed shafts, employing a *quill worm* driven by separate gearing that allows the feed worm on the inlet end of the shaft to revolve faster than the rest of the worms. Others use larger diameter barrels with a deeper channel (clearance between the barrel and the hub of the shaft) at the feed end. Sometimes *star wheels* are used to trap the incoming material within the barrel so it cannot back up into the feed hopper. A star wheel is a



**Figure 12.** *Twin-motor Super Duo Expeller 55 with conditioner (Courtesy of Anderson International Corp., Cleveland, Ohio).*

revolving disc, notched to intersect the first flight. It revolves in synchronization with the flight to block off the channel at the point where the material is most likely to backflow.

Anderson screw-presses are cooled by flowing a stream of oil over the drainage cages (112, 113). Process oil is cooled in a water-cooled heat exchanger to usually  $48.9^{\circ}\text{C}$  ( $120^{\circ}\text{F}$ ) and then caused to flow over all the exposed drainage bars at approximately  $132\text{--}189\text{ L/min}$  ( $35\text{--}50\text{ gpm}$ ) per screw-press. This immediately dilutes the hot freshly pressed oil with a much larger volume of cool oil. The flow of cooled oil flushes the surface of the barrels to rinse away any solids that might have passed out with the fresh hot oil. The cooled oil absorbs heat from the screw-press, keeping the barrel temperature within control. The flow of cooled oil also blankets the drainage surfaces with a fast moving layer of oil. This prevents the fresh hot oil from lingering on the barrel and becoming exposed to the oxidizing effects of air. An alternate method is to water cool the barrel housings themselves.

The wormshaft can also be water cooled. The central shaft holding the worms is bored and a tube is inserted. Water passes through a rotary seal, then flows through the bore in the shaft toward the feed end, and then out through the inserted tube, and finally exits through a second port in the rotary seal. The water draws heat from the hottest part of the shaft and distributes some heat to the cooler part (by the inlet).

The flow of water is adjusted to hold a desirable discharge temperature, usually 71°C (160°F). Sometimes the water flow is reversed, and sometimes the central tube is cut shorter so as not to transfer heat toward the feed inlet.

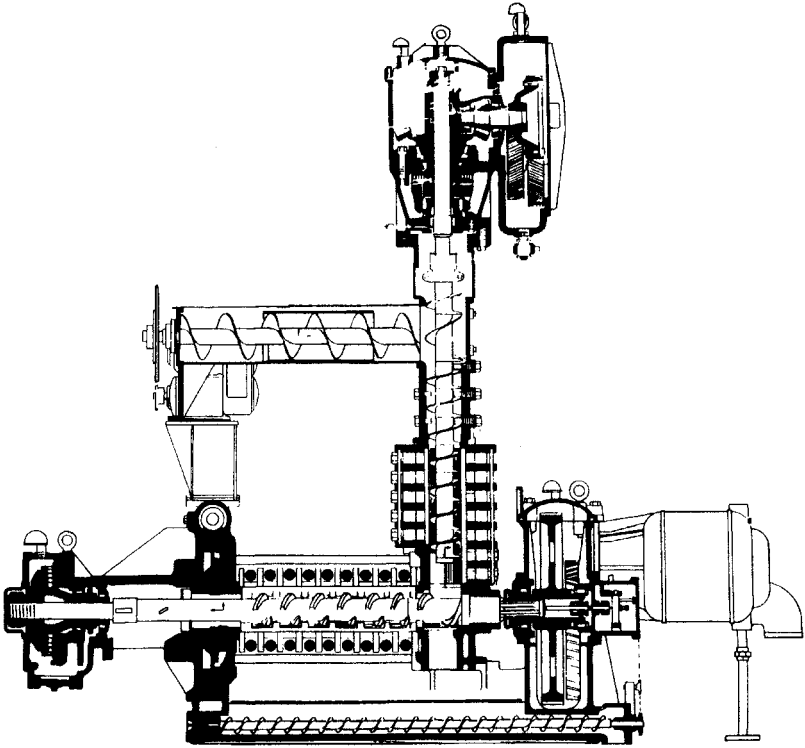
As discussed above, oilseed screw-presses fall into two categories: full-presses and prepresses. A full-press is designed to generate sufficient pressure—estimated at 96,500 kPa (14,000 lb/in.<sup>2</sup>) to remove as much oil as possible. For most oilseeds, the oil level can be reduced to between 3% and 5% remaining in the solids discharged. Animal residues can be reduced to 7–10% oil content. To accomplish this, wormshafts are designed to mechanically compact the material as it travels from worm to worm and to steadily increase pressure. Reducing channel depth (open space between shaft surface and inside diameter of the barrel) or decreasing pitch of successive worm flights or reducing the barrel diameter are the mechanical means for designing positive compaction into a wormshaft.

Some materials are more resistant to flow than others. Hull content in oilseeds, for example, increases the friction. Such materials do not slide easily through the channel in a screw-press. Those materials can be pressed with shafts having uniform channel depth and uniform pitch. Drag against barrel and shaft, augmented by choking at the discharge end, generates sufficient pressure for adequate pressing. Animal materials containing ground bone, or oilseeds with high fiber levels can be pressed with shafts of uniform channel depth and uniform pitch. Sometimes, one or more worms are replaced with unflighted segments. Drag then generates more pressure against the preceding worms than a flighted segment would.

Softer materials have considerably lower friction. A uniform-channel-depth, uniform-pitch wormshaft would generate very little pressure. This is where decreasing channel and decreasing pitch came into play. Another mechanical device to increase pressure is a “cone,” or a funnel-shaped segment, inserted between the wormshaft segments. Channel depth decreases as the oilseed flows across the cone’s end. The channel depth can be very thin at the cone’s discharge end, 3.17 mm (0.125 in) in some cases. Cones are obstacles for the preceding worms to overcome. The preceding worms have to generate higher pressure (and mastication) and, therefore, grind out more oil. The oil, however, does not escape from the barrel in the area where the cone is located. One seldom sees oil escaping through the drainage cage surrounding the cone. Instead, oil liberation is increased across the worms pushing the material over the cone segments.

An “ideal” wormshaft would draw in the feed material, trap it within the barrel so it cannot backflow, and then compress it. First-step compression (as air is trapped within the feed material) would force out air and bring the material into a densely compacted mass. Thereafter, mechanical compression should steadily increase compaction of the material while also compensating for loss of volume as oil escapes through the barrel walls (114). This is more difficult than it sounds because different oil- and fat-bearing materials have different coefficients of friction. Even the same material, with different preparation, can have varying coefficients of friction.

Most oilseeds and animal residues have a sufficient history of being pressed in continuous screw-presses. Therefore, all screw-press manufacturers have field-proven

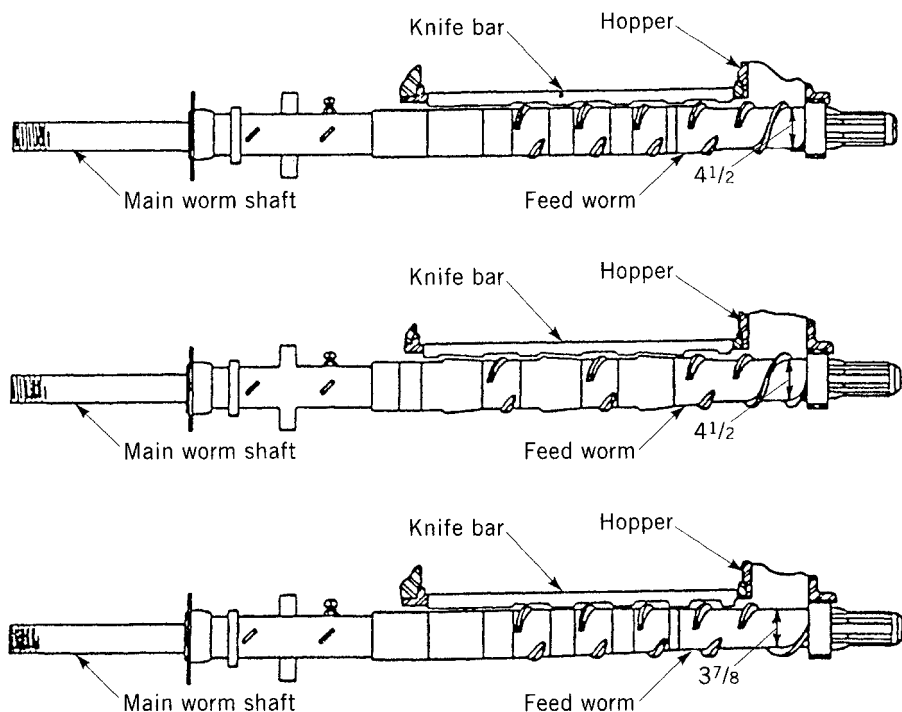


**Figure 13.** Sectional view of twin-motor Super Duo Expeller 55 with conditioner (Courtesy of Anderson International Corp., Cleveland, Ohio).

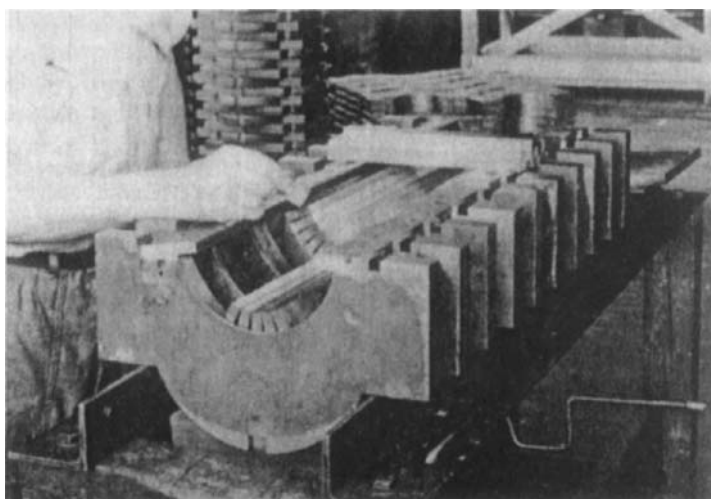
and optimized wormshaft configurations. No universal do-it-all configuration serves all applications. Screw-press manufacturers offer a variety of shaft configurations, each suitable for several materials of similar composition. Some materials require different shaft rotational speeds than others. Figure 13 shows a sectional view of a screw-press with shafts in place. Figure 14 shows several different wormshafts offered by one manufacturer.

The drainage barrel is assembled using rectangular bars fitted into a supporting frame (Figure 15). The individual bars are separated by spacing clips. Spacing clips are made in several thicknesses and allow for differing gaps between the drainage bars. Full-presses, because of the high pressure applied, generally use narrow spacers, 0.13–0.76 mm (0.005–0.030 in.). Prepresses, generating lower pressure at higher capacity and liberating a greater volume of oil (or water) generally use thick spacers, 0.5–1.5 mm (0.020–0.060 in.).

Despite the massive construction of screw-presses, the barrels are made of many small pieces, and these pieces must fit together properly through precision machining. The oil must flow out easily (requiring gaps as wide as possible), yet solids should not ooze out with the oil (requiring gaps as narrow as possible). This



**Figure 14.** Examples of three different worm arrangements of the Anderson main wormshaft (Courtesy of Anderson International Corp., Cleveland, Ohio).



**Figure 15.** Assembly of barrel bars with spacers in a barrel frame (Courtesy of Anderson International Corp., Cleveland, Ohio).

demands a compromise. Under ideal conditions, 2% of the solids escapes with the oil. Under poor conditions, 10% or more escapes. As barrels wear, the gaps enlarge and more solids escape. Also, if oilseed preparation is incorrect, especially if moisture level is too high, more solids escape.

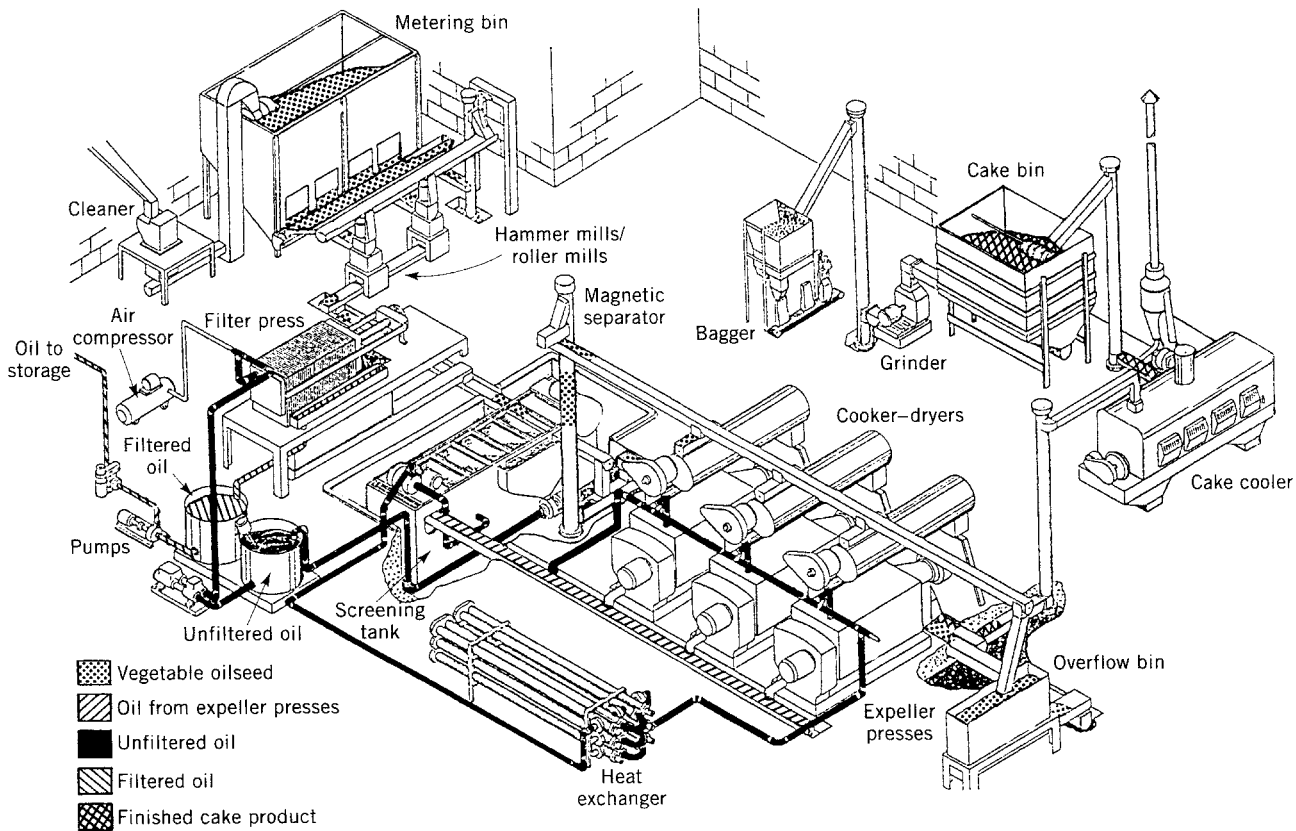
If the gaps enlarge, or if high moisture allows too much solids to escape through the bars, the pressure within the screw-press will drop to inadequate levels, because an excessive loss of solids reduces the volume under compaction. If the loss of solids is caused by wear, the screw-press performance will show several symptoms. Residual oil in the press-cake will increase as a result of loss of compaction. Solids in the oil will also increase. The press will begin to reject feed because the worn barrel and worn worms no longer “grab” the material, and capacity will fall off. Eventually the barrels must be reconditioned. Also, worn worm segments on the shaft must be replaced or resurfaced.

Reconditioning (relining) barrels is a delicate job. It must be done correctly to ensure the longest possible time between overhauls. As the revolving worms cause the material to slide over the barrel bars, it is critical that the material passes from the top of one bar directly to the top of the next bar. If any bar is cocked so that the sliding material encounters a protruding edge, that edge will scalp off a layer of solids that will pass between the barrel bars and escape with the oil, similar to the way a carpenter’s plane shaves off a thin layer of wood. The solids are much more abrasive than oil and fat, so those protruding bars will round off and wear faster than properly positioned bars.

Some manufacturers machine bevels along one longitudinal edge of each barrel bar. This bevel provides a flat surface that allows the bars to rock into correct alignment during relining. Bars without bevels (with rectangular cross sections) bear against the spacers only at the extreme inner edge of the bars. Bars with bevels are more likely to align into correct position than bars without bevels. The angle of the bevel depends on the diameter of the barrel and the thickness of the bar. Some barrel bars, after normal wear on one side, can be turned over and reused. These bars would have two beveled edges.

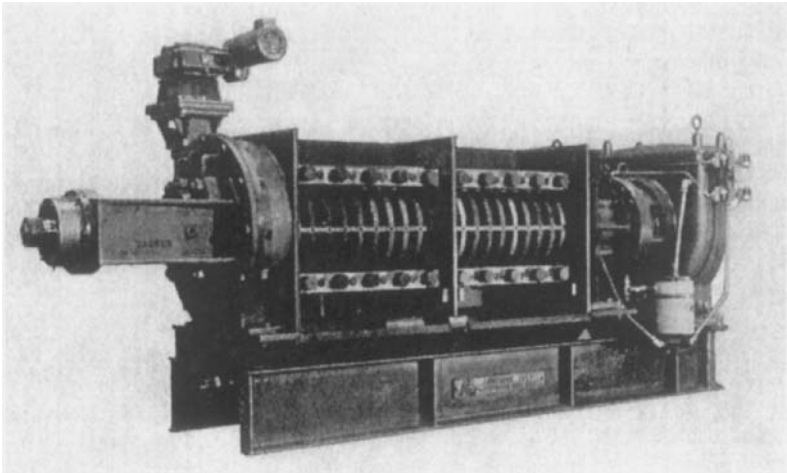
The liquid fat or oil coming from the press contains 2% to as much as 15% fat-free solids, which is usually separated out in a two-step procedure. First, the bulky solids are separated in a settling chamber equipped with a drag that collects the solids, passes them over a drainage screen, and then drops them into the conveyor taking fresh material to the screw-presses. The second step is to clarify the liquid in a manual or automatic plate-and-frame filter press.

The filtered solids from the filter press (filter press-cake) are dropped into a hopper equipped with a variable-speed screw that meters the filter cake into the fresh material. The feed to the screw-press, therefore, is a blend of fresh material, drained solids, and filter press-cake. The ratios of the blend ingredients should be kept constant. This will maintain the screw-press in steady-state operation. If all of the filter cake is recycled at once, for example, followed by several hours of no filter cake, the screw-press performance will change as feed composition changes. Figure 16 shows a typical screw-press operation with all of the above equipment in place.



**Figure 16.** Press room flow diagram (Courtesy of Anderson International Corp., Cleveland, Ohio).





**Figure 17.** Anderson Mega 11-66 prepress (Courtesy of Anderson International Corp., Cleveland, Ohio).

*Prepressing.* Some fat-containing materials are prepressed prior to hydraulic presses, as described earlier, and some are prepressed prior to secondary screw-presses. From the very beginning of screw-pressing, there were both high-pressure full-presses and low-pressure, higher capacity prepresses. In addition, there was also a separate line of prepresses made of nonrusting contact parts for dewatering steeped corn germ and the wet hulls from steeped corn. However, the major thrust of screw-pressing for oilseeds as well as rendering has been full-pressing. Figure 17 shows a modern prepress.

In the 1920s, solvent extraction plants were set up in Europe, then a decade later, in the United States. Oilseeds easily flaked, such as soybean, were direct extracted. Oilseeds not so easily flaked continued to be full-pressed, but in 1951, Dunning developed a process involving prepressing followed by solvent extraction (15). For this process, Dunning modified full-presses to run at faster shaft speeds, accept greater capacity, and generate less pressure so as to produce a prepressed cake in which the fat content equaled approximately one-fourth the protein content (115). The cake was then granulated, conditioned, and flaked. The preparation prior to screw-pressing, combined with conditioning of the partially deoiled cake, formed strong flakes that handled very well in a solvent extractor and resulted in very efficient extraction of oil from high oil-containing oilseeds.

As oilseed crushing plants increased their tonnage, full-pressing became more and more cumbersome. A new high-capacity plant requiring 20 full-presses would be better served by a single solvent extractor. Unlike soybean, most oilseeds cannot be direct solvent extracted. This new procedure (of prepressing) allowed high-capacity plants to use only a few screw-presses and still take advantage of solvent extraction. Solvent extraction is attractive for high-capacity plants: from several hundred tons per day up to two or three thousand tons per day. Full-press plants

are more attractive for low-capacity plants: less than 200 tons per day and, of course, for firms that do not want to work with flammable solvents.

## 5. SOLVENT EXTRACTION

### 5.1. Application

Although extraction with solvents is an effective method for the recovery of oil (or fat) from any material, it is most advantageous with materials low in oil. The minimum oil content to which oilseed press-cake can be reduced by mechanical expression is approximately the same for all oilseeds, about 2–3%. Consequently, the oil unrecoverable by mechanical expression, in terms of percentage of the total oil, increases progressively as the oil content of the material decreases.

Of all the common oilseeds, soybeans are solvent extracted most easily and, for some time, have preferentially been solvent extracted in the United States. In the crop year 1957–1958, for example, 93.2% of the soybean crop was solvent extracted (116). Cottonseed flakes disintegrate more readily than soybean flakes and are more troublesome to extract because of fines. Peanuts, flaxseed, and other high oil-containing seeds, because of their high oil content, disintegrate badly during solvent extraction. As a result of this, they are either prepressed in screw-presses or processed through slotted-wall extruders to remove a portion of the oil before being solvent extracted. Closed-wall extruders are used to form porous collets directly from flaked oilseeds of moderate oil content or from oilseeds of high oil content that have been prepressed. Slotted-wall extruders can be used to form porous collets directly from oilseeds of high oil content. Extrusion is extensively described in Section 3.4.

Solvent extraction is also used to extract fat from dry-rendered animal scraps and from garbage in garbage recycling plants. Solvent extraction may be used to obtain a low-fat meal in which proteins are not heat denatured. Thus, for example, cocoa is solvent extracted to produce a residue that may serve as a source of theobromine. Solvent-extracted meal is preferred for the manufacture of protein adhesives, fibers, or plastics, as there is much less heat denaturation of the protein in this meal than in that obtained by cooking and mechanical pressing. However, materials needing heat treatment are given special treatment. Soybean, for example, is “toasted” during desolventization.

The low temperature in solvent extraction of oilseeds should produce a better oil than oil from the high-temperature environment of screw-pressing. Solvent, however, extracts some nontriglycerides that are not pressed out by screw-pressing, so screw-press oil is generally of better quality than solvent-extracted oil. Although some animal fats are solvent extracted, most renderers full-press in screw-presses. Solvent extraction of oilseeds, on the other hand, is widely practiced. The section below is based primarily on research conducted with oilseeds. Some of it, however, would also apply to extraction of animal fats.

## 5.2. Principles and Theory

*Methods of Achieving Contact with Solvent.* Laboratory extraction in an ordinary Butt extraction tube is an example of solvent extraction in its simplest form. In this extraction procedure, the pure solvent is delivered continuously to the top of the mass of material to be extracted and is percolated through the mass by gravity until the removal of oil (or fat) is complete. Although this method is effective in the laboratory, it is highly inefficient. Complete extraction can be accomplished only by using a large volume of solvent compared with the volume of oil extracted, and the solvent must eventually be recovered from the oil. Even in the most efficient extraction plants, charges for steam and water for solvent recovery make up a substantial part of the operating cost (117). If the solvent–oil ratio is high, such charges may easily become prohibitive. A prime object in solvent extraction practice is, therefore, to reduce the solvent content of the final miscella or oil–solvent mixture to the lowest possible figure.

Efficiency can be improved by contacting the material with successive washes of previously used solvent. Each wash is recirculated through the material being extracted until equilibrium or near equilibrium is established between the oil content of the solid material and that of the solvent; that is, until all the solvent, both the solvent absorbed by the solids and the solvent in the free miscella, contain the same amount of dissolved oil. When this condition is attained, the free miscella is drained off and another wash of previously used solvent is recirculated through the material.

An extraction system set up to take advantage of solvent reuse is designated as countercurrent extraction. A battery of batch extractors is provided and the solvent is used to treat the contents of each extractor in succession. Each time a batch of miscella is drained from an extractor, it is sent to another extractor containing material previously extracted with a richer miscella.

Viewing the procedure from the aspect of solid material in a single-batch vessel, the solid material is subjected to multiple washes of leaner and leaner miscella and is finally extracted with fresh solvent. The solvent, on the other hand, is brought into contact with material of progressively increasing fat content until the solvent finally encounters fresh solid material. From there, the solvent leaves as full miscella. In this way, miscella is brought out of the system at a high oil content.

Although batch countercurrent extraction may theoretically approach the efficiency of continuous countercurrent extraction, charging separate batch vessels is cumbersome. Therefore, solvent extraction in high-capacity plants is carried out in continuous vessels that receive a continuous flow of solids in one end and a continuous flow of solvent in the other end. Such systems achieve the highest economy of steam, power, labor, and materials. Their adaptability is limited only by the mechanical difficulties involved in moving the solid mass and the liquid miscella in opposite directions, allowing free intermixing and providing for adequate separation of all the free-drained liquid from the solids before solids discharge.

If it is assumed in batch extraction that a constant volume of miscella is retained by the material after each drainage period, and if this volume is known, one may calculate the number of extractions required to reduce the oil content of the material to any given level (118–120). Actually, however, the retention of miscella is not constant but varies for different solvent–oil ratios. Solvent–oil ratio affects viscosity and surface tension of the miscella, which, in turn, affect drainage. This variation in miscella retention renders calculations highly involved, but Ravenscroft (121) has introduced a graphical method for estimating the number of extractions required for a given recovery of oil that is applicable in the case of variable oil retention. Ravenscroft assumes that miscella is retained only on the surface of the solid particles and refers to this retention as entrainment. His assumptions are also valid for miscella absorbed within the particles if there is equilibrium between the miscella within the particles and without. In continuous countercurrent extraction, differences in oil concentration in the miscella within and without the solid mass cannot be ignored, because here equilibrium conditions do not exist. Equations for extraction under conditions of nonequilibrium have been developed by Ruth (122) and Grosberg (123). More recently, Abraham et al. developed a computer model for the extraction of cottonseed flakes with hexane, isopropanol, and ethanol (124). The model uses laboratory benchtop data to determine the number of extraction stages and the solvent-to-flake ratio needed to obtain 1% residual oil in extracted meal using the three different solvents.

*Extraction Rates.* The design of large-scale solvent extraction vessels must accommodate the rate at which equilibrium is attained between the free miscella flowing past the solid particles and the miscella absorbed within the solids. Attainment of equilibrium may be quite slow, particularly as the oil content of the solid material drops to low levels. Investigations show that the rate at which equilibrium is approached (in effect, the extraction rate) is influenced by many factors, including the intrinsic capacity for diffusion of solvent and oil, which is determined primarily by the viscosities of the two; the size, the shape, and the internal structure of the solid particles; and, at low oil levels in the solids, the rate at which the solvent dissolves nontriglyceride substances that are soluble but dissolve less readily than the triglycerides.

In a homogeneous oil-impregnated material consisting of thin platelets of uniform thickness whose total surface area is substantially that of the two faces, the theoretical extraction rate, based on simple diffusion, has been given by Boucher et al. (125) as follows:

$$E = \frac{8}{2} \sum_{n=0}^{n=\infty} \frac{1}{(2n+1)^2} \exp -(2n+1)^2 \left(\frac{\pi}{2}\right)^2 \left(\frac{D\theta}{R^2}\right), \quad (1)$$

where  $E$  is the fraction of the total oil unextracted at the end of time  $\theta$  (in hours),  $R$  is one-half the plate thickness (in feet), and  $D$  is the diffusion coefficient (in. ft<sup>2</sup>/h).

Except at low values of  $\theta$ , the preceding equation takes the approximate form (126):

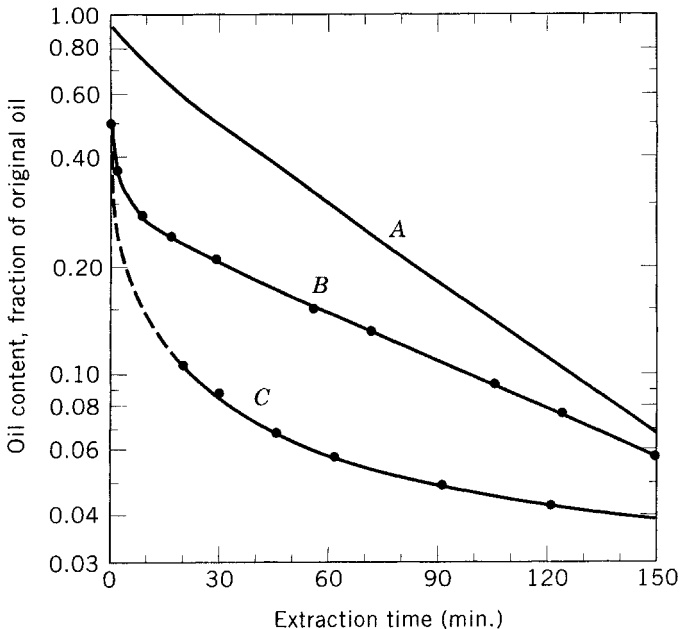
$$E = \frac{8}{\pi^2} \exp - \frac{\pi^2 D \theta}{4R^2} \quad (2)$$

or

$$\log_{10} E = -0.091 - 1.07 \frac{D \theta}{R^2}. \quad (3)$$

Hence, at the lower values of  $E$ , a plot of  $\log E$  against  $\theta$  gives a straight line with a slope dependent on the diffusion coefficient and the plate thickness. It is to be emphasized that the equation is valid only when all plates have the same thickness. Average plate thickness cannot be used for a material of nonuniform thickness.

Working with porous clay plates impregnated with phosphatide-free soybean oil and with tetrachloroethylene as a solvent. Boucher et al. (125) found that experimentally determined extraction rates checked closely with theory. A typical theoretical extraction curve is shown (curve A) in Figure 18. A lack of correspondence between extraction rates and Reynolds number of the flowing solvent, over a



**Figure 18.** Solvent extraction curves: A, theoretical curve for homogenous oil-impregnated platelets of uniform thickness (125); B, peanut slices, 0.66 mm thick, 14% moisture content, extracted at 25°C (77°F) with hexane (126); C, cottonseed flakes, 0.43 mm thick, 11.6% moisture content, extracted at 65.6°C (150.08°F) with hexane (127).

wide range of the latter, suggested that liquid-film resistance against transfer of oil to the solvent was inconsequential as compared with resistance against diffusion within the plates. The diffusion coefficient was simply a function of the product of the viscosities of solvent and oil. Under the particular conditions of the tests, it could be represented by the formula

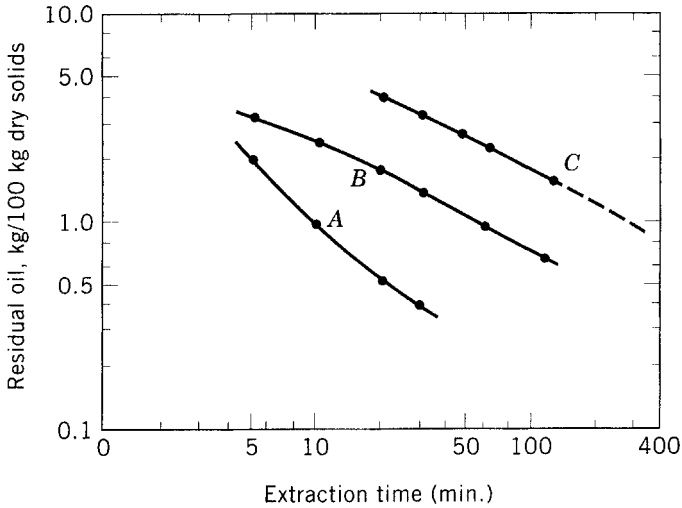
$$D = 12.96 \times 10^{-6} (\mu_o \mu_s)^{-0.46}, \quad (4)$$

where  $\mu_o$  and  $\mu_s$  refer to the viscosities, in centipoises, of oil and solvent, respectively. The numerical values in the formula are related to the structure of the plates and, therefore, can be considered specific only for the lot of plates used in the tests. Tests involving extraction with solvent-oil mixtures as well as pure solvents show that the diffusion coefficient is independent of the composition of the solvent, in terms of relative proportions of solvent and oil. It can, of course, be expected to increase with an increase in temperature or with the use of a less viscous solvent than tetrachloroethylene.

The experiments of Fan et al. (126) with peanuts carefully sliced with a microtome show that the relationships developed by Boucher et al. (125) are also applicable to at least one oilseed, provided that structural considerations are not complicated by crushing the seed to form flakes. A typical extraction curve is shown (curve *B*) in Figure 18. As required by diffusion theory, there is a linear relationship between the logarithm of the residual oil content and the extraction time after a short period has elapsed. During this period, however, much of the oil is extracted very rapidly.

Fan et al. (126) carried out a mathematical analysis showing that this deviation from theory with respect to rapidly extractable oil was caused by the opening of some oil-bearing cells in slicing the oilseeds, plus the occurrence of void spaces in the seeds after drying. Thus, Fan et al. agrees with Osburn and Katz (128) that the major obstacle to extraction is probably diffusion through the cell walls and that the initial rapid extraction is to be attributed to cell destruction. The proportion of easily extractable oil was found to decrease rapidly with increase in the slice thickness. In curve *B* of Figure 18, which represents the extraction of peanut slices 0.66 mm (0.026 in.) thick, the extraction curve became linear after about 76% of the oil was extracted. In other experiments with flakes of similar moisture content, there was linearity with 0.81 mm (0.032 in.) flakes after about 51% of the oil was extracted, and linearity with 1.02 mm (0.040 in.) flakes after about 30% was extracted. With flakes of constant thickness, there was a progressive decrease in the content of rapidly extractable oil with increase in the moisture content.

Fan et al. (126) found that the diffusion coefficient decreased considerably with increase in the moisture content. In the range of 10–22% moisture, it was about  $0.4 \text{ cm}^2/\text{s}$  per 1% moisture. With commercial hexane (Skellysolve B) at 24–26°C (75.2–78.8°F) and peanut slices with 13% moisture, the calculated diffusion coefficients averaged about  $7 \times 10^{-9} \text{ cm}^2/\text{s}$ .



**Figure 19.** Representative curves for solvent extraction of A, soybean flakes; B, flaxseed flakes; C, cottonseed flakes (127).

In the extraction of oilseed flakes formed commercially by rolling, there appear to be factors that still further complicate the extraction rate. Extraction curves not only reveal a very large fraction of easily extractable oil, but tend to be continuously concave upward from the time axis as is shown by curve C of Figure 18, constructed from the laboratory data of Wingard and Shand (127). In practice, extraction in the range of about 0.5–5.0% residual oil (on the basis of the dry, solvent-free meal) is so slow that it actually controls the overall extraction rate and extractor design (32). On a semilog plot, the concavity of the extraction curve is so great in this region that a nearer approximation to a straight line is obtained with a log-log plot as in Figure 19 (127).

Several explanations can be offered for the large deviation of curve C (Figure 18) from the form of curve A or curve B. King et al. (129) and Osburn and Katz (128) point out that structural heterogeneity (leading to the simultaneous operation of two different diffusion processes with different diffusion coefficients) could account for the shape of the curves. Analysis of soybean flake extraction curves by Osburn and Katz showed that, at 26.7°C (80°F), 70–90% of the oil was extracted with trichloroethylene at a diffusion coefficient of  $0.37 \times 10^{-6} \text{ m}^2/\text{h}$  ( $4 \times 10^{-6} \text{ ft}^2/\text{h}$ ), whereas the remaining 10–30% was extracted at a lower diffusion coefficient of  $0.46 \times 10^{-7} \text{ m}^2/\text{h}$  ( $5 \times 10^{-7} \text{ ft}^2/\text{h}$ ). Their work also showed that most of the readily available oil was derived from cells ruptured by rolling, whereas the difficult-to-extract oil came from undamaged cells. It seems unlikely, however, that such extensive cell destruction could occur. If cell destruction is not a sufficient reason, perhaps nonuniform flake thickness, or another random factor, is involved.

After extensive experimentation on the extraction of oilseed flakes, Karnofsky and Coats (32, 130–132) proposed that a slow final extraction rate is at least partly caused by decreased solubility of the last traces of oil. It is well known that

oilseeds subjected to repeated extraction with a solvent yield fractions of oil, toward the end of the process, that are much higher in phosphatides and other nontriglyceride materials than the first fractions (32, 133). These materials are obviously less soluble.

The difficulty of extracting the last portions of oil from oilseeds may be related to the chemical composition of the oil. This was suggested previously by Goss (134). Karnofsky's proposal is supported by the observation (130) that the last portions of oil are removed much more readily if the oilseeds are first given a soaking period, even in relatively strong miscella. It is further supported by the finding that no difficulty is encountered in recovering the last portions of oil from oilseeds reconstituted from extracted oil and oil-free solids. Karnofsky used his undissolved oil concept along with bench-top data to develop a method of calculating the retention time required for the counter-current extraction of oilseeds (131, 132).

Accepting that the last traces of oil are more difficult to dissolve does not nullify the basic conclusions to be derived from the preceding theories based on simple diffusion with free miscibility of solvent and oil. If free miscibility does not exist in the latter stages of extraction, this means simply that the effective concentration of solute is not the concentration of oil in the solid seed material but a lower concentration that is limited by the solubility of the oil in the solvent. The rate of diffusion will be less than observed in the earlier stages, not because the diffusion coefficient has decreased, but because the oil content of the solid material is no longer a proper measure of its instantaneous content of diffusible material. The diffusion or extraction rate will, for example, still be inversely proportional to the square of the flake thickness.

It may be noted that with simple diffusion, an increase in extraction temperature can be expected to increase extraction rate because it lowers the viscosities of solvent and oil. However, with incomplete solubility of the oil, an additional effect may come into play, that of increasing solubility as well as decreasing viscosity. This may account for some observations by Karnofsky (32). In one case, increasing the temperature from 37.8°C to 88.9°C (100°F to 192°F) reduced, by 80%, the time required to lower the oil content of cottonseed flakes to 3% using heptane. This is greater than would be predicted from a simple decrease in viscosity, according to the data of Boucher et al. (125). According to Wingard and Phillips (135), the time required to reduce oilseeds to 1% residual oil content varies with a power of the temperature that (with cottonseed, soybean, and flaxseed flakes using hexane as a solvent) ranges from -1.9 to -2.4. Hence, a log time-temperature plot yields a straight line.

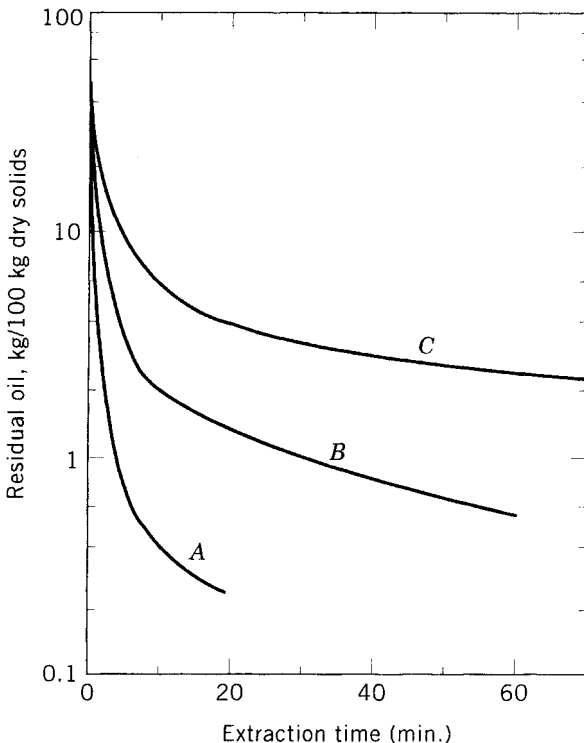
It is evident in commercial practice, and has been confirmed by closely controlled laboratory experiments, that different oilseeds differ markedly in the rate at which flakes of a given thickness can be extracted to low residual oil content. The relation of oilseed particle size to extraction rate has been clarified by a laboratory investigation reported by Coats and Wingard (136). They found that hexane extraction of flaked or cracked soybean, cottonseed, flaxseed, and peanuts conformed to the mathematical formula  $T = KD^n$ , where  $T$  is the time required to



reduce the material to a residual oil content of 1.0% (on a dry, solvent-free basis),  $D$  is the flake thickness or grit diameter, and  $K$  and  $n$  are constants. Consequently, a  $T/D$  plot on a log-log scale yields a straight line, with a slope equal to  $n$ .

Approximate values found for  $n$  were, for four samples of soybean flakes, 2.3–2.5; for two samples of cottonseed flakes, 1.5; for one sample of flaxseed flakes, 7; for one sample of peanut flakes, 3.2; for two samples of cracked soybean, 5.5; for one sample of cracked cottonseed, 4; and for one sample of cracked corn germ, 3.4. With  $T$  expressed in minutes and  $D$  expressed in units of 0.01 in, approximate values for  $K$  were: for soybean flakes, 6–20; for cottonseed flakes, 140 and 270; for flaxseed flakes, 3600; for peanut flakes, 1.4; for cracked soybean, 2.5 and 10; for cracked cottonseed, 40; and for cracked corn germ, 1.6.

Note that  $K$  in the equation  $T = KD^n$  is a measure of the ease of extraction of flakes 0.01 in. thick, whereas  $n$  is a measure of the influence of flake thickness on the extraction rate. Thus, soybean flakes extract more readily than cottonseed flakes of equivalent thickness. Cottonseed flakes, in turn, extract more readily than flaxseed flakes (Figure 20). The extraction rate of flaxseed is highly sensitive to flake or particle thickness, whereas that of soybean is less so. And cottonseed is less sensitive to flake thickness than either. The value of  $n$  for soybean flakes was substantially



**Figure 20.** Relative extraction rates of different oilseed flakes: A, soybean 0.19 mm (0.0075 in.); B, cottonseed 0.24 mm (0.0095 in.); C, flaxseed 0.19 mm (0.0075 in.) (32).

the same for different lots of beans of varying moisture content and flaked by different methods. The data of King et al. (129) obtained by trichloroethylene extraction suggested a value for  $n$  exactly in line with the results with hexane, as well as a comparable value for  $K$ .

There is evidence (129) that large seed particles rolled to form flakes of a definite thickness can be extracted more rapidly than small particles, presumably because they undergo greater internal disruption in the rolling process. Laboratory and pilot plant work on soybeans by Othmer and Agarwal (137) has led them to conclude that for hexane extraction of soybeans:

1. The oil extracted, the residual oil, and the rate of extraction are all independent of the concentration of oil in the solvent: that is, there can be no advantage in countercurrent extraction.
2. The rate of extraction is proportional to:
  - a. Residual oil<sup>3,5</sup>.
  - b. Flake thickness<sup>-3.97</sup>: that is, increasing flake thickness by 3 times decreases rate by 80 times.

Point 1 is also supported by Coats and Karnofsky (130) and, more importantly, is supported commercially in the "filtration extraction" process for cottonseed (138–140) in which a slurry of cooked cottonseed flakes and miscella is held for a time before the rich miscella is washed off on a continuous horizontal filter. Some practical and theoretical aspects of the solvent extraction of soybeans are discussed by Myers (141).

Any review of the literature on extraction uncovers several inconsistencies and contradictions that are largely because of the experimental techniques used. Results obtained under one method may not be obtained under another; consequently, the conclusions will differ, although each may be consistent with the data on which it was based. Some investigators mix solvent or miscella with the data on which it was based. Some investigators mix solvent or miscella with flaked oilseeds under conditions of good agitation, whereas others avoid agitation in the belief that a percolation-type lab extractor more closely parallels commercial plant conditions. Some use pure solvent and others use a combination of miscellas, such as would be used in a commercial plant. Some prefer to calculate the residual oil content of the extracted material from the oil level in the miscella rather than separate and analyze the extracted solids. Some consider solvent extraction as consisting of two parts: extraction proper and washing, where washing is considered inconsequential. They place primary emphasis on the attainment of equilibrium between oilseed flakes and miscella. Efficient washing, however, is required in a commercial plant; and if washing time is unduly long, it increases the total time the flakes are in the extractor just the same as if more time were needed for extraction proper.

Probably the largest change in the oilseed processing industry in the last 20 years has been the introduction of expander/extruders. As described in Section 3.4, their

use as a pretreatment prior to solvent extraction was slow to develop. However, because of their distinct advantages in reducing energy consumption and increasing throughput, they are now in general use throughout the industry. Pavlic and Kemper (142) listed some of the individual, beneficial characteristics of extruded collets as compared with flakes: (1) collet bulk density is approximately 50% higher; (2) **collets have greater internal porosity**; (3) they have a higher free oil content on their outside surface; (4) they release oil more quickly; (5) they fill an extractor bed more randomly; (6) they present less restriction to solvent flow; (7) they can withstand great force without impairing drainage; (8) they have fewer fines; (9) they drain approximately five times faster; and (10) their drainage is less affected by preparation problems. Except for Farnsworth et al. (101) and Watkins et al. (102), (see Tables 3, 4, and 5), very little data has been published on extruder setup and how extrusion benefits extraction. Table 3 shows, for cottonseed, some operating variables. Table 4 shows the residual oil contents after a bench-top solvent extraction of the respective meals. The data in Table 4 shows that most extruded collets extract to lower residual oil than flakes. Table 5 shows data for soybeans. Extruded collets do have one characteristic that could be detrimental to extraction. They drain approximately five times faster than flakes. This significantly reduces their contact time with solvent. This characteristic would only become important in shallow bed extractors. However, it could be overcome by operating the expander to make a more crumbly collet, or by crumbling a portion of the large collets, or by increasing miscella recirculation rate within the extractor stages.

### 5.3. Extraction Standards

Commercial solvent extraction equipment for oilseeds is expected to reduce the oil content of the desolventized solid residue to less than 1.0%, preferably 0.5%. In making guarantees on extraction equipment, manufacturers usually specify that the analysis be made on extracted material before toasting, as it is generally acknowledged that there is (for some materials, such as soybean) an increase in the apparent "oil" content (actually any solute dissolved by petroleum ether) during toasting. Moreover, as appreciable toasting takes place during the removal of solvent from extracted flakes in desolventizer toasters, the analysis should preferably be made on spent flakes that have been desolventized without the use of steam or heat. Unfortunately, this is seldom practical from a safety standpoint: thus, meal analysis is usually relied on.

In an operating extraction plant, the objective is to reduce the oil left in the solids to the lowest possible level. In practice, this is desirable; however, evidence shows that the last portions of extractable oil in the solids are not oils at all but phosphatides and other nontriglyceride solutes. Bull and Hopper (133) have reported a phosphatide content of 18.62% in the last 1.1% of solute extracted from a sample of soybean flakes with commercial hexane at 40°C (104°F). Karnofsky reported that a similar fraction had a refining loss of 81.5% (32).

**TABLE 3. Extrusion Conditions for Preparing Cottonseed Meats.**

Condition	Treatment						
	A	B	C	D	E	F	G
Die exit temperature. °C (°F)	87.7 (190)	87.7 (190)	97.7 (208)	105.5 (222)	111.1 (232)	112.7 (235)	104.4 (220)
Screw speed (rpm)	150	300	300	—	400	300	400
Flake feed rate (lb/hr)	560	375	460	460	560	400	340
Moisture content of feed (%)	12.0	12.0	7.5	7.5	7.5	7.5	24.0
Steam on jacket (turns)							
Feed end	0.0	0.0	0.5	0	0	0	0
Die end	0.0	0.0	1.5	0	0.75	0	1.0
Number of dies	3	3	3	3	3	3	1

Permission granted from AOCS to reprint from *The Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs* (102).

**TABLE 4. Residual Oil Contents of Extracted Cottonseed Meals.**

Type	Treatment							
	Flakes	A	B	C	D	E	F	G
Crude free oil (%)								
Initial	29.8	30.7	23.9	28.5	22.8	32.8	28.5	15.0
After 6 stages	2.77	1.44	1.08	0.82	0.70	0.80	0.77	3.16
After 8 stages	1.09	0.71	0.77	0.20	0.64	0.60	0.59	3.10
Acid hydrolyzed oil (%)								
Initial	28.9	28.2	22.6	27.4	22.5	32.0	24.3	17.1
After 6 stages	2.82	2.01	3.53	2.06	2.03	1.59	2.46	4.2
After 8 stages	2.99	1.96	2.84	1.74	2.05	1.55	2.48	3.7

Permission granted from AOCS to reprint from *The Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs* (102).

**TABLE 5. Residual Oil Contents of Extracted Soybean Meals.**

Type	Flakes	Pellets
Crude free oil (%)		
Initial	21.6	21.9
After 6 stages	1.83	0.37
After 8 stages	1.51	0.10
Acid hydrolyzed oil (%)		
Initial	19.8	20.8
After 6 stages	3.06	1.69
After 8 stages	2.78	1.70

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#### 5.4. Solvents for Extraction

MacGee, in 1947, published a comprehensive review of the early days of solvent extraction (143, 144). Many solvents, such as benzene, aviation gasoline, methanol, ethanol, isopropanol, carbon disulfide, diethyl ether, ethylenedichloride, carbontetrachloride, trichlorethylene, and the various petroleum naphthas were evaluated. However, by 1947, the most common solvents used in the United States were the light paraffinic petroleum fractions, such as the hexanes, heptanes, and pentanes. Hexane, because it was easily evaporated and left no residual obnoxious odors or tastes, was finally chosen. Later, this decision was further supported by Eaves et al. (145), who investigated the extraction of cottonseed by five commercial solvents (hexane, benzene, ethyl, ether, acetone, and butanone) and concluded that none compared favorably with hexane as an extractant for cottonseed.

*Hydrocarbon Solvents.* There are two types of hexane: normal, or *n*-hexane, and commercial, extraction-grade hexane, *n*-Hexane is pure and boils at 69°C (156°F). Extraction-grade hexane is not pure. The *n*-hexane content typically varies from approximately 48% to 98%, as shown in Table 6 (146).

As they are mixtures of several hydrocarbons, the various commercial grades of hexane have distillation ranges of from 1–5°C (2–9°F). Commercial grades with narrow distillation ranges are preferred.

The American-produced extraction hexanes are substantially free of nitrogen-containing or sulfur-containing compounds and unsaturated hydrocarbons. They are sufficiently stable to be reused indefinitely. Although the hexane-type naphtha is the most widely used and the one generally preferred for oilseed extraction, the heptane and pentane types can be used. Commercial heptane boils at a higher temperature, 88–99°C (190–210°F), which dictates hotter evaporator and desolventizer temperatures and higher energy usage. However, commercial heptane might be preferred for the extraction of castor oil, which is not freely miscible with hydrocarbons except at elevated temperatures. The pentane types, because of their lower boiling points, are easier to recover (a savings on energy costs) and find some use in

**TABLE 6. Examples of Specifications for Extraction-Grade Hexanes Offered for Sale in the United States.**

Property	Solvent			
	A	B	C	D
Gravity [15.56°C (60°F)]				
API	78.5	78.4	77.6	81.7
Specific	0.674	—	0.677	—
Density (lb/gal)	5.61	—	5.63	—
Distillation (°C)				
Initial boiling point	65.3	65.9	67.0	67.9
50%	66.4	67.0	68.0	68.4
Dry point	69.8	70.0	68.5	68.8
Flash point, tag closed cup °F	<0	—	-10.0	—
Kauri-Butanol (KB) value	29.7	—	30.0	28.0
Aniline point, °F	151.0	0	148.0	156.0
Color, Saybolt	+30.0	+30.0	+30.0	+30.0
Composition				
Olefins, ppm	6.0	—	—	—
Paraffins	89.0	—	—	—
Cycloparaffins, vol. %	11.0	—	—	—
Aromatics, vol. %	Nil	0.02	—	—
Dimethyl butanes, vol. %	—	2.07	1.0	0.1
<i>n</i> -Pentane, vol. %	—	0.04	—	0.2
Iso-Pentane, vol. %	—	0.91	—	—
2-Methyl pentane, vol. %	—	18.4	—	0.9
3-Methyl pentane, vol. %	—	17.9	—	0.8
2,4-Dimethyl pentane, vol. %	—	0.04	—	0.1
Methyl pentane, vol. %	—	—	15.0	—
Methyl cyclopentane, vol. %	—	10.7	14.0	0.2
<i>n</i> -Hexane, vol. %	48.7	47.8	71.0	97.7
Cyclohexane, vol. %	—	2.6	—	—
Benzene, wt. %	<001	0.30	0.004	Nil
Nonvolatiles, g/100 mL	<001	—	<0.1	0.35
Doctor test	Neg.	—	Neg.	Sweet
Cu Corrosion, 3 h @ 50°C (122°F)	—	1	1	1
Acidity of residue	Neutral	—	Neutral	—
Sulfur content, ppm	<2	—	<1	<1

Permission granted from AOCS to reprint from *The Proceedings of the World Conference on Edible Fats and Oil Processing* (146).

the extraction of heat-labile products, such as pharmaceuticals. Early studies by Ayers and co-workers (147, 148) as to the desirability of pure C<sub>5</sub>–C<sub>7</sub> hydrocarbons for the extraction of cottonseed convinced them of the distinct advantage of paraffinic hydrocarbons. In particular, they found the methylpentanes to be the best solvent.

As a result of the Clean Air Act of 1990 (Public Law No. 101-549, Nov. 15, 1990) and the designation of hexane as one of 189 hazardous air pollutants (149),

subsequent research has centered on looking at possible alternative solvents to hexane. This interest may center on the use of the pentane fraction, which is presently not considered a hazardous air pollutant, to extract cottonseed (150). A present-day commercially available methylpentanes-type naphtha is listed as having a typical boiling range of 55–61°C (131–142°F) (150).

*Halogenated Solvents.* A serious disadvantage of the naphtha-type solvents is their flammability. Precautions are required to avoid fire or explosion in the plants in which they are used. The proper safety measures are outlined by the National Fire Protection Association (NFPA) (151). As a result of the potential fire and explosion hazard involved when hydrocarbon solvents are used for extraction, there has always been a great deal of interest in nonflammable solvents. Halogenated solvents such as trichloroethylene were originally favored (152–154). During World War II, trichloroethylene was used in one large extraction plant in England. In the United States, however, its use was confined to a few small soybean extraction plants. Originally, it was thought that the use of a nonflammable solvent, such as trichloroethylene, would result in less danger of fire, less need for skilled labor, and lower initial investment cost. Experience showed, however, that trichloroethylene's vapors were flammable (155) and that trichloroethylene reacted with cysteine in soybean to give *S*-dichlorovinyl cysteine, which, when fed to cattle, caused aplastic anemia (156, 157).

In the early 1980s, when it appeared that hexane was in short supply, Johnson et al. described a 56-h pilot plant extraction of cottonseed using an alternative solvent, methylene chloride (dichloromethane) (158). This solvent extracted the oil and significantly reduced levels of the antinutrients gossypol and aflatoxin. However, because of environmental concerns, the commercial use of halogenated hydrocarbons in solvent extraction has not occurred.

*Water as a Solvent.* Water, of course, is nonflammable and immiscible with vegetable oil. Researchers have used water systems to extract oil and protein from soybeans, cottonseed, peanuts, sunflowers, and coconuts (159). Aqueous extractions usually consist of the following steps: grinding, extracting with aqueous solutions, separating the different fractions, then drying. The water used may be pH adjusted or may contain added chemicals, depending on the products desired. Although finding application in the commercial extraction of oil from palm, olive, and coconuts, aqueous processing has not been commercially accepted in the extraction of soybean or cottonseed because of approximately 10% residual oil, at best, in extracted solids and because of the energy-intensive separation and drying steps (160).

*Enzyme-Assisted Aqueous Extraction.* Enzymes are probably the most important additive or treatment used in aqueous extractions. The knowledge that enzymes in aqueous systems could enhance the extraction of vegetable oil was observed in the 1950s. However, it was not until the 1970s and 1980s that this knowledge was put to practical use in the development of pilot or industrial processes for the extraction of olive, rape, and coconut oils (160). As discussed earlier, efficient solvent extraction of vegetable oil is dependent on the destruction of cell walls. This is normally accomplished by physical means such as flaking and extrusion. Enzymes, because



they can digest the complex cell-wall structure of oilseeds, can significantly improve oil extraction (161). The digestion results in having all the components of the cell, i.e., protein, oil, and polysaccharides in a liquid state, where, with further processing, they can be individually separated out.

Domingues et al. (160), in their comprehensive review of enzymatic improvement of oil extraction, included discussion of various processes that also used hexane as a solvent. They reported aqueous enzyme processes that used hexane in a 1:2 hexane–water ratio. Additional hexane was added to the enzyme-treated and dried materials. Although enzymatic-assisted extraction is presently only economical in olive processing, because of the milder conditions used and, in most cases, higher quality oils obtained, it does have potential for future use.

*Acetone as a Solvent.* Acetone is an excellent solvent for cottonseed oil and for the antinutrients gossypol and aflatoxin. Acetone has found some commercial success in Sicily where Vaccarino used 96% aqueous acetone in a 50 ton/day batch process plant (162, 163). However, acetone has not had any other modern successes, probably because it produces a dark and hard-to-bleach cottonseed and because it imparts a disagreeable cat-like odor to the extracted meal.

*Alcohols as Solvents.* As a result of environmental concerns and inability of hexane to extract gossypol and aflatoxin, recent research on the solvent extraction of cottonseed has centered on developing processes using ethanol and isopropanol. The use of these alcohols to extract cottonseed and many other oilseeds is not new.

The Japanese in Manchuria in the 1930s actually built a plant that operated a short time, to extract soybeans using ethanol (164, 165). Interest in alcohol as a solvent was renewed in the 1950s (166–172) and again in the 1980s and 1990s by Karnofsky et al., Hron et al., and Lusas et al. Karnofsky et al. developed a four-step, energy-intensive process that could use either isopropanol or ethanol to extract soybeans or cottonseed. In their cottonseed process, they use 85% aqueous ethanol (by weight) to first extract fatty acids, nonoil lipids, aflatoxin, and gossypol. In a second step, 85% ethanol is displaced by 92% ethanol. In a third step, most of the oil is extracted and then removed by cooling, which precipitates the oil, after which the oil-depleted miscella is recycled back to the extractor. In a fourth step, the remaining oil is removed with pure 92% ethanol (173, 174). Corresponding concentrations of isopropanol can also be used.

Hron and co-workers (86–90), because of a shortage of hexane, searched for a biorenewable solvent alternative to hexane in the 1980s. They eventually developed a bench-top process for extracting oil, gossypol, and aflatoxin from cottonseed using aqueous ethanol (95% ethanol by volume) at atmospheric pressure (175). In the process, cottonseed meats are moisture-adjusted to 14% H<sub>2</sub>O, flaked to 0.229 mm (0.009 in), and then dried to 2% moisture. Drying is important to ensure that the ethanol does not absorb moisture from the incoming cottonseed and thereby lose its oil solvency (176). The dried flakes are then initially extracted with unheated 95% ethanol in a first stage to remove gossypol and then extracted with hot 95% ethanol in a second stage to extract oil and aflatoxin.

The ambient-temperature miscella is passed through an absorber to remove gossypol and is then recycled back to the first-stage extractor. The second-stage

hot miscella is chilled to 4°C (39.2°F) to reduce the oil's solubility in the alcohol. The miscella is then centrifuged into fractions that are further processed with reverse osmosis membranes and absorbers. These devices remove aflatoxin and gossypol and produce a crude oil fraction that is then miscella refined. Although the process is technically feasible, it would not be economical unless an oil mill has a continual, serious aflatoxin problem or the use of hexane is limited as a result of the Clean Air Amendment of 1990. Hron and co-workers noted during their research that, although aqueous 95% ethanol converts free gossypol into bound gossypol even at ambient temperature (89, 90), efficient extraction of bound gossypol, along with oil, can be accomplished only if the aqueous ethanol is acidulated with a tribasic acid and the cottonseed is extracted hot (89, 90).

Lusas et al. (91) at Texas A&M recently chose to research isopropanol over ethanol because of its greater oil solubility, its lower heat of vaporization, its reduced drying requirements for flakes and extruded collets, and its freedom from potential problems with government alcohol taxing agencies. Rather than using the common and easily distilled isopropyl azeotrope of 87.8% (by weight), they have centered their research on high-concentration (93–96%) isopropyl alcohols. They chose high-concentration solvents because the azeotrope has a maximum cottonseed oil solubility of approximately 12–14%, whereas the high-concentration solvents have been reported to dissolve up to approximately 48% oil. The much higher solubility of oil can significantly reduce the amount of solvent needed to extract oil to low levels in meals. Lusas et al. have developed a pilot plant process that consists of moisturizing dehulled meals to 12%, cold-flaking to 0.38 mm (0.015 in.), extruding through an Anderson expander at 88–93°C (190–200°F), and holding for 30 min. The collets are next re-extruded at 104–110°C (220–230°F). The resulting porous collets are then solvent extracted with high-concentration (95% isopropyl) alcohol, 1:1 solvent to meal ratio, at 77°C (170°F) in a simulated nine-stage countercurrent extraction. This process can reduce residual free gossypol in meals to approximately 220 ppm, which should broaden the meal's potential uses and improve its value. Oil is then removed from the alcohol, chill-separated at about 5°C (41°F), and desolventized in a thin-film evaporator. Caustic refining and bleaching of the resulting crude oils produced RBD (refined, bleached, deodorized) oils well within industry standards.

High-concentration isopropyl alcohol is recovered by the use of per evaporation techniques. Briefly, perevaporation is basically a membrane separation in which the mixture to be separated is vaporized and the vapors are passed over a membrane. The smaller molecule, in this case water, permeates the membrane into a vacuum area where it is condensed and removed. In this manner aqueous isopropyl alcohol solutions can be concentrated above the azeotropic concentration to approximately 99%, if desired (177). Texas A&M has also reported that their process works equally well on soybeans. Both alcohol processes are investigations into alternative procedures should petroleum-based solvents no longer be available. Neither process has been put into commercial operation.

When cottonseed first underwent direct extraction with hydrocarbon solvents, large amounts of free gossypol remained in the meal, causing a serious complication. As

explained earlier, several wet cooking methods were developed to bind the gossypol and detoxify the meal. Later, attempts were made to find a way of extracting gossypol. Another method, aside from acetone and the alcohols that looked promising, was extraction with a 53:44:3 mixture of acetone-hexane-water (178). This removed gossypol from the meal and transferred it to the oil, from which it could be removed by conventional alkali refining. Meals made by this method produced superior growth in chicks. The procedure was never accepted by the oilseed industry because of the difficulty of handling the three-component solvent and because of the presence of an off-odor in the meal. The off-odor was probably caused by the acetone and water in the extraction solvent (179).

*Supercritical Solvents.* Although it was known in 1879 that supercritical fluids had solvent properties (180), supercritical extraction was not extensively developed until the early 1980s. This method uses organic or inorganic compounds as solvent, at or usually above their critical temperature and pressure where they are known as supercritical fluids. In a supercritical fluid state, common gases such as carbon dioxide have the properties and extractive capacity of a liquid. The compound most used in supercritical extractions is carbon dioxide. Carbon dioxide can exist as a gas, liquid, or solid, depending on pressure and temperature conditions. However, at or above its critical point, CO<sub>2</sub> can only exist as a supercritical fluid.

The critical point is defined as the state at which a compound is at its critical temperature and pressure. For carbon dioxide, the critical temperature and pressure are 7387 kPa (1070 psig) and 31°C (88°F). Typical supercritical CO<sub>2</sub> extractions usually take place at pressures of 20,670–103,352 kPa (3000–15,000 psig) and 50–80°C (122–176°F), depending on the product being extracted. Normally, the higher temperatures and pressures extract more oil; however, changes in density of the supercritical fluid must be taken into consideration. Extractions are performed by circulating a supercritical fluid through a pressurized cell filled with pretreated material. As with hexane extractions, the amount of oil extracted can depend heavily on the method of preparation.

List and Friedrich (181) reported that essentially all of the oil could be extracted from 0.10 mm (0.004 in.) soy flakes, but only approximately 87% and 67% from 0.38 mm (0.015 in.) and 0.81 mm (0.032 in.) flakes, respectively, at the same temperature and pressure conditions. Extractions are usually performed batchwise. After extraction, by reducing pressure with and without temperature change, various liquid oil fractions can be separated out by precipitation. Feidrich and co-workers (182, 183) reported producing a better oil from soybeans using supercritical CO<sub>2</sub> than with hexane. Other oilseeds that have been successfully extracted using supercritical fluids are cottonseed (184), wet-milled and dry-milled corn germ (185), and rice bran (186). Although supercritical extraction is technically feasible because of the required high-pressure and the low-capacity equipment, it is not economically feasible. Supercritical extraction is presently limited to high-value materials such as coffee, hops, and natural flavors.

For comprehensive reviews of essentially all of the pure and mixed solvents associated with oilseed processing, see Johnson and Lusas (187). Lusas et al. (146), and Hron et al. (covering biorenewable solvents) (159).

## 6. TYPES OF EXTRACTORS

### 6.1. Early Extractors

*Batch Extractors.* The first solvent-extraction systems consisted of batch vessels charged with a material that was washed, batchwise, with progressively leaner miscellas, and, finally, with clean solvent. Plants of higher capacity used banks of batch vessels, loaded one at a time, and sequenced with the wash cycles so that the whole bank was handled in a countercurrent flow pattern. Batch extractors are no longer used for oilseeds, but some batch extractors are still used for special materials such as coffee, pharmaceuticals, and other high-priced materials.

*Total Immersion Extractors.* In a total immersion extractor, the solid material is immersed in and travels through a pool of solvent. The Hildebrand extractor of 1931 (188–190) is an early example. Hildebrand's extractor consisted of two vertical tubes connected at the bottom with a horizontal tube. All three tubes were filled with solvent. Three screw conveyors moved the solid material down one tube, across the bottom, and up the other tube. Solvent entered near the top of the solids discharge tube and overflowed the solids inlet tube. This flow pattern caused the extracted oil to move away from the solids in a countercurrent fashion. The screws, however, were rough on flakes and produced many fines.

Bonotto patented a different design in 1937 (191–194). He advised a single vertical tube, separated into several internal sections by horizontal baffle plates mounted on a slowly revolving central shaft. The plates had slotted openings that were arranged in a staggered helix from plate to plate. Fresh solvent was pumped in the bottom and flowed out the top. Flakes were introduced at the top and sank from plate to plate by gravity. Stationary scraper arms placed above each plate helped to prevent bridging and helped move the flakes through the slots. The V.D. Anderson Co. (Cleveland, Ohio) and Allis-Chalmers Co. (Elm Grove, Wisconsin) both supplied total immersion extractors of similar design in the 1940s and 1950s. Anderson's extractor employed stationary plates with rotating sweep arms. Pie-shaped openings in the plate were adjustable through external linkage.

Density of the flakes (compared with how fast the solvent rose up the column) put constraints on the efficiency of these columns. Under perfect conditions, all the solids would sink, and only solids-free miscella would flow out the top. One never reaches perfect conditions. Reasonable capacity requires appreciable rates of vertical ascent. Fines sink slower than flakes. Agitation creates fines and causes turbulence in the miscella. A major shortcoming of vertical immersion columns is excessive fines in the miscella. The miscella had to be filtered through the solvent-tight plate-and-frame filters, two filters in parallel, one on stream, the other being cleaned. Poor flake preparation caused short filter cycles. Cleaning the filters allowed solvent to escape and created a potential fire hazard.

Anderson improved their extractor with the addition of a fines separator on top (195, 196). The fines separator was a bundle of tubes through which the miscella ascended before leaving the extractor. The combined-cross sectional area of all the tubes was greater than the cross-sectional area of the column. This allowed the rate

of vertical ascent to decrease appreciably before the miscella left the extractor. Flakes were introduced through a separate spout in the center of the tube bundle to ensure that no flakes mixed with the exiting miscella. The diameter of each tube was small enough to dampen any turbulence caused by agitation within the extractor. This helped—it did increase filter cycle time—but the miscella still required filtering.

## 6.2. Percolation Extractors

Batch extractors mix a charge of solids and solvent and then let it drain. Immersion extractors cause the solids to pass through a pool of solvent. Percolation extractors carry the solids through a vapor-light chamber where solvent rains down through the solids, dissolving out the oil, similar to the way a coffee percolator works. There are five major types of percolation extractors: basket, rotary, perforated belt, sliding-bed, and rectangular loop (151).

*Basket Extractors.* The first percolation extractor was a basket extractor patented in Germany by Bollman in 1919 (196–200). Large baskets, supported by looped (or endless) chains, moved up one side of a vapor-tight enclosure and down the other side, like a slow-moving, up and down. Ferris wheel. As the empty baskets began the downward travel, they received a charge of incoming flakes from a hopper. So-called half miscella from the other side of the extractor was pumped onto the top basket and drained from that basket through the baskets underneath it concurrently and collected in a sump at the bottom of the extractor. The baskets then moved horizontally across the bottom of the enclosure and free drained of miscella before starting their ascent up the enclosure's other side. The miscella collecting in the sump under the downward-traveling baskets (so-called full miscella) was pumped to equipment that filtered it and separated the solvent from the oil. The solvent was then recycled back into the fresh solvent work tank.

Fresh solvent at the rate of approximately 1 kg of solvent per kilogram of oilseed was sprayed onto a basket near the top of the ascending column of baskets. From the top basket, the solvent percolated by gravity through the lower baskets in counter-current flow. The half miscella from this side was collected in a second sump in the lower part of the housing. A pump continuously withdrew the half miscella from that sump and sprayed it onto the topmost basket of the descending line as described above.

As the baskets traveled across the top, they were inverted momentarily to dump the extracted solids into a discharge hopper. The baskets then continued to the filling station to make another circuit through the extractor. These early vertical basket extractors (Figure 21) were tall and bulky, subject to chain breakage, and were difficult to service.

New designs, with baskets moving in a horizontal plane rather than vertically, corrected these shortcomings. These new designs permitted one-floor operation and allowed for more complete, multistage, countercurrent flow. Solvent passed through the last basket as final wash was pumped, as lean miscella, through the preceding basket. After passing through that basket into a sump, it was pumped

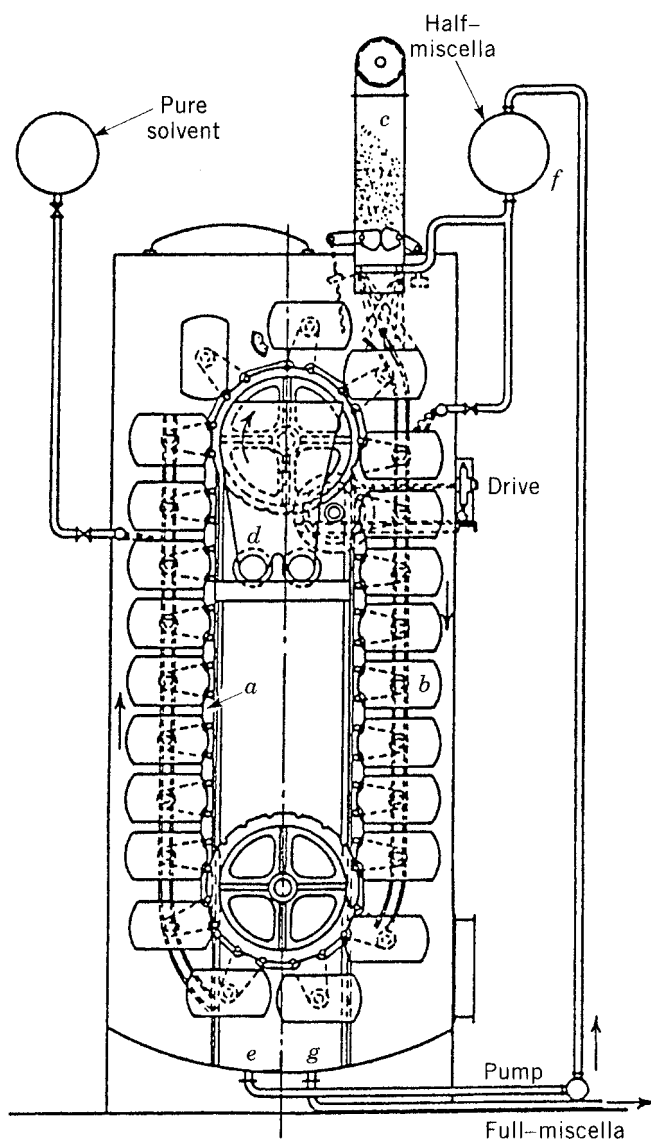


Figure 21. Early vertical basket extractor.

through the next preceding basket. A series of collecting sumps, pumps, and overhead spray heads under which the baskets traveled allowed these extractors to function in true countercurrent fashion.

An advantage of a multistage, countercurrent basket extractor, in contrast to the two-stage vertical unit, is that the rich miscella, after it has contacted the incoming flakes, can make one final pass through a basket where the flakes have already settled down to form a bed. This effectively filters the miscella and eliminates

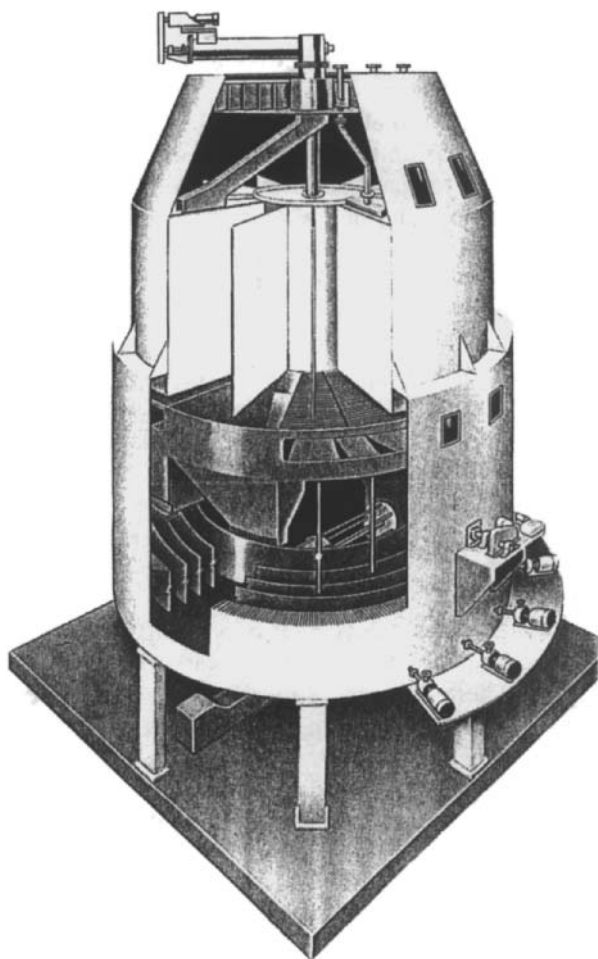
the need for the kind of filter presses required by immersion extractors. Usually, a liquid cyclone suffices to clarify the miscella before it goes to the evaporator, but it is important to remove the fines. Any fines left in the miscella may stick and burn onto the heater tube walls in the evaporator, needlessly darkening the oil.

Anderson Co. (Cleveland, Ohio) came out with a horizontal basket extractor (201) in which the baskets were pushed through a rectangular chamber by means of four externally mounted hydraulic rams. Each ram pushed a row of baskets in sequence to cause the baskets to travel, one-by-one, under each successive spray head.

*Rotary Extractors.* Rotary extractors come in two varieties. Both look similar, but the parts that move in one type do not move in the other type. One type uses a circular disk with pie-shaped compartment-like cells that slowly revolve, like a catrousel, above a nonrotating ring of sumps that collect miscella. Incoming flakes fall into each compartment as that compartment passes under the flake inlet spout. The cells then rotate under a circle of stationary spray heads located above the rotating disc. The heads spray miscella in a countercurrent fashion onto the flakes on the disc. The spent flakes, before leaving the extractor, pass under a spray of fresh solvent, then travel over a final drainage sump, and finally move over a discharge hopper where the floor of the cell swings open to drop out the marc (extracted solids). Continued rotation shuts the door and moves the now empty cell under the flake inlet. Called the Rotocel because the cells rotate (202–205), the first unit was commissioned by Blaw-Knox (now Dravco) (Pittsburgh, Pennsylvania) on soybean in 1950 at 275 mg/day (250 T/D) capacity (see vol. 2, ch. 11, “Soybean Oil,” Figure 11.3). A similar extractor, called Carrousel, is supplied by Krupp (Hamburg, Germany).

The other type of rotary extractor employs a circular disc of cells (or wedge-shaped baskets) that do not move. Instead, the flake inlet spout and fresh solvent and miscella spray heads revolve above the cells, and the drainage sumps and marc hopper revolve below the cells. Termed a *stationary basket extractor* (206) (Figure 22), this rotary extractor was supplied by the French Oil Mill Machinery Company (Piqua, Ohio) until they sold the solvent extractor part of their business to De Smet (North Humberside, United Kingdom) in 1999. De Smet, in 1997, had patented a similar extractor called the “Reflex Extractor,” but the stationary parts are rotating, and the rotating parts are stationary. De Smet does not build the “Stationary Basket Extractor,” nor does French Oil Mill Machinery. French Oil Mill Machinery, however, sells repair parts for existing Stationary Basket extractors and sells preparation equipment for solvent-extraction plants as well as a line of screw-presses.

All rotary extractors employ deep beds of solids to be extracted, sometimes as much as two or more meters deep. Hence, rotary extractors are also termed *deep-bed extractors*. There are several mechanical variations of these extractors. Two variations have already been mentioned. Another variation is where the individual cells have hinged bottoms that swing open whenever the cell is to be emptied. In this variation, the solids slide against a common one-piece bottom that has an opening to allow solids to fall through when extraction is completed. Termed *sliding plate*,



**Figure 22.** Stationary deep-bed extractor (Courtesy of The French Oil Mill Machinery Co., Piqua, Ohio).

this modification is thought to improve drainage because it “sweeps” the perforated bottom against the bed of flakes thereby minimizing the possibility of clogging the perforations with fines.

In rotary extractors, flakes are mixed with rich miscella as they are conveyed into the first-stage compartment. As the bed of material in stage 1 is formed, some oil is extracted. The miscella from this stage has picked up fines because of the slurry feed operation, so it is pumped through stage 2 compartment to filter out the fines. From stage 2, it leaves the extractor as full miscella. The lean miscella from stage 3 is pumped to stage 1 to form the feed slurry. Thereafter, the miscella is pumped from each stage sump in true countercurrent fashion, i.e., stage 3 gets miscella from stage 4 and stage 4 gets miscella from stage 5, etc. There are usually



five miscella stages, followed by a sixth stage during which fresh hexane makes the final wash. Usually, the solvent/miscella makes a single pass through each stage. All that flows out one stage is pumped directly into the preceding stage. If this is done, then it is critical that the entire surface of each cell is adequately contacted with incoming solvent/miscella.

Ideally, there should be a slight head of liquid on top of the solids in each stage. This ensures that all of the solids are in contact with liquid and that the liquid penetrates through the entire cross section of the bed as it percolates through the solid material. Any portions of solids missed will, of course, not be extracted. If the bed of solids allows too rapid a solvent flow, such that a head of liquid cannot be maintained, then the solvent/miscella should be made to fall into the cell as "curtain" or "water fall" distributed as a continuous sheet of liquid along the entire radius of the material in the wedge-shaped cell. Distribution heads are adjustable to provide for this. If a sheet of solvent does not drop on the outermost edge of the cell (like the pie crust of the wedge-shaped cell), then a significant amount of solids will not make adequate contact with the liquid.

Preparation, particle size, and moisture will influence how fast liquid flows through the bed. Preparation should be controlled so that the flow is not too fast. Worse than that, however, is too slow a flow. The cell will then "flood," and the liquid will overflow into the next cell. This could stop extraction in the flooded cell.

These extractors, like all extractors, have inspection windows through which the tops of each cell and the bottoms of each cell can be observed. There should be adequate coverage of the surface when the solvent/miscella is introduced. No cell should flood over the top. There should be some evidence of adequate drainage before the fresh solvent is introduced, and the fresh solvent should fall as a curtain covering the entire radius of the cell. There should be no "channeling" inside the bed where solvent/miscella is diverted away from portions of the bed "channels" through other portions. A hypothetical way this could happen (making it easier to explain) is to compare flakes with shingles. If, by chance, many flakes lay on each other horizontally, they can act as shingles and divert the solvent/miscella away from the material beneath them. Similarly, with extruded collets, if the collets are too large, or if solvent flow is too slow, the solvent may rush straight through the bed and not contact all the collets. If material preparation is wrong, especially if moisture is too high, or if too many fines are present, the material can bed down more tightly in some places than in others. The solvent/miscella, then, will channel through paths of least resistance.

When troubleshooting an extractor, it is wise to measure the oil concentration of the miscella coming from each stage. There should be a steady reduction of oil level from stage to stage down to a final concentration of 1% or 2% oil in the miscella produced by fresh incoming solvent. If miscellas from several stages have the same oil level, this indicates that something is wrong.

If a deep-bed rotary extractor is run so that the solvent/miscella makes only one pass through each stage, then preparation should be monitored to ensure that miscella does not rush through any stage without spreading across the surface or that no stage floods. Usually, on a deep-bed extractor, flooding is more likely to

happen than too rapid a percolation flow. Extrusion of the fresh oil-bearing material is helpful because extrusion agglomerates fines, producing porous collets that resemble gravel more than miniature shingles, and thereby permits a more free and more uniform flow of liquid.

After the material is rinsed with fresh solvent, it is allowed to free drain of liquid before leaving the extractor. Preparation, especially particle size, influences how much solvent the free-drained marc retains. Flaked materials retain from 30% to 40% by weight, depending on the quality of flake. Prepressed cake, if not granulated (and provided the ungranulated cake is porous enough to extract) can free drain to 20–25% solvent. Extruded collets (both from flakes and prepressed cake) can also free drain to 20–25% solvent (103). Substantial energy savings in desolventization can be realized if the marc drains to a low solvent level.

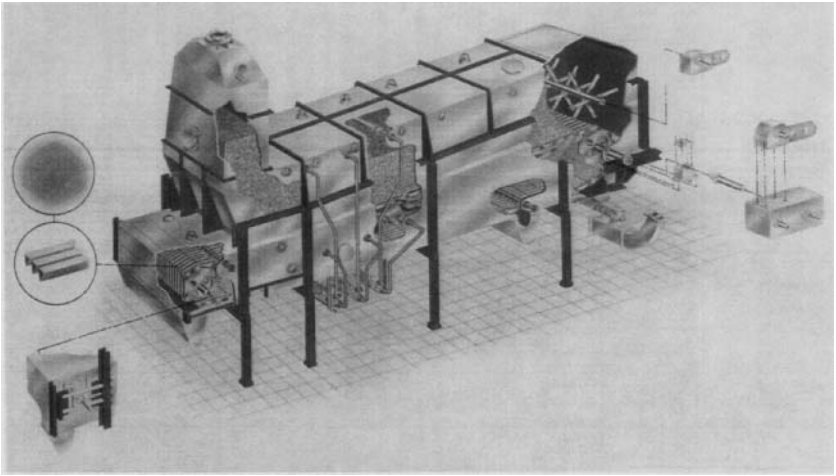
The solvent that drains out of the marc remains in the extractor, increasing the volume of miscella washing through each stage, and eventually winding up in the full miscella. If this extra solvent is not needed for extraction, the incoming solvent-to-meal ratio can be lowered. A lower ratio increases the miscella concentration by reducing the amount of solvent in the full miscella. This, in turn, reduces the energy costs for miscella desolventization (103). Extruded collets usually permit reduction of solvent-to-meal ratio because extrusion helps release oil, most of which rinses out in the first few stages and because extrusion produces collets that are very porous.

These energy savings are only a part of the many justifications for using extruders (101–103). The only concern, if solvent-to-meal ratio is reduced, is to make sure that all the solids make contact with the incoming solvent. This is important in the final wash that rinses out dissolved oil left in the marc by the last lean miscella wash. Replacing all absorbed lean miscella with oil-free solvent would significantly reduce final oil in the solids.

*Perforated Belt Extractors.* Another type of percolation extractor uses a moving, perforated belt underneath an extended bed of material. The belt is flexible and moves, with material riding on top, through the extraction chamber.

The leading supplier of perforated belt extractors is De Smet (Zaventem, Belgium). Jean Albert De Smet invented the company's first continuous extractor in 1946. De Smet's extractor is made of a series of wide, rectangular sections of either wire mesh or wedge-bar screens connected together to form a looped endless conveyor belt to carry the incoming solids through the stages in the extractor housing. Each stage consists of a sump beneath the belt and miscella spray heads above. Belt speed determines residence time. Incoming material, mixed with rich miscella, is deposited on the belt to form a bed of solids. Unlike a basket or rotary extractor, this bed is one continuous mass of material extending the entire length of the extractor.

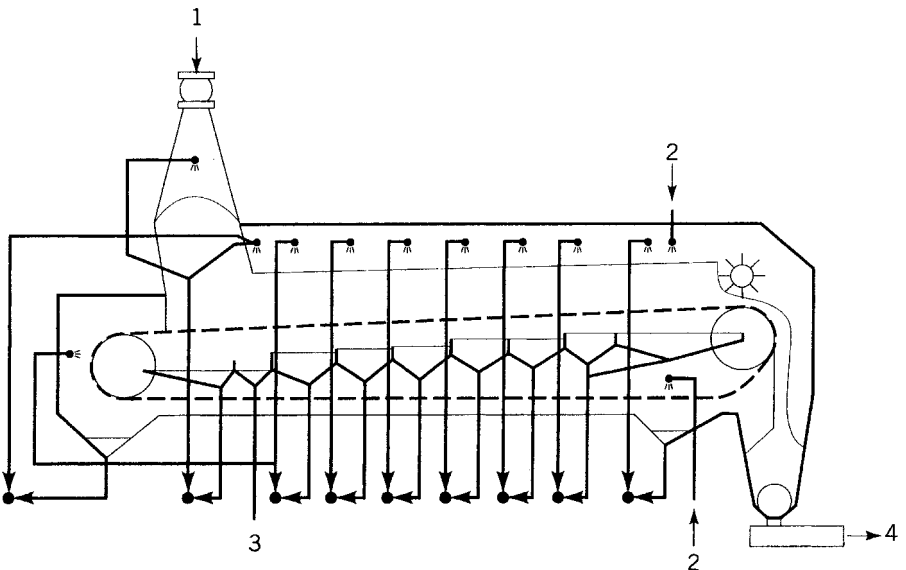
The De Smet extractor (Figure 23) has one moving part: the belt. The belt is set on a slight incline and carries the solids up the incline, throughout the extractor. This helps to ensure a more effective countercurrent flow of miscella through the solids. As a result of the incline, the miscella washes tend to permeate through the solids slanted toward the feed end (countercurrent to the solids flow). Rakes scrape



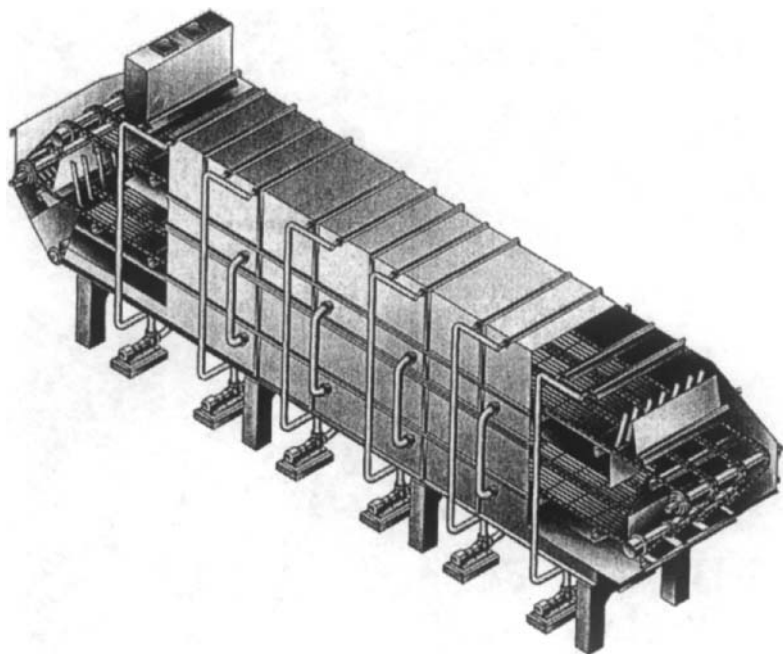
**Figure 23.** Perforated belt extractor (Courtesy of Extraction De Smet SA/NV., Zaventem, Belgium).

the solid's surface after each miscella spray. This builds ridges and keeps the surface open for a more rapid penetration of miscella.

The extractor can run as a deep bed or as a shallow bed. Usually, the bed depth is intermediate. There are several miscella stages and a final hexane wash (Figure 24). Each miscella sump pump recirculates miscella back onto the same stage the miscella came from, except for the final full miscella. Recirculation flows can be



**Figure 24.** Schematic of perforated belt extractor (Courtesy of Extraction De Smet SA/NV., Zaventem, Belgium).



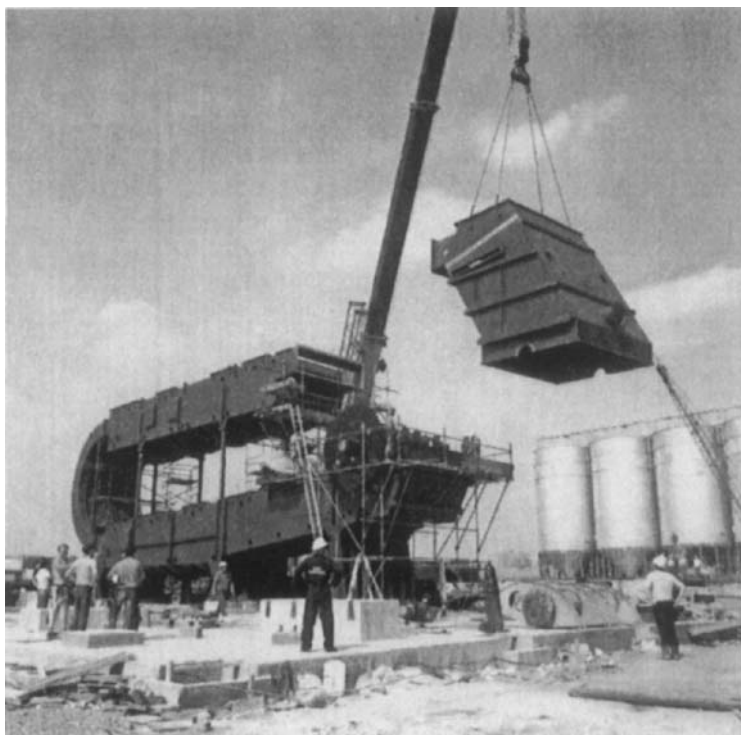
**Figure 25.** Sliding-bed extractor (Courtesy of Krupp Maschinentechnik., Hamburg, Germany).

adjusted to match percolation flows in each stage. Miscella moves from stage to stage by overflowing from one sump chamber into the preceding sump chamber, thereby traveling in opposite direction to the solids. As with other percolation extractors, rich miscella mixes with incoming flakes in stage 1. Final full miscella passes through stage 2 before leaving the extractor.

*Sliding-Bed Extractors.* Another type of percolation extractor slides the solids along a stationary steel plate. Special slotting in the plate allows miscella to pass through while retaining the solids on top. Manufactured by Krupp (Hamburg, Germany), who also manufacture a deep-bed rotary extractor, the sliding-bed extractor is shown in Figure 25.

The incoming solids form a continuous mat of material that is pulled along the slotted plate by drag bars spanned between sprocket-driven chains. There are two "plates." The solid material falls off the top plate, reforms into a fresh mat on the lower plate, and makes a second pass through the extraction chamber. The chained drag bars act like a looped endless conveyor. They pull the mat of material along the top plate, then follow the chains around a pair of sprockets, and, on the return pass, capture the material that reformed on the lower plate. The drag bars then pull the material along the length of the lower plate.

Fresh hexane enters on the lower bed, makes several passes, or stages, on the lower bed, then continues on the upper bed in true countercurrent fashion, similar to other percolation extractors. Krupp's sliding-bed extractor accepts flaked materials,



**Figure 26.** Rectangular loop extractor (Courtesy of Crown Iron Works., Minneapolis, Minnesota).

extruded collets, or prepressed cake. Depth of material on the bed can be adjusted during operation and is usually 0.5–1.3 m (20–50 in.).

*Rectangular Loop Extractor.* Another type of extractor drags the solids through a chamber shaped into a closed loop. Manufactured by Crown Iron Works Co. (Minneapolis, Minnesota), who sold its first commercial unit in 1949, it has been modified and enlarged several times. The extractor employs an “en masse” conveyor that moves the solid material through a closed housing of rectangular cross section. The housing loops back on itself, looking very much like a large letter O resting on its side (Figure 26).

The solid material is deposited as a shallow bed, usually less than 0.77 m (30 in.), and it travels a distance that is usually 50 times the bed depth. This allows ample room for solvent and miscella to leech through the bed in distinct stages without requiring the material to be partitioned into separate cells by means of divider plates.

Fresh material enters the inlet hopper on the upper level and makes its way into the drag conveyor chain just before the conveyor chain starts its downward pass to the lower level (Figure 27).

Rich miscella is introduced during the downward passage. The fresh material containing the miscella moves concurrently into the lower level. Then the miscella,

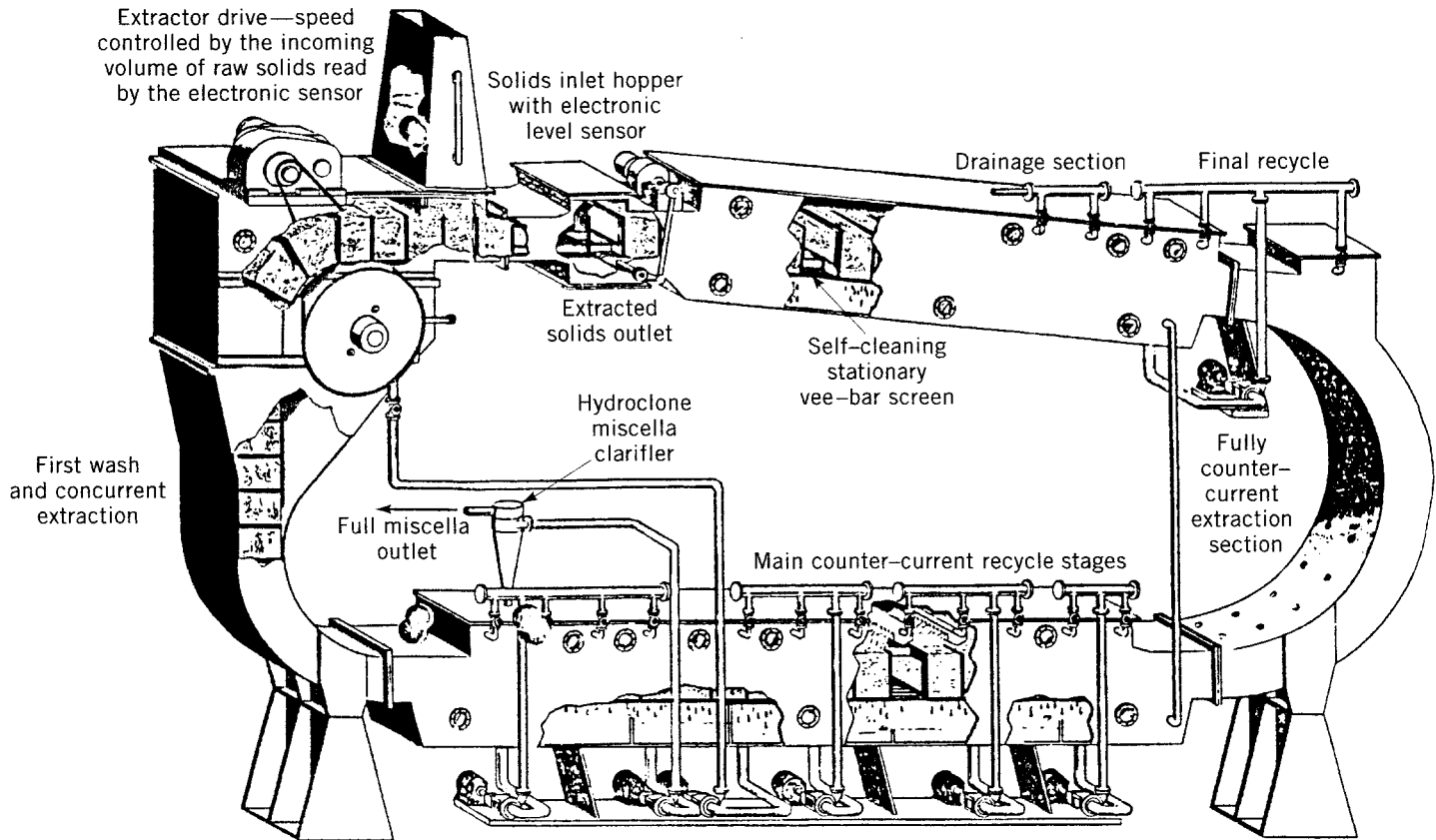


Figure 27. Internal view of rectangular loop extractor (Courtesy of Crown Iron Works., Minneapolis, Minnesota).

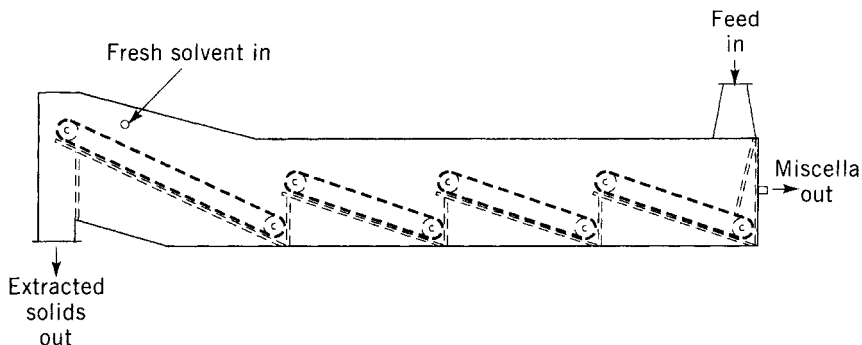
now containing additional freshly extracted oil, along with some fines, drains into the first-stage sump. From there it is pumped into the second stage where the now bedded-down material filters out most of the fines. Miscella draining from the second stage is pumped through a liquid cyclone and leaves the extractor as full miscella. Stages 3 through 7 function in true countercurrent fashion. The fresh solvent washes through the last stage just before solids discharge.

The drag conveyor slides the solid material along a slotted, vee-bar floor through which the miscella drains. After pulling the solids through stages 3, 4, and 5, the drag conveyor lifts the solids to the upper level, while turning the bed upside down, and guides the now inverted bed of solids onto the upper vee-bar flooring and through stages 6 and 7. Stage 7 is the final wash with clean solvent. After stage 7, the solids are pulled along a drainage section where they free drain to around 33% solvent by weight.

Rectangular loop extractors allow for external control of where the miscella from each stage goes. External valving permits each sump pump to return some of its miscella back to the stage it came from and some to the preceding stage. Sometimes all of the miscella is pumped back over the stage it came from. This allows the miscella to make multiple passes within each stage to increase contact time and to maintain an adequate head of liquid above the material. All countercurrent movement of miscella from stage to stage in these extractors could occur through overflow weirs from sump to sump.

Crown later changed the design reversing the direction of travel. Fresh material now enters at the beginning of travel on the upper level of the new (Model III) extractor. The first three stages are on the upper level. The bed of material then travels down to the lower level, turns over in transit, and passes through three more stages followed by a rinse with fresh solvent. The marc free drains on the lower level then discharges into the marc hopper just before the now-empty conveyor rises to the upper level. This relieves the extractor's drive from having to lift the solvent-laden material from the lower level to the upper level, thereby reducing horsepower consumption.

Crown also offers a Model IV extractor specifically designed for continuous extraction of powdery materials that cannot be extracted in percolation extractors (Figure 28).



**Figure 28.** Model IV extractor (Courtesy of Crown Iron Works., Minneapolis, Minnesota).

Percolation extractors provide for washing the solid material with solvent and for allowing the solvent and dissolved oil to separate from the solids through internal screens. Trying to do this with powdery materials would require screens with such fine mesh that the powder would blind the screen and prevent adequate solvent flow.

The Model IV does not use screens. Rather than rely on solvent moving through the material held stationary (relative to the solvent) against screens, the material itself is moved through a pool of solvent. An inclined drag conveyor slowly drags the solids up an inclined ramp, still submerged within the pool of solvent. The solids briefly rise above the solvent as the inclined drag conveyor rounds the top pulley. Then the solids free-fall through the solvent where they are captured by a second inclined drag conveyor. This gentle dragging, followed by free-fall, is repeated several times. Fresh solvent is introduced above the last inclined ramp, and full miscella is drawn off where the solids enter the extractor. The last inclined ramp pulls the solids above the pool of solvent to allow the solids to free drain before they leave the extractor.

The major requirement for operation of this extractor is that the densities of the solid material and the solvent be different enough so that the particles will settle out. The Model IV extractor reportedly is efficient in extracting finely particulate foods, pharmaceuticals, and spent bleaching earth (146).

## 7. RECOVERY OF SOLVENT

### 7.1. Recovery from Miscella

Normally, the full miscella is freed of finely divided solid material before the solvent is removed. The oil could be clarified after the solvent is removed (118), but fines complicate the operation of packed distillation columns and cause oil loss in the fines unless the fines are well washed. Solvent is removed from the miscella in a two-stage rising film evaporator, followed by a “packed” or “disc and doughnut” stripping column (207). Hot solvent vapors from the marc desolventizer condense against a miscella preheater or against the first-stage evaporator. Jacket steam provides heat for the second-stage evaporator. The solvent vapors from the evaporators are condensed, and the oil from the evaporators, still containing approximately 5% solvent, enters the stripping column, into which live steam is injected, countercurrent to oil flow. The oil forms a thin film, with large surface area, as it flows over the packing. This helps the steam “strip” the last traces of solvent. The stripping column is operated under vacuum—559–711 mm (22–28 in) Hg. This helps the solvent escape from the oil.

The solvent and steam vapors from the stripper are condensed together and go to a solvent–water separator, where the condensed liquids separate into lightweight hexane floating on the heavier water. The hexane overflows the top of the solvent–water separator and goes to the solvent work tank. Water, with some entrained hexane, syphons from the bottom of the solvent–water separator and goes to a water



**TABLE 7. Boiling Points (°C) of Mixtures of Cottonseed Oil and Commercial Hexane (208).**

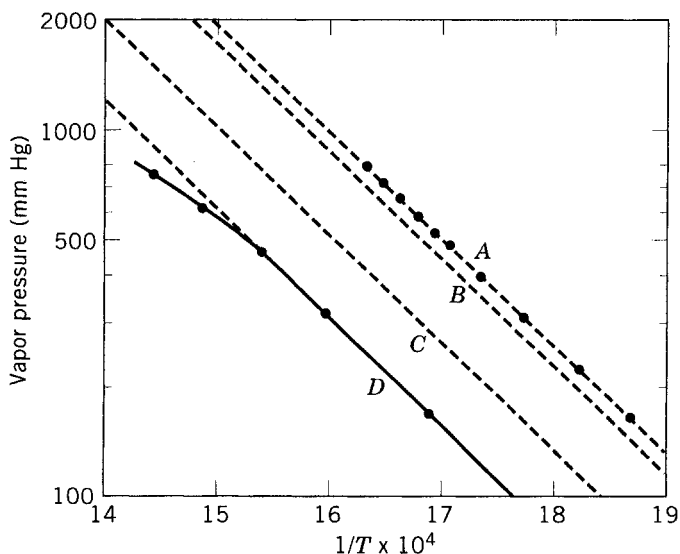
Oil in Mixture (wt. %)	Pressure kPa/(mm Hg)				
	101.3 (760)	81.3 (610)	61.3 (460)	41.3 (310)	21.3 (160)
0	66.7	60.0	51.1	40.6	26.7
50	70.0	62.8	54.4	43.9	27.8
60	72.2	65.6	56.1	45.6	29.4
70	77.2	69.4	60.0	48.9	32.8
80	85.6	77.8	67.8	55.6	38.9
85	93.9	85.6	75.0	62.2	45.0
90	110.6	98.9	87.2	72.8	53.9
92	120.0	107.8	95.0	80.6	61.1
94	133.9	121.1	106.7	88.9	68.9
95	142.8	131.1	114.4	95.0	73.9
96	—	—	123.3	101.7	80.6
97	—	—	132.8	110.0	87.8
98	—	—	—	120.0	97.2
99	—	—	—	133.3	109.4

stripper. All traces of hexane are removed from the water in the water stripper, and hexane-free effluent water leaves the system.

Care is taken not to overheat the oil, for prolonged heating of some oils, like cottonseed, “sets” the color and produces a permanently dark oil (158). The clarified, desolventized oil usually is degummed to remove the phospholipids. This ensures better oil quality if the oil is sold to firms that further process the oil. The gums are added to the marc going through the desolventizer. This raises the oil level of the extracted meal a few percentage points. Some plants may sell the phospholipids as crude lecithin.

Mixtures of dissolved oils and hexane or other hydrocarbon solvent exhibit a considerable negative deviation from the ideal; that is, the vapor pressure of the solvent is lower than that calculated from its molar concentration in the miscella and also that calculated for the pure solvent on the basis of Raoult's law. Below a solvent concentration of about 10% by weight, the boiling point becomes so high that steam stripping is essential in the final stages of solvent recovery. Boiling point and vapor pressure data on mixtures of commercial hexane with cottonseed and peanut oils have been published by Pollard et al. (208). Values for the boiling points of cottonseed-hexane mixtures at different pressures, as derived from Pollard's smooth data, are shown in Table 7.

Figure 29 plots vapor pressure curves for pure hexane, for commercial hexane experimentally determined, and for a 10% commercial hexane miscella, both as calculated for an ideal solution and as actually determined. The plots, which are on the conventional basis of log vapor pressure versus reciprocal of the absolute temperature to give straight lines, show that below a temperature of about 93.3°C (200°F)—which corresponds to a  $1/T$  value of 15.16—the actual vapor pressure

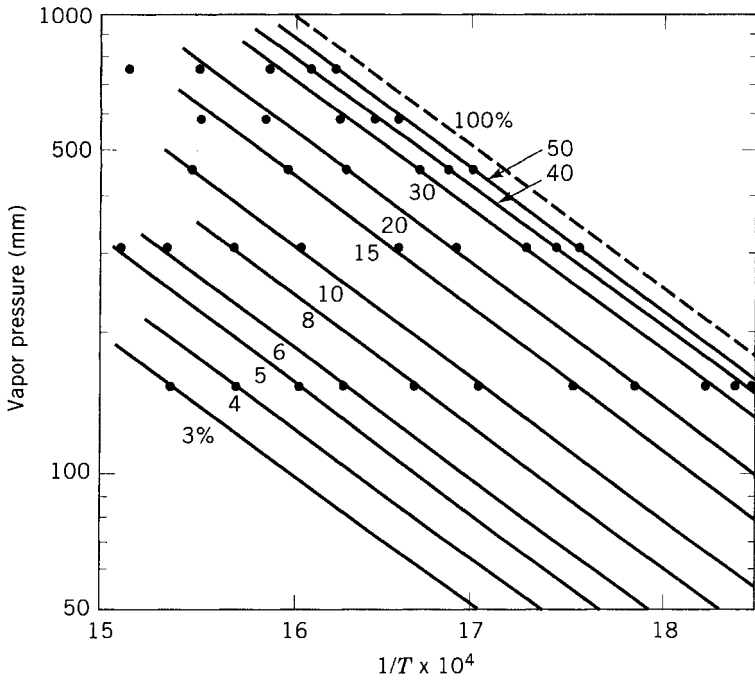


**Figure 29.** Vapor pressure curves: A, commercial hexane; B, pure hexane; C, 10% commercial hexane in mixture with cottonseed oil, ideal curve; D, same mixture, actual curve, according to data of Pollard et al. (208).

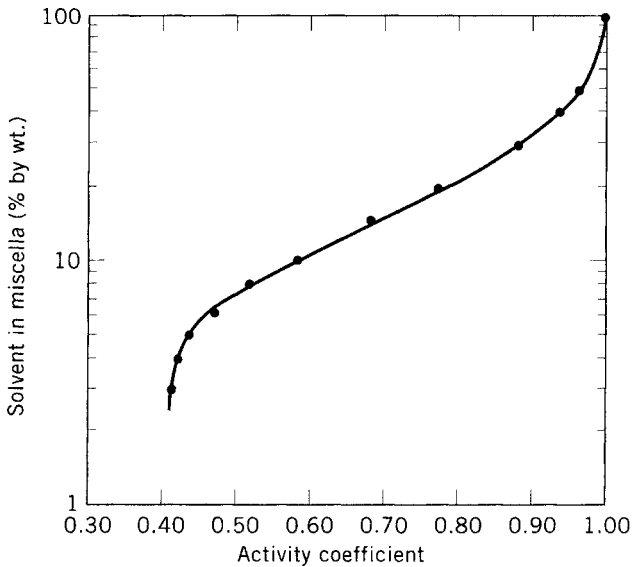
curve of the miscella is a straight line. The activity coefficient or the ratio of actual vapor pressure to ideal vapor pressure over the linear portion of the actual vapor pressure curve may be determined from Figure 29. At a  $1/T$  value of 16, for example, the actual vapor pressure is 310 mm, whereas the ideal vapor pressure is 520 mm; hence the activity coefficient is  $310/520 = 0.60$ .

Similar plots and calculations may be made for miscellas of other concentrations where experimental values have been determined at temperatures below  $93.3^{\circ}\text{C}$  ( $200^{\circ}\text{F}$ ); the graphical data are shown in Figure 30. Figure 31 shows activity coefficients in terms of the composition of the miscella. Data subsequently published by Smith and Wechter (209) on the vapor pressures of mixtures of soybean oil with hexane in the range of  $75\text{--}120^{\circ}\text{C}$  ( $167\text{--}248^{\circ}\text{F}$ ) and 2–36 mol% or 0.25–5.25 wt% (Figure 32) show activity coefficients generally between 0.50 and 0.60. For leaner miscellas, where boiling occurs at lower temperatures and steam stripping is not required, the data in Table 7 are directly applicable. The derived values in Table 7 were determined in a laboratory apparatus with vigorous stirring to prevent superheating the solvent. In ordinary apparatus, superheating may be expected.

Smith studied the effect of boiling point differences of various cuts of hexane on miscella (218). Smith also determined the vapor pressure of hexane–soybean oil solutions at high solvent concentrations (211). When making stripping calculations, one should be aware that the last portions of solvent to vaporize consist of higher boiling constituents in that particular cut of solvent.



**Figure 30.** Vapor pressure curves of commercial hexane in different concentrations by weight in mixtures with cottonseed oil, according to Pollard et al. (208).



**Figure 31.** Activity coefficients of commercial hexane mixed with cottonseed oil, according to the data of Pollard et al. (208).

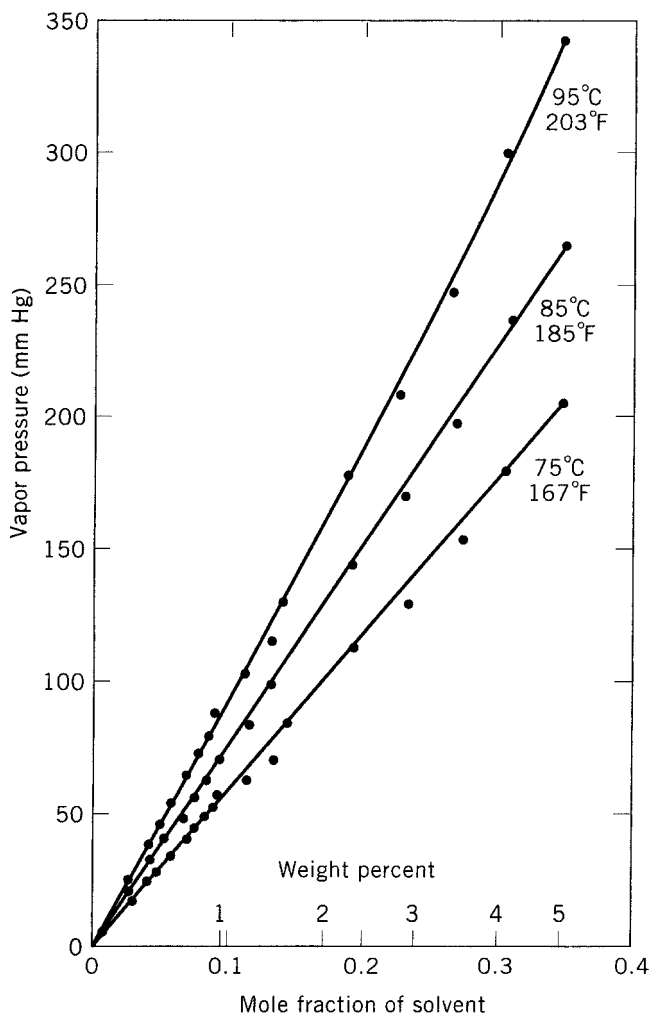


Figure 32. Vapor pressure curves for low concentrations of hexane in soybean oil (209).

## 7.2. Recovery from Marc

In the 1930s through the 1940s, especially in plants using immersion-type extractors, solvent was recovered from marc in a bank of horizontal cylindrical vessels interconnected so that the marc traveled through each vessel, dropping from a higher vessel to a lower one, and traveling in a zigzag fashion down through the bank of vessels. Originally developed in Germany, the desolventizer was sometimes called a *schneckens* (winding staircase) desolventizer. Each vessel was steam jacketed to provide heat and contained a revolving agitator to propel the marc. The last vessel employed a countercurrent flow of sparge steam to strip out the last traces of solvent.

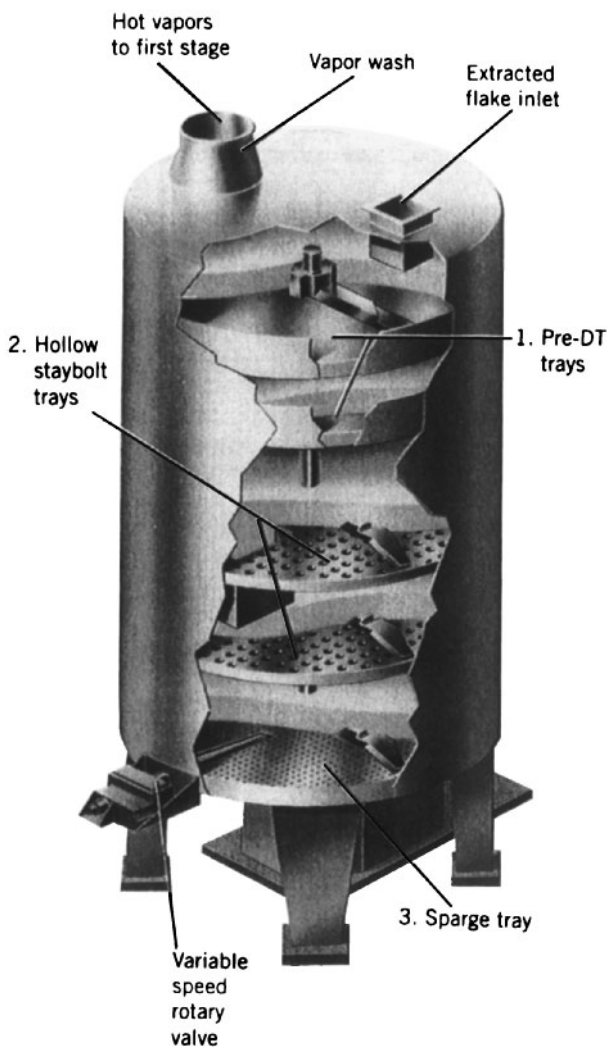
In the 1950s, with the advent of percolation extractors, a new type of desolventizer appeared (212). Termed the *desolventizer/toaster*, this vessel was shaped like a cylinder standing upright. Inside, it was divided into several horizontal sections, or "trays." A vertical shaft moved a sweep arm across the surface of each tray to keep the material agitated. The sweep arm also tripped a trap door built into each tray. This allowed product to fall from each tray to the tray below. The level in each tray was controlled by an adjustable linkage, activated by the material, that allowed the trap door to trip open only when the material was at the desired level. Heat was supplied by jackets on the tray bottoms and sometimes on the side walls. Sparge steam was used on the topmost trays to help flash off the solvent. The condensed sparge steam was absorbed by the solids, thereby, minimizing dust carry-over to the condenser. The sparge steam also partially cooked or "toasted" the solids. This was helpful for materials containing active enzymes, such as soybean, where the toasting inactivated urease.

Some stack desolventizers employ a true countercurrent flow of live (sparge) steam. Live steam is introduced under the bottom tray, which has a perforated bottom. The fresh steam migrates through the discharging material, stripping out the last traces of solvent. The steam then passes into the preceding tray through hollow staybolts that are dispersed throughout the tray (Figure 33). The steam migrates through the material on that tray and continues up through all the preceding trays. The top tray (sometimes more than one tray), called the *predesolventizing tray*, has a steam-jacketed bottom, thereby using indirect steam to flash off the surface solvent in the incoming marc.

This countercurrent desolventizer/toaster was originally developed in Germany by Heinz Schumacher and put into service in about 1982. Crown Iron Works Co. (Minneapolis, Minnesota), Extraction de Smet (Zaventem, Belgium), and Krupp Maschinentechnik (Hamburg, Germany) obtained licenses to build and sell this desolventizer in various countries of the world.

Another method of desolventization removes solvent at low temperature to avoid denaturing the protein. Called *flash desolventizing*, it exposes the marc to a recirculating stream of superheated vapor traveling at high velocity (Em. Corp., Des Plaines, Illinois) (213). Most of the solvent in the marc vaporizes and joins the superheated vapors, cooling them. Some recirculating vapor, roughly equal to what evaporated from the marc, is bled off through a rotary valve, scrubbed to remove fines, and condensed. The extracted material, still containing approximately 1% solvent, leaves the recirculation loop through a cyclone and rotary valve. The solid material then passes into a flake stripper where the remaining solvent is removed under conditions (low heat, low moisture) that do not denature or darken the protein.

High nitrogen solubility index (NSI), soy protein for manufacture of meat analogs is prepared in flash desolventizers. Some soybean extraction plants send part of their marc through a flash desolventizer to serve the high NSI soy flour market and the balance of their marc through desolventizer/toasters to serve the animal feed soy meal market. The first flash desolventizer was commissioned by EMI Corporation (Em. Corp., Des Plaines, Illinois) in 1960 (214).



**Figure 33.** Crown/Schumacher desolventizer-toaster (Courtesy of Crown Iron Works., Minneapolis, Minnesota).

### 7.3. Recovery from Effluent Streams

Aside from the two sought-after product streams in a solvent-extraction plant, there are two additional streams flowing out of the system: effluent air and effluent water.

*Recovery from Effluent Air.* Air enters the system trapped in the incoming material. Air also enters the system through leaks in the vessels, especially vessels operating under vacuum. Some vessels (the extractor, the desolventizer) are operated under a slight vacuum, 2.4–5 mm (1–2 in.) water column, to ensure that, if there

are leaks, air will leak in rather than solvent leaking out to cause a fire hazard. All this air leaves the system saturated with solvent vapors. If not properly handled, the effluent air could also contain solid particles.

Dust from the desolventizer is the source of solid particles. Desolventizer vapors are, therefore, usually sent through a cyclone-like device into which a mist of solvent is sprayed. The fines absorb the solvent, become heavier, and fall out of suspension. The “scrubbed” vapors continue to the condenser, whereas the solvent-laden fines return to the desolventizer.

Air is removed from the system by vent lines connected to all vessels likely to contain some incoming air: the extractor, the desolventizer, etc. A slight vacuum, induced by a vent fan, draws noncondensibles from all the condensers and vented vessels into a common header where they pass through a water-cooled vent condenser followed by some device to remove as much solvent vapors as practical and then pass through a flame arrestor before being released to the atmosphere. The simplest, least expensive device is a refrigerated condenser to lower the effluent air temperature. The air leaves the system saturated with solvent vapor; the lower the temperature, the less solvent in the air. A more effective method removes the solvent from the air by either absorption or by extraction.

Adsorption passes the air–solvent vapor mixture through a bed of activated charcoal. Most of the solvent vapors are adsorbed by the charcoal as the air, with some traces of solvent, passes through. When the charcoal is almost saturated with solvent, the air–solvent vapor mixture is sent to a second charcoal adsorber while the first one is cleansed with hot steam to strip out the solvent. The steam–solvent mixture is then condensed and sent to the solvent–water separator. Care should be exercised not to overload the adsorber with hexane vapors. Adsorption of hexane by carbon releases heat. If unmonitored and uncontrolled, the adsorber could catch on fire. This is especially likely to happen if a process upset sends a sudden surge of hexane vapors to the adsorber.

The other method, extraction, passes the air–solvent vapor mixture through a miniature solvent-extraction system using mineral oil as a solvent to “absorb,” or extract, the solvent vapor from the airstream. The mineral oil then goes to a steam stripper to strip out the solvent. The steam–solvent vapors are condensed and go to the solvent–water separator. The hot mineral oil from the stripper is chilled and goes back to the absorber where it picks up more solvent. Called *mineral oil scrubber*, the mineral oil is continuously recirculated through the absorber, the stripper, and the chiller.

The effluent air still contains some solvent vapor, but less than its saturation level. A well-functioning mineral oil scrubber will keep solvent loss below 1.89 L (0.5 gal) per metric ton of material processed.

*Recovery from Effluent Water.* All condensed mixtures of steam and solvent vapors go to a common solvent–water separator (mentioned above), where solvent overflows the top and water syphons out the bottom. The water, still containing traces of solvent, passes through a steam-heated *waste-water stripper*, where live steam raises the water temperature well above the boiling point of the solvent, 85°C (185°F) for hexane, which boils at 68.9°C (156°F). The driven-off solvent

is condensed and sent back to the solvent–water separator. The hot water goes to a large outdoor sump, and from there it leaves the system.

## 8. OBTAINING OIL FROM FRUIT PULPS

Fruit pulp oils of commercial importance are olive oil and palm oil. As these oils reside in the soft pulp of the fruit rather than in the kernel or seed, different techniques are used to separate the oil. Olive oil, being used primarily for cooking and as an oil topping on foods, is valued for its flavor. It is grown primarily in the regions surrounding the Mediterranean Sea and is probably the first oil produced by human effort. Palm fruit oil (not to be confused with palm kernel oil) serves the same markets as other vegetable oils and is grown on plantations in Malaysia, Indonesia, and parts of Africa.

### 8.1. Olive Oil

Olives are easily bruised, and, therefore, are carefully harvested, sometimes hand picked, to avoid any deterioration of flavor. They are normally processed within three days, otherwise the fatty acids increase and the oil quality deteriorates. Processing is done both in large modern plants and in small traditional oil mills. The olives are first washed, crushed or ground, and stirred into a thick paste (called malaxation) in agitated vats. The paste is then decanter-centrifuged or pressed to separate an oil–water emulsion from the solids (pumace). Pressing is done by spreading layers of the paste onto pressing bags and stacking the bags into a plate press. The bags are laid onto the bottom press plate in stacks of 25–50. Rigid plate guides are inserted after every fifth or sixth bag to ensure a more uniform distribution of pressure. When pressure is applied, the oil migrates from the paste into the cavities in the pressing bags and drips out of the press. The oil–water emulsion is centrifuged, and the separated oil is then clarified in a filter press using diatomaceous earth (215, 216). The modern use of a decanter centrifuge in recovering oil eliminates the labor-intensive steps of spreading the olive paste onto pressing bags and stacking the bags into a plate press. A decanter centrifuge also results in a more continuous process with higher productivity (215).

It has been found that a 5–10% increase in virgin oil yield can be realized by the addition of plant cell-wall degrading enzymes to the malaxer (217). The enzymes are usually added to early harvested (less ripe) olives. Olives harvested later, when they are more ripe, already contain internal enzymes that have the same effect as the added enzymes. Extraction produces three products: oil, an aqueous fraction that creates disposal problems, and a solids residue. The solid residue (pumace) obtained from pressing or centrifugation, contains 4–10% oil. The oil can be solvent extracted and refined. Solvent-extracted pumace oil is of low quality and is usually blended with a better quality virgin oil (215, 218).



## 8.2. Palm Oil

At one time, most of the palm oil on the market was extracted from fruit of wild-growing trees in Africa. Later, a superior quality oil was obtained from fruit grown on plantations in Indonesia, Malaysia, and Central Africa. Primitive extraction methods used for wild-growing fruit have been replaced by modern methods operating at much higher capacity for plantation-raised fruit.

The fruit grows in clusters on a central, branched stalk, similar to the way grapes grow, but the fruit is larger than grapes. The fruit consists of oily pulp surrounded by a tough outer skin and contains seeds (or kernels) imbedded in the pulp. Palm fruit oil is extracted from the pulp. Palm kernel oil is extracted from the seed.

The fruit is harvested in bunches, like grapes. The bunches are loaded into lorries or narrow-gauge railway cars that are taken to the extraction facility. The bunches are then loaded into sterilizing cars. Usually, some provision is made to allow sand, leaves, and other impurities to fall away as the sterilizer cars are loaded. The sterilizing cars are rolled into cylindrical sterilizing chambers. The chambers are horizontal cylinders open at one end (sometimes both ends) and having rail tracks for cages. The door is shut, and live steam is sparged into the chamber. The steam migrates through the bunches, bringing heat to all the fruit. The heat sterilizes the fruit to prevent bacterial or enzymatic activity from attacking the oil once the skin is broken. The length of time in the sterilization chamber depends on the maturity and average size of the fruit.

After sterilization, the palm fruit bunches pass through stripping/threshing equipment that removes the individual fruits from the stalks. Two types of stripping machines are available: drum strippers and beater strippers. The separated fruits are washed to remove sand and then go to a twin-screw-press to squeeze out the palm fruit oil. The oil is then clarified of moisture and solid matter that came out with the oil.

A typical twin-screw-press has two matching parallel wormshafts rotating in opposite directions and pushing the fruit through perforated cages against hydraulically operated conical chokes that apply back pressure. The liquid flows through holes in the cages. The solid residue flows over the cone chokes. This type of press is also used to press grapes and other soft and moist materials. A leading manufacturer is Usine de Wecker (Luxembourg), who supplied its first twin-screw-press in 1912 to press grapes for wine production.

The crude palm fruit oil, a mixture of oil, water, and solid impurities, is clarified in a continuous decanter, or in settling tanks, to remove the water and impurities. The cake from the screw-press consists of moist pulp solids, kernels (or seeds), and the outer skin of the fruit. The cake is sent to a pneumatic vertical column separator to separate the kernels from the fiber and cellular debris. The kernels are then "conditioned" by lowering their moisture level from about 16% to 10–12%. This causes the meats to shrink and pull loose from the shell. The kernels are then cracked and the meats are separated from the shells in one of two ways:

1. The cracked kernels are mixed into an aqueous slurry of clay or salt having a specific gravity of 1.18–1.20, where the kernels float and are skimmed off, and the shells sink.

2. The cracked kernels are mixed with water and pass through a liquid cyclone, where the heavier shells are pushed by centrifugal force against the outer wall and pass out the bottom, whereas the lighter meats float out the top.

The meats, now containing about 20% moisture, are dried to below 8% moisture and sent to storage for subsequent pressing in high-pressure screw-presses to produce palm kernel oil (219, 220).

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## REFERENCES

1. O. H. Alderks, in A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience, New York, 1948, ch. 15.
2. A. C. Wamble, in A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience, New York, 1948, ch. 14.
3. Staff editor, in *Proceedings of the World Conference on Oilseed and Edible Oil Processing*, *J. Am. Oil Chemists' Soc.* **60**, 189–248 (1983).
4. Several articles in A. R. Baldwin, ed., *Proceedings of the World Conference on Emerging Technologies in the Fats and Oils Industry*, AOCS Press, Champaign, Illinois, (1986).
5. M. A. Williams, "Extrusion of Rice Bran," in T. H. Applewhite, ed., *Proceedings of the World Conference, Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, AOCS Press, Champaign, Illinois (1989).
6. Several articles in D. R. Erickson, ed., *Proceedings of the World Conference on Edible Fats and Oils Processing: Basic Principles and Modern Practices*, AOCS Press, Champaign, Illinois (1990).
7. Several articles in T. H. Applewhite, ed., *Proceedings of the World Conference on Oilseed Technology and Utilization*, AOCS Press, Champaign, Illinois (1993).
8. M. A. Williams, *Lipids Industrial Applications*, Marcel Dekker, Inc., New York, 1996, ch. 5.
9. M. A. Williams, *Alternative Solvents for Extraction of Non-Petroleum Oils*, AOCS Press, Champaign, Illinois, 1996, ch. 6.
10. K. C. Rhee, in T. H. Applewhite, ed., *Proceedings of the World Conference on Oilseed and Vegetable Oil Processing Technology*, AOCS Press, Champaign, Illinois, 1993, pp. 346–351.
11. International Castor Oil Association. "The Processing of Castor Meal for Detoxification and Deallergenation," Technical Bulletin 1–1989. Ridgewood, New Jersey, 1989, pp. 58–62.
12. J. Horton and M. A. Williams, *J. Amer. Oil Chem. Soc.*, **66**, 227 (1989).
13. Several articles in *proceedings of the World Conference on Oilseed and Vegetable Processing Technology*, *J. Amer. Oil Chem. Soc.*, **66**, 221 (1976).

14. R. Stokes, L. M. Reid, R. P. Hutchins, and J. W. Dunning, *J. Amer. Oil Chem. Soc.*, **33**, 453 (1956).
15. J. G. Fawbush, *J. Amer. Oil Chem. Soc.*, **54**, 474A (1976).
16. J. M. Riddlehuber, *J. Amer. Oil Chem. Soc.*, **54**, 477A (1976).
17. A. Garcia-Sebrrato, *J. Amer. Oil Chem. Soc.*, **54**, 483A (1977).
18. P. Molabrigo, *J. Amer. Oil Chem. Soc.*, **54**, 485A (1977).
19. R. P. Hutchins, *J. Amer. Oil Chem. Soc.*, **53**, 279 (1976).
20. D. W. Petty, presented at Texas A & M University, College Station, Texas, Oct 22, 1979, unpublished.
21. L. A. Johnson, S. P. Clark, and E. W. Lusas, *Effects of Residual Linters on Oil and Protein Extraction from Cottonseed*, Final Report to USDA, Research Agreement No. 58-7B30-9-147. Texas A&M Research Foundation No. 4155, 1981.
22. L. A. Johnson, J. T. Farnsworth, E. W. Lusas, and C. Cox, *An Economic Analysis of Milling Cottonseed with Different Residual Linter Contents*, Final Report to USDA. Research Agreement No. 58-7B30-1-354. Texas A&M. TEES. Food Protein Research and Development Center, Reprint No's, 278, 288, 1983.
23. G. Florin and H. R. Bartsch, *J. Amer. Oil Chem. Soc.*, **60**, 193 (1983).
24. W. Fetzer, in T. H. Applewhite, ed., *Proceedings of the World Congress. Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, AOCS Press, Illinois, 1989, pp. 518-520.
25. G. D. Brueske, in T. H. Applewhite, ed., *Proceedings of the World Conference on Oilseed and Vegetable Oil Processing Technology*, AOCS Press, Champaign, Illinois, 1993, pp. 346-351.
26. J. R. Smith, in D. R. Erickson, ed., *World Conference Proceedings, Edible Fats and Oils Processing: Basic Principles and Modern Practices*, AOCS Press, Champaign, Illinois, 1990, pp. 324-330.
27. Z. Lebowitzky and C. Ruckenstein, in A. R. Baldwin, ed., *Proceedings of the World Conference on Emerging Technologies in the Fats and Oils Industry*, AOCS Press, Champaign, Illinois, 1986, pp. 208-212.
28. E. Chone, Y. Delaine, and O. Lapierre, in A. R. Baldwin, ed., *Proceedings of the World Conference on Emerging Technologies in the Fats and Oils Industry*, AOCS Press, Champaign, Illinois, 1986, pp. 236-242.
29. B. de Ramecourt, *J. Amer. Oil Chem. Soc.*, **53**, 256 (1976).
30. A. M. Goldovskii and M. Podol'skaia, *Maslob.-Zhir. Delo*, **10**(4), (1934).
31. J. O. Osburn and D. L. Katz, *Trans. Amer. Inst. Chem. Eng.*, **40**, 511 (1944).
32. G. Karnofsky, *Proceedings of a Six Day Short Course in Vegetable Oils*, American Oil Chemists' Society. University of Illinois, Champaign, Illinois, 1948, pp. 61-69.
33. W. R. Woolrich and E. L. Carpenter, *Mechanical Processing of Cottonseed*, Engineering Experimental Station, University of Tennessee, Knoxville, Tennessee, 1935.
34. A. C. Wamble, in A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience, New York, 1948, Ch. 14.
35. H. Schumacher, in T. H. Applewhite, ed., *Proceedings of the World Congress, Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, AOCS Press, Champaign, Illinois, 1989, pp. 37-40.
36. P. P. Hutchins, *J. Amer. Oil Chem. Soc.*, **33**, 457 (1949).

37. G. Karnofsky, *J. Amer. Oil Chem. Soc.*, **26**, 564 (1949).
38. J. P. Galloway, *J. Amer. Oil Chem. Soc.*, **53**, 271 (1976).
39. H. C. Dormitzer, *J. Amer. Oil Chem. Soc.*, **33**, 471 (1956).
40. C. B. Rose, *J. Amer. Oil Chem. Soc.*, **31**, 498 (1954).
41. W. C. Ault, *Progress in the Chemistry of Fats and Other Lipids*, vol. 5, Pergamon, New York, 1958, ch. 9.
42. R. J. Sims and L. Hilfman, *J. Amer. Oil Chem. Soc.*, **33**, 381 (1956).
43. F. Norris, *Bailey's Industrial Oil and Fat Products*, 4th ed., vol. 2, John Wiley & Sons, Inc., 1982, pp. 190–193.
44. W. R. Dayen, K. M. Christensen, and R. E. Morse, *Food Technol.*, **7**, 421 (1953).
45. J. E. Thompson, *Food Eng.*, **26**, 72, 102 (1954).
46. F. E. Sullivan, *J. Amer. Oil Chem. Soc.*, **36**, 70 (1959).
47. F. P. Downing, *J. Amer. Oil Chem. Soc.*, **36**, 319 (1959).
48. I. H. Chayen (to British Glues and Chemicals, Ltd.), U.S. Patent 2,635,104 1953.
49. R. Chayen, *Chem. Proc. Eng.*, **41**, 14 (1960).
50. N. J. Smallwood, in D. R. Erickson, ed., *World Conference Proceedings, Edible Fats and Oils Processing: Basic Principles and Modern Practices*, AOCS Press, Champaign, Illinois, 1990, pp. 246–249.
51. C. Greenfield, U.S. Patent 2,651,647, September 8, 1953.
52. C. Greenfield, U.S. Patent 3,076,715, February 5, 1963.
53. C. Greenfield, U.S. Patent 3,251,398, May 17, 1966.
54. R. R. Perry, A. G. Maran, and A. G. Schols: [to International Basic Economy Corp. (owners of V. D. Anderson Co.)]. U.S. Patent 4,259,252, March 31, 1981.
55. Staff editor, "High Tech Rendering," *Meat Processing*, 26–28 (May, 1983).
56. Staff editor, "U.S. Technology plus Dutch Determination Saves Energy," *Render*, 10, 11, 25. (Feb. 1980).
57. F. E. Deatherage, *J. Amer. Oil Chem. Soc.*, **23**, 327 (1946).
58. F. E. Deatherage (to the Kroger Grocery & Baking Co.), U.S. Patent 2,456,684, 1948.
59. K. E. Youseef, A. Y. Latfi, and A. A. Esmail, *Acta Agronomica Academiae Scientiarum Hungaricae*, **23**(1/2), 178 (1974).
60. K. E. Youseef, A. Y. Latif, and A. A. Esmail, *Food Sci. Technol. Agstr.*, **8**, 6N218 (1976).
61. I. A. Parfentjev, U.S. Patent 2,395,790, 1946.
62. H. L. Keil (to Armour and Company), U.S. Patent 2,423,102, 1947.
63. P. Halmbacker (to Paul Lewis Laboratories, Inc.), U.S. Patent 2,527,305, 1950.
64. I. Nakamoto, Japanese Patent 154,087, 1942.
65. C. E. Lane (to the Borden Company), U.S. Patent 2,550,570, 1951.
66. McGraw-Hill World News, *Food Eng.*, **24**(10), 143 (1954).
67. D. W. Freeman, in D. Yocum, ed., *Proceedings of the 11th Annual Meeting, Inland Commercial Fisheries Association*, Arkansas Game and Fish Commission, Little Rock, Arkansas, 1982, pp. 1–10.
68. G. B. Verburg and D. W. Freeman, *Aquaculture*, **41**, 171 (1984).
69. L. C. Berardi and L. A. Goldblatt, in I. E. Liener, ed., *Toxic Constituents of Plant Foodstuffs*, Academic Press, New York, 1969, ch. 8.

70. L. K. Arnold and D. J. Patel, *J. Amer. Oil Chem. Soc.*, **30**, 216 (1953).
71. H. D. Royce and F. A. Lindsey, *Ind. Eng. Chem.*, **25**, 1047 (1933).
72. A. C. Wamble and W. B. Harris, *Tex. Eng. Expt. Sta Bull. No. 136*, (1954).
73. P. H. Eaves, L. J. Molaison, E. F. Pollard, and J. J. Spadaro, *J. Amer. Oil Chem. Soc.*, **34**, 156 (1957).
74. J. W. Dunning and R. J. Terstage, *J. Amer. Oil Chem. Soc.*, **29**, 153 (1952).
75. W. H. King et al., *J. Amer. Oil Chem. Soc.*, **33**, 71 (1956).
76. I. Liener, in A. M. Altschul, ed., *Processed Plant Protein Foodstuffs*, Academic Press, New York, 1958, ch. 5.
77. J. C. Cowan, in B. E. Caldwell, ed., *Soybeans*, American Society of Agronomy, Inc., Madison, Wisconsin, 1973, ch. 20.
78. R. P. Hutchins (to The French Oil Mill Machinery Co.), U.S. Patent 2,695,495, 1954.
79. K. C. Rhee, in T. H. Applewhite, ed., *Proceedings of the World Conference on Oilseed Technology and Utilization*, AOCS Press, Champaign, Illinois, 1993, pp. 346–351.
80. M. C. Calhoun, J. E. Huston, C. B. Calk, B. C. Baldwin, Jr., and S. W. Kuhlmann, in L. A. Jones, D. H. Kindard, and J. S. Mills, eds., *Cattle Research with Gossypol Containing Feeds*, National Cottonseed Products Association, Memphis, Tennessee, 1991, pp. 39–51.
81. E. Eagle, L. E. Castillon, C. M. Hall, and C. H. Boatner, *Arch. Biochem.*, **18**, 271 (1948).
82. E. Eagle, H. F. Bialek, D. L. Davis, and J. W. Bremer, *J. Amer. Oil Chem. Soc.*, **31**, 121 (1954).
83. E. Eagle, H. F. Bialck, D. L. Davis, and J. W. Bremer, *J. Amer. Oil Chem. Soc.*, **33**, 15 (1956).
84. W. A. Pons and P. H. Eaves, U.S. Patent 3,557,168, 1971.
85. G. E. Mann, F. L. Carter, V. L. Frampton, A. B. Watts, and Charles Johnson, *J. Amer. Oil Chem. Soc.*, **39**, 86 (1962).
86. R. J. Hron, Sr., and S. P. Koltun, *J. Amer. Oil Chem. Soc.*, **61**, 1457 (1984).
87. G. Abraham, K. M. Decossas, R. J. Hron, Sr., and M. S. Kuk, *J. Amer. Oil Chem. Soc.*, **68**, 418 (1991).
88. R. J. Hron, Sr., M. S. Kuk, G. Abraham, and P. J. Wan, *J. Amer. Oil Chem. Soc.*, **71**, 417 (1994).
89. R. J. Hron, Sr., G. Abraham, M. S. Kuk, and G. S. Fisher, *J. Amer. Oil Chem. Soc.*, **69**, 951 (1992).
90. G. Abraham, R. J. Hron, Sr., M. S. Kuk, and P. J. Wan, *J. Amer. Oil Chem. Soc.*, **70**, 207 (1992).
91. E. W. Lusas, L. R. Watkins, and S. S. Koseoglu, *Inform*, **2**, 970 (1991).
92. J. W. Dunning, *Oil Mill Gaz.*, **54**, 20 (1950).
93. M. K. Thornton, *Oil Mill Gaz.*, **41**, 11 (1937).
94. C. W. Zies [to International Basic Economy Corp. (Owners of V. D. Anderson Co.)], U.S. Patent 3,108,530, October 29, 1963.
95. M. A. Williams, R. E. Horn, and R. P. Rugala, *Food Eng.*, **49**, 99 (1977).
96. M. A. Williams, R. E. Horn, and R. P. Rugala, *Food Eng.*, **49**, 87 (1977).
97. C. W. Zies [to International Basic Economy Corp. (Owners of V.D. Anderson Co.)], U.S. Patent 3,222,797, December 14, 1965.

98. M. A. Williams, *Agribusiness Worldwide*, 10–11, 16 (June, 1986).
99. S. Baer, M. A. Williams, and C. W. Zies [to International Basic Economy Corp. (Owners of V. D. Anderson Co.)], U.S. Patent 3,255,220, June 7, 1966.
100. M. A. Williams and S. Baer, *J. Amer. Oil Chem. Soc.*, **42**, 151 (1965).
101. J. T. Farnsworth, L. A. Johnson, J. P. Wagner, L. R. Watkins, and E. W. Lusas, *Oil Mill Gaz.*, **91**, 30 (1986).
102. L. R. Watkins, W. H. Johnson, and S. C. Doty, in T. H. Applewhite, ed., *The Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, AOCS Press, Champaign, Illinois, 1989, pp. 41–46.
103. E. W. Lusas and L. R. Watkins, *J. Amer. Oil Chem. Soc.*, **65**, 1109 (1988).
104. M. A. Williams, *Oil Mill Gaz.*, **91**, 25 (1986).
105. M. A. Williams [to Anderson International Corp. (formally The V.D. Anderson Co.)], U.S. Patent 4,901,635, February 20, 1990.
106. M. A. Williams, *Oil Mill Gaz.*, **95**, 10 (1989).
107. M. A. Williams, *Inform*, **1**, 959 (1990).
108. A. I. Nelson, W. B. Wijeratne, S. W. Yeh, T. M. Wei, and L. S. Wei, *J. Amer. Oil Chem. Soc.*, **64**, 1341 (1987).
109. F. Norris, *Bailey's Industrial Oil and Fat Products*, 4th ed., vol. 2, John Wiley & Sons, Inc., New York, 1982, pp. 201–207.
110. Valerius D. Anderson, U.S. Patent 647,354, April 10, 1900.
111. U.S. Trademark, Reg. No. 537,523, renewed May 22, 1991.
112. R. T. Anderson (to the V. D. Anderson Co.), U.S. Patent 2,365,442, December 19, 1942.
113. R. T. Anderson (to the V. D. Anderson Co.), U.S. Patent 2,420,927, May 28, 1947.
114. M. A. Williams, *Oil Mill Gaz.*, **96**, 38 (1991).
115. J. W. Dunning (to the V. D. Anderson Co.), U.S. Patent 2,551,254, May 1, 1951.
116. AMS "Market. Res. Rept. No. 360," U.S. Department of Agriculture (1959).
117. H. P. J. Jongeneelen, *J. Amer. Oil Chem. Soc.*, **53**, 291 (1976).
118. L. F. Hawley, *Ind. Eng. Chem.*, **9**, 866 (1917).
119. L. Silberstein, *Ind. Eng. Chem.*, **20**, 899 (1928).
120. S. D. Turner, *Ind. Eng. Chem.*, **21**, 190 (1929).
121. E. A. Ravenscroft, *Ind. Eng. Chem.*, **28**, 851 (1936).
122. B. F. Ruth, *Chem. Eng. Progr.*, **44**, 71 (1948).
123. J. A. Grosberg, *Ind. Eng. Chem.*, **42**, 154 (1950).
124. G. Abraham, R. J. Hron, Sr., and S. P. Koltun, *J. Amer. Oil Chem. Soc.*, **65**, 129 (1988).
125. D. F. Boucher, J. C. Brier, and J. O. Osburn, *Trans. Amer. Inst. Chem. Eng.*, **38**, 967 (1942).
126. H. P. Fan, J. C. Morris, and H. Wakeham, *Ind. Eng. Chem.*, **40**, 195 (1948).
127. J. O. Osburn and D. L. Katz, *Trans. Amer. Inst. Chem. Eng.*, **40**, 511 (1944).
128. M. R. Wingard and W. C. Shand, *J. Amer. Oil Chem. Soc.*, **26**, 422 (1949).
129. C. O. King, D. L. Katz, and J. C. Brier, *Trans. Amer. Inst. Chem. Eng.*, **40**, 533 (1944).
130. H. B. Coats and G. Karnofsky, *J. Amer. Oil Chem. Soc.*, **27**, 51 (1950).
131. G. Karnofsky, *J. Amer. Oil Chem. Soc.*, **63**, 1011 (1986).
132. G. Karnofsky, *J. Amer. Oil Chem. Soc.*, **64**, 1533 (1987).

133. W. C. Bull and T. H. Hopper, *Oil Soap*, **18**, 219 (1941).
134. W. H. Goss, *Oil Soap*, **23**, 348 (1946).
135. M. R. Wingard and R. C. Phillips, Abstracts of Papers, 41st Annual Meeting of American Oil Chemists' Society, May 1-3, 1950.
136. H. B. Coats and M. R. Wingard, *J. Amer. Oil Chem. Soc.*, **27**, 93 (1950).
137. D. F. Othmer and J. C. Agarwal, *Chem. Eng. Progr.*, **51**, 372 (1955).
138. K. M. Decossas, H. G. Many, O. J. McMillan, Jr., E. A. Gastrock, and E. F. Pollard, *Ind. Eng. Chem.*, **49**, 930 (1957).
139. H. W. Haines, Jr., G. C. Perry, and E. A. Gastrock, *Ind. Eng. Chem.*, **49**, 920 (1957).
140. D. F. Othmer and W. A. Jaatinen, *Ind. Eng. Chem.*, **51**, 543 (1959).
141. N. W. Myers, *J. Amer. Oil Chem. Soc.*, **54**, 491A (1977).
142. R. P. Pavlik and T. G. Kemper, *Inform*, **1**, 200 (1990).
143. A. E. MacGee, *Oil Mill Gaz.*, **51** (1947).
144. A. E. MacGee, *Cotton Gin Oil Mill Press*, (1947).
145. P. H. Eaves, L. J. Molaison, C. C. Blak, A. J. Crovetto, and E. L. D'Aquin, *J. Amer. Oil Chem. Soc.*, **29**, 88 (1952).
146. E. W. Lusas, L. R. Watkins, and K. C. Rhee, in D. R. Erickson, ed., *Proceedings of the World Conference on Edible Fats and Oils Processing: Basic Principles and Modern Practices*, AOCS Press, Champaign, Illinois, 1990, pp. 56-78.
147. A. L. Ayers and J. J. Dooley, *J. Amer. Oil Chem. Soc.*, **25**, 372 (1948).
148. A. L. Ayers and C. R. Scott, *J. Amer. Oil Chem. Soc.*, **28**, 348 (1951).
149. P. J. Wakelyn, *Cotton Gin and Oil Mill Press*, **93**(7), 12 (1991).
150. P. J. Wan, *Oill Mill Gaz.*, **100**, 32 (1994).
151. NFPA 36, *Solvent Extraction Plants*, National Fire Protection Association, **36**, Quincy, Massachusetts, (1993).
152. J. J. Duncon, *J. Amer. Oil Chem. Soc.*, **25**, 277 (1949).
153. O. R. Sweeney and L. K. Arnold, *J. Amer. Oil Chem. Soc.*, **26**, 697 (1949).
154. O. R. Sweeney and L. K. Arnold (to Iowa State College Research Foundation), U.S. Patent 2,497,700, (1950).
155. J. A. Price, *Proceedings of a Conference on Cottonseed Protein Concentrates*, USDA, ARS, 72-38, New Orleans, Louisiana, January 15-17, 1964, pp. 185-197.
156. J. J. McKinney et al., *J. Amer. Chem. Soc.*, **79**, 3932 (1957).
157. J. J. McKinney et al., *J. Amer. Chem. Soc.*, **81**, 909 (1959).
158. L. A. Johnson et al., *J. Amer. Chem. Soc.*, **63**, 647 (1986).
159. R. J. Hron, Sr., S. P. Koltun, and A. V. Graci, Jr., *J. Amer. Oil Chem. Soc.*, **59**, 674A (1982).
160. H. Dominguez, M. J. Nunez, and J. M. Lema, *Food Chem.*, **49**, 271 (1994).
161. F. M. Christensen, *Inform*, **2**, 984 (1991).
162. C. Vaccarino, *J. Amer. Oil Chem. Soc.*, **38**, 143 (1961).
163. C. Vaccarino, *Oleagineux*, **13**, 233 (1958).
164. M. Sato, T. Inaba, and K. Kitagawa, *J. Soc. of Chem. Indus. Jpn.*, **37**, 718b (1934).
165. S. Okatoma, *Contemporary Manchuria*, **3**, 83 (1937).
166. W. D. Harris and J. W. Hayward, *Tex. Agr. Expt. Sta. Bull. No. 121*, September 1950, p. 72.

167. W. D. Harris and J. W. Hayward, *J. Amer. Oil Chem. Soc.*, **27**, 273 (1950).
168. A. C. Beckel and J. C. Cowan (to the United States of America as represented by the Secretary of Agriculture), U.S. Patent 2,584,108, February 5, 1952.
169. F. C. Magne and E. L. Skau, *J. Amer. Oil Chem. Soc.*, **30**, 288 (1953).
170. R. K. Rao, M. G. Krishna, S. H. Zsheer, and L. K. Arnold, *J. Amer. Oil Chem. Soc.*, **32**, 420 (1955).
171. R. K. Rao and L. K. Arnold, *J. Amer. Oil Chem. Soc.*, **33**, 82, 389 (1956).
172. R. K. Rao and L. K. Arnold, *J. Amer. Oil Chem. Soc.*, **35**, 277 (1958).
173. G. B. Karnofsky and R. J. Hansotte (to Dravo Corp.) U.S. Patent 4,359,417, November 16, 1982.
174. G. B. Karnofsky, *Oil Mill Gaz.*, **86**, (1981).
175. R. J. Hron, Sr., M. S. Kuk, G. Abraham, and P. J. Wan, *J. Amer. Oil Chem. Soc.*, **71**, 417 (1994).
176. G. Abraham, K. M. Decossas, R. J. Hron, Sr., and M. S. Kuk, *J. Amer. Oil Chem. Soc.*, **68**, 418 (1991).
177. E. W. Lusas et al., *Inform*, **5**, 1245 (1994).
178. G. E. Mann, F. L. Carter, V. L. Frampton, A. B. Watts, C. Johnson, *J. Amer. Oil Chem. Soc.*, **39**, 86 (1962).
179. R. J. Hron, Sr. and M. S. Kuk, *J. Food Sci.*, **54**, 1088 (1989).
180. J. B. Hannay and J. Hogarth, *Proc. Royal Soc.*, **29**, 324 (1985).
181. G. R. List and J. P. Friedrich, *J. Amer. Oil Chem. Soc.*, **62**, 82 (1985).
182. J. P. Friedrich, G. R. List, and A. J. Heakin, *J. Amer. Oil Chem. Soc.*, **59**, 288 (1982).
183. J. P. Friedrich and E. H. Pryde, *J. Amer. Oil Chem. Soc.*, **61**, 223 (1984).
184. G. R. List, J. P. Friedrich, and J. Pominski, *J. Amer. Oil Chem. Soc.*, **61**, 1847 (1984).
185. G. R. List, J. P. Friedrich, and D. D. Christianson, *J. Amer. Oil Chem. Soc.*, **61**, 1849 (1984).
186. W. Zhao, A. Shishikura, K. Fujimoto, K. Arai, and S. Saito, *Agr. Biol. Chem.*, **51**, 1773 (1987).
187. L. A. Johnson and E. W. Lusas, *J. Amer. Oil Chem. Soc.*, **60**, 229 (1983).
188. K. Hilderbrand, German Patent 528,287, 1931.
189. K. Hilderbrand, German Patent 547,040, 1932.
190. K. Hilderbrand, U.S. Patent 1,961,420, 1934.
191. M. Bonotto, (to American M, Soya Products, Inc.), U.S. Patent 2,086,81, 1937.
192. M. Bonotto, (to Extractol Process, Ltd.), U.S. Patent 2,112,805, 1938.
193. M. Bonotto, (to Extractol Process, Ltd.), U.S. Patent 2,156,236, 1939.
194. M. Bonotto, (to Extractol Process, Ltd.), U.S. Patent 2,184,248, 1939.
195. L. F. Lanqhurst (to The V. D. Anderson Co.), U.S. Patent 2,441,200, May 11, 1948.
196. R. T. Anderson, U.S. Patent 2,548,333, April 10, 1951.
197. H. Bollman, German Patent 322,446, 1920.
198. H. Bollman, British Patent 156,905, 1921.
199. H. Bollman (to Hansa. Möhlenbau and Industric. A.C.), German Patent 670,283, 1939.
200. H. Bollman, British Patent 507,465, 1939.



201. C. W. Zies [to International Basic Economy Corp. (owners of V. D. Anderson Co.)], U.S. Patent 3,159,457, December 1, 1964.
202. G. Karnofsky, *J. Amer. Oil Chem. Soc.*, **26**, 570 (1949).
203. K. McKubbin and G. J. Ritz, *Cotton Gin Oil Mill Press*, **52**(6), 40 (1950).
204. K. McKubbin and G. J. Ritz, *Chem. Ind.*, **66**, 354 (1950).
205. G. Karnofsky, *Chem. Eng.*, **57**(8), 108 (1950).
206. E. D. Milligan, *J. Amer. Oil Chem. Soc.*, **53**, 286 (1976).
207. R. P. Hutchins, *J. Amer. Oil Chem. Soc.*, **33**, 457 (1956).
208. E. F. Polland, H. L. E. Vix, and E. A. Gastrock, *Ind. Eng. Chem.*, **37**, 1022 (1945).
209. A. S. Smith and F. J. Wechter, *J. Amer. Oil Chem. Soc.*, **27**, 381 (1950).
210. A. S. Smith, *J. Amer. Oil Chem. Soc.*, **28**, 356 (1951).
211. A. S. Smith and B. Florence, *J. Amer. Oil Chem. Soc.*, **28**, 360 (1951).
212. N. F. Kruse (to Central Soya Company, Inc.), U.S. Patent 2,585,793 1952.
213. O. L. Brekke, G. C. Mustakas, M. C. Raether, and E. L. Griffin, *J. Amer. Oil Chem. Soc.*, **36**, 256 (1959).
214. E. D. Milligan and J. F. Suriano, *J. Amer. Oil Chem. Soc.*, **51**, 158 (1974).
215. S. Latta, *Inform*, **2**, 99 (1991).
216. *Olive Oil Technology*, Food and Agriculture Organization of the United Nations, Rome, Italy, 1975.
217. F. M. Christensen, *Inform*, **2**, 984 (1991).
218. E. Fedeli, *J. Amer. Oil Chem. Soc.*, **60**, 356A (1983).
219. K. G. Berger, *J. Amer. Oil Chem. Soc.*, **60**, 158A (1983).
220. A. Southworth, *J. Amer. Oil Chem. Soc.*, **62**, 250 (1985).

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## *Storage, Handling, and Transport of Oils and Fats*

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### 1. INTRODUCTION

Damage to oils during storage, and the resulting loss in quality and yield, can occur because of deterioration-adverse chemical changes caused by the intrinsic nature of oils; contamination from natural substances associated with the source of the oil, traces of pesticides, and that introduced by oil processing or in transit; and adulteration-deliberate admixture of one oil with another.

#### 1.1. Deterioration of Oils and Fats

*Hydrolysis*, the reaction of fats and oils with water, and *oxidation*, the chemical reaction in which oxygen combines with another substance with the liberation of heat, are the two basic processes that result in the deterioration of oils and fats. Oxidation is mostly responsible for much more of the deterioration of fats and oils than hydrolysis.

Moisture promotes the splitting of triacylglycerols to form free fatty acids, mono- and diacylglycerols (Figure 1), which result in increase of refining losses directly related to the free fatty acid content of oils and fats. Essentially, hydrolysis

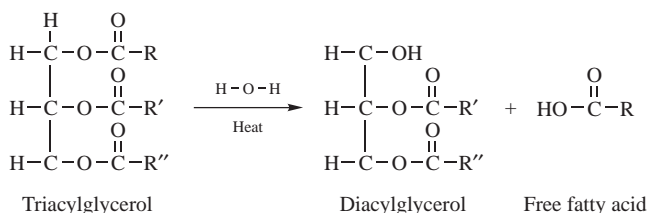


Figure 1. Hydrolysis of fats and oils.

is the reverse of making a fat molecule. This process typically requires a fat-soluble catalyst, high temperature ( $>100^\circ\text{C}$ ), and long time (several hours) (1). Partial hydrolysis of lipids typically occurs because of improper seed storage and handling, such as high moisture content, high temperature, and seed mechanical damage. Such reaction is usually catalyzed by the lipases naturally present in the oilseeds. The conditions used during oil extraction can also significantly affect such lipase catalyzed reaction (2). The deterioration of a fat or oil in the presence of oxygen is termed oxidative rancidity. The initial step in the oxidation of an oil or fat is the addition of oxygen at or near the double bond of a fatty acid chain to form unstable compounds generally designated as peroxides (Figure 2) (3).

There are three types of lipid oxidation: autoxidation, photo-oxidation (or photo-oxygenation), and enzyme-catalyzed oxidation. Lipid autoxidation is a free radical chain reaction that involves the initiation, propagation, and termination steps. The initiators for the initiation reaction are free radicals produced by (1) thermal dissociation of hydroperoxides, (2) metal-catalyzed decomposition of hydroperoxides, and (3) photosensitization of certain chemicals (4). The initial peroxides can either be impurities or formed between lipid and the very active singlet oxygen (1500 times more active than triplet oxygen), which is essentially photo-oxidation. Singlet oxygen is produced from the ordinary triplet oxygen by light in the presence of a sensitizer, such as chlorophyll and methylene blue. Singlet oxygen is added directly to the double bond by the concerted "ene" addition mechanism, and the peroxides formed are different from those formed by autoxidation (4). Photo-oxidation is not a chain reaction and is not affected by free radical quencher type of antioxidant, but

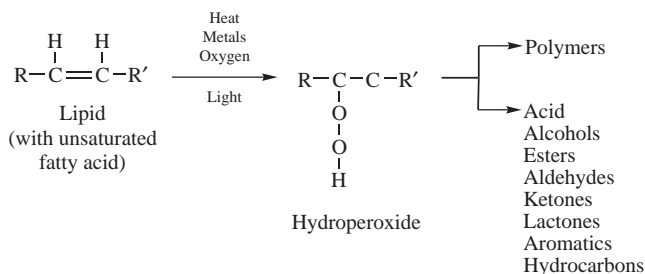
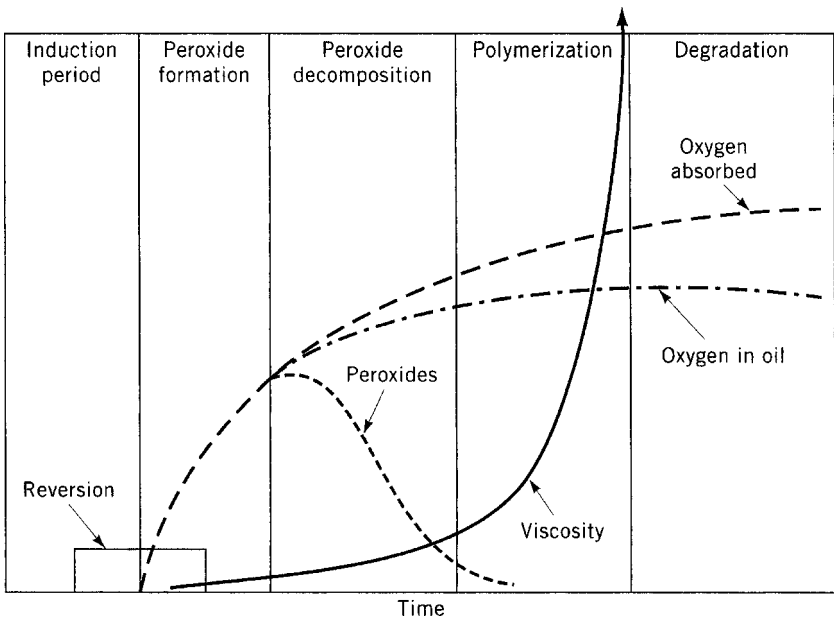


Figure 2. Oxidation of fats and oils.



**Figure 3.** Stages of progressive deterioration of fats through oxidation (5).

the reaction is inhibited by singlet oxygen quenchers such as carotenoids. Direct photo-oxidation of lipids is generally of less concern than autoxidation because light absorption at less than 220 nm cannot reach lipids unless they are exposed to sunlight or fluorescent light without proper protection. The peroxides initially present in the oil as impurities can also be produced by enzymatic oxidation. Various lipases and lipoxygenases are naturally occurring in plants or animals. The enzyme-catalyzed oxidation start with the hydrolysis of the lipid, and then only the polyunsaturated free fatty acids (pentadiene) are oxidized by lipoxygenases.

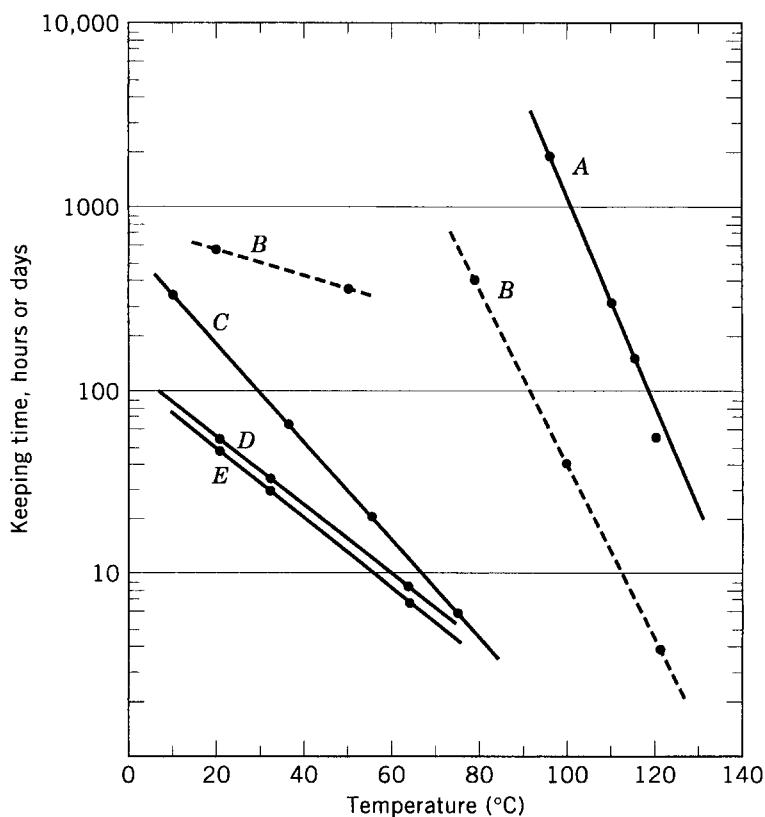
When the oxidation of an oil is followed experimentally, either by measuring the amount of oxygen absorbed or determining the peroxide value, the course of oxidation is defined by the oxygen absorbed in the oil, as shown in Figure 3 (5). During the initial or induction phase, oxidation proceeds at a relatively slow and uniform rate. Peroxides are formed during this period at a faster rate than they are destroyed so that their content increases in conjunction with the oxygen absorption. After a certain critical amount of oxidation has occurred, the reaction enters a second phase, characterized by a rapidly accelerating rate of oxidation. The point at which the sample begins to smell and taste rancid coincides with the beginning or early part of the second phase. As oil oxidation continues, with time, the peroxides that are formed decompose to generate volatile and nonvolatile compounds that contribute to flavor and odor deterioration of oils and fats. The extreme stages of oxidation, polymerization, and degradation are accompanied by rapid increase in the viscosity of the oil. There is considerable variation among different fats in the manner in which their oxidation and accompanying flavor deterioration proceeds. The

**TABLE 1. Relative Reaction Rate of Fatty Acids with Oxygen (6).**

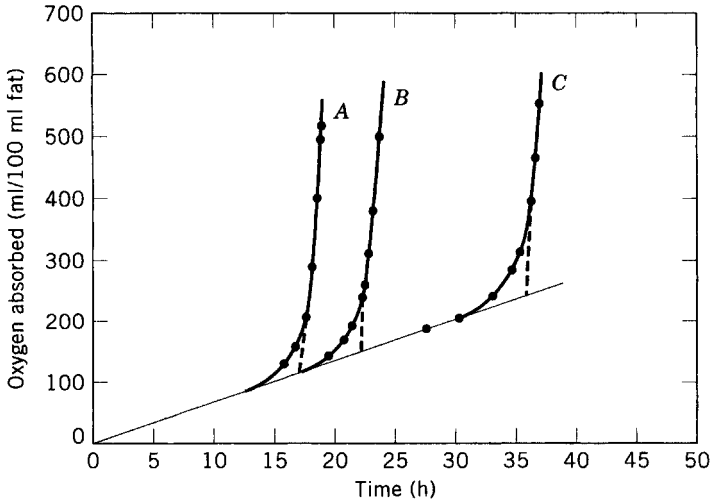
Fatty Acid	Approximate Relative Oxidation Rate (PV formation)
Oleic acid (C18:1)	1
Linoleic acid (C18:2)	10
Linolenic acid (C18:3)	25

amount of oxygen that must be absorbed to produce rancidity will vary according to (1) composition of the oil (2) presence or absence of antioxidants and pro-oxidants, and (3) conditions of oxidation.

Generally, oxygen absorbed will amount to about 15–150% of the oil by volume or 0.02–0.20% by weight. Fats high in oleic acid and low in linoleic acid will



**Figure 4.** Effect of temperature on the rate of oxidation of various fats: (A) Miscellaneous commercial shortenings aerated until rancid, (B) pure methyl oleate aerated to peroxide value of 500 meq/kg, (C) purified methyl esters of mixed soybean oil fatty acids aerated to a peroxide value of 500 meq/kg, and (D) and (E) commercial vegetable oil shortenings incubated at 65 °C and stored at 21 °C and 32 °C until rancid.



**Figure 5.** Oxygen uptake at  $90^{\circ}\text{C}$  of (A) corn oil, (B) corn oil plus 0.02% lauryl caffeate, and (C) corn oil plus 0.10% lauryl caffeate.

become rancid after the absorption of more oxygen than fats in which the ratio is reversed.

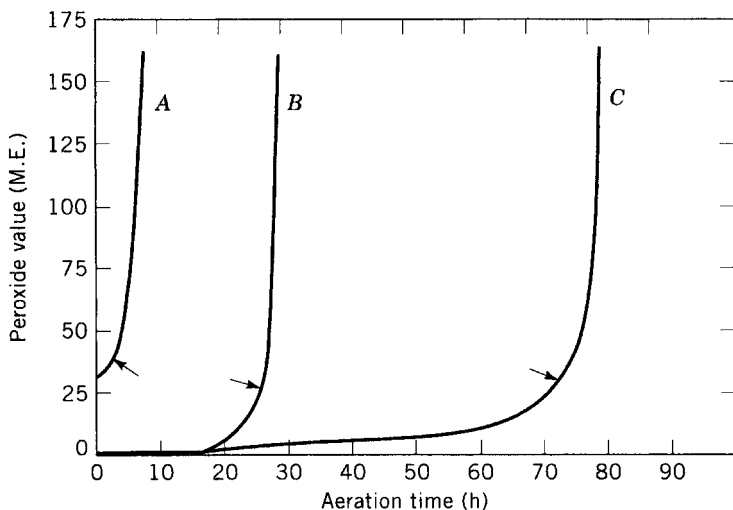
The relative oxidation rates of pure fatty acid esters based on peroxide formation are shown in Table 1 (6). This table is based on a common factor of one that has been arbitrarily assigned for the oxidation rate of oleic acid.

The rate at which oxygen is absorbed is markedly accelerated by heat and by exposure to light, particularly in the ultraviolet and near-ultraviolet range (7, 8). The effect of temperature on the rate of oxidative deterioration is shown graphically in Figure 4 (9). The coefficient of reaction increased markedly above  $60^{\circ}\text{C}$  ( $140^{\circ}\text{F}$ ).

As shown in Figure 5, oxygen uptake relates directly to the chemical reactivity of the edible oil. This effect becomes even more pronounced during the later stages of storage. The phenomenon of a reduced induction period, resulting in shortened product shelf life, is also shown in Figure 6 (10). For example, as shown by curve C, organoleptic rancidity occurs in an oil with good stability after 70 h of storage. Organoleptic rancidity occurs at a much more rapid rate in curves A and B which illustrate the effect of a reduced or shortened induction period.

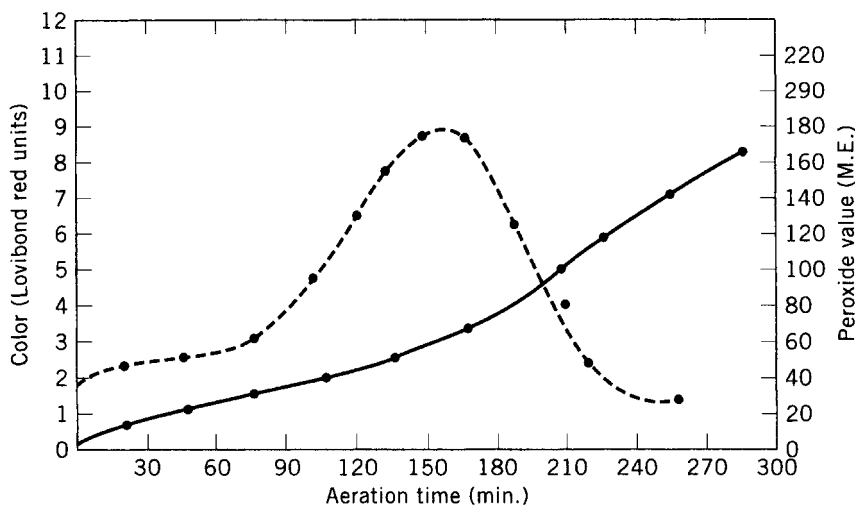
Oxidation has an important effect on the color of fats and oils. Although oxidation bleaches the carotenoid pigments, it tends to develop the color of other types of coloring material and, in certain cases, even develops colored compounds of quinoid nature from the fatty acids or acylglycerols of the oils. Cottonseed and, in certain cases, soybean oil are subject to marked darkening upon oxidation. The effect of oxidation or aeration on the color stability of oils is shown in Figure 7 (7).

Effects of temperature and oxygen concentration on oxidative deterioration during storage of crude sunflower oils, obtained by pressing and solvent extraction, were studied (11). Extracted oil showed a higher oxidative stability than pressed oil.



**Figure 6.** Stability tests (aeration at 110°C) of (A) hydrogenated peanut oil after oxidation to near rancidity, (B) oxidized oil after steam deodorization, and (C) hydrogenated peanut oil. (Arrows indicate points of organoleptic rancidity.)

This may be attributed to the presence of more polar protective antioxidants in the solvent-extracted product. As expected, oxidative deterioration was strongly dependent on temperature, oxygen availability, and the ratio of exposed surface to sample volume.



**Figure 7.** Effect of oxidation (aeration at 120°C) on the color of refined and bleached cottonseed oil.

## 1.2. Flavor and Odor Stability

Many fats and oils develop off-flavors during storage, which has been called *reversion*, that is, oil flavor reverting to the original flavor of the crude oil. However, as discussed by Smouse (12), this is more accurately called *retrogression* because the flavor retrogrades from a better to a worse condition. Also, aged oils do not develop off-flavors identical with that of the crude oils, but for any given type of oil, it is always specific and characteristic. Although all oils develop off-flavors, some do so at a faster rate than others. Soybean and marine oils develop “fishy” flavors, lard and tallow develop “animal” flavors, rapeseed and linseed oils develop “grassy/painty” flavors, and palm oil develops “painty/rancid” flavors. All of the efforts to prevent the development of off-flavors in oils are aimed at slowing down the rate at which they develop. Multivalent metals, such as iron or copper, act as pro-oxidants when present with triacylglycerols. Research at the U.S. Department of Agriculture (USDA) National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois showed that metal inactivators, which chelate or sequester these metals, were very effective at reducing the rate of reversion in soybean oil (13). This was a major improvement in processing for soybean oil stability and is practiced universally by the industry. Recent research (14) has indicated that citric acid addition to canola oil is important for flavor stability in the oil. A good discussion of the theories of soybean oil reversion is presented by Smouse (12), which includes 121 citations to pertinent research. Each theory is discussed in detail: linolenic acid theory, isolinoleic acid theory, oxidative polymer theory, phospholipid theory, nonsaponifiable theory, multivalent metals, and singlet oxygen. Each of the theories plays a role in soybean oil reversion.

## 1.3. Additives and Stability

Perhaps the most important additive of edible oils to enhance stability during storage and transport is citric acid or a similar metal chelating agent (15). Traces of metals in a reactive form act as catalysts that greatly promote the autoxidation of fats and oils. Concentrations of iron as low as 0.1 ppm or copper as low as 0.01 ppm can accelerate the deterioration of soybean oil (16). If an oil has higher concentrations of metals, it is necessary to first lower the content by a vigorous acid wash of the oil, for example, with very dilute phosphoric acid or by use of activated bleaching earth to absorb metals and metal complexes from the oil. A small amount of citric acid (i.e., 0.05% w/w, citric acid/oil) can be added to enhance the effectiveness of a bleaching earth. Some other acids that have been used to chelate metals are ascorbic, phosphoric, tartaric, and ethylenediamine tetraacetic acid (EDTA). It should be noted that citric acid does not improve the stability of olive oil (17).

Synthetic antioxidants effectively improve the stability of fats and oils with a low level of natural antioxidants, such as tocopherols, and with a low level of polyunsaturation. With oils that have a high content of polyunsaturated fatty acids and significant level of tocopherols, such as soybean oil, synthetic antioxidants



minimize the accumulation of peroxides but did not improve the flavor stability (18). The desired qualities of an antioxidant used to stabilize edible fats and oils are: (1) use in a product is safe (2) contributes no odor, flavor, or color to the product in which it is used (3) effective in the product at low concentrations (4) easily incorporated into the product (5) retained after cooking processes such as baking and frying (6) available at low cost for the application (19). Lauric-acid-containing oils are rather poor in their tocopherol content, as is the case with olive oils. However, palm oil, soybean, corn, and cottonseed oils contain higher levels of these natural antioxidants. Synthetic antioxidants were shown to be effective in the stabilization of virgin olive oil (17). Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary-butylhydroquinone (TBHQ) all improved the oxidative stability of the olive oil during storage at 50 °C (122 °F) in the dark but were not effective in preventing photo-oxidation of olive oil. It should be noted that addition of antioxidants to oils, which are already deteriorated, will not improve the quality or stability of the oil. Thus, it is of importance to produce a fresh quality material and immediately protect it with either nitrogen or antioxidants.

#### 1.4. Antioxidants as Processing Aid

Antioxidants are of extreme help in protecting the quality of the oils during transport, storage, and processing, and finally, when the oil is deodorized, these are partially removed. TBHQ plays a major role in providing protection by addition right in the crude oil at levels of 200 ppm together with 50 ppm of citric acid. This not only saves the oil from degradation but also improves the final yield of the processed oils, thus economics of addition of antioxidant can be very well balanced by improved yield with better profitability and less worries during processing.

#### 1.5. Contamination of Fats and Oils

Contamination begins in the fields and is possible at all stages of processing and application. The sources of contamination are several and some are found before the primary products are processed. All agricultural products are likely to contain evidence of the environment from which they come: dirt and dust, fragments of vegetable matter, insect parts, and so on. Other insoluble impurities appearing in crude oils will come as precipitates from the oils themselves. Most of the farm rubbish and the materials from the original plant and from the oil itself should have been removed before the oil is shipped, but some may carryover and some extra precipitation from the oil is possible.

Pesticide residues are the most common agricultural chemicals that contaminate oils and they are almost inevitable. Although edible oils are secondary products derived from the processing of crops, there will be some carryover of pesticide residues into the oil. In 1995, a list of persistent organic pollutants (POPs) were published (20). They were classified as (1) chlorinated pesticides, such as aldrin and DDT, and other industrial chemicals, such as hexachlorobenzene and

**TABLE 2. Polycyclic Aromatic Hydrocarbons (PAHs) Content of Some Crude Vegetable Oils (ppb) (20).**

	Light PAH	Heavy PAH
Coconut	992	47
Palm kernel	97	5
Rapeseed	30	4
Sunflower seed	66	12
Palm	21	1
Soybean	18	2

polychlorinated biphenyls (PCBs), or (2) bromine compounds from flame retardants, and (3) unwanted byproducts such as dioxin and polycyclic aromatic hydrocarbons (PAHs) from incineration. The PAH content of some crude vegetable oils is shown in Table 2. The light PAH is removable by deodorizer distillation, and the heavy PAH can be removed by adsorption by using activated carbon. Although there is no regulation at present about these PAHs, the desirable maximum content for heavy and total PAH is 5 ppb and 25 ppb, respectively.

Dioxin content in certain oil and its current and future maximum levels are shown in Table 3. Pilot-scale experiments have shown that dioxin can be sufficiently removed by activated carbon treatment (20). Similarly, PCBs that are present in foods in ppb level can be reduced by activated carbon or deodorization treatment of the oil.

Some oilseeds are dried in the field with hot gases from oil burners. Copra is often dried in ovens or kilns fired by coconut shells. In each case, contamination with diesel fuel fumes or polyaromatic hydrocarbons is inevitable. The only question is to what extent. Some hydrocarbons occur naturally in plants and appear in plant products. Mineral oil contamination of vegetable oil was also associated with toxic oil syndrome (20).

So long as uncoated mild steel tanks are accepted, there is a danger of mechanical contamination by rust flakes or powder or of the formation of iron salts by interaction with fatty acids in acid oils. Accordingly, such tanks are often coated with inert materials (plastics for food contact). The dangers of metal contamination can be eliminated altogether by using stainless steel.

**TABLE 3. Typical Dioxin Level (pictogram toxicity equivalent/gram) in Certain Oils and its European Union limits (20).**

	Typical Content	Current Max	Max After 2006
Vegetable fats	0.01–10	0.5	0.5
Crude fish oil	—	6.0	4.0
Refined fish oil	—	2.0	1.5

## 2. STORAGE AND HANDLING

### 2.1. Types of Facilities

Bulk storage and handling facilities are needed by (1) extraction plants and refineries, (2) industrial users of fats and oils, and (3) tank farm operations. The latter represent a distinct type of facility because they are not involved in the manufacture or use of edible oil products but merely store, handle, and ship them for national and international trade.

A typical bulk storage and handling facility for finished products is depicted in Figure 8. Key features include a plant for hydrogen production, hydrogenation facilities, deodorization equipment, storage tanks for partially processed and finished products, tank car and truck-cleaning facilities, and a loading line.

### 2.2. Crude Fats and Oils

Crude fats and oils may contain significant amounts of non-acylglycerol materials that should be removed prior to bulk storage, handling, and transport. These include gums (phospholipids), meal fines, moisture, seed fragments, and proteinaceous material. Seed fragments and cell tissue contain lipases that, in the presence of moisture, hydrolyze triacylglycerols into free fatty acids. Also, the combination of moisture, seed fragments, and mucilaginous material provides an ideal system for microbial growth that can lead to putrefaction and development of objectionable odors. These factors, coupled with the fact that crude fats and oils are usually stored



*Figure 8. Typical bulk storage and handling facility for finished products.*

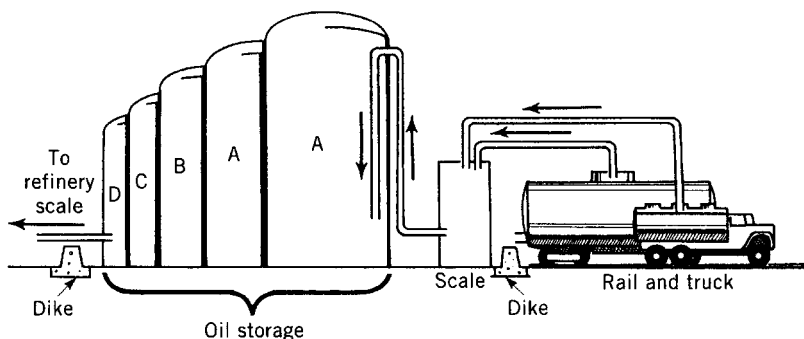


Figure 9. Ideal bulk storage system.

at temperatures conducive for enzymatic and microbial growth, emphasize the importance of removing non-triacylglycerol materials.

Removal of phospholipids is desirable because crude oil stored for extended periods is apt to deposit sludge in the bottoms of storage tanks, barges, or rail tank cars (21). Phospholipids (1–3%) represent the bulk of the fat-soluble materials in most crude vegetable oils and are particularly troublesome with soy oil. Soybean oil is the major commercial source of lecithin.

Storage of crude fats and oils is necessary to maintain sufficient quantities of oil to supply the refining operation. The objectives of such a bulk-storage system are also to maintain the quality of the incoming crude oil and to supply a uniform product to the refining operation (22–24). An ideal bulk-storage system is depicted in Figure 9. Key features include: (1) storage tanks and piping for each type of crude oil to be stored and handled, (2) an inventory control system that allows an accurate measure of crude oil coming into the refinery and to the refining operation, and (3) a spill prevention, containment, and control system. Items (1) and (2) are particularly important in preventing comixing, and mixing of old product with new where the deteriorated one could catalyze oxidation.

### 2.3. Quality Deterioration During Storage

The most serious types of quality deterioration occurring in bulk storage of crude fats and oils include comixing, contamination, chemical changes, and color increases (22).

*Coincidental Comixing.* Comixing occurs through the undesired mixing of different types of crude oils and can occur either through human error, faulty equipment design, or both. Comixing can be prevented by separating tanks and piping systems for each major type of crude oil handled. It can also be avoided by using strict operation procedures.

*Contamination.* Fats and oils with appreciable amounts of phosphatides and other easily hydratable materials, such as those from soybean, cottonseed, corn, rapeseed, etc., can be contaminated with moisture. Conditions of high humidity, warm oil, and cool temperatures can produce sweating within crude oil storage

tanks. In effect, water dripping into the oil causes an in situ degumming of the oil with an accumulation of sludge deposits on the bottom of the tank. The effect can be minimized by employing vent systems that restrict the flow of air in and out of the tank.

In the course of long-term storage, crude oils may be contaminated by oxidized or hardened oils that eventually coat the tank walls as a patch and provide uneven quality deterioration. This can be prevented by periodic cleaning of the tanks.

*Chemical Changes.* Primary considerations during crude oil storage and handling include preventing hydrolysis and oxidation of the crude oil. Storage of crude oil in the presence of moisture promotes hydrolysis, which, in turn, leads to increases in free fatty acids and lower yields of refined oil. Hydrolysis can be best prevented by ensuring that tanks are completely dry prior to pumping crude oil into storage and by keeping the oil as cool as possible throughout all handling operations.

Oxidation of crude oil may be prevented by excluding oxygen and air from the oil. One way is to fill storage tanks from the bottom, thereby minimizing exposure to the air as it enters the tank. A second, but more expensive, way to preserve crude oil quality is to blanket storage tanks with an inert gas such as nitrogen. However, inert gas blanketing is generally used more in conjunction with finished oil storage and handling. These techniques will be discussed in detail later.

Trace metals, in particular iron, copper, and nickel, accelerate the oxidative deterioration of fats and oils. Crude oil should not be in contact with iron, copper, or brass valves. One brass valve in a soybean oil refinery can lower the flavor stability of deodorized products. For proper handling, only stainless-steel tanks and valves should be used.

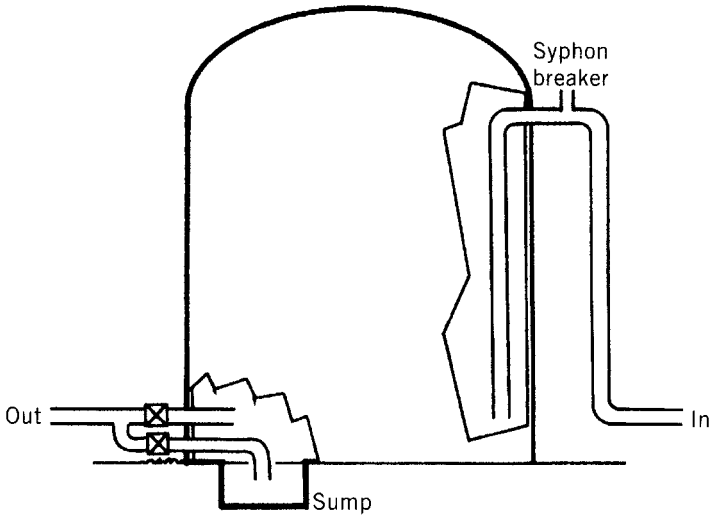
*Color Fixation.* Oxidation of crude fats and oils can also lead to *color fixation*. The most notable examples of color fixation are in cottonseed (25) and palm oils, where oxidation of gossypol and carotenoid pigments are involved.

Although not particularly sensitive to color fixation, the lecithin in crude soybean oil may darken excessively if the oil needs to be melted prior to unloading from rail tank cars or trucks. The use of high-pressure steam on heating coils should be avoided. Instead, low-pressure steam or, better yet, hot water should be used for this purpose. It is highly advisable to use longer time in slow and mild heating in order to give a gentle treatment to the oil in question.

## 2.4. Equipment for Storage and Handling of Crude Fats and Oils

*Tanks.* Crude oil storage tanks are usually constructed of welded iron, aluminium, or stainless steel. Their sizes vary throughout the industry depending on the refining capacity of the particular plant and the availability of incoming crude oil. Storage tanks with a capacity of 446,000 kg (1 million pounds) are not uncommon.

Crude oil storage tanks may be located in the open or in an enclosed area. If located outside, they should be set on concrete pads to protect them from surface water that eventually can cause rust and corrosion (26). A practical method for protecting crude oil quality involves painting outside storage tanks with aluminum or



**Figure 10.** Typical piping arrangement for a crude oil storage tank.

white paint. Such tanks may be as much as 11 °C (51.8 °F) cooler than those painted black (27). Most vegetable oils will not usually freeze in cold weather when held in large storage tanks. However, because storage tanks may be used for harder oils with higher melting points, the tanks should be equipped with heating coils to facilitate melting of bulk-stored fats and oils when required. We very highly recommend only stainless-steel tanks, as this investment will payoff in long run due to the absence of any variance in quality. These tanks should be built with nitrogen facility so that the oil is always protected under an inert atmosphere.

Successful refining is, in part, dependent on the supply of uniform crude fats and oils to the operation. Thus, storage tanks for crude fats and oils should be equipped with agitators to allow blending of crude oils and to assure uniformity.

Typical piping arrangement for a crude oil storage tank is depicted in Figure 10. Key features include an inverted U-shaped piping arrangement with a syphon breaker at the top. This lessens the possibility of oil in a full tank accidentally flowing to a tank containing less oil; also, it permits filling a tank from the bottom, thereby minimizing exposure of the oil to air. The storage tank can be emptied through one of two outlets: one is a short distance off the bottom and the second is from the sump. This arrangement allows any foreign material to settle to the bottom where it will not be picked up, and the tank can be cleaned by using the sump outlet (22).

## 2.5. Unit-Processing Operations

The purpose of this section is to outline the basic unit-processing operations required to convert crude fats and oils into finished products, such as cooking-salad oils, margarine, and shortening base stocks. Edible fats and oils are handled within

the unit-processing operation by pumping them through pipes. All edible fat and oil processing steps consist of independent operations that are usually not connected with each other by continuous flow. Between each processing step there may be one or more storage tanks. Generally, in each processing step, the oil is heated to process temperature, held for reaction, and cooled to preserve oil quality. Between processing steps, the oil is allowed to cool to a minimum or ambient temperature while being maintained in a fluid state to facilitate material handling by pumping (28).

The basic processing steps consist of (1) crude oil storage (2) degumming or caustic refining (3) bleaching (4) hydrogenation (5) fractionation or winterization (6) deodorization and (7) finished oil storage. Each step removes specific components from the oil. These materials may be classified as:

1. *Naturally occurring*: gums, phospholipids, pro-oxidant metals, pigments, color bodies, tocopherols, free fatty acids, and diacylglycerols
2. *New compounds formed during processing*: soaps, oxidation products (primary hydroperoxides and secondary decomposition products), polymers and their decomposition products, color bodies, triacylglycerol and diacylglycerol isomers, high-melting triacylglycerol, and *trans*-fatty acids
3. *Processing adjuncts*: hydrogenation catalysts, bleaching clay, metal scavengers that precipitate from finished oils, i.e., citric acid
4. *Contaminants introduced by processing*: moisture, trace metals, carbonaceous materials, and oil-insoluble materials

To produce an edible fat (an oil having the desired color, flavor and oxidative stability, and functional properties), naturally occurring and undesired compounds must be removed as efficiently as possible, with maximally preventing damage to the naturally occurring antioxidants and neutral oil fraction. Formation of new compounds, precipitated processing adjuncts, and contaminants are all undesirable.

A summary of typical handling operations and storage conditions for edible fats and oil products within the unit operations is shown in Table 4.

Virtually all edible fats and oils exhibit the best oxidative stability in their crude state. With increased processing, oxidative stability decreases and reaches a minimum after bleaching. Refined-bleached oil is the least stable, and extended storage of this oil is not desirable. Deodorization and treatment of the oil with metal inactivators or autoxidants cannot be relied on to correct faulty upstream handling and processing as peroxide buildup during crude oil storage, degumming, refining, or bleaching will ultimately have a detrimental effect on the storage properties of the finished oil. Storage and handling practices should be designed to compensate for the decreased oxidative stability.

## 2.6. Finished Oil

A review of finished-oil handling and storage in the industrial trade sector has been given by List and Erickson (31). For reviews of finished-oil handling and storage

**TABLE 4. Storage and Handling of Soybean Oil within the Unit-Processing Operations (23, 24, 26, 29, 30).**

Unit Processing Operation	Substance(s) Removed	Handling Operation(s)	Process Reaction Temperature °C (°F)	Oil Storage Temperature °C (°F)	Atmosphere
Crude oil storage	Oil insolubles	Pumping: tank cars, trucks, or barges to storage tanks	Ambient to 54 (130)	Ambient	Air
Degumming	Phosphatides Trace metals	Pumping: Storage tank to mix tank to centrifuge to storage or refining	70 (158)	Ambient	Air
Caustic refining	Free fatty acids Phospholipids Trace metals Pigments—color bodies	Pumping: Storage tank to inline mixer to centrifuge	74 (165)	Ambient to 50 (122)	Nitrogen or air
Water washing	Soaps	Pumping: From primary centrifuge to water wash centrifuge	88 (190)	Ambient to 50 (122)	Nitrogen or air
Drying	Moisture	Pumping: From water wash centrifuge to vacuum dryer to storage tank	82 (180)	Ambient to 50 (122)	Nitrogen or air
Bleaching	Pigments, soaps	Pumping: Storage tank to bleaching unit to filter to storage tank	105 (224)	Ambient to 60 (140)	Nitrogen or air
Hydrogenation	Olefinic bonds	Pumping from storage tank to hydrogenation converter	140–225 (284–437)	Approx. 10°C (180°F) above melting point	Nitrogen or air
Winterization	High melting triacylglycerols and waxes	Pumping from converter to chill tank through filter	4–5 (30–35)	60–66 (140–151)	Nitrogen or air
Filtration	Spent bleaching clay	Pumping from converter to filter to storage tank		Ambient to 60 (140)	Nitrogen or air
Deodorization	Pigments Unsaponifiables Free fatty acids Odor and flavor compounds	Pumping from storage tank to deodorizer	204–274 (400–525)	60–66 (140–151)	Nitrogen or air
Polishing filtration	Bleaching earth Citric acid Carbonaceous material	Pumping from deodorizer to polish filter to oil cooler to storage tank	50–70 (122–158)	60–66 (140–151)	Nitrogen or air
Finished oil storage	Oil insolubles	Pumping from storage tank to polish filter to packaging line or to tank cars or tank trucks		60–66 (140–151)	Nitrogen or air



within domestic and European refineries, articles by Wright (29) and Johansson (30) should be consulted. Weiss (32) has reviewed the bulk handling of shortening in the industrial and institutional sectors.

A finished oil is defined as that fat or oil product ready for use alone or as an ingredient without further processing. Assuming that raw materials are of high quality and previous operations are satisfactory, the result is a light-colored product, free from odors, flavors, hydroperoxides, and contaminants. The goal of finished-oil storage and handling is to preserve and maintain these quality factors (33).

A general scheme for the storage and handling of finished oils is shown in Figure 11. Oil coming from the deodorizer is passed through a heat exchanger and a polishing filter to remove any solid materials. After the polishing filtration, the oil is pumped through a cooler to a storage tank. Before packaging, the oil is generally filtered again to remove any solids picked up while in storage. Paper is generally used for this filtration (29).

Autoxidation increases markedly with temperature. Salad oils degrade rather quickly with increasing temperatures due to their high unsaturation. From this standpoint, it is desirable to handle the oil at temperatures as low as possible during discharge from the deodorizer into the storage tank. In practice, however, it is not desirable to cool the oil below 45 °C (113 °F) prior to the polish filtration for the following reasons: (1) moisture is apt to condense in the oil and (2) at lower temperatures the filtration rate decreases.

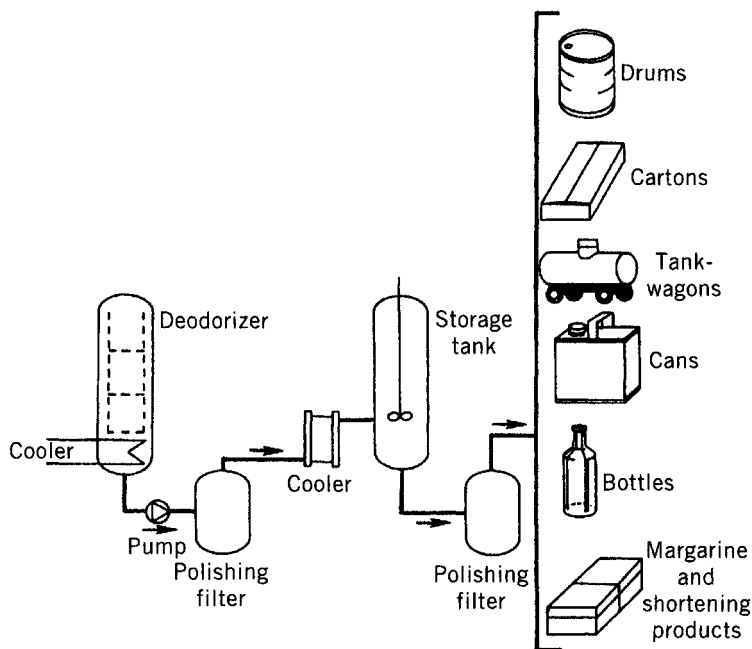


Figure 11. General scheme for the storage and handling of finished oils.

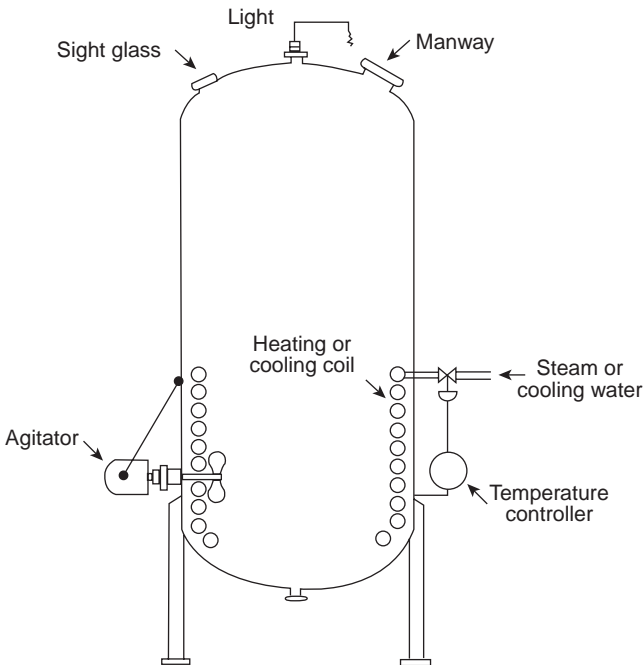
Further cooling before storage is generally considered optional. Where storage tanks are not blanketed with nitrogen, cooling is desirable. On the other hand, the use of nitrogen-gas blanketing may eliminate the need for cooling.

## 2.7. Storage Tanks for Finished Products

Storage tanks for finished fats and oils are preferably constructed of carbon steel, stainless steel, or aluminum. Finished-oil storage tanks vary considerably in size. Typically, they are designed to hold a given number of standard rail tank carloads of oil or a full operation batch of a deodorization vessel. A tank holding 500 metric tons of product is considered large, and a 10-metric-ton tank is considered small.

Finished-product storage tanks are usually closed and fitted with tank tops, internal heating or cooling coils, and an agitator to promote heat transfer and to prevent localized overheating. A typical finished-product storage is depicted in Figure 12.

Factors most likely to affect the quality of finished products to such an extent that they would require reprocessing or use as a subquality product include: (1) contamination from atmospheric adulterants, (2) internal contamination from water and soaps, (3) overheating, and (4) exposure to air and oxygen. Atmospheric adulteration is avoided by storing the product in completely closed tanks. Finished products held in storage tanks can be contaminated by water leaking from steam or cooling coils. Moisture in the oil can promote hydrolysis, particularly at elevated storage



**Figure 12.** Finished-oil storage tanks.

temperatures. Properly maintained coils are necessary. Overheating of bulk-stored oil is undesirable because soaps and pro-oxidant metals are much more active at elevated temperatures (34, 35). Finished-oil storage tanks are equipped with automatic temperature controllers to prevent overheating (Figure 12) (29).

## 2.8. Nitrogen Blanketing

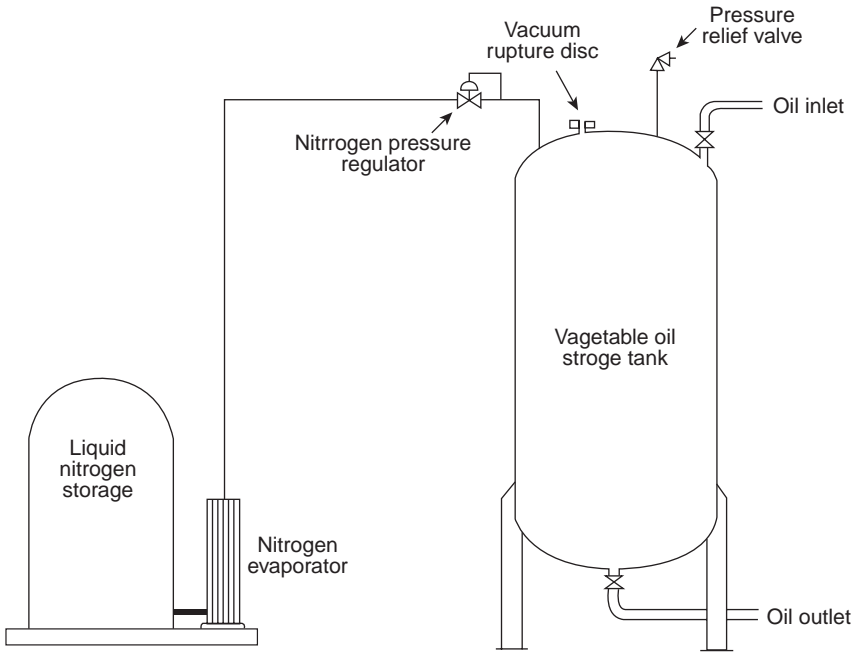
Oxidation has the most detrimental effect on the quality of finished products held in bulk storage. Thus, exclusion of oxygen during storage represents a highly desirable and practical method for preventing quality deteriorations (29, 36–38).

The usual procedure involves replacing air with nitrogen. Finished product is delivered from the deodorizer to the storage tank under a complete nitrogen blanket. Nitrogen can be supplied from a tank of liquid nitrogen or from commercially available nitrogen generators. Liquid nitrogen is distributed at a purity of 99.998%, in capacities of 1890–340,000 L (500–90,000 gal; 46,600–8,400,000 ft<sup>3</sup>). The tanks can be equipped for either gaseous or liquid withdrawal. For gaseous applications, a line of vaporizers—atmospheric, electric, steam, or hybrids—can be used to convert the liquid to gas, which is then discharged at the required pressure to use points.

Users of very large quantities of high-purity nitrogen [405,145 L (107,181 gal; 10,000,000 ft<sup>3</sup>) per month or more] can employ a cryogenic system, an air-separation system designed to filter, compress, purify, and liquefy intake air from the surrounding environment. Nitrogen is recovered from the liquefied air by fractional distillation. These systems range in capacities of less than 608 L (161 gal; 15,000 ft<sup>3</sup>) per hour to more than 12,154 L (3215 gal; 300,000 ft<sup>3</sup>) per hour at purities of 99.999% nitrogen or higher.

A self-contained, skid-mounted membrane air-separation system is commercially available that generates nitrogen at flow rates of up to 2431 L (643 gal; 60,000 ft<sup>3</sup>) per hour, at pressures up to 10.5 kg/m<sup>2</sup> (150 psig), and at purities ranging from 95% to 99.95%. The membrane systems are based on the selective permeability of the composite membrane fiber. Intake air is filtered, compressed, and cooled before entering the air-separation modules under pressure. Relative to oxygen, carbon dioxide, and water vapor, nitrogen does not readily permeate the composite membrane fiber and flows automatically into the pipeline distribution system at the specified volume, pressure, and purity.

A third method of on-site generation of nitrogen is the vacuum-pressure swing-adsorption process designed to separate nitrogen from air and generate between 405 L and 2026 L (107 gal and 536 gal; 10,000 ft<sup>3</sup> and 50,000 ft<sup>3</sup>) per hour at purities between 99.0% and 99.9% or higher. Offered as a skid-mounted system, a vacuum-pressure cycle combined with adsorbent vessels packed with synthetic zeolites cycle automatically to provide a continuous flow of nitrogen; while one vessel is purifying nitrogen to specifications, another is being regenerated. When intake air enters the active adsorbent vessel, the adsorbent selectively separates oxygen from nitrogen, which is produced to the specified flow and pressure.



**Figure 13.** Simple nitrogen-blanketing system.

Generally, these on-site methods are more cost effective than liquid nitrogen for purities lower than 99.5%; however, too low a nitrogen purity may lead to problems with stability of vegetable oils.

A simple nitrogen-blanketing system is shown in Figure 13. The nitrogen blanket is maintained by a pressure system controlled by a regulator. As the tank is filled with product, the pressure builds and the gas is vented to the atmosphere. Conversely, as the product is pumped from the tank, the pressure drops and replacement nitrogen gas enters the tank. It is common practice to equip storage tanks with a vacuum-relief valve or with rupture discs that prevent a vacuum and subsequent collapse of the tank. Nitrogen blanketing can be applied to multitank arrangements (Figure 14). The design is similar to single-tank systems and has the advantage of being less wasteful of nitrogen; as one tank is emptied, the gas can be displaced from another. When product is transferred from tank to tank, the inert gas is merely exchanged. Nitrogen pressures in such systems are kept at 1–15 psi. During filling or emptying, the pressures vary, but relief valves are set to release at pressures above 15 psi. Nitrogen-blanketed storage tanks will not support life and should be thoroughly purged before maintenance personnel attempt to enter the tanks. Signs warning of this hazard are normally displayed on nitrogen-blanketed tanks (29).

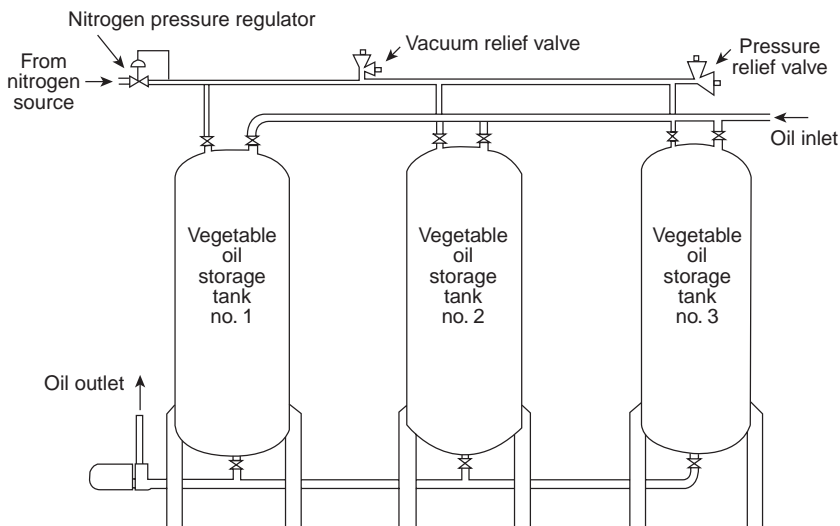


Figure 14. Multitank nitrogen-blanketing system.

## 2.9. The Industrial User Sector

Industrial users of fats and oils, such as those involved in the manufacture of fried foods and baked goods, often purchase their supplies in bulk because of cost advantages. Among these are elimination of cost of shipping containers, reduced shipping rates, and reduced labor costs due to more efficient unloading and handling of products within the user's plant. Other advantages include reduction in product waste and sanitary problems associated with small containers. Bulk handling also may reduce the amount of storage space needed, and this factor can be of economic importance in some plants.

On the other hand, bulk handling offers some disadvantages, including sizable capital investment for the purchase and installation of pumps, tanks, and other essential equipment. Operation of a bulk-handling system requires the attention of more skilled personnel than the simple unloading and handling of smaller containers. Another disadvantage is that the product may deteriorate before being used. This is particularly true of shortenings that must be held in a molten state. Melted products are generally more susceptible to deterioration than packaged semisolid products.

A prime requirement is that a minimum shipment, usually 13,392 kg (30,000 lb), be used within the storage life of the product. The storage life of shortenings varies with formulation, and the storage and handling conditions vary within the user's plant. Suppliers should be consulted to determine the storage life of shortening products.

Many types of fats and oils are amenable to bulk handling, including all salad and cooking oils, all types of frying fats, bread shortenings, and products normally fluid and pumpable at ordinary temperatures, such as "fluid" bread and cake

shortenings. Several types of products are not amenable to bulk handling because their physical or functional properties are dependent on proper plasticizing and tempering, and resultant crystal structure are altered by heat or melting. These include: (1) margarines and water–shortening emulsions that will breakdown under heat, with a separation of the fat and water phases, (2) baking shortenings used in the manufacture of cakes and icings, (3) shortenings containing significant amounts of lecithin, and (4) shortenings used in Danish pastry.

## 2.10. Equipment for Bulk Storage and Handling

Equipment required for bulk handling include: (1) tank cars or tank trucks and isotankers for transporting bulk shipments, (2) equipment for unloading the fats, (3) storage tanks, and (4) equipment necessary to deliver the product to the point of use within the user's plant.

Rail tank cars are rented by the supplier from companies leasing this type of equipment because railroads do not own or furnish equipment to haul liquid commodities such as fats and oils. An advantage of this arrangement is that the shipper of fat products can control the suitability, availability, and utilization of tank cars used for loading of products.

*Equipment for Unloading Tank Cars and Tank Trucks.* The unloading of tank cars, isotankers, or trucks requires fittings and adapters to connect the unloading line to the tank car, isotanker, or tank outlet. Tank cars generally require 10 cm (4-inch) outlets, whereas 7.6 cm (3-inch) outlets are standard for tank trucks. Flexible hoses made from carbon steel, neoprene, plastic, aluminum, or stainless steel (types 302, 303, 316) are suitable.

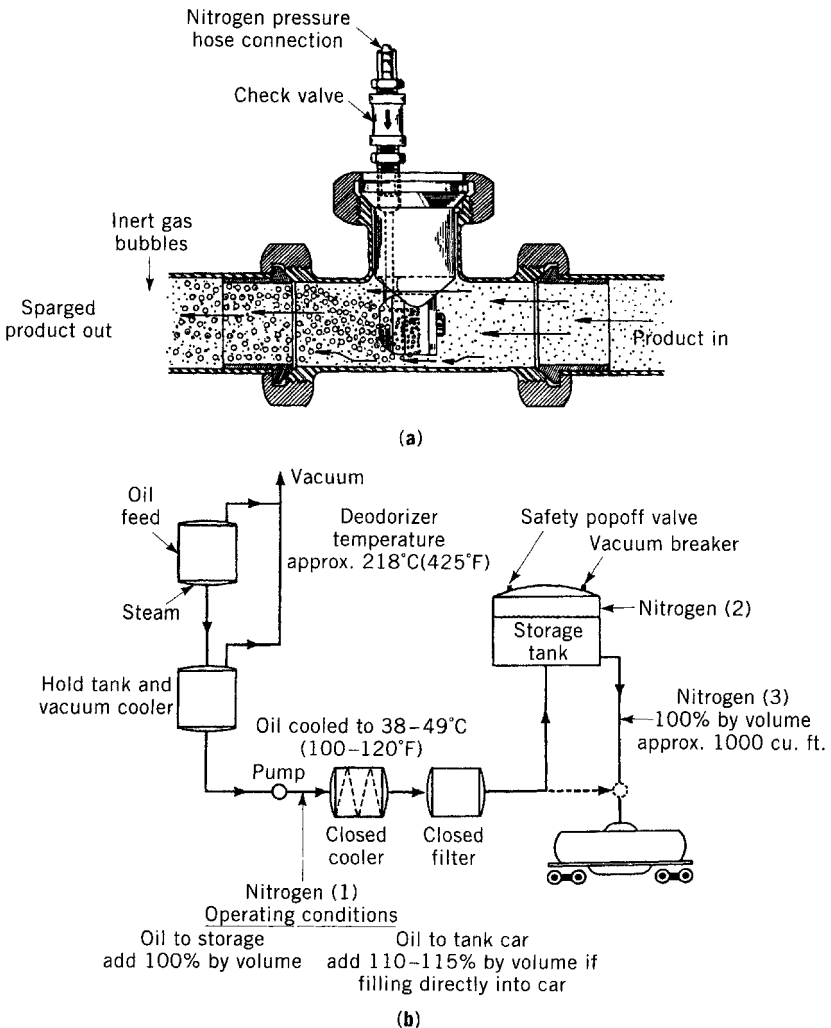
A basket strainer should be installed ahead of the pump to prevent damage from tramp metal. In addition, it is recommended that a filter be installed on the pump discharge to remove extraneous materials. In cases where it becomes necessary to pump partially solidified fats, the filter should be installed in such a way that it may be bypassed.

Excellent reviews of pumps for the edible oil industry have been published (39–42). Either centrifugal or positive-displacement pumps may be used, but the latter are preferable. Pumps should be constructed of carbon steel or stainless steel and have sufficient capacity to unload a 32,280-L (8000-gal) tank car in about 2 h or less. Factors governing pump selection include length of lines; restrictions, such as elbows and valves in the lines; height to which the fat must be pumped; type and temperature of fat being pumped; and whether the pump is located above or below the fat level in the storage tank. A relief valve must be installed to prevent damage to positive-displacement pumps in the event the discharge becomes blocked. Centrifugal pumps, however, do not require relief valves.

Certain utilities are required to unload liquid fats. These include pressurized air or nitrogen for clearing the connecting line and hot water or steam for melting solidified fats. It is also advisable to insulate the unloading lines, even if the shipment is hot and liquid on arrival. A drain valve should be installed at the lowest point in the line to prevent accumulation of fats, which can solidify and cause a blockage.

Tank cars require a level surface for unloading. However, complete unloading of tank trucks may require a slope toward the unloading end. The area underneath should be concrete and pitched toward a drain to receive wash water and maintain good sanitation.

*Sparging Techniques.* Sparging and nitrogen-blanketing techniques for protecting edible oils during truck and rail car shipments have been reviewed (22, 37). Sparging (Figure 15) represents a practical method for protecting finished oils from oxidative deterioration during shipment from refinery to destination. The technique is particularly useful when finished oils are loaded into tank cars and trucks.



**Figure 15.** Nitrogen-gas sparger and blanketing system for loading tank cars and trucks: (a) in-line sparger and (b) complete system.

The principle involved is saturation of the oil with nitrogen while it is completely free of air and oxygen. A sparger introduces minute bubbles of nitrogen into the oil stream; as the saturated oil falls into the tank car or truck, the effusing gas sweeps the headspace and, thus, removes most of the air and oxygen from the vessel (30). Another benefit of nitrogen sparging is the reduction of oxygen picked up from natural leakage of air into the tank car or truck. The gradual flushing action of the sparge gas reduces the reabsorption of oxygen because of the pressure differential between the liquid and the headspace above it (37). A nitrogen gas sparger and blanketing system for loading tank cars and trucks is illustrated in Figure 15. Nitrogen blanketing and sparging techniques have many other applications in protecting oil quality. A summary is given in Table 5.

### **2.11. Precautionary Measures in Bulk Handling**

In bulk-storage systems where positive-displacement pumps are used, special precautions are necessary on both sides of the pump. On the suction side, a double-side, double-check system should be used to avoid pumping the fat from a closed vessel, thereby preventing collapse of the tank and piping. Similarly, on the discharge side, checking for closed valves or relief valves is recommended to prevent danger to operating personnel, damage to equipment, and loss of product from blown fittings.

When it is necessary to heat fats to keep them fluid, heat should be applied to line, pumps, and tanks. Although insulation may be used to retain the necessary amount of heat, the ability to apply heat in emergency situations is most desirable. Occasionally, even when shortenings are held above their apparent melting point, hard fractions will separate or “seed” out or fractionation will take place. In effect, the composition of the fat changes in different levels in the tank and possibly can block the lines. Application of heat and agitation may be necessary to correct the problem. It is recommended that experimentation on the product be carried out at the projected temperatures and times to ensure that this problem will not occur under operating conditions in the plant.

Close control of temperature within handling systems is necessary because pumping rates are dependent on viscosity of the fat, which, in turn, is temperature dependent. Similarly, where volumetric means are used to control weights, it is absolutely essential to control temperature because the specific gravity is related to temperature. Graphs showing these relationships have been published by Erickson (33).

Cleaning procedures for bulk storage and handling systems are beyond the scope of this chapter. For a thorough discussion of this topic, the reader is referred to List and Erickson (31).

### **2.12. Packing of Institutional Shortenings and Margarines**

Industrial and commercial shortenings are packaged in 2.3-kg (5-lb) prints packed in 22.7-kg (50-lb) case, 22.7-kg (50-lb) plastic pails and cubes, 172.4-kg (380-lb)



**TABLE 5. Summary of Treatment Areas with Nitrogen to Protect Edible Oils (37).**

Area of Treatment	Method of Introducing Nitrogen	Classification	Approximate Usage (ft <sup>3</sup> )
Manufacturing pumping from deodorizer	In-line between cooler and storage tank	Sparging	Approx. 0.125 ft <sup>3</sup> /gal
Bulk oil storage	From sparger directly into headspace	Blanketing	Enough to maintain positive pressure
Filling of tank cars	In-line between storage and tank car	Sparging	1000 ft <sup>3</sup> /8,000-gal car
Tank car or truck	Into headspace	Blanketing	Undetermined filling
Customer's plant pumping from tank car to oil storage	Sparging in-line during pumping	Sparging	Approx. 0.125 ft <sup>3</sup> /gal
Storage in tanks	Directly into headspace plus nitrogen from sparger	Blanketing	Enough to maintain positive pressure
Pumping from storage to filler or header	Sparging in-line during pumping	Sparging	Approx. 0.125 ft <sup>3</sup> /gal
Filler bowl or header	Entrance into closed filler bowl or header pressure	Blanketing	Maintain slight positive pressure
Closing or capping machine	Shroud or purge technique	Blanketing or purging	Undetermined

open-end steel drums, or 907.2-kg (2000-lb) totes. Although attractiveness is not ignored, it is secondary to cost, protection, and convenience (32). Friction-top pails are convenient when only a part of the contents are used at one time because they are easy to reclose and store. The empty pails can be used for other purposes in the kitchen or bakery. Commercial shortenings are also packaged in corrugated paper boxes measuring about 30.5 cm (1 ft) on a side. Polyethylene film is used to line the box and prevent leakage. This type of packaging is popular because it is cheap, the contents are easily removed, and the package can be readily disposed of by folding flat. Storage problems are minimal because the package takes up little space. Plastic cube liners are usually colored because they melt in hot fat if they are accidentally dropped into a fryer, and the color makes them easy to detect and retrieve. A potential disadvantage of cube packaging is that melted cube liners cause foaming, darkening, and smoking of the frying fat (32).

Drum-packaged pourable shortenings may be emptied upright with a hand-operated pump or emptied by gravity through a spigot with the drum placed horizontally on a rack. Similarly, totes may be pumped or emptied by gravity from a valve and hose connection.

Fully hardened fats are sold as flakes or powdered materials and are packaged in multiwalled paper bags holding 22.3 kg (50 lb), in fiber drums holding 44.6–89.2 kg (100–200 lb), or larger bulk containers.

Margarines intended for institutional and industrial trade are often packaged in pails holding 13.4–22.3 kg (30–50 lb) and in cubes. Open-end steel drums [208.7 L (65 gal)] hold 181.5 kg (400 lb) of margarine. It may also be available in 2.3-kg (5-lb) prints or sheets packed in 22.7-kg (50-lb) box.

### 2.13. Storage of Shortenings Effect on Functional Properties

Excellent discussions of shortenings are given by Weiss (32), Hartnett (43), and Thomas (44); these should be consulted for information on formulation, processing, and end uses. Shortenings may be classified as solid, fluid, liquid, and powdered. Five factors govern the quality of shortenings: (1) triacylglycerols and emulsifier composition (2) processing conditions (3) tempering conditions (4) usage temperature and (5) storage conditions.

*Solid Shortenings.* Solid shortenings are the most generally useful products of their class because they may be used for both frying and baking. The crystal structure is an important consideration in solid shortening compositions. For example, for optimum creaming ability (or the ability to incorporate air into a cake batter), the shortening must be stable and in the  $\beta'$  form and characterized as being smooth and creamy. These products can be adversely affected by improper and nonuniform storage conditions. As reflected by pound cake volume, general-purpose solid shortenings will show no loss in creaming ability over storage temperatures of 4.4–21.1 °C (40–70 °F). Beyond that, a marked loss of creaming ability is observed. Prolonged storage of solid shortenings at high temperatures causes the lower melting-fat fractions to liquefy; upon cooling, they solidify into crystal forms that are less functional (43).

Solid shortenings require no refrigeration during storage. However, they will absorb odor. They should be stored in a cool, dry place away from odor-producing materials.

*Fluid Shortenings.* Fluid shortenings are pourable, opaque products containing suspended solids that are hardfats or emulsifiers, depending on whether the product is to be used for frying or baking. Like solid shortenings, proper storage temperatures are important in preserving the functional properties of the product. Storage of fluid shortenings at temperatures below 18.3 °C (65 °F) will cause the product to set up and lose fluidity. Loss of fluidity can be reversed by mild warming. Storage at temperatures beyond 35 °C (95 °F) will cause either partial or complete melting of the suspended solids. This, however, is not a reversible phenomenon because cooling will cause formation of large crystals that will not remain in suspension. If the product is formulated with hardfats as the suspended solids, improper storage might be of little consequence because they will merely settle to the bottom of the container upon cooling. On the other hand, if the solids are emulsifiers, settling out will upset the balance of the product, with the upper portion under-emulsified and the bottom part over-emulsified. Thus, the product would not perform as intended (32).

*Liquid Shortening.* The liquid shortenings include regular cooking oils, salad oils, and products resulting from fractionation of semi-hardfat. These products are not ordinarily emulsified. Ordinary cooking and salad oils pose no unusual storage problems because they possess no crystal structure to protect and have no suspended solids. If they are stored at temperatures low enough to cause solidification, they can easily be returned to clear liquids by returning the product to ambient storage conditions.

## 2.14. Factors that Contribute to Oxidative Deterioration of Finished Products

The concept and discussion of atmospheric oxidation were introduced earlier. As a result of the importance of preventing oxidation in finished products, a more thorough discussion follows. It should be pointed out that the effects of atmospheric oxidation apply to all fats and oils regardless of their stage of processing.

Five factors contribute to the oxidative deterioration of fats and oils: (1) oxygen or air (2) heat (3) light (4) pro-oxidant metals and (5) time.

Although the elimination of air prevents oxidative deterioration, complete elimination of air is impractical. The solubility of oxygen in soybean oil is quite high (2.1 mL/100 mL) at 22–23 °C (45) and is sufficiently soluble to yield a peroxide value of 18 (46), assuming complete reaction. Flavor deterioration in soybean oil occurs at peroxide values considerably lower than 18. Published data show that soybean oil can have poor flavor at a peroxide value of 1 (47).

Even when using nitrogen blanketing, sufficient oxygen already may be present in the oil to promote oxidative deterioration (see section on sparging). A more practical approach is to minimize the incorporation of air into the oil by proper handling procedures.

Faulty handling procedures to be avoided are as follows:

1. Allowing a liquid fat to cascade or fall through air into storage or holding tanks. As mentioned previously for crude fat and oil handling, a more desirable procedure is to fill the tank from the bottom with subsurface entry.
2. Sucking of air into the suction side of pumps or lines caused by faulty pump seals or fittings.
3. Whipping air into a fat through improper agitation within storage tanks. Creation of whirlpools or vortexes should be avoided.
4. Minimize blowing of lines with air, which in turn may bubble air through the fat being held in the storage tank.

Heat accelerates the reaction of atmospheric oxygen with edible oils. For deodorized products, it is estimated that the speed of oxidation is doubled for each 15°C (59°F) increase in temperature over the range 20–60°C (68–140°F) (31). A rule of thumb for handling is to keep the fat no warmer than necessary to facilitate handling by pumping. For hydrogenated fats, a holding temperature 5.6°C (10°F) above the melting point is considered sufficient. Design of bulk-storage systems should take into account the use of low temperature whenever possible. For example, short insulated lines will permit lower storage temperatures and, thus, prolong the quality of bulk-stored fats.

Localized overheating is detrimental to fat quality and should be avoided. All storage tanks with heating devices should be equipped with a mechanical agitator. Power agitation will not only minimize fat damage from localized overheating but will save time and heating costs as well. If agitation is temporarily out of service, the temperature differential between the fat and the heating medium must be kept minimal.

All edible fats and oils deteriorate under the effects of light. Normally, light-induced deterioration is not of concern in the oil-processing operation or at the user level because processing, handling, and storage are carried out within closed systems. However, light deterioration is an important factor in the storage stability of liquid oils packaged for retail trade.

The importance of avoiding metallic contamination cannot be overstressed. Copper and iron are strong pro-oxidants capable of lowering the flavor and oxidative stability for fats and oils at levels of 0.01 ppm and 0.1 ppm, respectively (48). Copper or copper-containing alloys should never be used in equipment used for handling and storing fats and oils. The prevention of iron contamination is somewhat more difficult, because most of the industry uses black iron for construction of tanks, pumps, and lines. However, through proper treatment and cleaning of black iron equipment, iron contamination can be kept to a minimum.

Any fat or oil will deteriorate with prolonged time even if stored and handled under ideal conditions. Unhydrogenated or lightly hydrogenated oils, which do not require heat for keeping them liquid, have greater resistance to deterioration than do shortenings. The latter, however, will keep for 2–3 weeks in a melted

condition. Shortenings should not be allowed to solidify and then be reheated during use. Bulk-storage systems should be designed with a maximum turnover time of 2–3 weeks or within the storage life of the product. The mixing of fresh shipments with those already in storage should be avoided. Small quantities of old products in a tank may hasten the deterioration of new product mixed with it. Shipments should be scheduled so that mixing of old and new products does not occur. An auxiliary tank can be installed to hold the remainder of the old shipment and permit inspection and cleaning of the receiving tank.

## 2.15. Transport of Fats and Oils

During transport, the quality of oils may be impacted by oxidation, adulteration from other oils, moisture condensation, contamination with foreign matter (dirt, rust, mould, etc.), and overheating. Transport of fats and oils is designed to preserve quality in the delivery to the product formulator in the most cost-effective manner. The advantages and disadvantages of bulk handling of fats and oils are detailed in Table 6 (33,49). The economics of bulk shipment are the major advantages.

Movement of fats and oils may be as the crude, partially refined, or finished oils and may be by rail tank cars, tank trucks, barges, and tanker ships. Rail tank cars are usually of the standard 60,566-L (16,000-gal; 120,000-lb) or the jumbo 232,170-L (61,333-gal; 460,000-lb) size. Standard cars are designed with a thermal expansion area of about 2% of the total capacity as a dome in the car. However, the rated capacity of the jumbo cars does not include any expansion space. Rather, these cars are equipped with a permanently installed bar marker located in the passageway, above which the quantity of oil should not be loaded. Heating coils can be installed for handling higher melting fats.

Tank trucks typically have 20,412-kg (45,000-lb) capacity. Generally, tank trucks are made of stainless steel but have no standard shape or construction. Heating coils can be installed when carrying oils that might solidify during shipment. Trucks are provided with a bar marker to indicate maximum load level, are insulated, and carry 4.6 m (15 ft) of 7.5 cm (3-inch) hose and the fittings required to unload the oil to a storage tank.

**TABLE 6. Bulk Handling of Oils (33, 49).**

Advantages	Disadvantages
Container packs unnecessary	Greater capital investment in handling facilities
Lower freight charge per ton	More highly skilled (paid) individual operators
Lower labor handling costs in transit and at destination	A larger quantity purchased at one time
Less loss due to smaller overall holdup in handling facilities (average loss estimated at 0–5%)	Holding time of the commodity is longer and, possibly as a melted solid, places demands on planning
Cost savings in smaller storage area	Bulk shipping requires sanitation and security procedures
No cost for package disposal	

A barge generally has three compartments with a total capacity of 1,270,072 kg (2,800,000 lb). The compartments are fitted with steam coils to melt oils and fats that have solidified during transport. Barges are used to transport crude and crude-degummed oils along major rivers and, in the United States, shipping time is seldom longer than 7–10 days.

Shipment by tanker is the most economical means of ocean transport of fats and oils. A typical shipment ranges from 1814 to 9072 metric tons (2000 to 10,000 short tons). Tankers are compartmentalized and inert-gas blanketing or sparging is not employed because the gas can escape. Rather, the compartments are filled to capacity to minimize headspace air and reduce oxidative deterioration. Heating is not required to unload liquid oils, but to facilitate unloading of palm, coconut, and tallow, heating is required.

Centrifugal pumps with capacities of up to 794,934 L/h (210,000 gal/h) are used for handling nonsettling fats and oils. Positive-displacement pumps are preferred for unloading crude and degummed oils because they will maintain their prime during the operation, which requires an operator to squeegee the remaining foots oils to the tank outlet during the final pumping. Both types of pumps are used to load tankers.

## 2.16. Temperatures during Shipment and Unloading of Fats and Oils

Extremes of temperature of an oil during shipment can have a deleterious effect on quality because oxidative deterioration and hydrolysis are accelerated by temperature. The International Association of Seed Crushers has established recommended safe limits of temperature during the voyage and on discharge for the principal oils in transport (Table 7) (50).

**TABLE 7. Recommended Temperatures (°C): Principal Oils (50).**

Oil	During Voyage		On Discharge	
	Min.	Max.	Min.	Max.
Sunflower	Ambient	Ambient	Ambient	20
Soybean	Ambient	Ambient	20	25
Safflower	Ambient	Ambient	Ambient	25
Peanut	Ambient	Ambient	20	25
Rapeseed	Ambient	Ambient	Ambient	20
Maize	Ambient	Ambient	Ambient	20
Palm	32	40	50	55
Palm stearine (RBD)	40	45	60	65
Palm olein (RBD)	25	30	50	55
Coconut	27	32	40	45
Fish	20	25	30	35
Palm fatty acid distillate	42	50	67	72

## 2.17. International Codes and Previous Cargo Listing for Shipment of Fats and Oils

During recent years, international fats and oils associations and the Fats and Oils Committee, Codex Alimentarius, have been quite concerned about previous cargos carried in tankers for shipment of edible oils. A listing of acceptable previous cargos has been promulgated by the Federation of Oils, Seeds and Fats Associations (FOSFA) (51). This information is also available from NIOP (National Institute of Oilseed Products), a U.S. association. The listing includes five generic items that greatly extend the coverage of the list: acid oils and fatty acid distillates; animal, marine, and vegetable oils, other than cashew shell nut oil and tall oil; beverages; dairy products; and fatty acid esters. The same organization has assembled a list of 42 items banned as immediate previous cargos, including isocyanates, phthalates, transformer oils of the PCB type, lube oil additives, uncured epoxy resins, and all leaded products (52). Another concern has been the establishment of a standard reference document concerning advice on the storage and transport of edible oils. Such a document has been developed under the leadership of the Malaysian Palm Oil Board (MPOB) and adopted as an international code of practice by Codex Alimentarius (53, 54). Advice is given on storage tanks and ship's tanks, pipelines, heating, loading, unloading, and cleaning.

## 2.18. Oil Spill

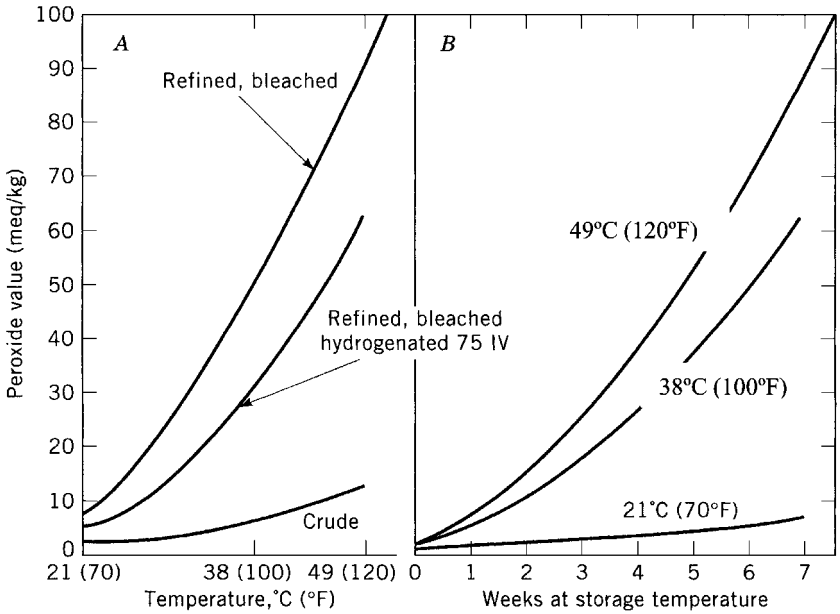
Under Section 311 of the Clean Water Act, the U.S. EPA amended the Facility Response Plan requirements in the Oil Pollution Prevention regulation for nontransportation-related facilities. The main purpose of these amendments is to provide a more specific method to plan response resources that can be used by an owner or operator of a facility that handles, stores, or transports animal fats and vegetable oils. EPA issued this rule in response to legislation requiring the Agency to issue regulations (55).

Spill-prevention procedure is beyond the scope of this chapter. For a thorough discussion of this topic, the reader is referred to List and Erickson (31).

# 3. DETERIORATION PROCESSES

## 3.1. Oxidative Deterioration During Long-Term Storage

Significant amount of knowledge of oxidative and related forms of deterioration occurring in long-term storage of soybean and cottonseed oil (56) was obtained from studies conducted at the U.S. Department of Agriculture (57–61) between the late 50s to the early 70s. A most interesting and informative study reported the oxidative stability of soybean oil at various processing stages, and the results were related to commercial storage conditions (38). As oxidative deterioration is an important consideration in the processing, storage, and handling of soybean and other oils, these studies are reviewed briefly here.



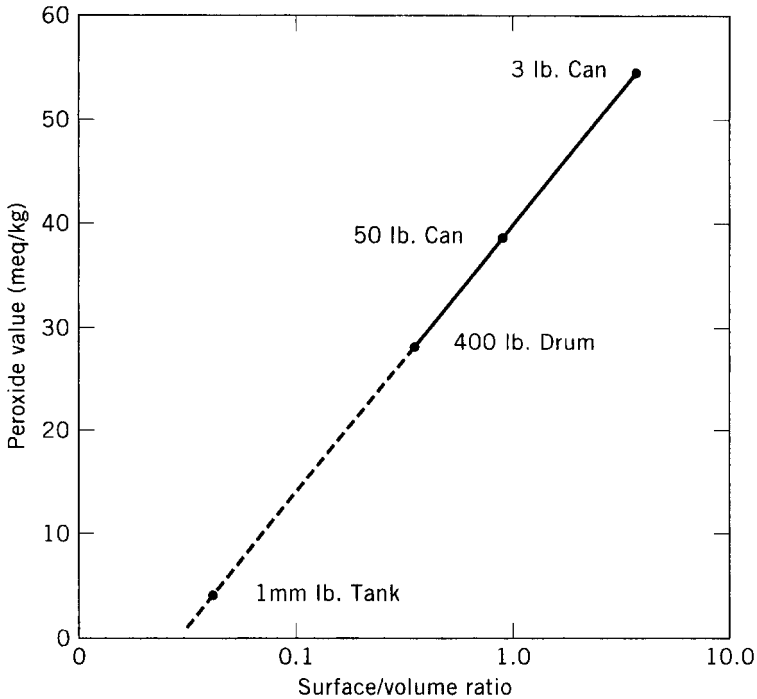
**Figure 16.** Effects of processing and storage temperature on oxidative stability as measured by peroxide value. (A) 1-qt container held for 7 weeks in storage. (B) Refined and bleached oil in 1-qt size container.

*Effects of Processing and Storage Temperature (38).* A fresh, non-degummed soybean oil was refined, bleached, and hydrogenated to an iodine value of 75. Samples from each processing stage were placed in closed 0.946-L (1-qt), 22.3-kg (50-lb), and 208.2-L (55-gal) containers and stored at 21.1°C, 37.8°C, and 48.9°C (70°F, 100°F, and 120°F). The effects of processing and storage temperature on oxidative stability as measured by peroxide value are depicted in Figure 16, parts A and B, respectively.

Part A [0.946-L (1-qt), samples] shows crude oil to be most stable; refined and bleached are most prone to develop peroxides under accelerated storage conditions. Part B shows the marked effect of temperature on peroxide development in refined, bleached oil. Peroxides developed very slowly at 21.1°C (70°F) but increased markedly at 37.8°C (100°F), and 48.9°C (120°F), probably because oxygen diffuses more rapidly into the reaction interface at higher temperatures. The oxidation rate doubles for every 11.1°C (20°F) increase in temperature.

The effect of container size on the oxidative deterioration of refined, bleached soybean oil stored at 48.9°C (120°F) for 5 weeks is shown in Figure 17. The results clearly demonstrate that oxidation is not only a function of time and temperature but also of the surface area. Figure 17 shows that as the container size increases, the surface-to-volume ratio decreases and peroxide development is an approximately linear function of surface-to-volume ratio.





**Figure 17.** Effect of container size on the oxidative deterioration of refined, bleached soybean oil stored at 49°C for 5 weeks.

Extrapolation of the laboratory data to a storage tank of 9.2 m (30 ft) in diameter with an oil capacity of 453,597 kg (1 million pounds) would predict that the refined, bleached soybean oil would reach a peroxide value of 3.5 after 5 weeks storage at 48.9°C (120°F). However, laboratory conditions are seldom, if ever, duplicated in commercial storage tanks. For example, under constant-temperature conditions in the laboratory, little or no oil movement results from temperature gradients. However, field storage tanks are subjected to continual day and night conditions that cause development of sizable temperature gradients and produce considerable internal oil movement. Such movement would be expected to increase the quantity of oil available at the surface and to accelerate oxygen diffusion. Thus, the rate of oxidation in field storage tanks would be somewhat faster than at similar temperatures in the laboratory.

Attempts were made to relate the laboratory studies to commercial practice (38). Refined, bleached soybean oil (1.5 million lb) was thoroughly mixed in a nitrogen-blanketed tank, after which one-half was moved to an air-blanketed tank. Storage conditions are given in Table 8. The two half-filled tanks, one blanketed with air and the other with nitrogen, were stored at ambient conditions for 5 months, after which samples were hydrogenated, deodorized, and subjected to organoleptic evaluations. Flavor stability also was determined on the pilot-plant-produced shortening stock made from the two lots of stored oil.

**TABLE 8. Conditions of Plant Storage Test on Refined, Bleached Soybean Oil (38).**

Conditions	Nitrogen	Air
Oil temperature		
Range	15.6–33.9 °C (60–93 °F)	17.8–30.6 °C (64–78 °F)
Average	23.9 °C (75.0 °F)	23.9 °C (75.0 °F)
Average O <sub>2</sub> content of tank headspace	1.4%	21%
Peroxide value		
Initial	1.0	1.0
Final	1.5	5.0

The plant-scale samples were placed in storage beginning in mid-summer and thus were exposed to the highest temperatures during the early part of the storage test. The observed peroxide values were 5.0 for the air-stored sample and 1.5 for the nitrogen-stored sample. The study of effects of nitrogen- and air-storage conditions on flavor quality of finished deodorized oils (salad oil and hydrogenated oil) showed a decreased flavor score with storage time at 60 °C (140 °F) for all products. However, the products made with the oil stored in air had a greater rate of flavor score reduction than those from the oil stored under nitrogen. The results clearly demonstrate that even low levels of oxidation products formed while soybean oil is held in bulk storage, although not necessarily harmful to initial quality, have a deleterious effect on shelf life. These results are in accord with Evans et al. (62), who showed that autoxidation of soybean oil prior to deodorization has a detrimental effect on flavor and oxidative stability.

*Nitrogen Packaging.* A study of the impact of use of nitrogen in consumer packages on soybean oil stability was completed by Evans et al. (61). Eight lots of commercially processed soybean salad oils, representative of those available to American consumers, were obtained for the study. Each lot consisted of 20–40 samples packaged in screw-cap cans and bottles. Headspace gas analysis (63) showed that air, pure nitrogen (0.0% oxygen), and nitrogen containing low levels of oxygen were used to package the samples. All samples were stored in the dark at two temperatures, 25.6 °C and 37.8 °C (78 °F and 100 °F), and flavor evaluation was conducted by a 20-member sensory panel (64).

The effects of nitrogen versus air packaging on the flavor deterioration of hydrogenated-winterized soybean oils (HWSBO) stored in the original containers at 25.6 °C and 37.8 °C (78 °F and 100 °F) for 1 year showed that the initial flavor scores of the nitrogen-protected samples (average 7.5) was similar to the air-packaged samples (average 7.9). However, during 1-year storage, nitrogen-packaged samples only showed loss of approximately one flavor score unit at both 25.6 °C and 37.8 °C (78 °F and 100 °F). By contrast, air-packed samples deteriorated rapidly at both storage temperatures. The effect of storage temperature is pronounced. One sample dropped to a flavor score of 6 in approximately 18 weeks at 25.6 °C (78 °F), whereas equivalent deterioration occurred in less than 6 weeks at 37.8 °C (100 °F). It was found that hydrogenation alone, without added antioxidants, metal scavengers,

and nitrogen packaging, is not sufficient to ensure adequate shelf life of hydrogenated-winterized soybean oil, particularly at elevated storage temperatures.

Oxygen in the headspace of bottled soybean oil greatly affects the storage stability. As the oxygen content of the headspace gas increased, the flavor scores of the oils decreased. A nonhydrogenated oil, protected with antioxidants but with incomplete nitrogen protection (2% oxygen), deteriorated to a flavor score of 5.0 after 18 weeks of storage at 37.8 °C (100 °F). Even so, its stability was greater than that of an air-packaged sample of hydrogenated oil. After several months of storage, loss of flavor score occurred for both hydrogenated and nonhydrogenated samples unless they were protected with nitrogen. The improvement in storage life offered by 100% nitrogen (0% oxygen) over 98% nitrogen (2% oxygen) is obvious. Nitrogen sparging and nitrogen blanketing have been shown to increase the induction phase of the oxidation, and to reduce the rate of accelerated phase of oxidation. The benefits of nitrogen as a protective measure are used widely in the oil industry.

A relatively recent study compared the effects of light, temperature, and nitrogen sparging on the stability of nonhydrogenated and hydrogenated soybean oils in plastic and glass containers (65). Prior research demonstrated that the most effective methods of maintaining the quality of liquid soybean oil was the use of amber glass containers to reduce light exposure and nitrogen packaging to minimize the oxidation of the oil (61). Properties such as oxygen permeability, impact resistance, clarity, and flavor transmission from the bottle material to the food have also been considered by other researchers (66).

Oil samples were packaged in glass and polyvinylchloride (PVC) bottles and sparged with nitrogen for 1 min, then sealed and aged at 25.6 °C (78 °F) for 4, 6, 9, and 12 months. The results of these evaluations indicated no significant difference in the sensory evaluation of the oils aged in the long-term ambient temperature tests regardless of the packaging material used for bottling. In these long-term ambient-temperature studies, HWSBO or SBO show no differences in oil deterioration between glass and PVC packaging. Oils packaged with nitrogen in the headspace treatment in PVC bottles had equivalent flavor stability compared with oils packaged in a similar manner in glass bottles. The conclusion was that PVC bottles were acceptable alternatives to packaging oils in clear glass. The results of this research had a significant impact on the edible oil industry, which, in 1985, converted all consumer soybean oils to translucent plastic bottles.

### 3.2. Other Deterioration During Extended Storage

Prime concerns in storage of crude and once-refined soybean oils include increases in moisture and volatile matter, color after refining or bleaching, peroxide value, free fatty acids, and refining losses. Aside from possible effects on flavor and oxidative stability, many of these quality factors also affect the commercial value of oil in trading channels. With the exception of peroxide values and refined color, the other quality factors above are written into specifications for oils sold under trading rules of the National Soybean Processors Association (NSPA) (67).

To study the changes in these quality factors, Baumann et al. (59, 60) obtained thirty-six 189.3-L (50-gal) drums of crude, crude-degummed, and once-refined oils. These samples were stored outside (Beltsville, Maryland), exposed to atmospheric conditions, for 4–6 years. The drums were stored under the following conditions to simulate general conditions of storage in large field tanks: painted aluminum, fitted with breathers, and tilted at an angle to permit moisture to drain. Some drums were completely filled to exclude air (designated no-breather drums) and were opened only for sampling. Other drums were half filled (designated half-filled drums).

The study showed that probable changes in the characteristics of once-refined, degummed, and crude oils can be predicted for various periods of storage. Formulas were developed based on statistical interpretations that, in most cases, predicted oil characteristics after storage. In general, moisture and volatile matter contents were not affected by oil type and storage conditions. Color of the refined oils was affected by these treatments. Peroxide values showed that once-refined oils were the least stable to oxidation, followed by crude-degummed, with crude oil being the most stable. Oils stored in no-breather drums had the lowest peroxide values, and oils stored in half-filled drums had the highest peroxide values. Storage of once-refined oils had only a slight effect on free fatty acid content after the 4-year storage period, it increased from 0.058% to 0.135%. As observed with peroxide formation, the increase in free fatty acids is dependent on access to air and to temperature within the storage drum.

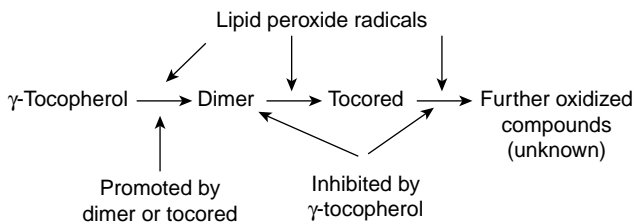
### 3.3. Color Reversion

Under certain conditions, soybean oil that is refined, bleached, and deodorized to yield a pale light-colored product becomes darker upon storage. This phenomenon, known as color reversion, has been studied in detail by Japanese scientists (68, 69). The degree of color change is dependent on the nature of the raw soybeans, refining and deodorization conditions, storage conditions, temperature, exposure to light, and contact with air during storage. The tendency to undergo color reversion is variable. Some oils require only a few hours to revert, whereas others require several months. In either event, the color becomes paler after it reaches a peak.

The moisture content of the soybeans, going to the extractor, appears to be a key factor in promoting color reversion in fully refined oils. The tocopherol content of extracted oil is markedly affected by the beans' moisture content. Over the normal moisture range (7–12%), the tocopherol content of crude soybean oil remains normal, but in wet beans, the tocopherol content is markedly reduced (68). Subsequent work (69) showed that tocopherol [2, 7, 8-trimethyl-2 (4', 8', 12' -tridecyl-2)-chromane, 5, 6-quinone] is the precursor of the reversion color, and that this material reaches a maximum in soybeans with moisture contents of 15–18%. This phenomenon was observed by Shukla (unpublished observation) in beans originating from Argentina and was highly related with the moisture content and the ways beans were treated during storage and voyage to Europe.

Other work (70) indicated that no change in the  $\alpha$ -tocopherol content of the crude oil was observed by elevating the moisture content of soybeans, but a

decrease in  $\gamma$ - and  $\delta$ -tocopherols occurred. The decrease in  $\gamma$ - and  $\delta$ -tocopherols was accompanied by an increase in dimeric oxidation products. The latter effect could be reversed by decreasing the moisture content of the beans. Thus, it appears that color reversion involves oxidation of  $\gamma$ -tocopherol to a dimeric product that, in turn, is converted to tocored. The following mechanism was suggested for color reversion (70):



#### 4. CONCLUSIONS AND FUTURE PERSPECTIVES

This chapter deals with problems involved in treating the vegetable oils at every aspect of production and highlights the sources of errors and how to rectify these with proper management. All attempts should be made to produce the final product with extremely limited destruction, thus keeping the active ingredients and still providing the freshness of the product to be used as ingredient in the food chain. Modern processing technologies provide foods with consistent quality and hygiene. Newer technologies will help to create much cleaner raw materials, thus avoiding the application of complicated processing methods. Thus, foods should be manufactured with simple, greener technologies rather than the complicated chemical technologies as used in the early days (71).

#### REFERENCES

1. F. D. Gunstone, in F. D. Gunstone, J. L. Harwood, and F. B. Padley, eds., *The Lipid Handbook*, Chapman & Hall, New York, 1994, pp. 594–597.
2. C. Adhikari, H. E. Snyder, T. W. Kwon, and P. K. Clark, *J. Amer. Oil Chem. Soc.*, **69**, 1141 (1992).
3. A. E. Bailey, *Industrial Oil and Fat Products*, Interscience Publishers, New York, 1951, p. 967.
4. E. N. Frankel, in *Lipid Oxidation*, The Oily Press, Dundee, Scotland, 1998, pp. 13–22.
5. E. Perkins, *Food Tech.*, **21**, 611 (1967).
6. F. D. Gunstone and T. P. Hilditch, *J. Chem. Soc.*, 836 (1945).
7. G. R. Greenbank and G. E. Holm, *Ind. Eng. Chem.*, **25**, 167 (1933).
8. G. R. Greenbank and G. E. Holm, *Ind. Eng. Chem.*, **33**, 1058 (1941).
9. S. W. Thompson, Proceedings of the Committee on Food Research, in Conference on Deterioration of Fats & Oils, Quartermaster Corps Manual 17-7, 1945.

10. A. E. Bailey and R. O. Fuege, *Oil Soap*, **21**, 286 (1944).
11. G. H. Crapiste, M. I. V. Brevedan, and A. A. Carelli, *J. Amer. Oil Chem. Soc.*, **76**, 1437 (1999).
12. T. H. Smouse, in D. B. Min and T. H. Smouse, eds., *Flavor Chemistry and Fats and Oils*, American Oil Chemists' Society, Champaign, Illinois, 1985, pp. 85–116.
13. H. J. Dutton, A. W. Schwab, H. A. Moser, and J. C. Cowan, *J. Amer. Oil Chem. Soc.*, **25**, 385 (1948).
14. K. Warner, E. N. Frankel, and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **66**, 558 (1989).
15. Law Kia Sang, *Oleagineaux*, **39**, 89 (1984).
16. J. C. Cowan, *Food Tech.*, **19**, 1413 (1965).
17. A. K. Kiritsakis, C. M. Stine, and L. R. Dugan, *J. Amer. Oil Chem. Soc.*, **60**, 1286 (1983).
18. T. L. Mounts, K. A. Warner, G. R. List, J. P. Friedrich, and S. Koritala, *J. Amer. Oil Chem. Soc.*, **55**, 345 (1978).
19. P. P. Coppen, in J. C. Allen and R. J. Hamilton, *Rancidity in Foods*, eds., Elsevier Applied Science Publishers, London, 1983, pp. 67–87.
20. K. Berger, *INFORM*, **13**, 475 (2002).
21. G. E. Halliday, *Oil Soap*, **14**, 103 (1937).
22. J. P. Burkhalter, *J. Amer. Oil Chem. Soc.*, **53**, 332 (1976).
23. R. A. Carr, *J. Amer. Oil Chem. Soc.*, **53**, 347 (1976).
24. R. A. Carr, *J. Amer. Oil Chem. Soc.*, **55**, 765 (1978).
25. H. L. Vix, E. F. Pollard, J. J. Spadaro, and E. A. Gastrock, *Ind. Eng. Chem.*, **38**, 635 (1946).
26. G. M. Neumunz, *J. Amer. Oil Chem. Soc.*, **53**, 334 (1976).
27. A. R. Baldwin, *Processing Soybean Oil—Some Practical Considerations for Preserving Flavor Quality*, Proceedings, Regional Oil Conference, Tehran, Iran, Sponsored by Soybean Council of America, 1964, pp. 30–33.
28. B. F. Brooks, *J. Amer. Oil Chem. Soc.*, **55**, 772 (1978).
29. L. M. Wright, *J. Amer. Oil Chem. Soc.*, **53**, 408 (1976).
30. G. M. R. Johansson, *J. Amer. Oil Chem. Soc.*, **54**, 410 (1976).
31. G. R. List and D. R. Erickson, in D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soy Oil Processing and Utilization*, American Soybean Association and American Oil Chemists' Society, Champaign, Illinois, 1980.
32. T. Weiss, *Food Oils and Their Uses*, AVI, Westport, Connecticut, 1982.
33. D. R. Erickson, *J. Amer. Oil Chem. Soc.*, **55**, 815 (1978).
34. N. W. Zeils and W. H. Schmidt, *Oil Soap*, **22**, 327 (1945).
35. R. E. Beal and E. B. Lancaster, *J. Amer. Oil Chem. Soc.*, **28**, 12 (1951).
36. T. W. Eselgroth, *Food Eng.*, **23**, 72 (1951).
37. Air Reduction Sales Co., "Treatment and Protection of Edible Oils to Preserve Quality and Increase Storage Life," *Technical Data Proceedings Bulletin*, Air Reduction Sales Co., Madison, Wisconsin, 1961, pp. 1–16.
38. L. H. Going, *J. Amer. Oil Chem. Soc.*, **45**, 632 (1968).

39. W. D. Harris, *J. Amer. Oil Chem. Soc.*, **30**, 579 (1953).
40. Anonymous, in W. J. Mead, ed., *The Encyclopedia of Chemical Process Equipment*, Reinhold, New York, 1964, pp. 759–769.
41. R. T. Sheen, in W. J. Mead, ed., *The Encyclopedia of Chemical Process Equipment*, Reinhold, New York, 1964, pp. 769–775.
42. W. E. Wilson, in W. J. Mead, ed., *The Encyclopedia of Chemical Process Equipment*, Reinhold, New York, 1964, pp. 775–788.
43. D. I. Hartnett, *J. Amer. Oil Chem. Soc.*, **54**, 557 (1977).
44. A. E. Thomas III, *J. Amer. Oil Chem. Soc.*, **55**, 830 (1978).
45. L. Aho and O. Wahroos, *J. Amer. Oil Chem. Soc.*, **44**, 65 (1967).
46. J. C. Cowan, *Food Technol.*, **19**, 1413 (1965).
47. H. J. Dutton, A. W. Schwab, H. A. Moser, and J. C. Cowan, *J. Amer. Oil Chem. Soc.*, **25**, 385 (1948).
48. C. D. Evans, A. W. Schwab, H. A. Moser, J. E. Hawley, and E. H. Melvin, *J. Amer. Oil Chem. Soc.*, **28**, 68 (1951).
49. H. B. W. Patterson, *Handling and Storage of Oilseeds, Oils, Fats and Meal*, Elsevier Applied Science, London, 1989.
50. Anonymous, *Oils Fats Int.*, **9**(5), 27 (1993).
51. FOSFA International List of Acceptable Previous Cargoes, Federation of Oils, Seeds and Fats Associations, Leatherhead, England, 1991.
52. FOSFA International List of Banned Immediate Previous Cargoes, Federation of Oils, Seeds and Fats Associations, Leatherhead, England, 1988.
53. K. G. Berger, ed., *Recommended Practices for Storage and Transport of Edible Oils and Fats*, Palm Oil Research Institute of Malaysia, UK Liaison Office, Brickendonbury (MRPRA), Hertford, United Kingdom, 1985.
54. *International Code of Practice for Storage and Transport of Edible Oils and Fats in Bulk*, Codex Alimentarius FAO/WHO, Rome, 1989.
55. Federal Register, **65** (127), 40776 (2000).
56. R. R. King, *Oil Soap*, **18**, 16 (1941).
57. L. A. Baumann, *J. Amer. Oil Chem. Soc.*, **36**, 28 (1959).
58. L. A. Baumann, Marketing Research Report No. 532, Agricultural Research Service, U. S. Dept. of Agriculture, 1959, pp. 1–56.
59. L. A. Baumann, Marketing Research Report No. 795, Agricultural Research Service, U. S. Dept. of Agriculture, 1967, pp. 1–22.
60. L. A. Baumann, D. O. McConnell, H. A. Moser, and C. D. Evans, *J. Amer. Oil Chem. Soc.*, **44**, 663 (1967).
61. C. D. Evans, G. R. List, H. A. Moser, and J. C. Cowan, *J. Amer. Oil Chem. Soc.*, **50**, 218 (1973).
62. C. D. Evans, E. N. Frankel, P. M. Cooney, and H. A. Moser, *J. Amer. Oil Chem. Soc.*, **37**, 452 (1960).
63. C. D. Evans and E. Selke, *J. Amer. Oil Chem. Soc.*, **49**, 106 (1972).
64. K. Warner, in E. G. Perkins, ed., *Analysis of Fats, Oils and Lipoproteins*, American Oil Chemists' Society, Champaign, Illinois, 1991, pp. 344–385.
65. K. Warner and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **61**, 548 (1984).

66. R. Logsett and D. E. Parson, *J. Amer. Oil Chem. Soc.*, **53**, 45A (1976).
67. *NOPA Year Book and Trading Rules, 1992–1993*, National Oilseed Processors Association, Washington, D.C., 1992, pp. 77–78, 82–84.
68. M. Komoda and I. Harada, *J. Amer. Oil Chem. Soc.*, **46**, 18 (1969).
69. M. Komoda, N. Onuki, and I. Harada, *Agric. Biol. Chem.*, **30**, 906 (1966).
70. M. Komoda, N. Onuki, and I. Harada, *Agric. Biol. Chem.*, **31**, 461 (1967).
71. V. K. S. Shukla and E. G. Perkins, *Inform*, **9**, 995 (1998).



# 5

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## *Packaging*

Vance Caudill

Packaging systems for oil and fat products have become more complex as the demands for containing, protecting, and identifying products have increased for manufacturers. The packaging system must be designed to produce an economical product that satisfies regulatory guidelines, consumer demands, and production requirements. This Chapter discusses the design methodology for implementing an integrated oil–fat packaging line and the major line components and includes a description of typical edible oil lines and bulk filling lines. A further discussion of the design methodology explores the three phases involved: conceptual–definition phase, preliminary engineering, and final engineering.

### **1. CONCEPTUAL DESIGN OF PACKAGING SYSTEMS FOR OIL PRODUCTS**

During conceptual design–definition, the project parameters are determined by establishing the packaging requirements and identifying the desired packaging equipment. This first phase of an oil packaging project is possibly the most critical. Other phases are implemented only as successfully as the information is collected and goals are defined. The team selected to perform the integration of the packaging system is an integral part of this conceptual design. This team must create a conceptual definition plus a project schedule. As Figure 1 illustrates, the team must collect information from several diverse areas and combine these data into

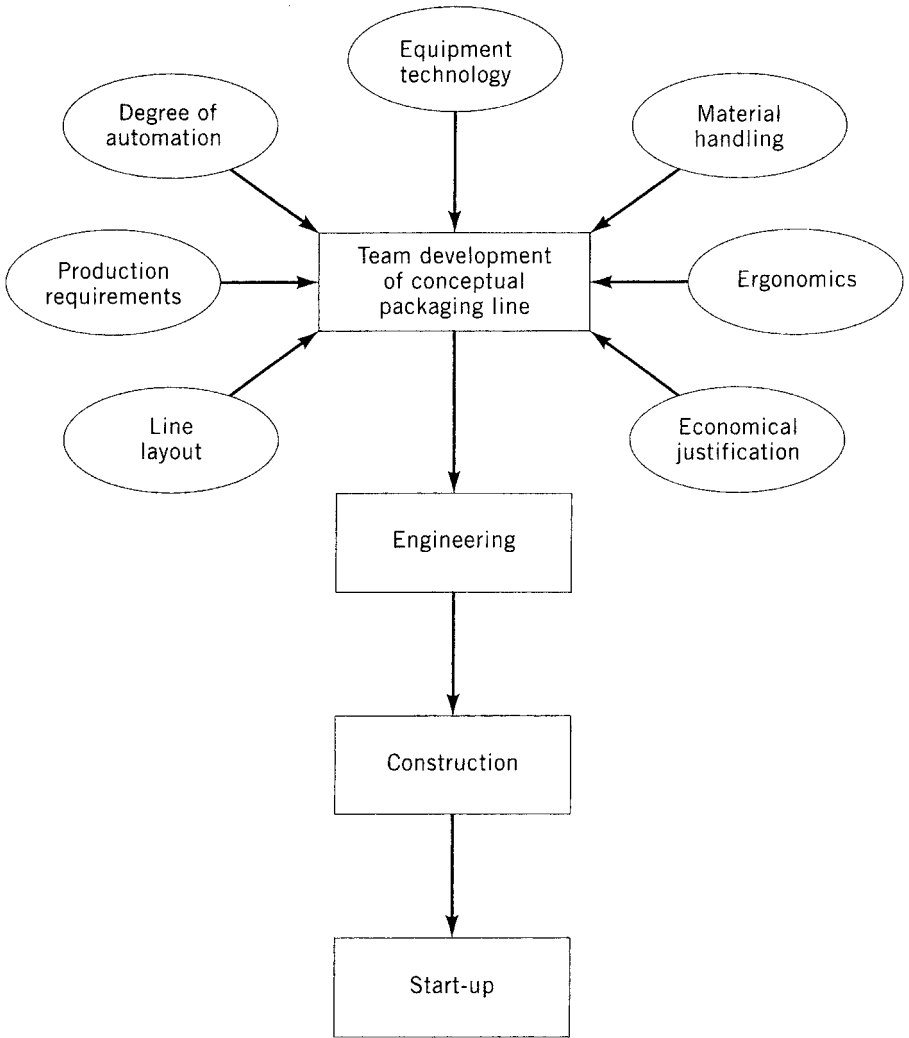


Figure 1. Team development of a conceptual packaging line.

a project. Once the definition, schedule, and concepts are completed, the information is communicated to management. The project team will direct and involve the other disciplines in programming and the remaining project phases.

### 1.1. Definition of Operations and Requirements (Programming)

The definition and concepts for the packaging project starts with establishing general comments about the objectives of the project. This information can be transferred through a conceptual programming procedure. In a nonjudgmental

atmosphere, issues, questions, and concepts are raised to identify goals, facts, and problem statements. It is necessary to have defined goals and objectives to accomplish the successful integration of all packaging issues. Early communication with various parties (production, vendors, marketing, engineering firm, construction firm, etc.) becomes critical in defining project schedule, design criteria, process flow diagrams, general arrangement, and desired equipment. The project team translates the goals and requirements from the programming sessions into a feasible definition of the packaging project. Once these issues are documented, a conceptual project report and scope are issued to management for review and then to the project team for developing the project schedule.

## **1.2. Project Schedule**

A project schedule is composed of a series of events or work efforts. These events can be graphically developed to illustrate the project plan. A project schedule must be established to ensure the timely completion of the project. Events that must occur during various phases of the project can be represented by blocks as illustrated in Figure 2. The order of events are connected by lines in which one event must be completed before proceeding to the next work effort. For example, programming should be completed before vendors are contacted and vendor information is collected. This schedule can also contain a critical path and review sessions, thus supporting management and the project team in planning, monitoring, and controlling resources and time. The critical path becomes the series of events that control the timing of the schedule. Reviews are events that occur at the end of a work effort and/or initiate the next series of events.

## **1.3. Product Characteristics**

To determine the type of equipment and packaging, the designer must determine and define characteristics of the product or products in the conceptual phase. For example, oils, oil bases, and fats contain a large percent of molecules sensitive to oxidation. These products (liquid oils, margarine, shortening, etc.) often have points of unsaturation or double bonds within their molecules due to the absence of hydrogen atoms. These double bonds are susceptible to oxidation. Exposure to reactive environmental compounds, such as oxygen, liberates off-flavors or undesirable compounds (1). This type of product deterioration can often be prevented or minimized by limiting the exposure of the product to oxygen or metal ions through the selection of the packaging materials. Thus, the requirements of the product dictate the package construction and consequently the equipment.

## **1.4. Design Criteria Development**

A design criterion provides a basis and rationale for sizing and selecting the packaging equipment. The design criteria for an oil packaging operation start with statements about:

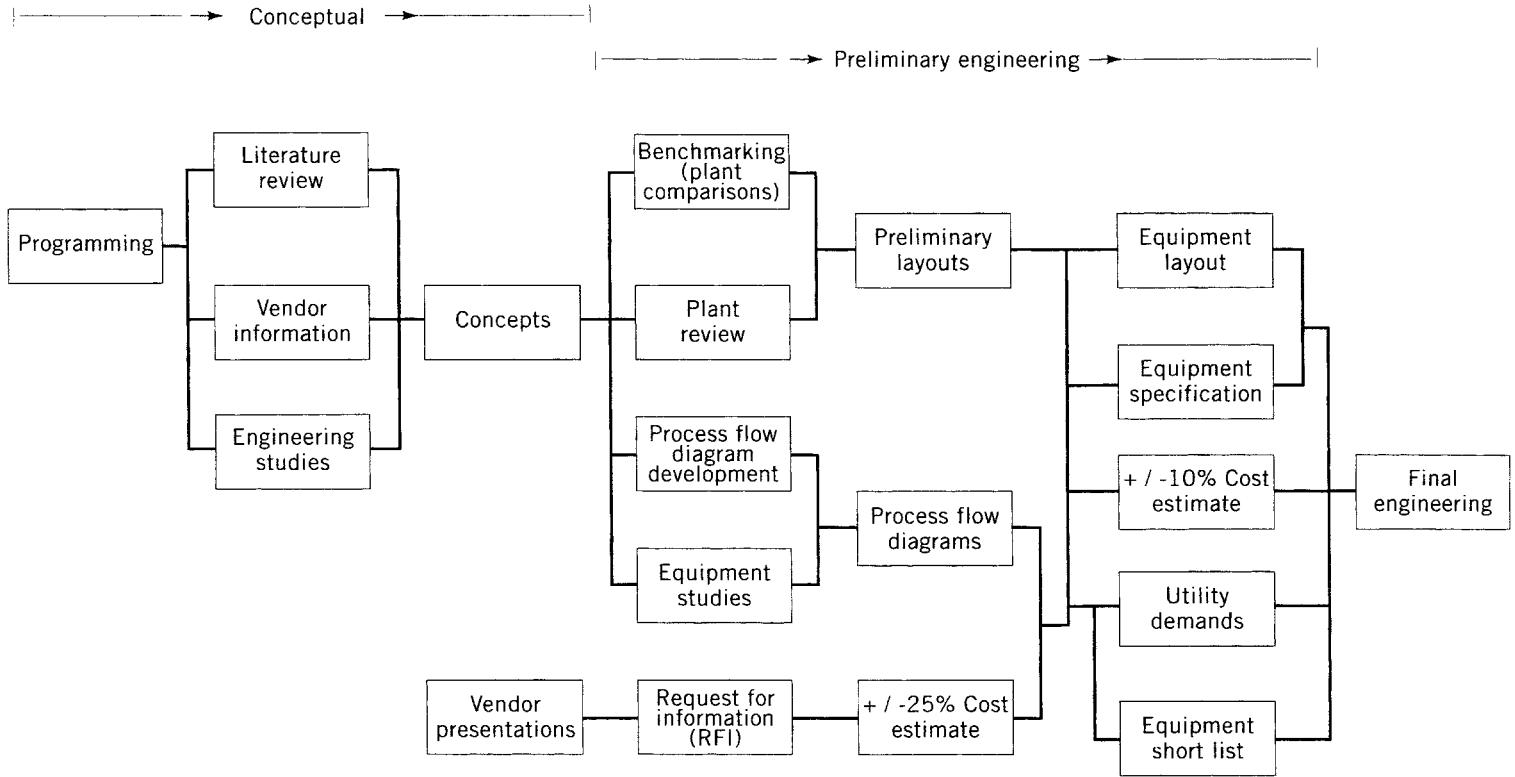


Figure 2. Event diagram of conceptual and preliminary engineering.

- Production matrix
- Production rates
- Product interactions
- Operator interface
- Secondary material handling
- Documentation
- Labeling and coding requirements
- Packaging sizes
- Operating parameters

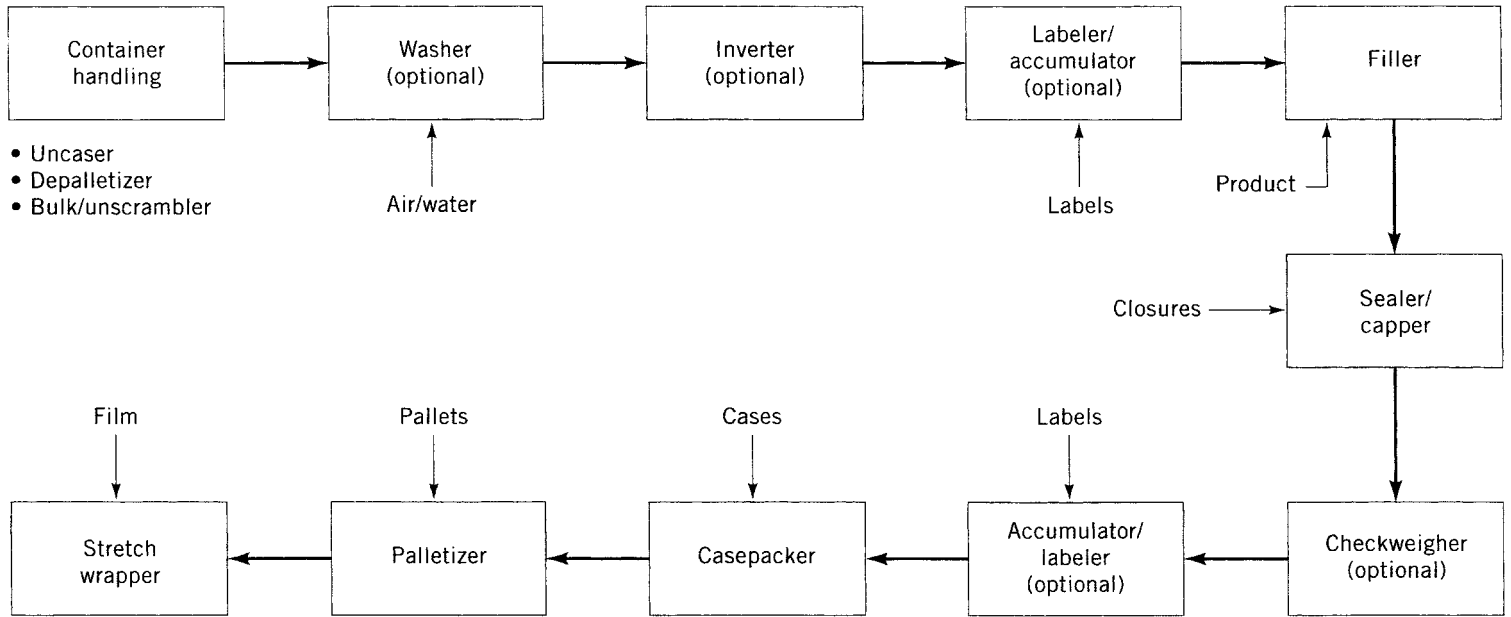
The project team synthesizes the design criteria into working documents and diagrams. These documents describe the equipment requirements and ensure proper sizing of the equipment and definition of design requirements. From these working documents, preliminary process flow diagrams and equipment layouts are developed. These diagrams and layouts are based on operating experience in production facilities and proper engineering practices concerning the relationships between individual pieces of equipment.

### **1.5. Process Flow Diagrams**

The process flow diagrams (PFDs) are schematics that identify the steps of the operation in proper sequence. A conceptual diagram should identify the flow of material from line staging to the operating areas and then continue the flow to all the associated packaging equipment (Figure 3). Further development of the PFDs illustrates the fundamental size and shapes of various components and controls input–output requirements. These requirements are based on flow of materials, heat and material balances, unit operations, storage, and future expansion (2). The process flow diagrams of the packaging system should be completed before initiating vendor or equipment selection. Development of equipment specifications and detailed piping drawings as well as equipment layouts should also be postponed, pending completion of the process flow diagrams.

### **1.6. General Equipment Layout**

An equipment layout affects the construction and manufacturing costs and must be engineered to prevent future problems in production. General arrangements (GAs) of the packaging operation should be completed to provide direction for the project team and possible vendors. Preliminary layouts are developed to illustrate the following.



**Figure 3.** Preliminary process flow diagram in block form.



*Figure 4. Liquid oil filling line.*

- System interface points
- Space requirements
- Ergonomics
- Construction issues
- Manufacturing flow
- Maintenance assessment
- Future expansions or alterations

As the requirements of any two plants are not exactly alike, there is no one ideal plant layout. However, some guidelines exist for edible oil packaging areas; Figure 4 illustrates a typical packaging operation. The general arrangement of equipment in this room is most important from the standpoint of flow of product, ease of operation, and cleaning of the equipment. The GA will indicate whether the layout will be a straight line or in the form of an L or a U, depending on the location of the filling machines with respect to the warehouse and the finished product loading docks.

### **1.7. Equipment Selection**

The next element in designing a packaging operation is the development of preliminary specifications for budgetary pricing of equipment and the selection of multi-vendors per system. Vendors are often asked to present their system to the project

team, and a selection is made reducing the number of equipment bids to three or four candidates. Specified features or goals of equipment should be established. These features include speeds, capacities, and ideal relationship between machines. This will narrow the reviewing process to exclude equipment vendors that cannot satisfy the project goals. As equipment vendors and control points are further refined, a more accurate general plant layout, equipment specifications, and process flow diagram will be developed in preliminary engineering (3).

## **2. PRELIMINARY ENGINEERING**

The next phase is preliminary engineering, in which process and instrumentation diagrams are developed with identification of critical process control points. The design requirements for an integrated packaging system are complex and technically demanding for any company. An understanding of the operations and packaging technology as well as a working knowledge of engineering principles and governmental regulations are mandatory to achieve optimal equipment selection. This will result in an effective process that is established at a desirable cost. Planning and team development are the tools necessary to produce an efficient and cost-effective integrated production facility that is completed on time. Planning is the most important step in developing the desired process or package. A logical design procedure is the only effective method to produce an operating packaging line.

### **2.1. Packaging Equipment Selection Procedure**

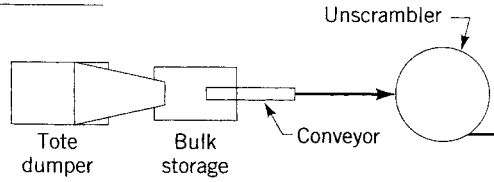
General arrangements and process flow diagrams are provided for the vendors to assist in their equipment proposals. These documents illustrate the fundamental requirements and morphology of various equipment within a defined operation or area. The general arrangements are essential for equipment selection, because they develop optimal placement of the processing equipment within the total manufacturing area. Figure 5 represents a general arrangement that can be developed during preliminary engineering. This oil packaging line has three possible methods for handling the containers. Consequently, multiple reviews and modifications of these drawings will occur before final issue to the vendors. Special design requirements of a particular manufacturing process will establish the sanitation for both equipment and surrounding areas. This must also be taken into consideration when selecting equipment. By determining the type of production equipment and analyzing the design factors that are involved in a plant layout, a detailed proposal involving project costs can be prepared for management's approval.

### **2.2. Control System Requirements**

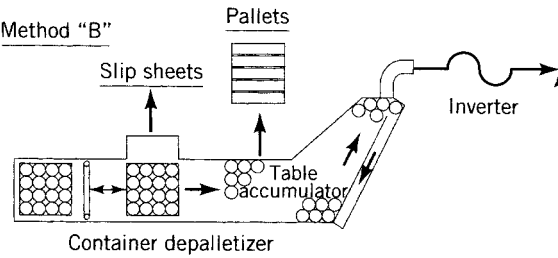
Automation of controls for an existing or new packaging system are expensive. Many managements in the oil industry find it difficult to justify automated systems



Method "A"



Method "B"



Method "C"

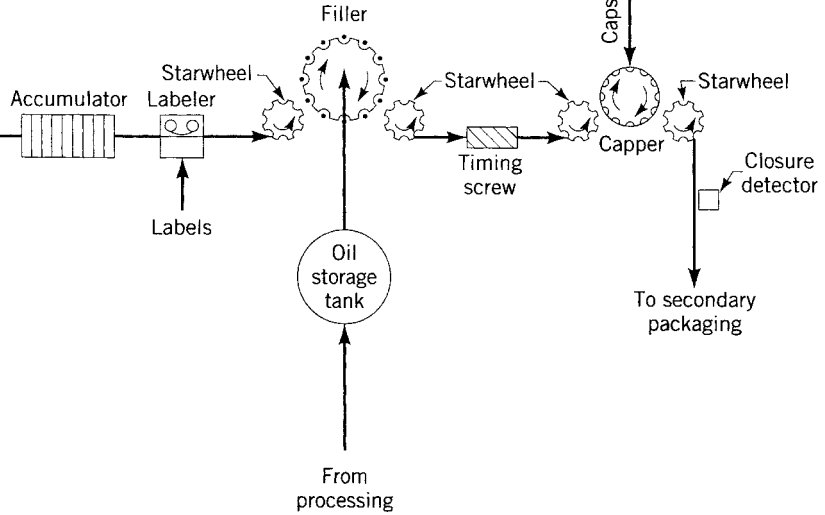
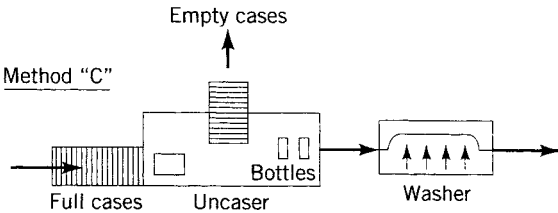


Figure 5. Preliminary flow diagram for container handling.

based solely on process improvement. It is useful to establish other cost-saving applications of an automated monitoring and controlling system such as the following:

- Improvements in inventory
- Improvements in purchasing
- Preventative maintenance
- Line efficiency

Several published articles discuss the recent advances in instrumentation and control (4–6) and may aid in the process of justification. Automation has rapidly developed in cases where documentation is required by U.S. Food and Drug Administration (FDA) regulations. These same regulations will continue to force development of computer systems for the oil industries and help justify the use of automated control systems.

Once a control system is desired, a detailed control specification is required as a guideline to select possible control systems for the various packaging equipment vendors. This functional specification is developed during preliminary engineering and is a “living document” intended to describe the requirements for the systems to be integrated. When accompanied by logic diagrams or logic flow charts, this document should contain all the data required to facilitate final engineering and system programming. Once the type of package, equipment, production monitoring points, and marketing requirements are decided, management must determine the degree of automation desired and select an engineering firm or engineering staff to orchestrate the new integrated system. An engineering firm should be contracted to study possible software and hardware systems based on a developed business objective. For example, if the project is financially justified, then cost becomes a primary consideration versus a costly state-of-the-art facility to develop new market shares.

### 2.3. Regulations

The *Federal Register* details many of the requirements for plant construction. It is important to become thoroughly familiar with each step in the development of the plant, process, and package before attempting to evaluate the system in terms of compliance with guidelines pertaining to the specific project. A review of the guidelines outlined in the *Federal Register* should occur at all phases of construction. Some of the more critical guidelines to be followed are contained in Title 7 (Agriculture, Vol. 40, Part 58) and Title 21 (Food and Drugs, Vol. 51, Parts 108, 110, 113, 114 and 131). The Federal Food, Drug, and Cosmetic Act Part 110 contains the regulations under the current heading “Good Manufacturing Practice (cGMP) in Manufacturing, Processing, Packing, or Holding Human Food.” These are commonly referred to in all phases of a food project, because they have the force and effect of law. The cGMPs are detailed guidelines that specify standards for all food manufacturing functions, including processing, packing, and storing of

raw materials, processed ingredients, packaging materials, and finished food products. Because the FDA customarily does not issue standards or approve products or equipment, the cGMP is the closest thing to regulatory standards for building and operating a food manufacturing facility.

## 2.4. Project Cost Estimates

Management, manufacturing, and marketing must work together to determine and target the type of packaging system, the product sales, and potential revenue. A cost estimate incorporated into a study of the feasibility of a project must be done in the early stages of planning. In some cases, this would determine whether to build, modify, or retrofit an existing facility. The feasibility study involves sending a qualified representative of the organization to similar packaging facilities to observe the latest developments in control strategy, equipment, flexibility, and consequent project cost. The representative should also document the possible requirements for future plant production and incorporate this information into the project's cost report. The feasibility study should summarize the results and equipment costs developed from engineering production capabilities (7). The objective is to estimate accurately the benefits and costs that the packaging project will provide to the corporation.

## 3. FINAL ENGINEERING

Detailed schematics, equipment specifications, and construction details of the various systems are developed during the final stages of project implementation. As demonstrated in Figure 6, several parameters are orchestrated simultaneously. Obviously, with this degree of detail, the project involves input from several disciplines. The final engineering phase includes development of the following:

- Detail general layout
- Process instrumentation diagram
- Isometric drawings of the packaging systems
- Equipment and control specifications
- Installation specifications
- Construction schedules
- Contractor scope of work

In some cases, construction and startup are separate phases of a project and are not included in final engineering. In oil packaging projects, this is normally part of the final phase. Thus, concise definition and agreement on the contents of final schematics and specifications cannot be overestimated, as both system capability and overall project cost can be traced to this definition.

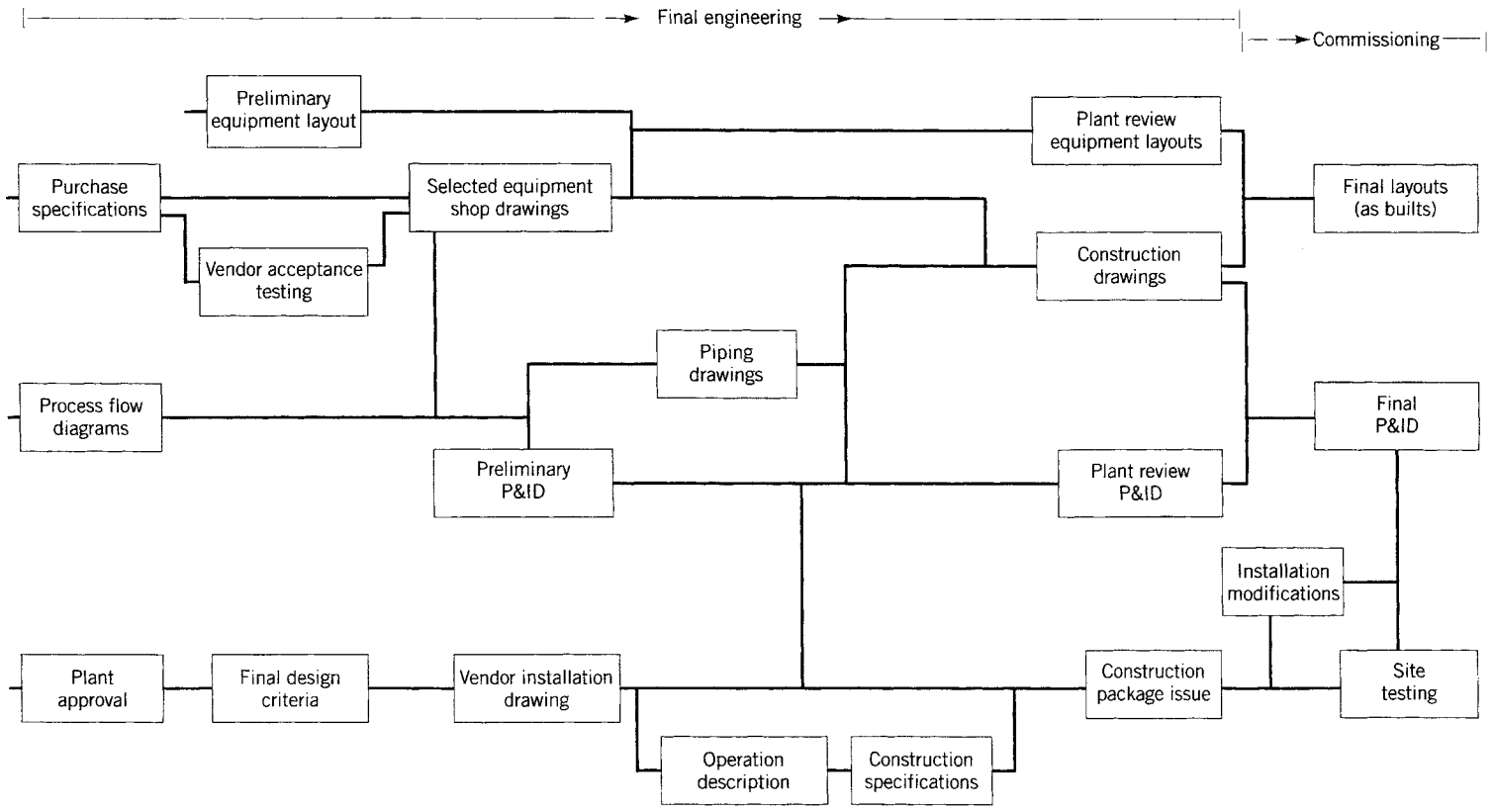


Figure 6. Event diagram of final engineering.

### 3.1. Detail Schematics

Detail schematics are the final process flow diagrams, general arrangements, process instrumentation diagrams, and piping drawings used for construction. The final process flow diagram should present a detailed, accurate, and ordered flow of raw material or ingredient through each manufacturing phase, including the storage of the finished products. Selected vendors and shop drawings must be incorporated into the flow diagram. These diagrams will be used to develop the process and instrumentation diagrams (P&ID, or in some definitions, piping and instrument diagrams). An instrument schematic should illustrate the detailed piping, electrical, and control requirements of the packaging line.

Instrumentation symbols and identification must be agreed on by all team members. The lead sheet to the P&IDs shows the instrument symbols, pipeline designation letters, and process–utility service symbols. These schematics, along with the general arrangement layout, will ensure effective communication of the installation requirements during construction and equipment hookup. The final general arrangement should leave adequate space around the packaging equipment to permit cleaning and maintenance. When equipment is placed adjacent to walls, a minimum of 46 cm should be kept clear between the equipment and the wall (8). More distance is desirable and, in some cases, mandatory because of minimum aisle requirements. Life safety codes based on occupancy levels will establish the requirements for many main aisles.

### 3.2. Equipment Specification and Quote Analysis

Equipment specifications are detailed design documents that describe the requirements and procedures for vendors to quote on a piece of equipment. The following list outlines the requirements and information that should be formatted in an equipment specification:

Scope of specification	General overview of the specification
Performance criteria	Product characteristics, capacities, accuracy requirements, and efficiencies
Project execution	Schedule; contact responsibility
Description of system	Equipment design features; operator interface
Maintenance issues	utilities available at the site; maintenance schedule; spare parts
Instruments, controls, and electrical system	Control of processing parameters; operator interface requirements; level of control
Safety and inspection	Performance acceptance testing; verify safety features
Shipping procedures	Delivery method; equipment identification

Detailed equipment and functional specifications are produced to aid in the selection of equipment and control strategy.

After analyzing all the factors that are involved in the quoted packaging equipment, the vendor quote is evaluated against the specification. Design features are listed and compared with other vendor equipment quotes. Two to four vendors are selected to receive and quote on the specification. The response to the specification and quoted cost then allows the equipment to be purchased according to the specification.

### **3.3. Construction and Startup**

Once the project is under construction, a project cost control system should be implemented. This system provides a status report listing construction status, current budget, committed cost, expenditures to date, cost to be committed, final cost, and variance from budget. This cost system provides management with a method to plan and track the various phases of a packaging project.

Site construction of the project requires organizing the construction bid packages, selecting contractors, and writing subcontracts. These contracts include a specific scope of work for the various construction and installation tasks and the implementation of appropriate procedures for expediting the project. An equipment installation document is then prepared for the construction contractor (9). This document verifies that equipment is properly connected to the defined utilities (air, electrical, water, sanitary drains, product, cleaning systems) and that the contractor has a planned schedule of events. Arrival of the equipment will initiate the startup and testing phase for the equipment.

## **4. PACKAGING SYSTEMS COMPONENTS**

### **4.1. Packages, Containers, Closures**

No packaging system can be functional without a compatible material that will deliver both good machine throughput and provide a sufficient packaging barrier. Any packaging material that is used for an edible oil packaging project must have a low initial microbial load (must be clean) and provide a barrier compatible with the oil product. Product shelf life and stability are directly related to the inherent ability of a packaging material to affect the transmission of oxygen and moisture. It is important to determine the desired shelf life of the product and then select the material that will provide that shelf life. For example, it may be preferable to use a low-cost material with a low oxygen barrier property when packaging edible oils for institutional trade. In this case, the turnover is rapid and the desired shelf life is only a few weeks. The same oil product packaged in a high oxygen barrier material would allow a retail shelf life of several months. Therefore, although the same products are processed in a like manner and the packaging systems are nearly the same, the final products will have completely different shelf life characteristics based

**TABLE 1. Barrier Properties of Some Common Packaging Plastics (11).**

Plastics	O <sub>2</sub> Permeability <sup>a</sup>	CO <sub>2</sub> Permeability <sup>a</sup>	WVTR <sup>b</sup>
PE			
Low density	300–600	1200–3000	1–2
High density	100–250	350–600	0.3–0.6
PP			
Unoriented	150–250	500–800	0.6–0.7
Oriented	100–160	300–540	0.2–0.5
PS	250–350	900–1050	7–10
PET	3–6	15–25	1–2
PVC			
Unplasticized	5–15	20–50	2–5
Plasticized <sup>c</sup>	50–1500	200–8000	15–40
PVDC	0.1–2	0.2–0.5	0.02–0.6
EVOH			
0% RH	0.007–0.1	0.01–0.5	—
100% RH	0.2–3	4–10	—
Ionomer	300–450	—	1.5–2
Nylon 6	2–3	10–12	10–20
PC	180–300	—	10–15

<sup>a</sup>Unit in (cm<sup>3</sup> mil)/(100 in<sup>2</sup>/day atm) at 25°C.

<sup>b</sup>Unit in (g mil)/(100 in<sup>2</sup>/day) at 38°C, 90% RH.

<sup>c</sup>Values depend greatly on plasticizer content.

Source: Data are taken from several sources.

on the packaging material used (10). See Table 1 for barrier properties of common packaging plastics (11).

Another consideration is the product–container compatibility. For an oil product intended for an extended shelf life, an appropriate container choice would be a multiple layer container, as the layers are composed of high- or low-density polyethylene on the outside, ethyl vinyl alcohol in the middle, and high-density polyethylene on the inside (Figure 7). High-density polyethylene on the inside is a suitable plastic for contact with edible oil and provides an adequate moisture barrier. Low-density polyethylene is not a suitable inner layer material due to the high rate of migration allowed. The middle layer is the oxygen barrier layer, and the outer layer should be resistant to water. Another multiple layer container is the combination of paper and plastic materials. There are a variety of combinations possible for these containers, and their use depends on the properties of the product, container, and packaging equipment. This combination is commonly used in composite cans for shortening. Normally, the equipment vendor has experience in selecting the appropriate packaging material, and the packaging barrier is developed between supplier and manufacturer. The primary packaging materials that are successfully being used include styrene, polyethylene, polypropylene, poly(vinyl chloride), Saran, paper, aluminum foil, and combinations of these materials. Two processes widely used in forming barriers are plastic coextrusion and lamination. Plastic coextrusion is the combining of two or more polymer layers during a one-step process of film or

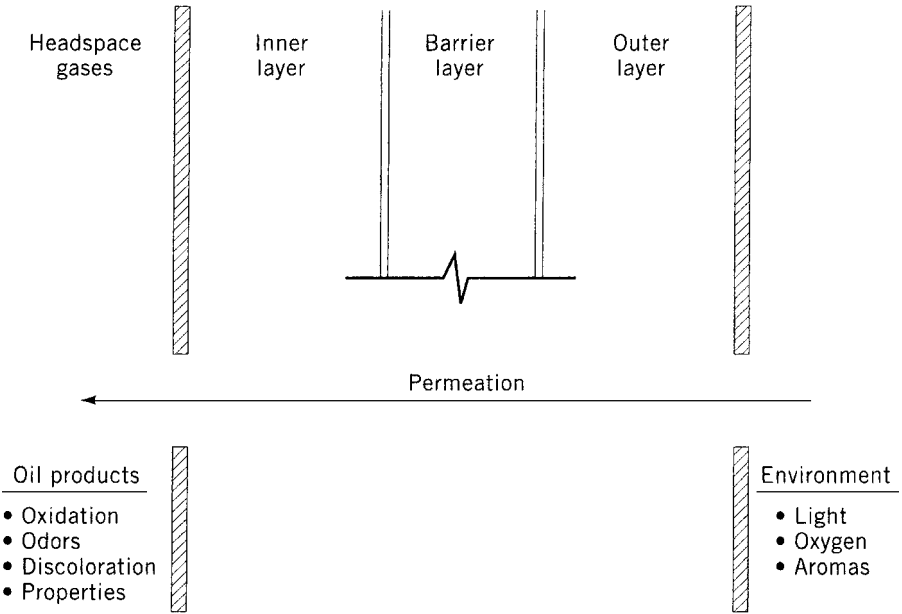


Figure 7. Container-product interaction.

sheet extrusion. Lamination involves an adhesive or extrusion-bonding process that brings together two or more materials that have been preformed (12).

There are three major forms of plastic packages used in oil packaging projects: blow-molded containers, such as plastic oil bottles; thermoformed packages, such as trays, tubs, cups, and inserts; and injection-molded fittings, closures, and lids. The basic techniques of plastic blow molding were derived from those used in the production of glass bottles. Air is forced under pressure into a sealed molten mass of material that is surrounded at the right distance by a cooled mold of the required shape. The pressure of the air causes the molten mass to move out to the mold walls where it cools on contact. Finally, the mold is opened and the molded container ejected. Thermoformed operations are used primarily for containers that have limited depth in the shape. This operation heats a sheet of multilayer plastic film that is in a softened state. A mold then exerts a force on the sheet and forms it into a container. A wide range of polypropylene sheets are available for thermoforming. Polypropylene is selected for the compatibility with thermoforming, acceptability as a food contact surface, resistance to heat, low water transmission, and ability to be extruded in a sheet with another material (13). To reduce oxygen transfer, this sheet has an oxygen barrier. Ethylene vinyl alcohol is often the material used as an oxygen inhibitor. The use of a single material such as poly(ethylene terephthalate) (PET) has also been used for thermoforming; however, this material is more often blow molded (13).

Injection molding is a method to make complex shapes rapidly, normally fitment or closure. The main function of a closure is to keep the container sealed until the



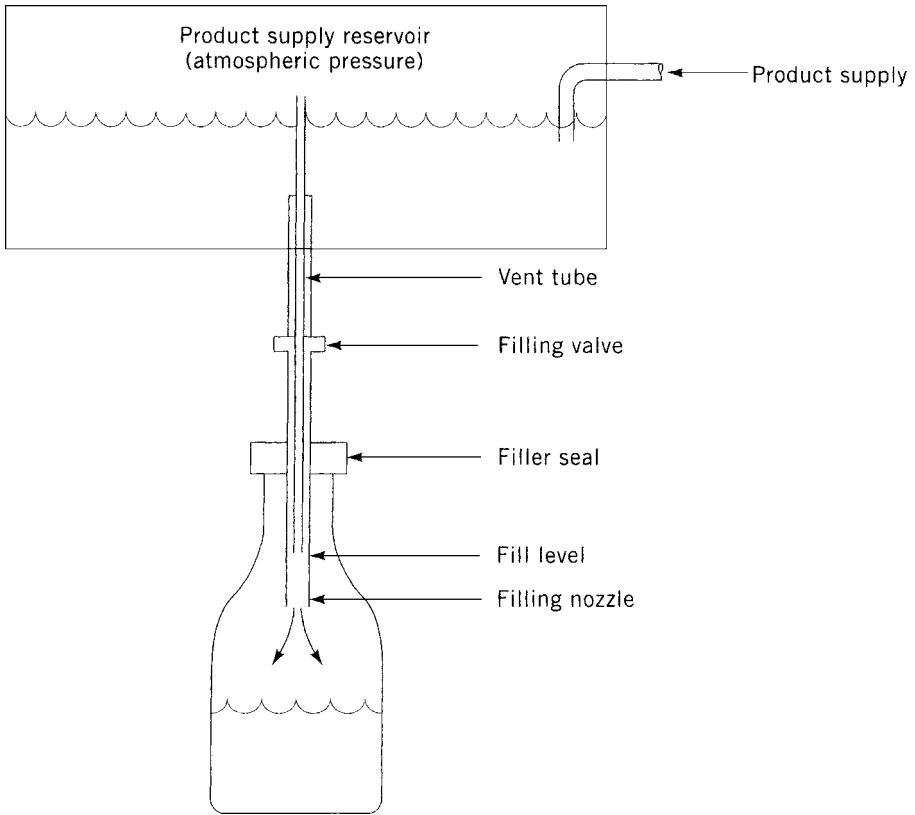
contents are required for use. This means that the contents cannot escape and external environments cannot enter the container. The degree of tightness required by the seal of oil products is one that provides a completely hermetic seal. It must be possible to open the container by breaking the seal without difficulty and also to reseal the closure when only part of the contents is used. Some containers will have an aluminum foil membrane sealed to the bottle opening by heat before capping or by induction after capping. Capping can be accomplished by using either a press cap or screw cap. Alternatively, the closure can serve as a dispensing device, such as an orifice or spout. The closure must neither affect nor be affected by the contents of the container. It should be inert to any conditions to which it may be exposed during processing, storage, or normal end use. The closure may need to provide a safety seal to verify whether it has been removed before use, thus giving the consumer assurance that no one has tampered with the contents.

## 4.2. Fillers–Sealer

The filler is the focal point and the most critical component on any production line. This piece of equipment will always adversely affect the production when it is not operating. Filler downtime can never be recovered; therefore, it should be minimized. All other equipment on the line can be sized or adequately positioned to allow the filler to continue running when downtime occurs. In addition, the performance of the filler with respect to filling parameters remains constant when the filler operates without interruption. This constant performance is critical when determining equipment line speed and relationships. The rate-limiting factor of the whole line depends on the performance of the filler.

The selection of the filler speed is based primarily on product characteristics, production volume, shift–time structure, package sizes, and other operating factors. Once the filler speed has been selected, the capacities of the other production equipment can be calculated to maximize filler run time (14). Equipment before the filler in the production line is required to have enough capacity to ensure a constant supply of product to the filler and adequate recovery from upstream machinery interruptions. Similarly, equipment down line from the filler must have added capacity to recover available accumulation during downstream interruptions. Selection of the type of filler to be used depends on the desired method of filling. There are two basic methods of putting liquid oil into a container. The first, and most common, is to fill to a level, and the other method fills by a premeasured rate.

*Level Control Filler.* Plastic bottles can be manufactured clear; thus for consumer appeal, they are filled to a level. This means the volume may vary but will appear to be equal due to the visually perceived common level in the containers. Flow is by gravity through a nozzle into a sealed container, whereas air equilibrates via a vent tube (Figure 8). When the rising liquid reaches the air vent port, flow stops. There is no overflow of product as in a pressure or pure vacuum filler. Aeration is at a minimum, perceived fill level is extremely accurate, and the filler is relatively simple and easy to maintain. Size consistency of the blow-molded container is critical to control, because this dictates the amount of product being sold.

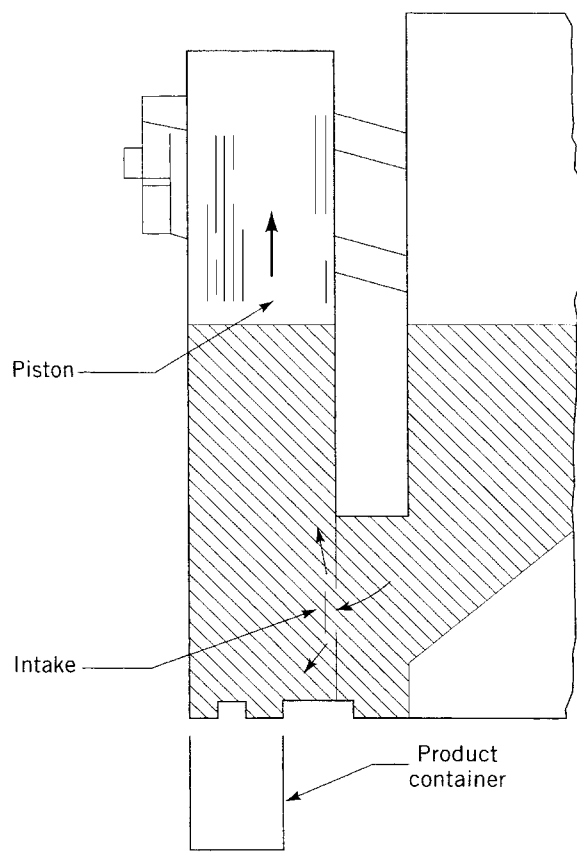


**Figure 8.** Gravity filling system.

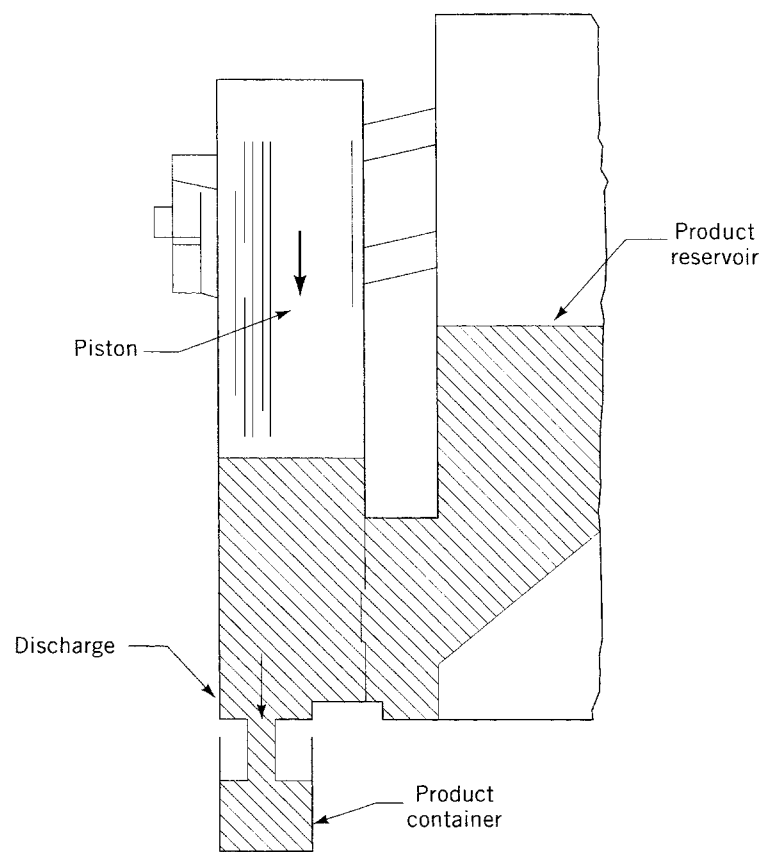
*Premeasured Volume Filler.* The second method is to fill a premeasured volume. For containers that do not allow the product to be visible for comparison, such as a composite can for shortening, the filler transfers a premeasured volume of product into the container. There are three types of premeasured volume fillers.

For a piston premeasured volume filler, the fill cycle consists of an intake stroke and a discharge stroke (Figure 9). During the intake stroke, the piston rises and draws product from the supply tank into a calibrated cylinder, which measures the volume of product. When the cylinder is full, the intake port closes and the discharge port opens. The fill takes place as the piston moves downward and delivers the premeasured volume of product into the container below. Most fillers are mechanically operated and simple. These fillers can handle a wide range of product viscosities and a broad range of speeds (15).

Other premeasured volume methods deliver a volume (or mass) by controlling the pressure and time of the delivery. Pressure is used to fill the container for higher viscosity products. Product is pumped under pressure through a filling valve and then via a product tube transferred into the container. A sensing air tube is built into the product tube to monitor air pressure in the container. Low-pressure air



(a)



(b)

Figure 9. Volumetric piston filling.

(25-cm H<sub>2</sub>O) flows through the sensing air tube into the container while it is being filled. When the rising liquid reaches the sensing air tube port, it stops the flow of sensing air, which triggers a controller to close the product valve causing product flow to stop. A nonturbulent product flow reduces aeration to an extremely low level. Fill is rapid, and fill level accuracies are excellent. This is the ideal method for high-speed filling of low-viscosity products into plastic containers. Most fillers are mechanically operated and simple. These fillers can handle a wide range of oil product viscosities and a broad range of speeds.

Timed flow systems measure the volume delivered to the container by controlling the amount of time product is permitted to flow. Flow is by pressure from a product pump. Delivered volumes are determined by a precise measurement of flow time through a controlled orifice while the delivery pressure remains constant. Volume can be increased or decreased also by changing the rate of flow to the metering pump. By using more than one metering pump, multistage filling of different products in the same container can be performed. This type of filler has few moving parts that are in contact with the product and can be cleaned or steamed in place.

*Net Weight Filler.* Net weight scale filling involves the use of a microprocessor and highly accurate weigh scales that “tare” an empty container and then control precise liquid flow into it (Figure 10). The computer logic monitors fill weights and makes continual adjustments to the flow, producing overall average fills with virtually zero error. No dynamic seals are required. Rates are slower than most methods; however, filler cleanability is superior to other types of systems. A product fill rate of 80 bpm is an acceptable rate for net weight filler. Two types of net weight systems are available: linear with inline weighing and rotary with off-set weighing.

Linear load cells are located parallel to the travel of vials. Vials are transported over the load cells, tared, filled, and controlled to a monitored weight. Production rate increases can be accomplished by additional load cells and filling nozzles.

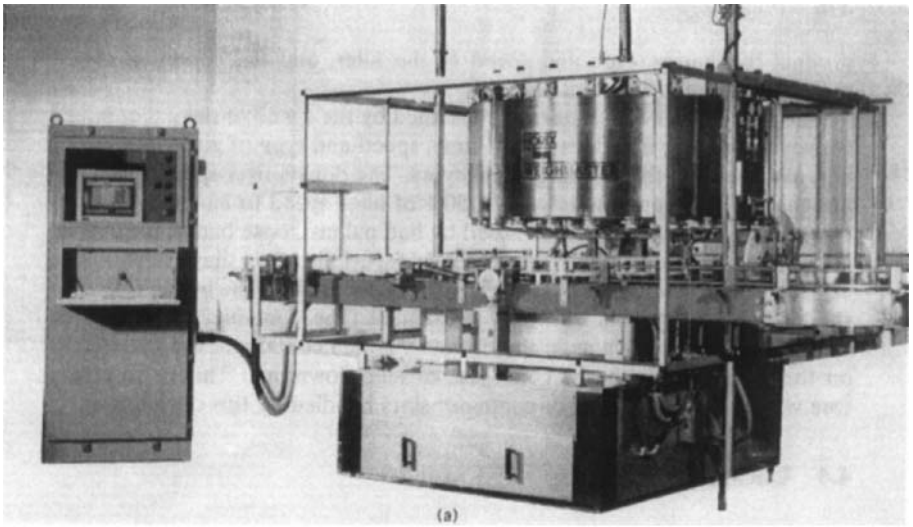
Rotary load cells are located in a semicircular pattern. Containers are transported by physical placement onto load cells. Future addition of load cells is limited; however, the unit has rapid changeover, small size, and rapid response to taring of the vials.

This type of filler is a growing type of system in filling oil-based product since the expense of the product justifies the cost of a net weight filler.

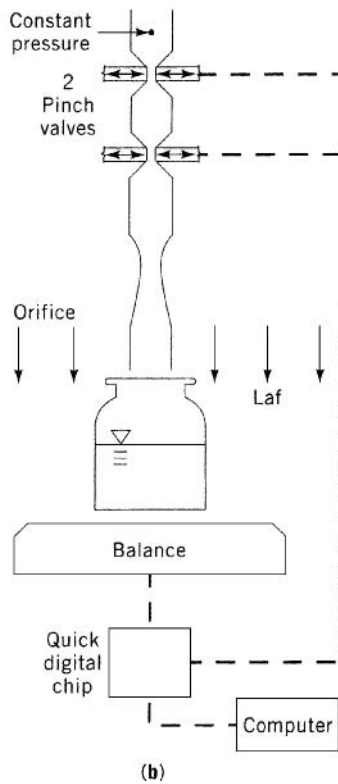
### 4.3. Depalletizers

The depalletizer in a packaging line accepts stacked bottles on pallets that have dividers and discharges one container at a time in single file. Sizing of the depalletizer for unsorted containers must be analyzed with respect to the product mix ratios, type and speed of the filler, and the condition of the pallets, conveyors, and containers.

The speed of a depalletizer is determined by the speed of the pallet infeed conveyor entering the elevator, lift-time, speed and type of pallet tier sweep bar, and speed of the discharge conveyors. The depalletizer speed should be designed to be



(a)



(b)

**Figure 10.** Net weight filler.

approximately 140–150% of filler speed to handle periodical downtimes. Downtime can be caused by bad pallets, loose bands, protruding nails, loose boards, or leaning pallet loads, especially when they are unloaded directly from a truck and set on the infeed conveyor. Thus, the infeed conveyor should hold five pallets of empty containers. The container load must be straightened to avoid jams in the elevator. Fallen containers will cause jams on the single-file discharge conveyor, causing downtime. This is especially true when there is a range of container sizes handled by this depalletizer.

#### **4.4. Uncaser**

A typical returnable glass uncaser is a belt type machine, with the belts picking the bottle up by the neck from a half depth case and placing them on a tabletop bottle conveyor. Typical downtime is caused by bad cases, either due to incorrect dimensions (poor taping or glue sealing of flaps), excessive moisture, or warping. Minor mechanical downtime is normally caused by off-timing and belts jumping off pull-eyes. As with a depalletizer, the uncaser speed should be sized to keep an accumulation table located before a washer full under normal operation or have the ability to fill the conveyor–washer quickly after possible downtimes. With a speed of 130–140% of filler speed and a possible design downtime of 5 min, the uncaser should be designed to fill an empty accumulator in 10–15 min with the filler running. The purpose of this bidirectional covered accumulator is to prevent filler downtime for minor washer or uncaser problems.

#### **4.5. Conveyors–Accumulators**

Conveyors between various equipment must be sized to run at a rate 110–115% faster than the capability of the highest speed equipment that it feeds or from which it receives containers or cases. Conveyors must also be capable of running 140% faster than the highest speed equipment to create adequate gaps between units when separation is required. For improved container handling, conveyor systems should be run as slow as possible with as few interface points as possible between equipment components. This will result in less container-to-container pressure and reduced maintenance and downtime. It is sometimes desired that a conveyor system be capable of handling multiple size containers, i.e. those with a major difference in product volume, 100 mL versus 1.5 L. The speed of these conveyor systems, especially the single-file speeds, needs to be variable. These conveyors should have soft starts with the variable drives and transfer points. Selection of the conveyor manufacturer becomes a critical design feature and must be addressed in the preliminary engineering phase.

Case conveyors should be long enough to accumulate the cases rather than having an extra operator to stack them off the system when the palletizer shuts down. The cost of the accumulation conveyor must be compared with the extra labor cost incurred by hand stacking the cases. Return on investment criteria should be used to make this decision. Case conveyors should be at least 10% faster than the

case packer or palletizer to create gaps between cases, minimize jamming on curves, and provide some accumulation. Reasons for the faster conveyor speed are to accommodate some case packers that may eject multiple cases at the same time or to allow for on-line case labeling.

A container accumulation table should provide 5 min of surge capacity when it is full. The length—size of the accumulator is determined by the amount of container required when the uncaser or palletizer systems shut down. The sizing of an accumulator is determined by the length and frequency of expected upstream and downstream stoppages. Experience shows that the vast majority of stoppages last 2–5 min. Accordingly, the accumulation table should be designed to hold 5 min of filler production. The infeed of the accumulator should have three conveyor sections with one of the conveyors closest to the table running slower than the others to reduce bottle tippage due to speed changes. The rationale is to keep the filler functioning until the stoppage is removed or repaired (16).

#### **4.6. Washer**

In selecting a washer design for temperature control, motor sizing, compartment size, and so on, the designer should allow the speed of the filler to determine the rate and capacity. Based on typical washers, fillers, and periodical jams that take 2–3 min, the washer is sized for a rate that is 130–140% of filler speed to recover lost operator time.

Two bidirectional accumulation tables should be installed as close to the washer as possible, one before and one after. The infeed accumulator should remain full as often as possible. The purpose of the discharge accumulator is to keep the washer running when the uncaser or depalletizer is down. The accumulation table following the washer will remain empty as long as the speeds of the washer and filler are the same and running at rated line speed. When the filler is down, the infeed conveyor to the washer should shut down, and the remaining containers in the washer should be stored on the discharge accumulation table. This sequence will minimize washer cycling. This ability to recover accumulation on the discharge table has a direct effect on line efficiencies. An alternative method of accumulating containers is by on-line conveyor accumulation. This requires additional conveyor lanes or extended lengths. This is not recommended, due to additional transfer points and difficulty in ejecting fallen containers.

#### **4.7. Labelers**

Several types of labeling systems are available for packaging systems. Online application of labels are an efficient, cost-effective means of identifying and decorating packages. Labels consist of face stock material coated with an active adhesive on one side. The label is carried on a silicone-coated backing paper to allow for easy release on demand. Pressure-sensitive labels are supplied in sheet, roll, or fanfold form. A roll form, with a predetermined specification for automatic application, is the most common due to the ease and efficiency of handling.

A variety of standard and specialized applicators exist for pressure-sensitive labeling. Standard portable head applicators provide top, side, and bottom labeling. These applicators use one of several basic methods available for application of automatic pressure-sensitive labels. In a tamp-blow method of application, the label is dispensed from the backing web and held in position by vacuum on a tamp pad. The tamp pad is indexed 0.8–1.5 cm away from the product, and then the label is applied to the product by a gentle blast of air. Tamp-blow applications are used for a large array of packages that have irregular, curved, or recessed surfaces.

The term *print/apply* refers to the ability to print identifying information directly onto a label just before its application. Serial numbers, date codes, lot codes, and S.K.U. numbers are examples of such printed information or identifiers.

Automatic container handling and orientation are often integrated into a labeling system. The labeling system can control the containers by use of the accumulation conveyor, adjustable side rails, gate stop mechanism, rotary table, pneumatically operated pushing cylinders, and the reject station. The system can also rotate the container after labeling by 90° or 45° for proper alignment approaching the case packer.

#### 4.8. Case Packers

The case-packing function is one area of the packaging line that can benefit greatly from automation, since this function is one of the most labor-intensive areas of a packaging line. Case packers can be high-volume, automatic machines that can handle products more gently than human hands.

Automatic case packers do not require a full-time operator, although an attendant is needed to replenish materials at periodic intervals. The case packer automatically opens and positions the case for loading, accumulates the product, packs, and seals the case. The case can be sealed with adhesive (either hot melt or cold glue) or pressure-sensitive tape. A hot melt system is normally the preferred method to seal cases. An operator is required to replenish the case magazine and sealing materials at regular intervals. Some packagers add auxiliary case magazines to their case packers. An auxiliary magazine can increase the blank case capacity twofold or threefold, so that the operator has more time between magazine refills to attend to other tasks. Styles and types of case packers vary, but they can be grouped into four basic categories: side load, top load, wraparound, and bottom load (17).

Side load case packers are generally used for packing cartoned products into corrugated cases (although tubes, cans, and other containers may be packed in this manner). However, for oil, side loaded case packers could increase the stress of handling the product, which may result in product damage.

Glass and plastic bottles are most commonly packed by top load case packers. Top load packers can pack bottles into corrugated cases, plastic cases, corrugated trays, plastic trays, full- or half-depth cases, or nested carriers. The complexity of the cases and product matrix determines the justification for a top loader.

The wraparound concept is that of the “tight-pack” case, meaning that the products are packed tightly. The tight pack is designed to eliminate product movement



that can cause label damage or carton scuffing. The operation of the wraparound packer is somewhat like that of side load case packers. The product feeds by conveyor and is staged into case loads. At the same time, a case blank is indexed from the magazine into loading position. The product load is transferred onto the case blank, and the case is folded around the load. Adhesive is applied to the flaps, and they fold to seal the case. A disadvantage with a wraparound case packer is the increase in case inventory that can result from installing this type of case packer.

In using a bottom load case packer for a packaging operation, the containers are accumulated and unitized into the selected case pattern. As the unitized load is indexed onto the lift platform, a case blank is unfolded from the case magazine over the product. The lift platform raises the product into the opened case. The case is indexed out of the loading area and is top and bottom sealed by a hot melt glue system. The bottom load case packer is the best type of case packer for containers that require stacking, pads, or automatic case insertion.

#### 4.9. Palletizers

Palletizers are available in high-level, low-level, or robotic versions. In high-level palletizers, the case or product enters the palletizer via an overhead conveyor. In a low-level system, the case or product enters the palletizer at floor level or approximately 75–90 cm off the floor. Robotic palletizers generally operate at floor level and involve mechanical placement of the cases and/or rolls.

A high-level palletizer offers the advantage of bringing the case in near the ceiling. The cases can be conveyed to the palletizer just under the ceiling, freeing the floor underneath for other operations or storage. A high-level palletizer usually requires a metal platform, catwalk, and stairs for an operator to have access to the pattern-forming station. The pallet discharge height is at floor level. The pallet with the load raises and lowers to the correct tier height with each tier formed. The case infeed and pattern forming is done at the high level.

In the low-level palletizer, both the case or product infeed and the full pallet discharge are at floor level. The floor level infeed permits the case pattern to be formed at floor level and the palletizer to be close coupled to the discharge end of the packaging line. It also eliminates the necessity of elevating the case from floor level at the case packer to ceiling height. The low level eliminates the need of having metal platforms, catwalks, or stairs. It also discharges the full pallet at floor level. A pattern for a tier is formed at low level and transferred to an elevating platform that raises the tier to the proper stack height. The pallet being loaded is not raised or lowered, but it stays at floor level. This reduces the overall weight being lifted to the height of one tier. The low-level palletizer provides ease of operator supervision of the machine, because all the mechanical operations used in maneuvering the cases into their perspective unit loads are done at ground level.

Palletizers that are designed to receive the cases at either a high or a low level require the cases to be formed on a stripper plate. Once the layer is formed on the stripper plate, it is lowered to the pallet that is stationed on a floor level pallet conveyor. A sweep arm strips the cases off onto the appropriate layer on the pallet as

the stripper plate slides out from underneath. The stripper plate is tapered and is equipped with a powered roll conveyor section to ensure a positive transfer of the cases as they exit onto the pallet. Based on which style palletizer is selected, the stripper plate will either index to the top of the machine to receive product for a high-level palletizer operation, or if a low-level palletizer is selected, the stripper plate will stay at ground level at the height of the infeed conveyor to receive product.

Palletizers can be furnished with automatic pallet dispensers, glue systems, automatic bottom-pad dispensers, and automatic tier-sheet dispensers. Palletizers offered are required to have a programmable logic controller (PLC) for multisize cases. It is possible to program different tier and pallet patterns into a PLC unit, and thus change over from one size to the other by means of selector switches. A palletizer can automatically arrange the cases for each tier, dispense the pallet, or insert the tier or tie sheets. These functions can alternatively be performed manually by an operator.

Robotic palletizer systems have the ability to orient the cases and to pick and place the trays, pads, and slip sheets. The robotic palletizers' rate of operation will be considered too slow for most oil production lines. However, robotic palletizing systems will become more common for oil packaging lines based on the following issues:

- Improvements in the design of end effectors that will allow multiple case placement
- The reduction in equipment prices for robotic systems
- Increases in the number of acceptable equipment suppliers
- The using of multiple robots for loading a single pallet

#### **4.10. Stretch Wrappers**

Stretch wrapping is the method of unitizing and protecting pallet loads or groupings of individual containers of product by applying layers of plastic film. The film is mechanically stretched to increase its yield and create a tension force around the load.

There are basically two approaches in stretch wrapping: turntable and overhead rotary arm. The turntable stretch wrap system is accomplished by rotating the load on a mechanically driven platform and applying multiple layers of film. With overhead rotary arm systems, the unit load remains stationary and the film application unit spirals around the load. On some units, a top platen can also be added to the rotary arm system to ensure that cases and the top slip sheet on a bulk pack remain stable during the stretch wrap operation.

Fully automated conveyor systems are available for turntable and overhead rotary arm models. Product loads can be fed into the infeed conveyor system by the automated palletizer system or manually by fork truck. The loads are staged

on the infeed conveyor system and automatically sequenced into the wrapping station. The load is wrapped and then discharged on the exit conveyor system. An overhead platen stabilizes the unit load during the stretch wrapping process. This turntable method provides the flexibility to accommodate loads of varying heights. Stretch wrappers must be able to provide a rugged state-of-the-art stretch wrapper that includes structural steel construction, powered prestretch film delivery system, factory startup assistance, and PLC documentation as standard features.

#### 4.11. Line Efficiencies

Packaging lines operating in many different oil industries across the country are currently performing at mechanical efficiencies that are far below what was projected and anticipated during conceptual design. Efficiency claims for packaging machinery, particularly for new equipment, are stated so high that they rarely, if ever, perform at such plateaus. In most cases, efficiency ratings projected by an equipment manufacturer, even after a thorough checkout test is performed, are unrealistically high for average plant conditions and production requirements.

The term *mechanical efficiency*, as used by production departments, identifies the mechanical reliability in a percentage of all the components that make up the packaging line. The components of a packaging machine must start, run, stop, reset and restart, reliably, thousands of times before actual packaging of the product is complete. The basis on which many machinery manufacturers state that their machine has an efficiency rating of 100% is often based on a time-limited test run of the customer's product under a controlled condition and not under actual production requirements. It is critical that packaging machine manufacturers understand and state the reliability of all the components of a machine, how many components compose each machine, and how the components interact with each other throughout the whole machine cycle.

A typical packaging machine is composed of numerous parts or assembly blocks. These parts are not complicated and are reliable; however, when several of these assembly blocks are combined together in the building of the machine, the blocks as a whole become more complicated, and the machine's overall efficiency rating is adversely affected. It must be understood and emphasized that in determining mechanical efficiencies, many components or building blocks are necessary to assemble a complete machine.

This knowledge of the machine is needed before any calculations are made on mechanical efficiency. During machine analysis on new equipment, evaluations on machine reliability and calculation of an efficiency rating should be done when equipment is under consideration for purchase. This is one of the key factors to consider during the purchasing of a packaging machine, and this calculation must be as realistic as possible from a true in-operation situation. The starting of a production schedule with accurate mechanical efficiencies to calculate into that schedule will greatly improve scheduling accuracy in terms of labor requirements, length of production run, and total production output. Proper preventative maintenance,

operating procedures on the equipment, and startup and shutdown for breaks, lunch, and shift changes are all extremely important and have a big impact on operating efficiencies (18).

## 5. EDIBLE OIL OPERATIONS

Edible oil operations use glass or plastic containers in a variety of sizes and shapes. Glass containers are rarely used in this market category; occasionally glass containers will be found on import oils. Plastic containers dominate this packaging operation, the result of a packaging transformation that has occurred in recent years.

### 5.1. Low-Viscosity Products

Plastic bottles are either received in bulk or in reshippers, whereas glass bottles are usually received in reshippers. A reshipper is a shipping case that is used for both returning the empty containers and shipping the filled containers. Plastic and glass reshippers are decased by inverting the reshipper unscrambler to orient and convey the containers. Containers for plastic bottles that are preformed outside the plant are received in bulk handling containers. The bulk handling containers either are pallet loaded, which requires a depalletizer, or are in bulk boxes, which require an unscrambler. Another option would be to have an in-house blow-molding operation. The containers pass through an unscrambler or depalletizer for loading onto a single-lane conveyor. The container can be oriented so that a label can be placed on a side of the container, or the label may be placed after filling. These containers are then processed through a bottle washer or bottle inverter, which usually is an air-jet type. The containers are filled with or without nitrogen sparging, capped, labeled, and conveyed to secondary packaging (19). A general process flow diagram for a packaging line is illustrated in Figures 5 and 11.

Filler downtime typically is due to star-wheel jams, irregular shaped containers, and timing adjustments. The accumulation table between the washer and the filler provides the filler with bottles during these periods. The washer speeds need to be fast enough to refill the table in a reasonable time. However, the filler needs to be slightly slower than the washer, since the accumulation table after the washer should normally be empty.

In addition to prolonging the life of the equipment, reducing maintenance, and reducing downtime, it is recommended not to operate the equipment continuously at the full manufacturer's rated speed. The only exception is the filler. Table 2 provides a guideline to line component speed in regard to the filler and type of container.

As typical downtime is 1–2.5 min, the accumulator should be sized for 2.5 min of filler speed. Thus, under normal conditions, the accumulator should remain empty. When the filler system goes down, the washer should ramp down accumulator quantity. The frequent stoppage of the fillers is due mainly to poor container handling. As the state of the art progresses and the equipment becomes more

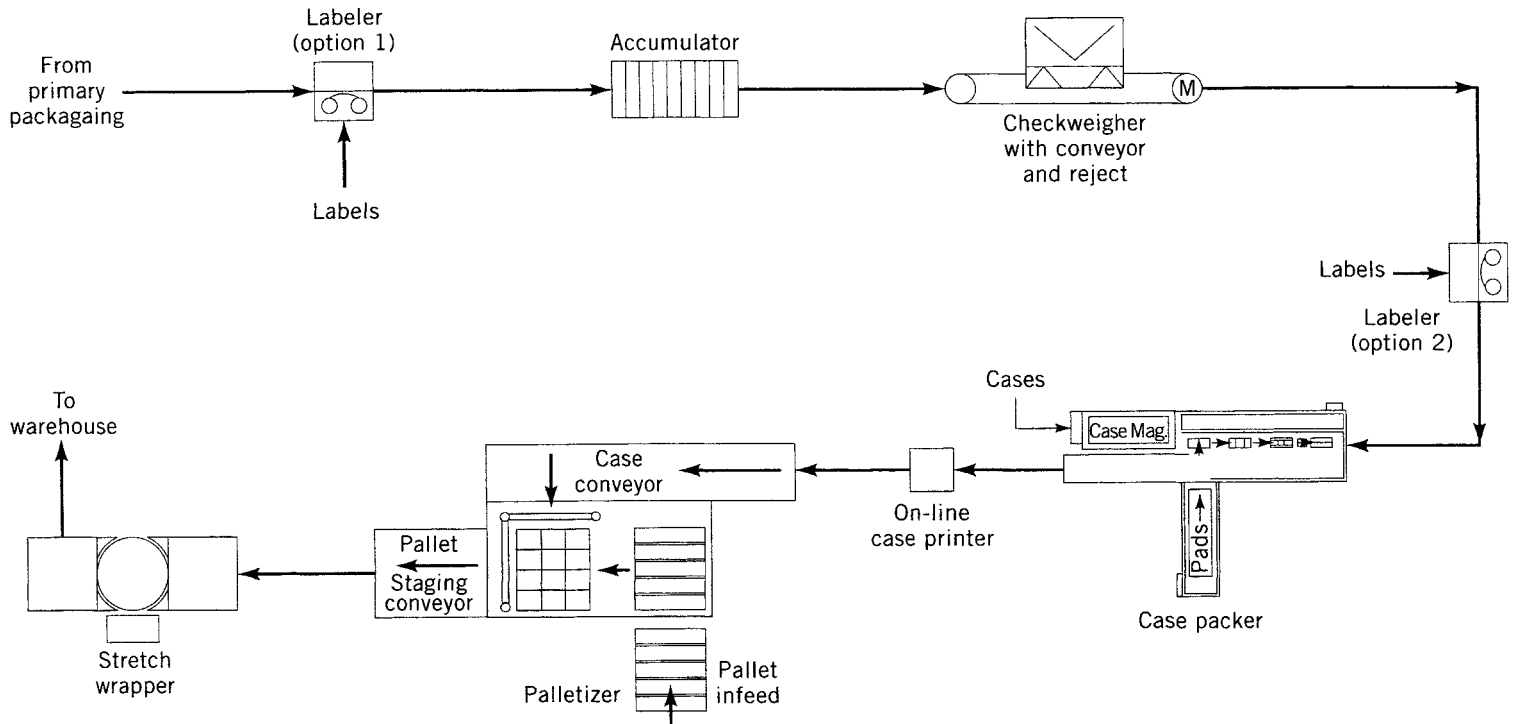


Figure 11. Flow diagram for secondary packaging operation.

**TABLE 2. Line Component Operating Speed as a Function of Filler Speed.**

Equipment	Primary Container Type	Speed as a Percent of Filler
Depalletizer or unscrambler	Bulk plastic containers	140–150
Uncaser	Glass bottles in cases	130–140
Empty container accumulator		2.5–5 min
Inverter	Plastic containers	140
Washer	Glass bottles	110–120
Filler		100
Sealer	Plastic containers	100–110
Capper	Glass bottles	100–110
Accumulator		5 min
Case packer	Plastic containers	130–140
Recaser	Glass bottles	120–130
Palletizer	Cases	140–150
Stretch wrapper	Pallets	150

efficient, improvements in filler technology may enable the elimination of the accumulator from the layout.

The filler and capper can be specified to package and handle different bottle sizes. The filler bowl that contains the edible oil is constructed from 304 or 316 stainless steel that has a highly polished outer finish to promote sanitary operations. A clean-in-place (CIP) system is designed with the necessary connections for interior spray ball cleaning of the bowl and high velocity cleaning of the pipes and valves. Temperature probes are located in the bowl to indicate CIP solution temperatures and for product temperature during packaging (20). The fillers can operate at rates of 500 containers/min and may have 8–56 filling stations.

Although as a general principle, equipment upstream and downstream from the filler should be sized faster than the filler for accumulation and recovery, in the case of this washer it is preferable not to cycle the machine. The washer speed, therefore, needs to be variable to run slower than the filler when the accumulation table after the washer starts to fill.

## 5.2. Thermoset Fat Products

Margarine, mayonnaise, shortening, and other high fat products are filled as high viscosity products or at elevated temperatures. Margarine packages consist of wrapper sticks, squirt bottles, and tubs. Margarine packed as four individually wrapped 0.25-lb (0.133-kg) sticks is still a common product. The line components for soft margarine are dispenser, former, wrapper, cartoner, labeler, case packer, and palletizer. Wrapping and cartoning are usually done by a single machine. This unit must be adjusted based on the characteristics of the margarine, such as product temperature and emulsion firmness. Direct injection systems for forming the margarine have product recirculation loops to return the oil through a heat exchanger to control product temperature. The correct product filling pressure is controlled by the

margarine oil formulations and the filling molding temperature. The speed of a filler varies between 90 and 300 packages/min (21). Increasing the product pressure during filling–molding results in a more dense fill–mold. The margarine mold is wrapped by either paper or foil. The four sticks are cartoned by pushing them against the carton blank. A dense mold can be wrapped and cartoned easily; however, too much density will result in a crumbly mold or stick. Too soft a mold will alter under this pressure, and the carton will be irregular.

Mayonnaise is usually packaged in glass containers, whereas peanut butter and salad dressings are usually packaged in plastic containers, in the range of 0.2–1.0 L (8, 16, and 32 fluid oz) and sometimes in 3.79 L (1 gal) for institutional trade. Mayonnaise is still packaged in glass to allow the product to be retorted (heated to preserve) after container sealing and because of the sensitivity to oxidation. However, some of the small sizes for mayonnaise have been converted to plastic. Other oil and oil-based products are also sensitive to oxidation; thus after being sealed in the container, some products will oxidize until the headspace oxygen is used up. Therefore, headspace is often flushed with an inert gas such as nitrogen. The extent of oxidation after flushing is not sufficient to result in off-flavor formation. However, a loose lid or seal that has been warped or overheated will leak and admit oxygen from the atmosphere, causing rancidity. Oxidation or moisture loss from the product through such leakage will also cause the product to be rejected by the consumer (22). Filling equipment is normally volumetric volume (Figure 8). A product that has been flushed with a nitrogen–carbon dioxide mixture may create a headspace vacuum when the carbon dioxide is absorbed by the product. Overfilling the container will cause the lid to force the product onto the closure threads or the heat seal to fail. Obviously, overfill is to be avoided.

The multiple straight-line or rotary-head liquid margarine–shortening fillers are of either the timed flow or volumetric action (piston), which can fill up to 300 tubs/min. These fillers require preformed cups or tubs and lids that can be molded by specialized machines as follows. The printed plastic sheet material is unwound from a reel and travels through a heating section to soften the material. An indexing clamp frame allows the warm sheet to be molded to the shape of the cups–tubs or lids. Vacuum can assist the pressure (induced by mold operation) to “thermoform” the container. The filling machine accepts the preformed cups–tubs and lids in separate stacks. After filling the cups–tubs, lids are mechanically pressed into place, resulting in a closed product container. Closure of tapered tubs can be done by heat sealing a plastic and aluminum laminated film to the tubs–cups. The filled containers are separated by cutting the lids out of the film. Machines that thermoform the container, fill product into the container, and seal the container are termed *form/fill/seal-type machines*. Thermoform filling has become more common in margarine packaging.

Metal, composite, or plastic containers received on unitized pallets are usually packed with a shrink or stretch wrap shroud to maintain integrity of the load. Manual or automated depalletizers are available that orient the cans onto single-lane conveyors. The cans are inverted for cleaning and enter the filler. Filling is accomplished by timed flow or piston-type, volumetric action fillers. The end or side of

the container may be ink jet printed with information such as code date, product code, and/or inspection establishment number. If a seaming operation is used, then the roller must be kept precisely adjusted to maintain package integrity. If a heat sealing method is used, then precise temperature pressure controls are required to monitor integrity. Upon leaving this operation, the containers pass onto a single-lane conveyor, the intent of which is to provide sufficient control for labeling. The containers are packed manually or automatically into shipping cases, then palletized.

## 6. BULK PACKAGING OPERATIONS

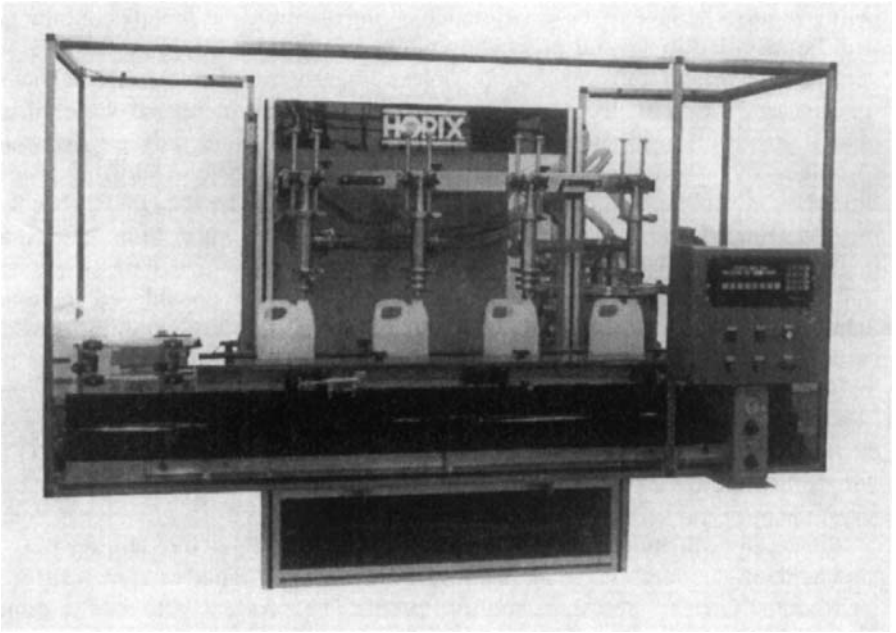
Bulk systems using flexible packaging were developed several years ago. These systems have primarily packaged products such as shortening, but many packaging vendors believe that successful packaging of edible oils in bulk containers will be installed in several processing plants in the near future.

The concept of bulk packaging of raw ingredients significantly reduces packaging costs and provides other benefits, such as increased warehouse and transportation efficiencies, reduced storage costs, and reduced disposal problems of the spent container. Manufacturers who use solid shortenings or liquid oil in sufficiently large quantity, on the order of at least 44,000 kg/week, most frequently purchase their shortening supplies in bulk. Shipments are made in 55,000–100,000 kg capacity tank trucks or 125,000–350,000 kg capacity rail car tankers. There are a number of factors to be considered in bulk handling installations. Bulk deliveries must be in a liquid or semiliquid state to be easily removed from the carrier. Tank trucks or rail tankers usually have no facilities for heating the shortening and must be insulated to prevent the product from solidifying. An insulated tank filled with hot shortening cools at the rate of 0.2–1.0°C (0.5–2.0°F)/h and will remain fluid for 15–20 h. The length of time depends on filling temperature, environmental conditions, oil crystallization point, and discharge methods (21).

On receipt, the melted shortening or liquid oil is pumped into storage tanks and held until needed. The bulk product should be maintained at a temperature of about 6°C (9°F) above its melting point. The product held in the tank should be flushed with nitrogen gas to prevent the slow deterioration and color darkening of the product while in storage. The low storage temperature and nitrogen blanketing help retard deterioration, but the storage life of a heated oil product is limited. The bulk storage tank of product should be used in about 2 weeks for best results. The capacity of the storage tank should be based on this use rate. Storage tanks are fabricated in many shapes, round or square, conic or flat-bottomed, tall or short. The design selected depends on space requirements, which are often critical in a general equipment layout. A stainless-steel tank for food products is recommended. Other oil base products can be stored in tanks of fiberglass fabrication (23).

Another method of handling institutional containers is in the form of flexible bags in drum or wooden totes. Bags for above a few liters are presterilized using  $\gamma$  radiation and are provided to the user with an overseal on the spout, which





*Figure 12. Bulk filling operation.*

protects the sterility of the bag. Bag size varies from 9.5 L to 1127 L. The presterilized bags are fitted to the filler in a chamber that prevents contamination from entering the bag when the cap is removed from the spout. Figure 12 illustrates a bulk packaging system commonly used for institutional oil products. Difficulty arises in ensuring that the chamber is free of microorganisms when the spouts are being exposed in the chamber. Hydrogen peroxide is often used to sterilize the chamber and spout caps. After removal of the cap, a fill valve moves into the spout, the bag is filled, and the cap is replaced. The bags are placed in a tote, a fiber drum, or other bulk container before filling.

The bulk edible oil by-products are pumped or dumped into a processing tank. This food oil becomes an ingredient in a consummable product. Nonedible oil can be used in the original container or transferred to a distribution vessel in either a bulk form or in a liquid state that is metered into the production operation.

## 7. SUMMARY

Planning is the foundation of any project. It is the most important step in developing the optimal plant. Engineering should be initiated during the conceptual stages of the project. A subsequent study of regulations—plant site, plant room arrangements, material of construction, construction issues, process flow diagrams, general arrangements, equipment specifications, and vendor qualifications must be

sufficiently developed before a successful production implementation. Processing and packaging systems should be examined in regard to consumer requirements, new technologies, and energy use.

## 8. FUTURE CONSIDERATIONS

Energy issues are very important in plant design, processing, and packaging. Plant site selection and material of construction will incorporate the advantages of energy conservation such as passive solar design. Other energy studies will optimize material flow through the plant. Online testing of oil product for foreign substances with greater sensitivity will also be a future trend. For oil production operation, flexible processing and bulk packaging will become increasingly important, since this technology offers lower processing cost, less space, and lower container cost. Containers and other packaging materials will be designed for a secondary usage, e.g., incineration for energy or recycling into other usable plastic products.

## REFERENCES

1. N. N. Potter, *Food Science*, AVI Publishing Co., Inc., Westport, Connecticut, 1973.
2. J. R. Backhurst and J. H. Harker, *Process Plant Design*, Heinemann Educational Books Ltd., London, 1973.
3. V. E. Caudill, *Ten Steps to a Successful Aseptic Packaging Line*, Prepared Foods, Highland Ranch, Colorado, 1992.
4. R. H. Caro, *Food Technol.*, **62**, 62–66 (1991).
5. J. Foggiato, *Solid State Technol.*, **1**, 178–179 (1986).
6. J. White, *Material Handling in Integrated Manufacturing Systems*, ISA, Research Triangle Park, North Carolina, 1992, pp. 46–59.
7. M. Peters, *Plant Design and Economics for Chemical Engineers*, McGraw-Hill Book Co., Inc., New York, 1958.
8. J. W. Harper and J. A. Jones, *Dairy Technology and Engineering*, AVI Publishing Co., Inc., Westport, Connecticut, 1976, pp. 153–155.
9. H. L. M. Lelieveld, *J. Soc. Dairy Technol.*, **38**, 179–186 (1985).
10. S. J. Palling, *Developments in Food Packaging*, Elsevier Applied Science Publishers, London, 1980.
11. F. J. Francis, ed., *Wiley Encyclopedia of Food Science and Technology*, vol. 3, 2nd ed., Wiley, New York, 2000.
12. S. L. Rosen, *Fundamental Principles of Polymeric Materials*, Wiley-Interscience, New York, 1982.
13. V. E. Caudill in Ph.D. dissertation, Rutgers University, New Brunswick, New Jersey, 1989.
14. J. F. Hanlon, *Packaging Engineering*, McGraw-Hall Book Co., Inc., New York, 1984.
15. K. W. Clarke, *Packaging Encyclopedia: Filling Liquids and Semi-Liquid*, vol. 12, New York, 1976.

16. J. L. Harper, *Elements of Food Engineering*, AVI Publishing Co., Inc., Westport, Connecticut, 1979.
17. F. A. Faine, *Packaging Users Handbook*, AVI Publishing Co., Inc., New York, 1991, pp. 319–321.
18. V. E. Caudill in Y. H. Hui, ed., *Engineering: Plant Design, Processing, and Packaging*, vol. 3, VCH Publishers, Inc., New York, 1993, pp. 295–329.
19. G. Hoffman, *The Chemistry and Technology of Edible Oils*, Academic Press, Inc., New York, 1989, pp. 333–335.
20. T. J. Immholle, *Engineering for Food Safety and Sanitation*, Technical Institute of Food Safety, Crystal, Minnesota, 1984.
21. T. H. Applewhite, ed., *Bailey's Industrial Oil and Fat Products*, vol. 3, Wiley, New York, 1985, pp. 335–338.
22. K. Ito, *Food Technol.*, **78** (1980).
23. 3-A Sanitary Standards Administrative Council, *Materials Used as Product Contact Surfaces in Dairy Equipment*, 3-A Sanitary Standards Administrative Council, Ames, Iowa, 1975.

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## *Adsorptive Separation of Oils*

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Adsorption is a complex chemical process employed in the refining of vegetable oils whereby nontriacylglycerol oil impurities are removed by sorbent materials after alkali or physical refining. The process involves “bleaching” or the adsorptive reduction of contaminants by any means, including surface attraction, molecular/ionic bonding, oxidative catalysis and controlled by the chemistry of the vegetable oil constituents, the physicochemical characteristics of the adsorbents, and the process parameters or conditions wherein they interact. This chapter reviews the theory of adsorption and its importance in the industrial evaluation and control of adsorption oil refining.

### **1. DEFINITIONS**

#### **1.1. Adsorption**

A general definition of adsorption is the “preferential partitioning of substances from a gaseous or liquid phase onto the surface of a solid substrate. The adsorbing

phase is the adsorbent, and the material concentrated, or adsorbed, at the surface of that phase is the adsorbate” (1). Patterson (2) defined adsorption as “a phenomenon wherein the local concentration of a substance at the surface of a solid or liquid becomes greater than the concentration throughout the bulk. Thus, adsorption is a concentration of solute at an interface surface.”

A definition of adsorption applying specifically to vegetable oil processing would be “the assimilation of solute oil impurities by the surface of the adsorbent” (3). Adsorption was later more fully described, as it applies to fats and oils as, “the physical and chemical interaction of a fat or oil to improve oil quality whereby detrimental contaminants are concentrated onto a sorbent and subsequently removed prior to finishing processes” (4).

In adsorption oil processing, the time required for maximum adsorption is also important with respect to minimizing catalytic side reactions, such as heat-cleavage of carotenoids, hydrolysis, oxidation, isomerization, and polymerization reactions of the oils (5).

## 1.2. Absorption

Absorption is distinguished from adsorption by defining it as the filling in of the intergranule void between and within particles. It is thus a filling of three-dimensional space in or between adsorbent particles, whereas adsorption is purely a surface phenomenon.

## 1.3. Filtration

Filtration is the separation of suspended particles of solids from fluids (liquid or gas) by use of a porous medium (6). It is therefore a sieving process whereby solids are separated from a solvent and does not involve solutes interacting with surfaces. In discussing oil processing, adsorption can be confused with filtration. For example, it could be argued as to whether soaps and phospholipids were separated by adsorption or filtration. Under low-solubility conditions (low temperature and high concentrations), soaps and phospholipids tend to be separated more by filtration than adsorption, and *visa versa*. Expressed simply, adsorption is described as the assimilation of oil-soluble impurities, whereas filtration is the removal of solid particulates and insoluble contaminants.

## 2. MATHEMATICAL MODELS

From a practical perspective, an isotherm is a graphical representation of how much of a specific contaminant is effectively removed by a given weight of sorbent, and it is a means to this end. It allows the refiner to judge between sorbent efficiencies, *i.e.*, which clay will be more economically efficient. However, from a theoretical interest in studying the nature of adsorption, it is a means to empirically determine the manner by which an adsorbate is removed by the sorbent and mathematically define the adsorptive nature of a sorbent.

## 2.1. Langmuir Isotherm

**2.1.1. Theory** An isotherm is the expression of the relationship between the partial pressure of an adsorbate gas, or solute concentration in solution, and the surface coverage of the adsorbent at a constant temperature (7). The Langmuir isotherm has been used to describe the oil pigment adsorption and adsorption of other minor oil solutes during oil processing. However, it was originally developed by Irving Langmuir in 1916 to describe gas adsorption. The model describes a reversible monolayer, chemisorption adsorption onto distinct sites of an idealized adsorbent, at equilibrium. The isotherm expresses the hypothetical relationship between gas pressure and the proportion of adsorption sites occupied by an adsorbate at a fixed temperature. The model assumes that the adsorbate is bound to a fixed number of energetically equal, specific sites, each adsorbing one molecule with no interaction occurring between molecules on adjacent sites. The reaction can be expressed as



where  $S$  represents the nonadsorbed species,  $A$  represents the empty adsorption sites, and  $SA$  is adsorbed species on specific sites. The equilibrium constant  $K$  is expressed as

$$K = [SA]/[S][A]. \tag{2}$$

If  $\theta$  = the fraction of the total surface sites that are occupied ( $0 < \theta < 1$ ), then  $[SA]$  is proportional to the surface covered by adsorbed molecules and  $\theta$ . Furthermore,  $[S]$  is proportional to the number of unoccupied sites ( $1 - \theta$ ). If  $p$  is gas pressure or solute concentration, the equilibrium constant  $b$ , which is the adsorption coefficient, can then be expressed as

$$b = \theta/(1 - \theta)p. \tag{3}$$

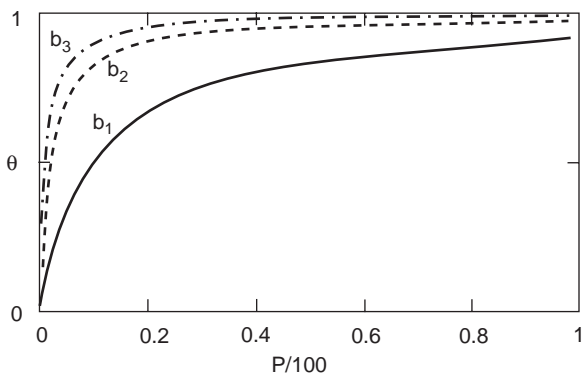
When solved for  $\theta$ , the equation now becomes

$$\theta = bp/(1 + bp). \tag{4}$$

The equation is true when  $bp \gg 1$  and  $b$  is the adsorption coefficient, expressed as the ratio of the rate constants of adsorption and desorption, i.e.,  $k_a/k_d$ . When considering  $n$  = number of sites occupied and  $n_m$  = maximum number of sites, the equation can then express  $\theta$  in terms of  $n/n_m$ :

$$n/n_m = bp/(1 + bp). \tag{5}$$

The number of adsorbed species, temperature, and pressure or solute concentration will determine the position of the equilibrium, i.e., how much solute is adsorbed relative to how much remains in solution. Adsorption will increase with solute concentration and decreasing temperature. Figure 1 shows plots of the



where  $b_3 > b_2 > b_1$

**Figure 1.** Langmuir isotherm at different equilibria,  $b$ .

number of sites occupied ( $\theta$ ) plotted against solute concentration or gas pressure ( $p$ ), at different equilibria ( $b$ ). Equation (5) can be applied to test empirical data for Langmuir behavior rewritten as the equation of a straight line as follows:

$$p/n = 1/bn_m + p/n_m. \quad [6]$$

The plot of  $p/n$  against  $p$  will then produce a straight line, with slope  $1/n_m$ . The Langmuir isotherm has been applied to pigment adsorption from vegetable oil in the form:

$$X_e/(x/m) = 1/a.b + X_e/a, \quad [7]$$

where  $x$  = amount of adsorbed solute,  $X_e$  = amount of unadsorbed solute,  $m$  = grams of adsorbent,  $a$  is the surface area of the adsorbent ( $m^2/100$  g), and is  $b$  is a constant of the intensity of the adsorption. Although Langmuir behavior is observed if a straight line is obtained when  $X_e/(x/m)$  is plotted against  $X_e$ , this is not definitive proof of a simple, reversible monolayer, chemisorption adsorption mechanism; i.e., simply conforming to the Langmuir equation does not constitute absolute proof of the mechanism (7).

### 2.1.2. Application of the Langmuir Isotherm to Oil Pigment Adsorption

The Langmuir equation has been used to describe adsorption of  $\beta$  carotene from solution onto activated bleaching clays (8) and free fatty acid from isoctane solution by acid-washed rice hull ash (9). Likewise, isotherm analysis of the commercial bleaching of rubber and melon seed oil by Fullers earth, activated carbon, and Fullers earth/activated carbon mixture (10) followed Langmuir behavior at  $55^\circ\text{C}$  and  $80^\circ\text{C}$ , suggesting the possibility of monolayer adsorption with little competition. This behavior was not observed at  $30^\circ\text{C}$  where the isotherm no longer applies, because of desorption. The amount of adsorbent was kept constant while varying

the amount of oil. The work further showed that Langmuir constant  $1/a$  decreased with rising temperature for all adsorbent systems used, indicating increasing availability of adsorption sites with elevated temperatures. Therefore, all sites were not energetically equal. A later study (11) used isotherm analysis to investigate pigment adsorption efficiencies of a variety of vegetable oils using bentonite, montmorillonite, and sepiolite under industrial bleaching conditions. The work showed that the Langmuir equation held true for sepiolite but did not explain the adsorption of rapeseed, soybean, wheatgerm, and safflower oil pigments on montmorillonite and standard activated clay. Reportedly, the applicability of the Langmuir isotherm depended on whether or not adsorption was by chemisorption. In this study, the Langmuir isotherm was more widely applicable in various oil types to adsorption by fibrous sepiolite than to powdered and spherule sepiolite. Heat of adsorption data suggested that adsorption to powdered and spherule sepiolite was by physical adsorption, which would explain the limited application of Langmuir isotherm. A later study Boki et al. (12) compared the applicability of Langmuir isotherm behavior with pigment adsorption efficiencies of various attapulgite (palygorskite) clay adsorbents in bleaching soy and rapeseed oils. Attapulgite drying affects the preservation of an open clay structure and active surface area. At lower temperatures (100–200°C), moisture loss begins, and at high temperatures (500–800°C), dried attapulgite loses all of the tightly bound moisture with breakdown crystal structure and loss of surface area. Unprocessed attapulgite is known as high volatile material (HVM). Relatively mild heating removes most pore and interlayer moisture forming regular volatile material (RVM) attapulgite. More severe heating removes all of this water, resulting in low volatile material (LVM). Boki et al. (12) found that low-temperature dried and high-temperature dried attapulgite had a lower  $a$ -constant-value, that is, isotherm gradient, (6.62–6.95) (Equation 7) than RVM matter adsorptive, pharmaceutical grade, and cosmetic grades (8.45–9.25), indicating a greater active surface area in the latter, which is most probably because of the greater moisture content. The volatile matter content of the pharmaceutical grade and cosmetic grades was not stated. There was little difference in the intensity of the adsorption of the low-temperature dried, high-temperature dried, pharmaceutical grade, and cosmetic grades attapulgites, as shown by the  $y$ -intercept (8.24–9.17), but the intercept of regular volatile matter attapulgite was considerably smaller (4.38). A subsequent study (13) showed that the adsorption of  $\beta$  carotene to attapulgite and sepiolite from hexane solution in the presence of diglycerides followed a Langmuir relationship. They deduced that  $\beta$  carotene was adsorbed as a monolayer with no competitive adsorption from diglycerides. Again, it should be realized, however, that conformity to the Langmuir equation does not constitute absolute proof of the mechanism (7).

In studying the behavior of adsorption behavior from miscellas, Kun-She-Low et al. (14) showed that  $\beta$  carotene adsorption from crude palm oil hexane miscella by regenerated bleaching clay followed a Langmuir isotherm with constants  $1/a$  and  $1/(a.b)$ , of 0.25 and 0.02, respectively. Transfeld (15) used Langmuir isotherms to investigate two-stage and three-stage counter-current bleaching operations where both complete mixing and separation of oil and adsorbent are achieved at each



stage. A significant improvement in decolorization was possible than with batch bleaching. He showed that the advantage of the counter-current bleaching technique is enhanced as the Langmuir equilibrium constant is reduced, enabling less clay to be used, relative to conventional bleaching. This is because of the steep rise of the isotherm, which is at the point of most practical value. Transfeld also reported that 40% savings were found when commercially bleaching dark oil with highly active clay.

In summary, Equation 6 is a useful means of comparing adsorption behavior of adsorbents in various oil systems based on adsorption constants. Caution, however, should be taken in using the equation to deduce details of the adsorption mechanisms.

## 2.2. Freundlich Isotherm

**2.2.1. Theory** The Freundlich isotherm developed in 1924 by Freundlich (16) is widely used to study oil processing adsorption. It is applied as an empirical expression to describe the reversible adsorption of a single solute from aqueous solution at equilibrium at a fixed temperature, and it is expressed as

$$x/m \propto c, \quad [8]$$

where  $x$  = amount of solute adsorbed (mg),  $m$  = grams of adsorbent,  $c$  = residual concentration (mg/ L) of solute at adsorption equilibrium, and

$$x/m = Kc^n. \quad [9]$$

$K$  is a constant indicating adsorption capacity, and  $n$  is a constant of the energy of adsorption. The constant  $n$  is sometimes expressed as  $1/n$  to standardize notation so the constant varies only between 0.1 and 1.0 (10). At low solute concentrations, the amount of adsorbate binding increases rapidly as solution concentration increases, but at high concentrations, the amount adsorbed approaches a constant value. However, a disadvantage of the equation is that it does not allow for a limitation in the adsorption capacity. Theoretically the amount adsorbed may be infinite. Furthermore, the success of the Freundlich isotherm in describing oil pigment adsorption to bleaching clay is paradoxical, because it describes a reversible adsorption. Carotene pigment binding to bleaching clay is irreversible, but chlorophyll is reversible under certain conditions.

Nevertheless, empirical data are evaluated for Freundlich behavior by using the equation in its logarithmic form, as the equation of a straight line:

$$\log (x/m) = \log K + n \log c, \quad [10]$$

where  $\log (x/m)$  is plotted against  $\log (c)$  and the slope is  $n$  and the intercept is  $\log (K)$ . The Freundlich equation is valid for any color measurement method, providing the units are additive and proportional to the oil pigment concentration.

Lovibond color values have traditionally been used by industry, but optical absorbance and subsequent calculation of pigment concentration can be applied (17).

### 2.2.2. Application of the Freundlich Isotherm to Oil Pigment Adsorption

Practically,  $K$  is a good indication of the decolorizing power of a bleaching clay and  $n$  is the characteristic manner of adsorption. If two adsorbents have differing capacities,  $K(K_1 \neq K_2)$ , but adsorb in the same manner, i.e., have a similar  $n$ , the relative amounts of adsorbent needed will be inversely proportional to  $K$  values. Consider the following for bleaching earths A and B. For A:

$$x/m = 0.5c^{0.5}. \quad [11]$$

For B:

$$x/m = 1.0c^{0.5}. \quad [12]$$

Twice as much earth will be needed if A is used rather than B to bleach to a specified color. The  $n$  value is used to determine the range of decolorization within which the adsorbent is most effective. An adsorbent with a high  $n$  will be relatively effective at initially binding oil pigments but relatively inefficient at bleaching oil to a low-color value. The opposite is true for an adsorbent with a low  $n$  value. A high  $n$  value is desirable but not at the expense of  $K$ . Both  $K$  and  $n$  can vary with process variables, including adsorbent, temperature and moisture content, oil source, and other minor oil components as well as bleaching technique. Less adsorbent is usually needed to process oil in a plant relative to laboratory bench bleaching systems. This is because of the buildup of filter cake during the separation of oil and adsorbent results in additional bleaching as the adsorbent capacity is increased. The phenomenon is called the "press bleaching effect," resulting from a new adsorption equilibrium and reduced residual oil pigment level.

Proctor and Toro-Vazques (18) reviewed the nature and history of the use of the Freundlich isotherm in studying adsorption in oil processing. Hassler and Hagberg (19) pioneered the application of the Freundlich isotherm to adsorption bleaching of cottonseed oil by Fullers earth and active carbon by measuring Lovibond units before and after processing. The log/log plot of the Freundlich isotherm showed Freundlich adsorption behavior that allowed comparison of adsorbents based on the  $K$  and  $n$  constants. The gradient of the plot showed the  $n$  constant value that was used to compare adsorption efficiencies and established the value of the Freundlich isotherm in comparing adsorbent performance. The effectiveness of the Freundlich isotherm is interesting as it was originally developed in a single solute system where residual solute was measured on equilibrium, whereas Hassler and Hagberg (19) used a simple multicomponent oil system, taking Lovibond color measurements, both before adsorption and after achieving equilibrium. The work showed that adsorbent contact with a lightly colored oil will have reserve adsorption capacity for additional bleaching of darker oils. Hence, a countercurrent procedure can be effective where a fresh crude oil is initially contacted with partially

used bleaching clay. The used clay can still adsorb a significant amount of pigments from unbleached oil. This observation was used to explain the "press bleaching effect" whereby addition oil decolorization occurs as oil is filtered through the clay during separation. During this process, the adsorbent showed additional adsorption capacity with further lightening of the oil as the bleaching clay cake builds up during filtration, i.e., press bleaching (20).

Hinners et al. (21) showed that Lovibond values alone cannot adequately describe adsorbent capacity by isotherm analysis. The adsorption of each pigment in the oil should be evaluated independently. In addition, the ratio of the pigment concentration in the oil changes after adsorption to various degrees depending on the adsorbent type and concentration. The authors obtained oil chlorophyll adsorption data by measuring chlorophyll concentration before and after processing to produce Freundlich isotherms to determine the adsorbent capacity of various bleaching earths. They found the slopes  $n$  were similar and therefore deduced that the adsorption sites were similar. They reported that the constant  $K$  was proportional to the bleaching capacity of the clay, and larger values indicated the ability to bleach to low residual pigment levels. Hinners et al. (21) reported that a large  $n$  number is beneficial as it describes an ability to efficiently adsorb pigments from highly colored oils. The  $n$  value indicates the pigment concentration range over which the adsorbent is most effective. A high  $n$  value should not be compromised at the expense of  $K$ . The findings thus described how the Freundlich equation and constants could be used to determine the bleaching clay doses and types needed to meet a specified oil color.

Stout et al. (22) used Freundlich log/log plots to survey the performance of a wide range of bleaching clays and silicates to decolorize cottonseed and soy oil. They found that acid-activated clays had a lower temperature range for optimum performance, 100–106°C, than natural clays (180–250°C) and were 1.5–2.0 times more effective than natural earths in decolorizing oils. King and Wharton (23) observed that chemical reactions could occur that during industrial oil bleaching could affect pigment adsorption. Atmospheric bleaching produced lower  $K$  and  $n$  values than vacuum bleaching, implying oil oxidation catalyzed by the clay. This effect resulted in colored oxidation products that darkened the oil and decreased the Freundlich constants. This scenario is very plausible because Lovibond values were used, and the study could not distinguish between natural oils pigments and colored oil oxidation products.

Gutfinger and Letan (24) used Freundlich log/log plots to measure phospholipid adsorption and pigment reduction of degummed soy oil when exposed to Fullers earth or Tonsil TM clay. Oil phospholipids were measured as specific concentrations ( $\mu\text{g/g}$ ), but pigments were measured as subjective Lovibond values. All adsorptions occurred according to a Freundlich isotherm, with the exception of pigment adsorption to Fullers earth. This exception was explained as being caused by oxidation on contact of the oil with Fullers earth resulting in oil darkening, as mentioned above (23). Log/log isotherm plots were limited to only three data points, however. With such a small number of points, linearity is almost assured. As in previous studies, the Freundlich equation was used for the simplicity of the expression

and the value of the constants in comparing adsorbents. There has, however, been little attempt to understand why the Freundlich isotherm is applicable to multicomponent oil systems that are prone to oxidation and catalysis when Freundlich's original work was conducted with a single stable solute system.

Nevertheless, experimental measurement of Freundlich constants continues to be a popular means to distinguish between adsorbent performance and bleaching systems. In addition, they show that temperature promotes access to further adsorption sites. In the report previously discussed by Achife and Ibemesi (10), they described adsorption according to both Freundlich and Langmuir isotherms. There were deviations in Freundlich plots at high oil concentrations, outside the adsorption saturation point where desorption has become significant. The constant  $1/n$  ranged between 0.1 and 1.0 with varying temperature dependence depending on the bleaching agent used. There was a decline in  $1/n$  with Fullers earth with increasing temperature, whereas the opposite occurred when using a Fullers earth/activated charcoal blend. No clear trend emerged with activated charcoal. No detailed explanation of any of the data was offered. The constant  $K$  increased with increasing temperature for both oil systems when using Fullers earth and activated charcoal, indicating increasing access to adsorption sites. Little change in  $K$  with increasing temperature was observed, but not explained. Boki et al. (11) described the use of isotherm analysis to investigate pigment adsorption efficiencies of a variety of vegetable oils using bentonite, montmorillonite, and sepiolite under industrial bleaching conditions. They found that that the Freundlich isotherm was more applicable to the systems than was the Langmuir isotherm. They reported, however, an inflection in the Freundlich log/log plots with a higher gradient at lower  $\log X_e$  and  $\log x/m$  values with a lesser gradient after the inflection. This effect was explained by reference to the work of Kulkarni and Jatkar (25) who attributed it to physical adsorption at lower pigment concentrations and chemisorption at higher concentrations. They also suggested that adsorption occurs initially in smaller pores, progressing to larger pores as smaller pores become saturated. The Freundlich isotherm also was found to be more applicable than the Langmuir isotherm (12) in describing the adsorption of carotenoid pigments from rapeseed and soybean oil onto standard bleaching clays, synthetic adsorbents, and attapulgites, with inflections in the Freundlich log/log plots also being found in these systems.

Additional research has been done on the use of Freundlich isotherm to investigate adsorption of pigments from oil/hexane miscellas. Feuge and Jansen (26) compared miscella bleaching with conventional bleaching and found that commercial grade-activated bleaching clay reduced the oil color of a 30% oil miscellas more than by conventional processing and was independent of the clay dosage (0.5–8%). This was shown by using the Freundlich isotherms to compare the different methods. The bleaching of both oil and oil/hexane miscellas at 25°C produced similar  $n$  values, but processing in hexane produced a three-fold increase in  $K$  values, indicating that hexane did not affect the mode of adsorption, but increased the maximum pigment adsorbed. Conventional oil processing at 110°C produced a larger  $K$  value and a significantly lower  $n$  value. Feuge and Jansen proposed that miscella bleaching was more effective at initially removing pigment than

conventional bleaching and proposed a counter-current bleaching procedure with 30% oil miscellas. Proctor and Snyder (17) used Freundlich isotherms to attempt to understand the theory of pigment adsorption from oil miscellas onto silicic acid. Adsorption occurred according to a Freundlich isotherm, but propan-2-ol added to soy oil/hexane miscella and water deactivated silicic acid reduced the maximum amount of pigment binding. They proposed that lutein, the major pigment in soy oil, did not occupy all of the available adsorption sites because of competition with propan-2-ol and other oil components, e.g., triacylglycerol. The hypothesis was tested by an isotherm study with a soy lutein-rich, low triacylglycerol extract in hexane prepared from crude soy oil. The isotherm produced was a line close to the vertical axis. Isotherm studies with pure lutein in hexane showed that lutein was totally bound to silicic acid, as shown by a vertical isotherm. Therefore, triglyceride and other oil components were proposed to be competitively limiting pigment adsorption in miscellas and probably in other oil adsorption processing systems, based on molecule polarity. Minyu and Proctor (27) demonstrated the effect of function group and carbon chain length on the competitive adsorption of soy oil lutein on silicic acid. They studied the effect of an equimolar homologous series of added alcohols and acids on adsorption isotherm behavior. Minor differences in the Freundlich isotherms were found between members of a homologous series. A molecule's ability to reduce lutein adsorption was related to its ability to form hydrogen bonds, rather than its polarity. This was shown by the reduction in lutein binding to silicic acid on addition of equimolar three carbon molecules with various functional groups, as alcohol > acid > ketone > ester. Chapman and Pfankoch (28) used single-component isotherms and ideal solution theory (IDST) to study coadsorption of the chlorophyll-based pigment, protoporphyrin IX, fatty acids, and triglycerides from dichloromethane onto acid-activated bleaching clay. Data from single component systems showed that the clay had a much greater relative affinity for protoporphyrin than for either fatty acids or triglyceride. Isotherms from a binary solute system data calculated by IDST showed the ability of a high concentration of lipids to dramatically suppress protoporphyrin binding by competitive adsorption, however.

Miscella oil refining typically uses 40–80% oil (29). However, most miscella isotherm studies have been done with dilute oil miscellas (<40%) and short contact times (<20 minutes). Toro-Vasquez and Rocha-Urbe (30) investigated the interaction between oil components in determining Freundlich isotherm behavior. They showed the interactive effect of oil miscellas concentration (60–100%) and ethanol (0.5 and 25%) on the carbon adsorption of peroxides, unsaturated carbonyls, free fatty acids, and carotenoid from sesame seed oil miscellas. They found that adsorption was greater in more dilute miscellas. Toro-Vasquez and Mendez-Monteolvo (31) conducted later Freundlich isotherm studies on competitive adsorption of minor oil components in a similar system. They showed that free fatty acids adsorption was reduced in oxidized oil because of competition from carbonyl compounds produced from lipid oxidation, while free fatty acids reduced carotenoid adsorption. This is in agreement with the findings of Chapman and Pfankoch (28) who also

noted that fatty acid inhibited the adsorption of the pigment protoporphyrin IX onto bleaching clay from dichloromethane.

Langmuir and Freundlich isotherms were originally developed to explain adsorption under limited conditions. Langmuir plots are often favored because they are the best one-parameter isotherm; nevertheless, they ignore interactions between adsorbate molecules. Freundlich isotherms are the best two-parameter plot, but they have no physical basis in explaining why the equation is actually effective (32). Nevertheless, these equations are of practical value in studying vegetable oil processing systems by providing a comparative basis of adsorbent, bleaching system performance and in gaining an understanding of the theoretical basis of adsorption. The isotherms have proven useful in summarizing the main factors influencing the outcome of pigment adsorption but not in determining the mechanism of adsorption.

### 2.3. Practical Application of Adsorption Isotherms

Obstacles to practical application of the Langmuir and Freundlich isotherm theories include the following: (1) These isotherms do not effectively address adsorption versus degradation and competitive adsorption; (2) the conclusions are not all inclusive; i.e., adsorption constants and coefficients do not hold true in all cases within similar oil types let alone across different oil types; (3) the process has so many variables that the additive variance is commonly too great to prove any subtle difference between clays other than a vastly different level of activity (this problem is especially true when using log vs. log plots with incremental changes on the order of 0.1%); and (4) the adsorption constants and coefficients have limited use for the refiner.

Although reports are regularly published on adsorption bleaching and adsorption isotherms, they rarely provide examples of how these isotherms can be used practically by commercial refiners. Dijkstra (33), however, offers a few perspectives in which the pure mathematical approach to adsorption isotherms might be of practical use to the refiner.

The first perspective concerns bleaching options for oil blends such as those supplied by refiners to margarine plants and shortening packing facilities. Such blends are made up of several components such as one or more hardstocks and one or more liquid oils that have to meet color and sensory specifications. This means that these blends will have been deodorized and previously undergone an adsorption treatment. Dijkstra pointed out the following possible ways that exist to arrive at a fully refined oil blend:

1. Neutral, but not yet bleached, components could be mixed in the required proportions, and this mixture could then be bleached and deodorized to yield a fully refined oil blend of the right composition.
2. On the other hand, the refiner could also bleach individual neutral components on their own and keep each bleached component in its own intermediate

storage connected to the weighing scales used to make up the blend. Deodorizing this blend of bleached components would also lead to a fully refined oil blend of the required composition.

3. Instead of deodorizing blends as mentioned under 1) and 2), the refiner could also choose to bleach and deodorize certain components, as for instance liquid oil, and blend this fully refined component with a fully refined (i.e., neutralized, bleached, and deodorized) hardstock or blend of hardstocks prepared via route 1) or 2).

The particular question Dijkstra raised in this context was which of the above routes requires the least bleaching earth. By using the Freundlich isotherm, he showed mathematically that bleaching a blend of components to a certain residual color requires exactly the same amount of bleaching earth as bleaching the individual components to a given residual color, provided only a single adsorbent (type of bleaching earth) is used and the same adsorbate (e.g., chlorophyll) has to be removed from each component.

On the other hand, if the adsorbates to be removed from the various components differ in nature such as chlorophyll versus polyaromatic hydrocarbons (PAH) and if therefore different adsorbents have to be used such as activated bleaching earth for the chlorophyll and active carbon for the PAH, the situation changes. Then the same mathematics referred to above demonstrate that bleaching components separately requires less adsorbent than bleaching their blend. Accordingly, if a blend contains a certain proportion of coconut oil and if this oil on stock requires an active carbon adsorption treatment, minimal adsorbent usage results from treating the coconut oil on its own with active carbon. Using active carbon on "diluted" (*viz.* blended) coconut oil would be a waste of active carbon.

In the above illustration of the usefulness of the Freundlich adsorption isotherm in solving practical problems, the assumption was made that in route 2), each component was bleached to the same residual color. Not surprisingly, Dijkstra then asked whether bleaching components to different residual colors in such a way that their blend would have the desired color would require less or more bleaching earth than bleaching all components to the same, final blend color. Dijkstra simplified this very general question to a particular, but in a representative situation, a 50/50 blend of only two components, bleached to 0.5 the final blend color and 1.5 the said color, respectively. He then showed mathematically that bleaching to different residual colors requires more adsorbent than bleaching all components to the same final blend color or *vide supra* bleaching the blend of component to this final blend color. Similarly, the Freundlich isotherm merely states that the adsorbate (coloring matter) loading on the adsorbent (bleaching earth) depends on the residual adsorbate concentration (color after bleaching) of the oil. It depends in such a way that an increased adsorbate loading corresponds to an increased residual color. Hence, it is not surprising that bleaching oil to a lower than required final color (0.5 of the final blend color) requires more additional earth than can be saved by bleaching the other component to only 1.5 of the final blend color.

However, Dijkstra states that current industrial bleaching processes are scaled up laboratory experiments. Accordingly, spent bleaching earth has been in adsorption equilibrium with bleached oil and thus has a much lower adsorbate loading than if this spent earth had been in equilibrium with nonbleached oil. He then raises the question of how much bleaching earth could possibly be saved if spent earth were to be in equilibrium with nonbleached earth as in a truly counter-current process with respect to the present, cocurrent processes.

In this illustration of the usefulness of the adsorption isotherms, Dijkstra used the Langmuir adsorption isotherm to mathematically quantify this potential savings. By introducing a variable, indicative of the extent of color removal, he demonstrated that the relative potential savings increased strongly with the extent of color removal. As was only to be expected, this relative potential savings also depends on the Langmuir constants  $a$  and  $b$ , and quantifying the savings required these constants to be quantified. Using these estimates of these constants and assuming 90% color removal, Dijkstra calculated 80% potential savings if spent earth were in equilibrium with nonbleached oil rather than with bleached oil. His estimate illustrates how adsorption isotherms can be used to solve industrial problems with the provision that developing practicable mathematical solutions requires laboratory bench work and pilot or plant trials for verification.

In a simplistic and most practical approach, aside from the pure mathematical models, an isotherm is basically a plot of the concentration of contaminant(s) remaining in solution after bleaching versus the dosage of sorbent used. Assuming that all variables are constant, the sorbent that reaches a target specification for a given contaminant at the lowest dosage has the best performance under the given set of process conditions. Bleaching efficiency of the process, however, includes weighing the performance of the sorbent with respect to its cost efficiency.

### 3. MECHANISMS

The first step in the adsorption process involves attracting and attaching contaminants to the sorbent surface in two ways. First, by chemisorption, where the contaminant is bonded directly to the surface by ionic or covalent bonding. Second, by physisorption, where the contaminant is, instead, held by physical (i.e., van der Waals) forces or the molecular sieving properties of the sorbent. A contaminant that undergoes any exchange of electrons between the contaminant molecule and the sorbent surface would be fundamentally defined as being chemisorbed. In contrast, physisorbed molecules, governed by polarization (i.e., van der Waals) forces, do not share or undergo any significant change in electronic structure (2, 32). In general, bleaching conditions in the refining process favor chemisorption over physisorption as the bleaching process progresses from high contaminant concentrations and low temperatures ( $<100^{\circ}\text{C}$ ) to higher temperatures and increasingly lower contaminant concentrations. Bleaching temperatures above  $100^{\circ}\text{C}$ , combined with a sorbent's chemical and physical properties (discussed in Chapter 5), improves adsorption kinetics as primary and secondary side reactions are



propagated. Carotenes and hydroperoxides, for instance, once adsorbed onto bleaching clays, tend to undergo catalytic oxidization, as evidenced by the formation of secondary oxidation products (i.e., aldehydes and ketones). In these instances, adsorption is irreversible. Ionically bound contaminants, including soaps, chlorophyll, phospholipids, and trace metals, however, can be adsorbed and desorbed without molecular degradation.

## 4. MECHANICS

### 4.1. Processing Conditions that Affect Adsorption

**4.1.1. Sorbent Efficiency vs Bleaching Efficiency** Adsorption mechanics or kinetics are influenced by the adsorptive potential of a sorbent, the degree of oil/sorbent interaction, and the processing parameters governing the conditions under which the interaction occurs. Sorbent products, including clay minerals, amorphous silicates, and activated carbons, are both physically and chemically modified to enhance the adsorption potential or bleaching activity to remove detrimental contaminants from the oil during the bleaching stage of the refining process. Sorbent efficacy is dependent on many parameters including those listed in Table 1. Most of these variables are generally understood and are discussed elsewhere. Bleaching efficiency is, however, interdependent on the degree of activation and the processing conditions of the oil prior to and during contact by reaching a balance between the beneficial and detrimental effects of the many variables. Consideration should be given to the final oil quality versus the cost of producing it by weighing the effect of every processing variable.

**4.1.2. Dosage vs Contact Time** The dosage and length of contact time needed to bleach have an indirect relationship that is strongly influenced by the degree of agitation and oil temperature. Refiners achieve rapid dispersion of sorbent by using adequate agitation and controlling oil temperature well above the viscosity break of the oil, typically around  $85^{\circ} \pm 5^{\circ}\text{C}$ . Under optimal conditions, adsorption

**TABLE 1. Key Bleaching Clay Characteristics.**

	AM	M
Surface Area (BET, m <sup>2</sup> /g)	120–160	200–340
Total Pore Volume (cc/g)	0.25–0.45	0.20–0.50
Effective Pore Volume (cc/g)	0.20–0.30	0.12–0.33
Density (lb/ft <sup>3</sup> )	24–33	38–46
pH	2.4–7.0	2.5–7.0
% Free Moisture	8.0–15.0	10.0–16.0
Average Particle Size	30–40	30–40

Clay Sorbents:

A/M = Attapulgit/Montmorillonite Natural Occurring Blends.

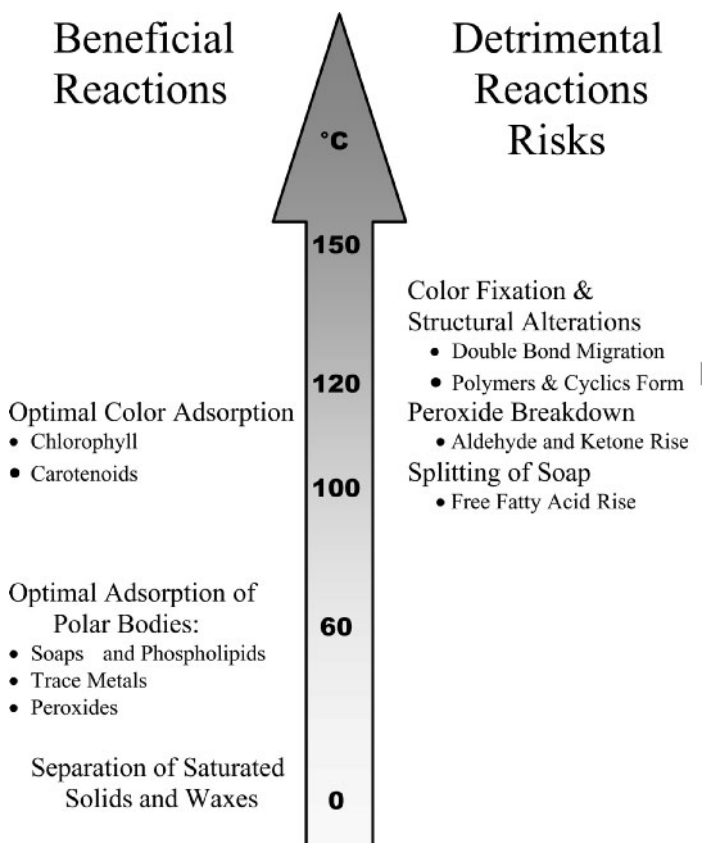
M = Montmorillonites.

equilibrium for colorants can be reached within 5 minutes with a maximum adsorption, for most contaminants of interest, achieved in less than 30 minutes (2, 5, 34, 38).

**4.1.3. Acidity** Acidity in the bleaching process can originate from the sorbent, with regard to surface and residual acidity in the sorbent, and the addition of citric or phosphoric acid to the oil. Chapter 5 addresses the fundamental relationships between the physiochemical properties of bleaching clays (including surface acidity and residual acidity) and their adsorbent efficacy. It is important to note that acidulated sorbents such as activated bleaching clays and carbons can be categorized as a solid acid with varied adsorptive efficiency depending on the number of surface Brönsted acid sites, and the type and amount of residual acid or extractable/titratable acid remaining in the sorbent. Citric or phosphoric acids are generally added to the bleaching process to benefitiate the sorptive activity of the sorbent and therein improve the efficiency of the process (39–42).

**4.1.4. Moisture** The influence of moisture on the adsorption process is dependent on the source and respective level of moisture in the system. Moisture is introduced into the bleaching process from the adsorbent moisture, oil moisture, and water added during processing. Efficacy of the bleaching process, as already explained, is dependent on the adsorbent efficacy of the sorbent in conjunction with the oil processing conditions, including the amount of water in the oil/clay slurry. Moisture effectively improves the efficacy of the bleaching process with levels up to 1.0%, with optimum levels ranging between 0.2% and 0.3%. This level of moisture is an order of magnitude higher than the contribution from the sorbent used during bleaching, however. Sorbent efficiency for bleaching clays and carbons is indirectly affected by the amount of free moisture present in the given sorbent. A lower percent of free moisture levels in the adsorbent translates to more active material introduced to the system. Inversely, silica hydrogels lose activity with moisture loss and must maintain at least 40% free moisture to maintain efficacy.

**4.1.5. Temperature and Vacuum—Air vs No Air** Temperature has a direct influence on the kinetics of adsorption. In general, reaction rates tend to double for every 10°C rise in temperature (i.e., van Hoff rule). This rule holds true for both beneficial and detrimental reactions. Optimal temperature ranges from 85°C to 120°C depending on oil type, clay activity (i.e., residual and surface acidity), and the balance achieved between the byproducts formed from adverse reactions and the desired oil quality (Figure 2). The presence of air at elevated temperatures provides ideal conditions for oxidation reactions at the oil/sorbent interface, e.g., peroxidation, hydrolysis, and structural alterations, such as migration of double bonds, polymerization, cyclization, and decomposition. Zchau (2000) (38) states that “If bleaching earths were simply adsorbents, the best color reduction would be expected at low temperatures. . .and shift toward desorption with high temperatures. . .this is not observed.” The commercial reality is the continuous challenge to



**Figure 2.** Influence of temperature on adsorption.

optimize adsorptive efficiency of the process conditions. This includes adsorbent selection and the control of oil processing conditions to produce quality oil while balancing process efficiency by controlling material, energy, and labor costs.

## REFERENCES

1. International Adsorption Society. (2002). *Adsorption*. Available: <http://ias.vub.ac.be/General/Adsorption>.
2. H. B. W. Patterson, *Bleaching and Purifying Fats and Oils*, American Oil Chemists Society, Champaign, Illinois, 1992.
3. D. D. Brooks and D. Shaked, *Bleaching Difficult to Bleach Palm Oil*. PORIM, 1997.
4. D. D. Brooks and S. Azzarello. *Bleaching Options for Refining Palm Oil*. Latin America AOCs Convention, Costa Rica, 2001.
5. T. Mag, *Bleaching, Theory and Practice: Edible Fats and Oils Processing: Basic Principles and Modern Practices*, AOCs, Champaign, Illinois, 1990, pp. 107–116.

6. T. Vermeullen, *Adsorptive Separation: Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 1, 3rd ed., Wiley, New York, 1980, pp. 531–544.
7. S. J. Greg and K. S. W. Sing, *Adsorption, Surface Area and Porosity*, Academic Press, London, 1997, pp. 43, 197–199.
8. L. E. Khoo, F. Morsingh, and K. Y. Liew, *J. Am. Oil Chem. Soc.*, **56**, 672 (1979).
9. A. Farook and S. Ravendran, *J. Am. Oil Chem. Soc.*, **77**, 437 (2000).
10. E. C. Achife and J. A. Ibemesi, *J. Am. Oil Chem. Soc.*, **66**, 247 (1989).
11. K. Boki, M. Kubo, N. Kawasaki, and H. Mori, *J. Am. Oil Chem. Soc.*, **69**, 372 (1992).
12. K. Boki, H. Mori, and N. Kawasaki, *J. Am. Oil Chem. Soc.* **71**, 595 (1994).
13. K. Boki, N. Sakamoto, and K. Minami, *J. Am. Oil Chem. Soc.*, **78**, 733 (2001).
14. K. S. Low, C. K. Lee, and L. Y. Kong, *J. Chem Tech. Biotech.*, **72**, 67 (1998).
15. P. Transfeld, *Inform*, **11**, 776 (2002).
16. H. Freundlich, *The Elements of Colloidal Chemistry*, translated by G. Barger, Dutton and Company Publishers, New York, 1924, pp. 58–61.
17. A. Proctor, and H. E. Snyder, *J. Am. Oil Chem. Soc.*, **64**, 1163 (1987).
18. A. Proctor and J. F. Toro-Vazques, *J. Am. Oil Chem. Soc.*, **73**, 1627 (1996).
19. J. W. Hassler and R. A. Hagberg, *Oil Soap*, **16**, 188 (1939).
20. J. H. Henderson, *J. Am. Oil Chem. Soc.*, **70**, 831 (1993).
21. H. F. Hanners, J. J. McCarthy, and R. E. Bass, *Oil Soap*, **23**, 22 (1946).
22. L. E. Stout, D. F. Chamberlain, and J. M. McKelvey, *J. Am. Oil Chem. Soc.*, **26**, 120 (1949).
23. R. R. King and F. W. Wharton, *J. Am. Oil Chem. Soc.*, **26**, 201 (1949).
24. T. Gutfinger and A. Letan, *J. Am. Oil Chem. Soc.*, **55**, 856 (1976).
25. B. S. Kulkari and S. K. K. Jatkar, *Kolloid-Z*, **89**, 54 (1939).
26. R. O. Feuge and H. J. Jansen, *J. Am. Oil Chem. Soc.*, **28**, 429 (1951).
27. J. Minyu and A. Proctor, *J. Am. Oil Chem. Soc.*, **70**, 575 (1993).
28. D. M. Chapman and E. A. Pfannkoch, *J. Am. Oil Chem. Soc.*, **69**, 1009 (1992).
29. G. C. Cavanagh, *World Conference Proceedings on Edible Fats and Oil Processing, Basic Principles and Modern Practices 1990*, American Oil Chemist's Society, Champaign, Illinois.
30. J. F. Toro-Vasquez and A. Rocha-Urbe, *J. Am. Oil Chem. Soc.*, **70**, 598 (1993).
31. J. F. Toro-Vasquez and G. Mendez-Montealvo, *J. Am. Oil Chem. Soc.*, **72**, 675 (1995).
32. R. Masel, *Adsorption I: The Binding of Molecules to Surfaces: Adsorption and Reaction on Solid Surfaces*, Wiley, New York, 1996, pp. 108–234.
33. A. J. Dijkstra, *Bleaching Revisited: Technology Award Address*, 2nd European Federal Lipid Congress and Expo, Strasbourg, Germany, 2002.
34. A. D. Rich, *J. Am. Oil Chem. Soc.*, **41**, 315 (1964).
35. A. D. Rich, *J. Am. Oil Chem. Soc.*, **44**, 298A (1967).
36. U. I. Brimberg, *Fette Seif. Ans.*, **83**, 184 (1981).
37. U. I. Brimberg, *J. Am. Oil Chem. Soc.*, **59**, 74 (1982).
38. W. Zschau, *Bleaching: Introduction to Fats and Oils Technology*, 2nd ed., AOCS, Champaign, Illinois, 2000, pp. 158–178.

39. S. Brophy, D. D. Brooks, A. Brophy, and G. R. Goss, *Chlorophyll Removal from Canola Oil: A New Concept*, American Oil Chemical Society Annual Meeting, Cincinnati, Ohio, 1989.
40. U.S. Patent 5,004,570 (April 2 1991), D. D. Brooks et al., *Oil Bleaching Method and Composition For Same*.
41. U.S. Patent 5,151,211 (Sept. 29 1992), D. D. Brooks et al., *Oil Bleaching Method and Composition For Same*.
42. W. L. Siew, *Inform*, **12** 1183 (2001).

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## *Bleaching*

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### 1. INTRODUCTION

Traditionally, the term *bleaching* has been used to denote the process of reducing colored pigments, primarily various pheophytins and carotenoids, in fats and oils by treatment with powdered substances called *bleaching earth* or *bleaching clay*. After heating and mixing, the bleaching clay is removed by filtration; the resultant oil is brighter, less colored, more stable (1, 2), and in the case of soybean oil, more flavorful (3). Although appropriately descriptive of the reduction in color intensity that occurs during the bleaching process, the term tends to imply a chemical effect when, in fact, the predominant action involves an adsorptive interaction between these pigments and the clay surfaces. Furthermore, we know bleaching clays interact with other noncolored oil constituents such as soaps, free fatty acids, (FFAs) phospholipids, and peroxides, and the role they play in these processes is at least as important as the role they play in color reduction.

Although bleaching clays do exhibit some chemical and catalytic behaviors, notably, the conversion of soaps and triacylglycerols into free fatty acids (4) and

the decomposition of hydroperoxides, respectively (5), the major operative mechanism for color reduction occurs via pigment adsorption. Because of this and the fact that other sorbent materials besides bleaching clays can be used during the bleaching process, other terms have been suggested (6–8). Of these, the term *adsorptive purification* seems perhaps most appropriate and can be used interchangeably with the term *bleaching* to describe adsorptive interactions between a sorbate molecules (e.g., chlorophyll and  $\beta$ -carotenes) and adsorption sites on sorbent surfaces.

Adsorptive purification of the type we are talking about here (sometimes called *physorption*) involves a relatively weak interaction between the sorbate molecule and the surface active sites in the sorbent. The association depends mostly on van der Waals forces, which are primarily caused by electronic and/or electrostatic interactions between electron-rich or electron-poor regions of the sorbate molecule and receptive sites on the sorbent surface. Molecules bound by such weak forces can be removed by various solvent extraction techniques; in contrast, molecules that actually form covalent bonds with the surface (*chemisorption*) usually cannot.

## 2. BACKGROUND AND HISTORICAL PERSPECTIVE

Bleaching and the use of bleaching clays in adsorptive purification processes have a long history of use. In fact, *fulling*, the process of removing lanolin from lamb's wool using natural bleaching earths called fuller's earths is mentioned in the Bible (Mark 9:3). The use of fuller's earth in the adsorptive purification of fats and oils in the United States dates from about 1880 when William B. Albright (of N.K. Fairbanks & Co., Chicago, IL) learned about the use of clays to decolorize olive oil and subsequently developed a process using it for filtration and color improvement of cottonseed oil (9). Natural (nonactivated) bleaching clays are still used today for processing lightly colored oils, but they have been replaced by the use of acid-activated bleaching clays in the case of more highly colored or difficult-to-process oils.

The first commercial acid-activated clays were produced about 1905–1909 in Germany (10, 11) and in 1922 in the United States (12). Clays susceptible to acid-activation are found on every continent, and companies making these products are distributed worldwide. Although there has been some consolidation in the number of producers in recent years, world production of bleaching clays nevertheless amounts to about 700,000 tons/year (13), up from the 500,000 tons/year capacity of the early 1990s. Depending on grade, natural bleaching clays currently sell in the range \$200–\$250/ton and acid-activated bleaching clays in the range \$250–\$350/ton.

Although not as extensively used as bleaching clays because of cost and higher oil retention, porous activated carbon also has an equally long history in the adsorptive purification of fats and oils, with patents dating back to the early 1920s (14). Typically, 10–20% activated carbon is used in combination with 80–90% bleaching clay. Currently, there is renewed interest in the use of activated carbon in fats and oils processing because of its ability to adsorb polyaromatic hydrocarbons (PAHs)

and other polycyclic contaminants. Activated carbons used in fats and oils processing currently sell in the range of \$1000–\$2000/ton.

More recent additions to the arsenal of sorbent materials used for fats and oils purification are the amorphous silica hydrogels. Their use, originally discussed in a mid-1960s paper by Bogdanor and Welsh (15), was patented (16–19) and commercialized by the mid-1980s. Silica hydrogels are selective for adsorption of soaps (and associated metal ions) and phospholipids, but not for color bodies. The basic process for employing silica hydrogel in the adsorptive purification of fats and oils was first described in 1986 (20). Silica hydrogels are relatively expensive, typically selling in the range \$900–\$1200/ton. Furthermore, as their moisture content is high (i.e., 50–70%), the economics of their use must be carefully weighed against the advantages they offer.

Advancements in processing and equipment, driven by the imperative for producing better quality and more economic products, have occurred over the years. Nowhere is this more apparent than in the many changes that have occurred in the adsorptive purification process and the ancillary equipment used to carry it out.

Historically, the bleaching process was a batch process conducted at atmospheric pressure. In this process, refined oil was treated with bleaching clay in a stirred vessel and heated to bleaching temperature for a prescribed period of time. When the process was completed, the hot oil and clay were separated using filtration. Bleaching under atmospheric conditions, however, hastened oxidative processes that can lead to darkening or “color reversion” in the processed oil after a period of time. Vacuum batch bleaching was found to alleviate this condition because oxygen was excluded from the system. Vacuum batch bleaching is still used today in smaller operations or in operations where oil types are frequently changed.

As the size of plants has increased and become more specialized, and the oil being processed is of a single type, or rarely interchanged, continuous or semicontinuous (vacuum) bleaching has become the method of choice. Better efficiency, reduced operational costs, and the production of very high-quality oils are among the advantages offered by these systems. Continuous countercurrent bleaching, a more recent variation of this process, results in more efficient use of the bleaching earth (21) and cuts bleaching costs (22).

### 3. ADSORPTIVE PURIFICATION AGENTS—DESCRIPTION/PREPARATION/PROPERTIES

#### 3.1. Bleaching Clays—Natural and Acid-Activated

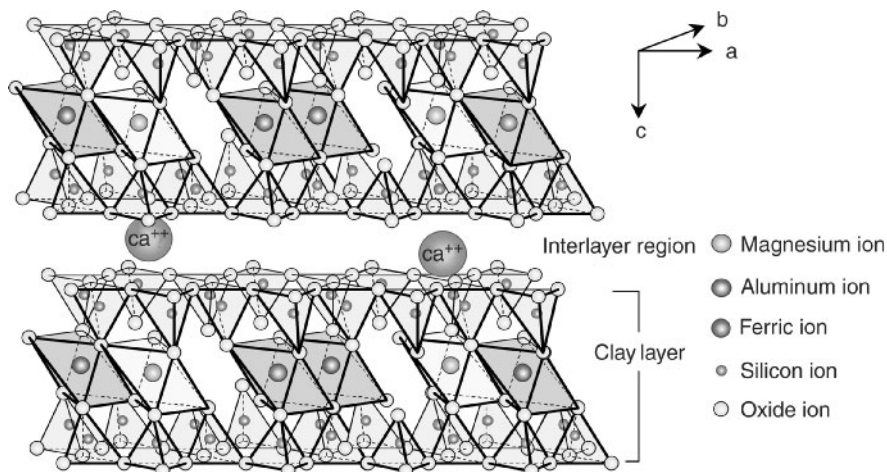
Bleaching clay, bleaching earth, and fuller’s earth are all terms used to denote certain clay minerals that can be used in their natural or acid-activated states to clarify and reduce color intensity of fats and oils. Although there are 7 major clay groups and at least 33 different specific clay minerals (23), only 2, calcium montmorillonite (sometimes called *calcium bentonite*) and a particular naturally occurring mixture of calcium montmorillonite and attapulgite called *hormite*, play a significant commercial role as bleaching clays (13).



Even though the terms *bentonite* and *montmorillonite* are often employed interchangeably, to be perfectly correct, *bentonite* denotes a clay ore in which the mineral *montmorillonite* is the principal component (12). Because the conditions for its formation have been ubiquitous over geologic time, bentonite has been found on every continent except Antarctica (24). Generated by the devitrification and weathering of volcanic glasses, bentonite typically forms when volcanic ash falls into water bodies (lakes, shallow seas) that are slightly alkaline or by leaching permeable, but poorly drained volcanic glass-bearing strata with slightly alkaline drainage water. Water, at any rate, is essential for the transformation of volcanic glasses into montmorillonite. In addition, magnesium must always be present in the parent rock or in the subsequent leaching solution for montmorillonite to be formed (25).

Hormite (mixed montmorillonite/attapulgite) clays are much less common and are found in only a few places around the world. In the United States, they occur almost exclusively in a small area in southern Georgia and northern Florida. At one time, an arm of the sea extended across this region from the Atlantic Ocean to the Gulf of Mexico. Sediments washing into this sea were gradually changed into the mixed montmorillonite/attapulgite assemblage that is mined and processed today (26). Deposits containing montmorillonite range from a few millions of years (27, 28) to about 140 million years (29) in age. Deposits containing hormite clay mined in Georgia and Mississippi are much younger, probably in the range 10–15 millions of years (26).

An idealized three-dimensional representation of calcium montmorillonite structure at the atomic level is shown in Figure 1. The 2:1 layered structure is composed of upper and lower layers of silicon oxide tetrahedra linked in hexagonal arrays to form two-dimensional silicate sheets extending in the a,b-directions. Sandwiched between these sheets are partially filled two-dimensional sheets composed of

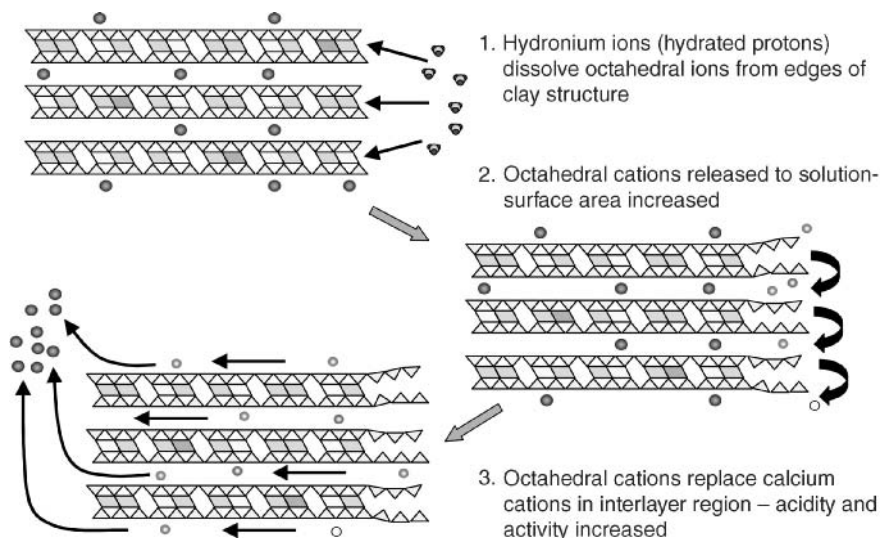


**Figure 1.** Three-dimensional representation of the atomic structure of Ca-montmorillonite. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

magnesium (yellow), aluminum (pink), and occasional ferric oxide octahedra (orange) also extending in the a,b-directions. Tetrahedral and octahedral sheets share common linkages through oxide bonds, so the basic 2:1 structure forms a series of clay layers that repeat in the c-direction. A montmorillonite particle, under the microscope, looks like a corn flake composed of 7–14 layers stacked one on top of another and extending 1–2  $\mu\text{m}$  across (30). Because magnesium and ferrous ions in the octahedral layer only possess +2 charges and aluminum and ferric ions possess +3 charges, not all six nearest-neighbor oxide ions (net  $-3$  charge) are completely charge balanced. As a result, montmorillonite clay layers possess negative surface charges. In the case of calcium montmorillonite, calcium cations residing in the interlayer region balance the net negative charge on the clay layers, thereby ensuring overall charge neutrality.

**3.1.1. Preparation by Acid-Activation** Acid-activation is the process whereby the adsorptive powers of natural bleaching clays are enhanced. For montmorillonite, the process consists of “cooking” 25–30 wt% clay slurry in water and mineral acids (usually sulfuric acid or hydrochloric acid) for a number of hours, and then filtering, washing, drying, and grinding to desired specifications. Typically, high levels of acid (i.e., >45 wt %) relative to clay are required to make the more active grades.

The acid-activation process can be understood by considering the structural and chemical changes that occur when the montmorillonite structure is subjected to reaction by strong acid (Figure 2). During the acid-activation process, acidic protons dissolve magnesium, aluminum, and ferric ions from the octahedral layer. Two important consequences are as follows: (1) Mobile alkali and alkaline earth cations



**Figure 2.** Clay activation by acid dissolution and cation replacement. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

( $\text{Ca}^{+2}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ) in the interlayer region are replaced (exchanged) by more acidic metal cations ( $\text{Al}^{+3}$ ,  $\text{Fe}^{+3}$ ,  $\text{Fe}^{+2}$ ,  $\text{Mg}^{+2}$ ) leached from the octahedral layer, and 2) surface area and porosity are increased by the dissolution process that opens up previously inaccessible sites within the clay structure. These consequences, discussed in more detail in Section 4.4.3, constitute the key aspects of the acid-activation process.

Hormite clay is naturally more susceptible to acid-activation than montmorillonite; lower levels of acid are required and can be directly applied (i.e., by simple spraying or pugging). Consequently, the slurry technique described above is not required, and the cooking, filtration, and washing steps can be eliminated (31). Data in Table 1 compare the response of hormite and montmorillonite clays with acid-activation.

In this work, montmorillonite and hormite were subjected to acid-activation over a range of acid dosages. As expected, the montmorillonite clay exhibited steadily improving adsorption capacity for carotene and chlorophyll with increasing degree of acid leaching up to the 90% level. At the same time, surface area and porosity were substantially increased (over 400% and 450%, respectively). Clearly, however, montmorillonite only treated at 10% acid dosage is no competition for typical commercial acid-activated bleaching clays (compare bleaching data for 10% acid dosage montmorillonite with bleaching data for Gr 105 and Gr 160 bleaching clays). In contrast, the hormite clay achieves maximum activity by 10% acid dosage (and even lower dosage levels according to the patent). In the case of hormite, surface area and porosity are only modestly increased by acid-activation (67% and 19%, respectively); probably because crude hormite starts out with relatively high surface area and porosity. This natural porosity may, at least in part, explain its pronounced susceptibility to acid activation. As it is porous to begin with, extensive acid-leaching to achieve adequate porosity is unnecessary. Therefore, only low levels of acid are needed to replace native cations in cation exchange sites with acidic hydronium ions (hydrated protons).

As already discussed, bentonite ores require relatively high acid levels to achieve adequate activity and extensive leaching takes place during the activation process. Excess residual acid and acidic salts formed during activation must be washed off and separated from the product by filtration. Different types of filtration equipment may be used in the filtration and washing steps, but the essential objective is to lower the levels of unused (residual) acid and excess acidic salts (primarily aluminum sulfate). If high levels of either are left in the product, bleached oil quality will be impaired because of generation of undesirably high levels of free fatty acids and contamination by metallic ions. Finally, of course, the leachate solution (acidic salts and residual acid) is a waste stream that contains materials harmful to aquatic life and therefore must be neutralized or otherwise disposed of in an environmentally acceptable manner. This constitutes an additional expense of producing bleaching clays from bentonite ores.

The way hormite clay responds to the acid-activation process allows manufacturers to skip certain manufacturing steps that are required when producing acid-activated bentonite. Because of its susceptibility to acid, the activation proceeds

**TABLE 1. Hormite vs. Montmorillonite: Response to Acid-Activation and Comparison of Bleaching Efficiency in the Treatment of Caustic Refined Soybean Oil (0.5 wt% Clay Dosage) (31).**

Clay	% Acid Dose*	Lovibond Red Value	Chlorophyll (ppb)	Peroxide Value (meq/kg)	FFA (Wt %)	Surface Area (m <sup>2</sup> /g)	(cc/g)	pH
Montmorillonite	0	13.9	660			59	0.09	8.6
	10	13.6	520			83	0.12	3.5
	20	13.4	381					
	30	10.2	232			252	0.25	2.9
	45	8.0	85			286	0.36	3.4
	60	3.8	37			297	0.43	3.8
	90	2.6	15			237	0.5	2.9
Hormite	0	3.5	160	4.2	0.039	133	0.32	6.6
	10	2.2	54	0.6	0.040	161	0.32	2.8
	20	2.1	52	0.5	0.051			2.6
	30	2.1	54	0.5	0.042	188	0.31	2.6
	45	2.2	62	0.5	0.040			2.5
	60	2.1	60	0.6	0.044	223	0.38	2.5
	90	2.2	74	1.3	0.043	205	0.38	2.5
Filtrol Gr 105		8.0	88	6.2	0.047			
Filtrol Gr 160		3.8	34	3.4	0.049			
Starting Oil		15.2	715	9.7	0.045			

\*% Acid Dose = (wt. of pure acid × 100)/(wt. of dry clay).

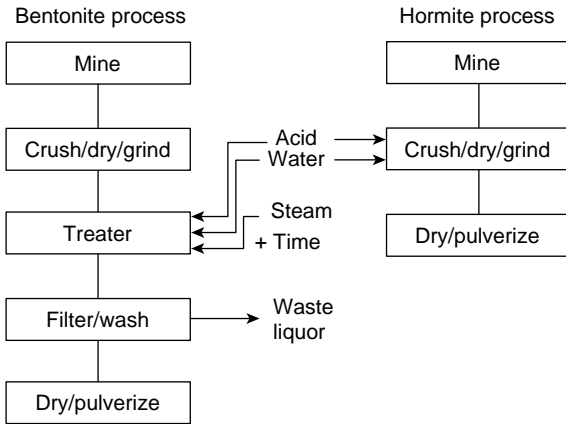


Figure 3. Bentonite versus hormite acid-activation processes.

very rapidly and requires substantially less acid. Consequently, residual acid levels are low, and subsequent washing and filtration steps are unnecessary. These features—low acid requirements, fast activation, absence of requirements for washing and filtration—are distinctive and clearly advantageous processing characteristics of this clay.

The final steps in manufacturing bleaching clays, regardless of type, are drying and grinding operations. Of course, natural bleaching clays, whether prepared from montmorillonite or hormite, only require drying and grinding. Although different methods and equipment for drying and grinding may be employed depending on the manufacturer, these parameters are vitally important in determining performance and are discussed in detail in Section 4.4.3 (*vide infra*).

Processing differences between bentonite and hormite clays are shown in Figure 3.

TABLE 2. Typical Properties of Commercial Bleaching Clays.

Property	Clay Type	Montmorillonite		Hormite	
		Acidified	Natural	Acidified	Natural
Free Moisture (wt%)		10–15	10–15	10–17	13–16
pH (2% slurry)		2.5–5.5	6–8	2.7–3.5	5–8.6
Residual Acidity (mg KOH/g)		1–10	<1	1–10	<1
Surface Area (m <sup>2</sup> /g)		150–350	50–100	120–160	90–130
Pore Volume (cc/g)		0.3–0.35	0.05–0.15	0.3–0.38	0.25–0.32
Bulk Density (lb/ft <sup>3</sup> )		34–50	57–63	23–33	24–31
Bulk Density (kg/m <sup>3</sup> )		550–800	960–1200	370–530	385–500
Wt% passing 200 mesh (75 μm)		70–100	70–100	>99.5	>99.5
Wt% passing 325 mesh (45 μm)		60–75	60–75	74–82	83–87
Particle Size (Ave. diam., μm)		15–35	15–35	30–35	35–38
Oil Retention (wt%)		30–40	25–35	32–45	34–45

**3.1.2. Typical Properties** Typical properties for commercial bleaching clays are listed in Table 2. It must be kept in mind that bleaching clay manufacturers produce a broad range of product grades that possess a correspondingly broad range of properties. Also, factors such as starting source clay and intended application will affect the final properties, so these properties will have limited value to the end user in terms of choosing one material over another. However, when used in combination with certain performance evaluations (see Section 4.4.3), they can be extremely useful to both producer and customer for the purpose of monitoring product quality and variability.

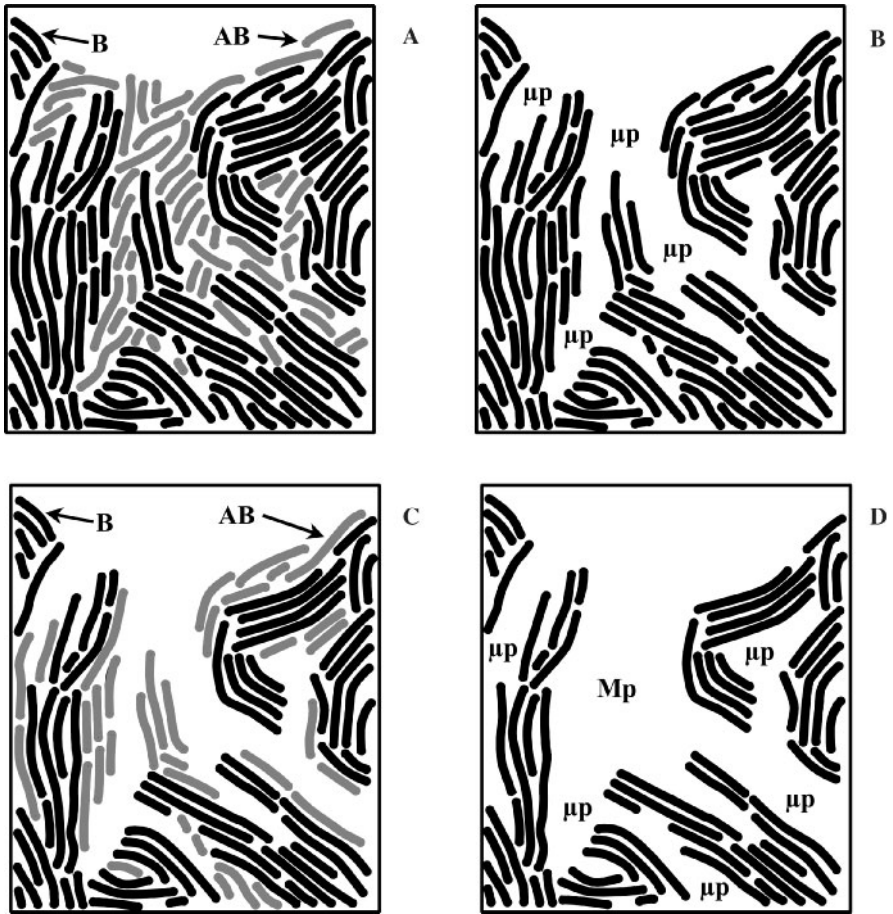
## 3.2. Activated Carbon

Powdered activated carbon (PAC) can be derived from a number of different agricultural commodities, including waste soy hull (32–36). Commercial activated carbon, however, is manufactured from only a few of these, including wood and sawdust, peat, lignite, nutshells (including coconut shell), and pits (37). Related materials include animal charcoal, gas black, furnace black, lamp black, and activated charcoal, but these are not used in fats and oils purification and will not be further addressed.

Activated carbon possesses extremely high surface area (38), often in excess of  $1000 \text{ m}^2/\text{g}$ . Much of that surface area is, however, associated with micropores—that is, pores  $<20 \text{ \AA}$  ( $<2 \text{ nm}$ ) in diameter. The surface area associated with mesopores—pores 20 to  $500 \text{ \AA}$  (2 to 50 nm) in diameter—is considerably lower (typically in the range  $10\text{--}100 \text{ m}^2/\text{g}$ ). Most liquid-based applications (including fats and oils purification) involve the adsorption of high-molecular-weight contaminants whose molecular dimensions prevent penetration into micropores; therefore, activated carbon containing significant mesoporosity is most desirable in these applications (39).

**3.2.1. Preparation** There are two basic processes for producing activated carbon: chemical activation and steam treatment. In chemical activation, the raw carbon source and acid (usually phosphoric acid) are first comixed and carbonized together at temperatures around  $500^\circ\text{C}$ . The chemical agent is then removed by washing, which leaves a highly porous structure. In steam treatment, the carbon source is first carbonized and then treated with steam at temperatures of about  $1000^\circ\text{C}$ . Under these conditions, some of the carbon and water react to generate gaseous carbon monoxide and hydrogen. Porosity develops as the more reactive carbon basal planes within the matrix are volatilized. The progressive steam activation process can be visualized by referring to Figure 4.

**3.2.2. Typical Properties** The basal planes depicted in Figure 4 are small graphite-like plates composed mostly of aromatic carbon atoms with some functional groups (carbonyl, carboxylic acid, lactone, furan, ketone, and alcohol moieties) at their edges. Flat or somewhat bent, these graphite-like plates are about  $3.5 \text{ \AA}$  in thickness and a few nanometers in length and width. The type of porosity that develops depends to some extent on the starting raw material (39).



**Figure 4.** Generation of porosity in powdered activated carbon by steam treatment. Note: For clarity, all planes are oriented in such a way that they are viewed from the edge. In reality, any orientation will be found. A. Structure of carbonized raw material showing basal planes (B) and active basal planes (AB). B. Development of microporosity after active basal planes are gasified. C. Subsequent thermal treatment generates additional active basal planes. D. Development of mesoporosity and additional microporosity after more active basal planes are gasified. B = basal plane; AB = active basal plane;  $\mu p$  = micropore; Mp = mesopores. (Courtesy Norit Nederland B.V.)

Figure 5 shows the porosity as a function of pore radius for a series of PACs made from different starting raw materials. Macroporosity is largely absent in the case of PACs because they are already very finely ground. Coconut shell-based activated carbon (B) possesses only microporosity. It is suitable only in applications where low-molecular-weight compounds are to be removed; the presence of high-molecular-weight compounds readily leads to plugging of the entrances to the micropores. Although chemically activated wood-based carbon (C) possesses

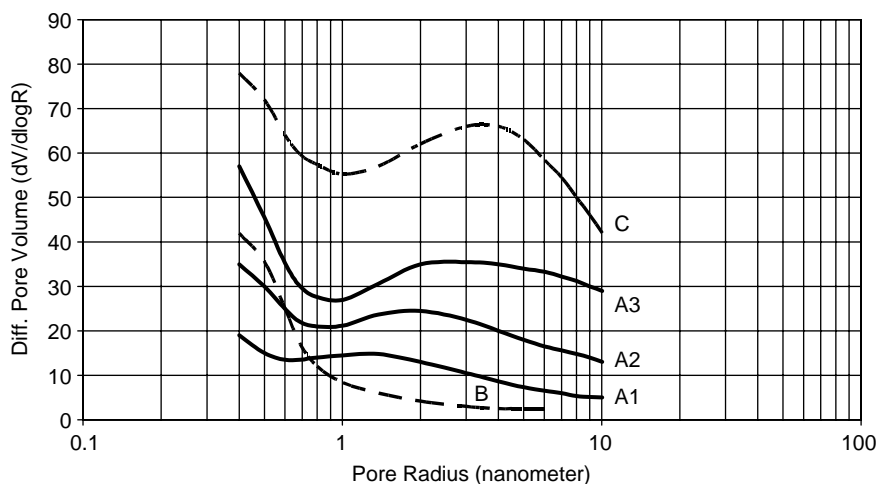


Figure 5. Typical pore size distributions for powdered activated carbons.

excellent mesoporosity and is sometimes better at chlorophyll removal, it fails completely at removing polyaromatic hydrocarbons (PAHs) and dioxins and has an oil retention almost twice that of the peat-based activated carbons. For this reason, peat-based activated carbon with porosity distribution similar to curve (A2) has been recommended for adsorptive purification of PAHs and dioxin (40). Table 3 lists typical properties of powdered activated carbons.

### 3.3. Amorphous Silica Hydrogel

As with powdered activated carbon, amorphous silica hydrogels are used in conjunction with bleaching clays in the adsorptive purification of fats and oils. Silica hydrogels possessing average pore diameters greater than about 60 Å exhibit high

TABLE 3. Typical Properties of Commercial Powdered Activated Carbon (PAC).

Property	Powdered Activated Carbons (Typical)
FM (wt%)	2–10 max.
pH (water extract)	4.3–7
Surface Area (m <sup>2</sup> /g)	650–1300
Pore Volume (cc/g)	0.9–1.2
Bulk Density (lb/ft <sup>3</sup> )	30.5–32
Bulk Density (kg/m <sup>3</sup> )	490–514
Wt% passing 100 mesh	96–98
Wt% passing 325 mesh	>70
Oil Retention (wt%)	70–150
Water solubles (wt%)	1.5 max.



capacity and selectivity for the adsorption of phospholipids and soaps (and associated metals ions) from glyceridic oils. The presence of significant moisture (i.e., >30 wt %) keeps the structure open during the initial stages contact and aids filtration from the bleached oil (16).

**3.3.1. Preparation** Amorphous silica can be prepared by any number of different routes, but typically it starts with aqueous sodium silicate solution (“water glass”), which is subsequently destabilized by the addition of acids or certain inorganic salts to yield silica hydrogels. Silica gels and/or precipitated silicas are derived from these hydrogels after appropriate washing and drying. Other, more exotic forms of preparation can also yield amorphous silica, but cost precludes their use in this application. Biogenic silica (rice hull ash) has also been prepared and evaluated, but found to be inferior to synthetic forms (41, 42).

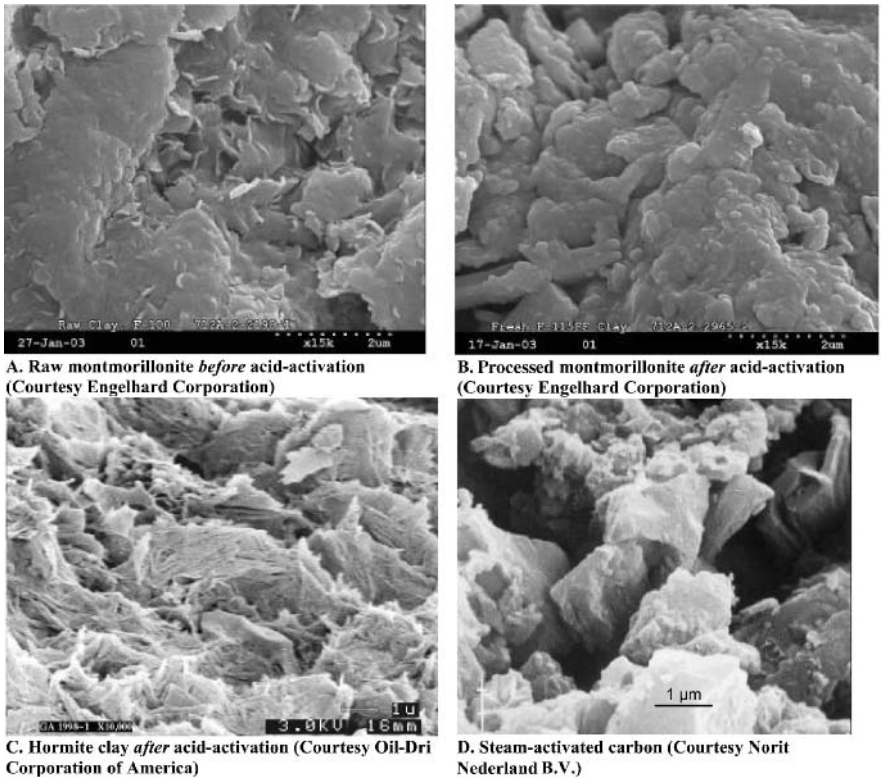
Conditions employed during preparation are extremely important in determining final product properties; generally, these are more important than the actual manufacturing process. Specifically, superior phospholipid adsorption is attained when the amorphous silica hydrogel is prepared in such a way that it possesses a high proportion of its total porosity (and associated surface area) concentrated in pores whose average diameter falls in the range 60–350 Å. Practically speaking, such silicas will generally have surface areas in the range 100–1200 m<sup>2</sup>/g and total pore volumes in the range 0.9–1.9 cc/g. A second important requirement of silica hydrogel used for adsorptive purification of fats and oils is that it possess substantial quantities of water (typically >60 wt %) in its pores. The presence of the water, although it seems not to affect phospholipid adsorption, significantly improves the filterability of the silica from the oil, even after use at oil temperatures that would cause the water content to be substantially lost during the treatment step.

**3.3.1.1. Typical Properties** Typical physical properties for commercial amorphous silica hydrogels are shown in Table 4. Chemical purity is high; typically >99.5% SiO<sub>2</sub>, <0.1% Al<sub>2</sub>O<sub>3</sub>, <0.01% Fe<sub>2</sub>O<sub>3</sub>, with only traces of alkali and alkaline earth oxides on a volatile free basis. By definition, amorphous silica is noncrystalline; it contains no crystalline silica forms (i.e., quartz, cristobalite, tridymite).

**TABLE 4. Typical Properties of Commercial Amorphous Silica Hydrogels.**

Property	Amorphous Silica Hydrogels (Typical)
LOI (wt% @ 1750°F)	50–70
pH (water extract)	4.5 (2.5)*
Surface Area (m <sup>2</sup> /g)	500–1000
Pore Volume (cc/g)	1.5–1.9
Bulk Density (lb/ft <sup>3</sup> )	30–32
Bulk Density (kg/m <sup>3</sup> )	490–514
Wt% passing 100 mesh	98
Oil Retention (wt%)	~65

\* Acidified silica hydrogel.



**Figure 6.** (plates A–D): Scanning electron micrographs of the major adsorbent types at 10,000–15,000 magnification.

### 3.4. Scanning Electron Micrographs of Adsorbents

See Figure 6 (plates A–D). Note difference in morphology between raw (Plate A) and acid-activated (Plate B) montmorillonite. Most probably this is related to the generation of hydrated amorphous silica sheets after leaching the central octahedral layer. The resulting single-layered silica sheets are much less rigid than the 2:1 clay layers comprising the original platelets and assume a disordered “fuzzy” morphology. Plates C and D are scanning electron micrographs of acid-activated hormite and steam-activated carbon, respectively. Because so little acid is used in hormite activation, no change in its morphology is observed after acid-activation.

## 4. TRACE CONSTITUENTS IN LIPID OILS AND FATS

Edible oils and fats contain a variety of naturally occurring trace constituents, including the following:

- Color pigments (carotenoids/pheophytins)
- Dissolved metals (metal ions)

- Free fatty acids (and corresponding soaps after alkali refining)
- Phospholipids (lecithin/cephalin/phosphatidic acids/etc.)
- Primary oxidation products (peroxides/hydroperoxides)
- Secondary oxidation products (aldehydes/ketones)
- Natural antioxidants (tocopherols/tocotrienols)
- Sterols and triterpene alcohols

More recently, certain non-natural, exogenous contaminants in fats and vegetable oils have become an issue (43). Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins, and aflatoxin, to name the more serious offenders, can find their way into fats and oils by a variety of pathways. In the case of PAHs, direct drying of seed materials (44, 45) is a major pathway. Dioxins, released to the environment as unintentional byproducts of combustion processes, are mainly found in animal and fish fats as a result of ingestion of dioxin-contaminated feeds, soils, and sediments (46) by these animals. PCBs were used as dielectric fluids in electrical equipment and as flame-retardants until their production was banned (USA—1977; Europe—1985); they are generally not found in edible oils.

Because PAHs, PCBs, and dioxins are long-lived and resist biodegradation, they have polluted the biosphere on a worldwide basis. The concern with these contaminants is that they tend to *bioaccumulate* in human and animal fat tissues where they can exert long-term, deleterious health effects (47, 48). PAHs are subdivided by molecular weight and size of molecule into two subcategories: light PAHs with 3–4 fused benzene rings (Mol. Wt. 178–228) and heavy PAHs with 5–7 fused benzene rings (Mol. Wt. 278–326). Typical levels by oil type and origin (49) were recently reported (Table 5).

Aflatoxin B1, B2, G1, and G2 are related mycotoxins that compromise the auto-immune system at low levels and can cause cancer at higher levels; the toxins are

**TABLE 5. PAH Levels in Crude Oils.**

Crude oil	Origin	Light PAH (ppb)		Heavy PAH (ppb)	
		Range	Media	Range	Media
Fish	South America	11–2383	486	1–149	29
Fish	North America	3–23	13	1–6	3
Fish	Japan	17–1935	584	2–900	49
Fish	Scandinavia	38–109	85	5–8	6
Coconut	Philippines	234–4563	2264	15–130	79
Soybean	United States/Argentina	34–141	80	2–6	4
Sunflower	Eastern Bloc	8–118	39	1–6	3
Rapeseed	Europe	3–276	38	1–20	6
Palm kernel	Indonesia/Ivory Coast	6–81	28	0–10	4
Palm	Indonesia/Ivory Coast	2–42	15	0.7–10	3.5
Cottonseed	United States	40–59	50	2–6	4

elaborated under certain conditions by particular moulds that can grow on grains, seeds, and nuts (47). If present during processing, they can contaminate oils that are derived from these sources.

Regulations on maximum allowable levels for these contaminants have been legislated in various countries: heavy PAHs—5  $\mu\text{g}/\text{kg}$  and for sum of heavy and light PAHs—25  $\mu\text{g}/\text{kg}$ , German Society for Fat Science (45); dioxins—0.75 pg TEQ<sub>WHO</sub>/g fat in the EU (50) where TEQ<sub>WHO</sub> means Toxic Equivalence expressed as the sum individual toxicities for 17 toxic polychlorinated-*p*-dibenzo dioxins and furans as identified by the World Health Organization; aflatoxin B<sub>1</sub>—5  $\mu\text{g}/\text{kg}$  (51) in the EU. Regulatory limits for PAHs, PCBs, and dioxins have not yet been set in the United States; the Food and Drug Administration (FDA) has established an action level of 20 ppb for aflatoxins in human foods (52).

#### 4.1. Effect of Lipid Constituents on Oil Quality; Determination of Oil Quality

Trace constituents in oils are removed (or destroyed) in varying degrees during refining, bleaching, and deodorization steps. Figure 7 summarizes the various steps in oil processing and the major constituents removed in each of them. The main objectives of oil processing are to enhance appearance, flavor, and oil stability and to ensure safety for human consumption.

Phospholipids, if not removed from oils before deodorization, can lead to dark-colored oils and serve as off-flavor precursors (53, 54). Chlorophyll (55), pheophytins and pyropheophytins (56), and metal ions (57, 58) are prooxidants that decrease oil stability. Iron and copper at levels as low as 0.01 and 0.1 ppm, respectively, are capable of lowering flavor and oxidative stability (59). Free fatty acids, besides representing a refining loss, have also been shown to act as prooxidants (60) and to lower smoke points (61) of oils during frying. Linolenic acid has

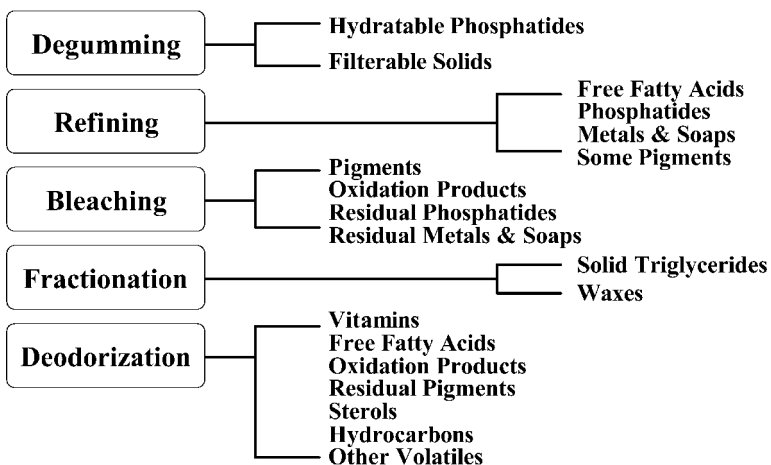


Figure 7. Major impurities removed by different processing steps.

been identified as a precursor of off-flavors in soybean oil (62). Hydroperoxide decomposition yields numerous lower molecular weight compounds (aldehydes, ketones, hydrocarbons, alcohols, lactones, furans) that can adversely affect odor and flavor characteristics (63–65). Notably, many of these compounds can be detected organoleptically when peroxide values are still low (single digit)—well before actual *oxidative rancidity* develops. Volatile peroxides and hydroperoxides can themselves impart off-odors (66). Soaps, because they are strongly adsorbed by bleaching clays, negatively affect bleaching performance (67).

The effect of carotenoids is mostly cosmetic in nature; high levels of carotenoids cause the oil to be too reddish or yellowish for present-day consumers who prefer lightly colored oils. Tocopherols and tocotrienols are natural antioxidants; it is desirable that they be left in the oil to the maximum extent possible during processing.

The American Oil Chemists' Society (AOCS) has developed and published over 400 methods to measure important lipid quality parameters. These tests are usually specific to the form, the type, and the stage of processing that the lipid is in. Levels of specific contaminants in oils can be determined by using one or more of the *Official Methods and Recommended Practices of the American Oil Chemists' Society* (68).

## 4.2. Distribution and Concentration of Trace Constituents as a Function of Oil Type

Table 6 contains typical data on the distribution and concentrations of trace constituents commonly found in soybean, palm, and canola, three oils that when taken

**TABLE 6. Typical Levels of Common Trace Constituents in Some Crude Vegetable Oils.**

Component	Type of oil			
	Soybean	Canola	Palm	
Free Fatty Acids	0.3–0.7%	0.3–1.0%	2–5%	
Phosphatides (lecithin/cephalin/ phosphatidic acid)	1–3%	1–1.5%	0.05–0.1%	
Sterols/triterpene alcohols	0.3–0.5%	0.5–0.97%	0.1–0.2%	
Tocopherols (natural antioxidants)	0.15–0.2%	0.06–0.1%	0.06–0.1%	
Carotenoids (red/orange pigments)	40–50 ppm	25–90 ppm	500–800 ppm	
Chlorophyll/pheophytins (green pigments)	1–2 ppm	5–30 ppm	nil	
Peroxides (meq O <sub>2</sub> /kg oil)	<10	<10	1–5	
Metals	{ Iron Copper	1–3 ppm	1–5 ppm	4–10 ppm
		0.03–0.05 ppm	0–0.03 ppm	0.05 ppm
Soaps (after caustic refining)	0.01–0.04 ppm	0.06–0.09 ppm	Not applicable	

together constitute the majority ( $\sim 70\%$ ) share of the worldwide vegetable oils market (69). Coincidentally, the highs and lows (circled values) exhibited by these three do a good job of spanning the extremes exhibited by the other oils. Highest phosphatide levels are found in soybean (and canola) oils; very low levels are seen in palm oil. Chlorophyll and pheophytins can be high in canola oil (especially depending on harvest conditions), whereas levels in soybean and palm oils are typically low. Carotenoid levels are highest in palm oil; high free fatty acid levels are also common with this oil (because of improper or rough handling during harvesting).

Although not specifically mentioned in the above table, waxes are found in some oils (e.g., canola, corn, pomace-olive, rice bran, and sunflower). Gossypol, a yellow-pigmented, highly functionalized  $\beta, \beta'$ -dimer of two dihydroxy- $\alpha$ -naphthol units unique to cottonseed oils is present in crude oils at levels in the range 0.25–0.47% (70). Because gossypol exhibits toxic effects at higher concentrations, its level in refined-bleached-deodorized oil is regulated to less than 450 ppm.

### 4.3. The Use of Adsorbents for the Removal of Lipid Constituents (and Contaminants)

Some trace lipid constituents (i.e., phospholipids, free fatty acids, metals, and waxes) are mostly removed during pre- or post-bleach processing steps. Gossypol is almost completely removed by alkali refining. However, bleaching clays (12, 71) or other sorbents (20) are needed to remove color bodies, peroxides, and aldehydes (72, 73) as well as remaining traces of metals, soaps, and phospholipids in the bleaching step (71). Color bodies, soaps, phospholipids, and metals are removed by adsorption, but peroxides are reduced via catalytic decomposition (5, 73). Because many of these compounds cannot be removed from the oil by steam distillation alone, the use of purification adsorbents is crucial to the ultimate success of the deodorization step. Cowan (3) has shown that refined/deodorized oils processed without the bleaching step possessed lower flavor scores both initially, and more significantly, after aging as compared with refined/bleached/deodorized oils. Likewise, these sorbents play an essential role in purification of hydrogenation feedstocks by ensuring removal of traces of soaps and phospholipids, which can act as catalyst poisons (74).

Acid-activated bleaching clays are more adsorptive and more chemically active than natural (or neutral) bleaching clays; they are usually required for oils high in chlorophyll because this pigment tends to be the most difficult to remove. Also, because they can be used at relatively lower dosages, they reduce spent filter cake and concomitant oil losses (75). Nevertheless, for some applications, particularly in the case of palm oils where only red and yellow pigments and free fatty acids need to be removed, natural bleaching clays still have their place. It should also be noted that acid-activated clays may, depending on residual acidity levels and processing conditions employed during use, cause a slight increase in free fatty acid levels (2, 4) of bleached oils via acid-catalyzed hydrolysis of triacylglycerol ester bonds (76).

As noted earlier, pure silica hydrogel possesses high capacity and selectivity for phospholipids, soaps, and metals (20) but low capacity for color pigments (chlorophylls, pheophytins, and carotenoids). Although it is possible to increase chlorophyll adsorption by acidifying the hydrogel before contact (17–19), some of the acid always leaches back into the oil during the bleaching step and causes R/Y “color fixation” problems in the subsequently deodorized oil (77). Consequently, it cannot be used alone and bleaching clay must still be used to ensure high-quality RBD oil. A recently patented approach (78) using hormite clays treated with selected complexing agents achieved similar objectives afforded by acidified silica hydrogel but with added advantages such as improved bleaching of red colors, more effective chlorophyll removal, and reduction of peroxide values.

Adsorbents also assume a key role in removing undesired exogenous contaminants from fats and oils. Aflatoxin, although not a common contaminant in fats and oils, will at any rate be completely removed by bleaching clay, which binds this toxin very strongly (79, 80). Dioxins and polyaromatic hydrocarbons, if found in oils, can be removed by treating with activated carbon and bleaching clay blend (typically one or two parts carbon to ten parts blend, depending on degree of contamination).

Many other materials, including synthetic aluminas, aluminum carbonates, aluminum silicates, magnesium silicates, various forms of attapulgite and sepiolite (81–83), alumina-pillared acid-activated montmorillonite (84), synthetic mica montmorillonite, HY-zeolite, zirconium phosphate (85), mica, kaolin, and synthetic hectorite (86), have been evaluated for their ability to purify virgin fats and oils, but none were as good as acid-activated bentonite.

#### 4.4. Fundamentals of Adsorbents and Adsorptive Purification

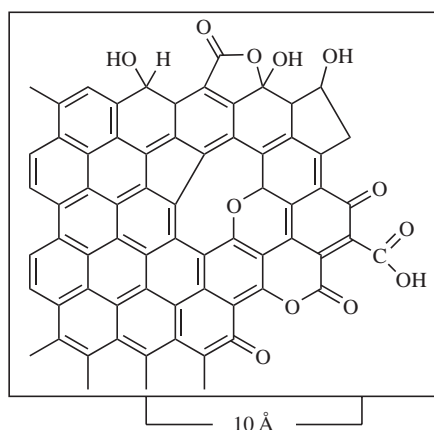
To understand how adsorbent properties affect performance, we need to understand the adsorbent surfaces. Adsorption is a surface phenomenon governed by surface chemistry. Both bleaching clays and silica hydrogels are richly endowed with surface hydroxyl groups; these play a major role in adsorbate-adsorbent interactions. Silica hydrogels possess mainly Si-OH hydroxyls. Bleaching clays exhibit a more diverse array that includes Si-OH, Mg-OH, Al-OH, and Fe-OH hydroxyls. In the case of bleaching clays, additional surface hydroxyls are generated during acid-activation when octahedral layers disrupted by the leaching process leave behind hydroxylated segments (“silica tails”) derived from remnants of the tetrahedral layers (87). Also, exchangeable interlayer cations can function as very strong surface active sites.

The adsorption process is primarily dictated by the interaction between these various surface species and the adsorbate molecules (color bodies, phospholipids, soaps, etc.) Depending on the energy barrier involved, an organic adsorbate may undergo actual bond breaking–bond making to form a covalent bond with the adsorbent surface (*chemisorption*), or it may interact through hydrogen bonding or van der Waals forces (*physisorption*).

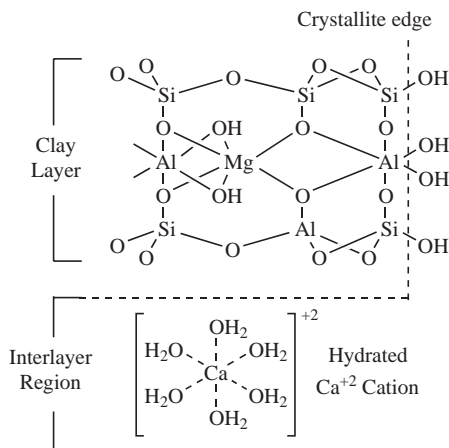
**4.4.1. Surface Characteristics of Different Sorbent Types** Figures 8–11 present reasonably probable representations for surface active sites associated with the three major adsorbent types used in fats and oils processing.

Activated carbon, in keeping with the enhanced complexity of carbon-based chemistry, possesses an extensive array of surface moieties, including hydroxyl groups, carboxylic acid groups, aldehyde groups, keto groups, cyclic ethers, and lactone groups.

**4.4.2. Surface Interactions with Adsorbed Molecules** Surface hydroxyls can interact with highly polar O, N, and S atoms by means of hydrogen bonding

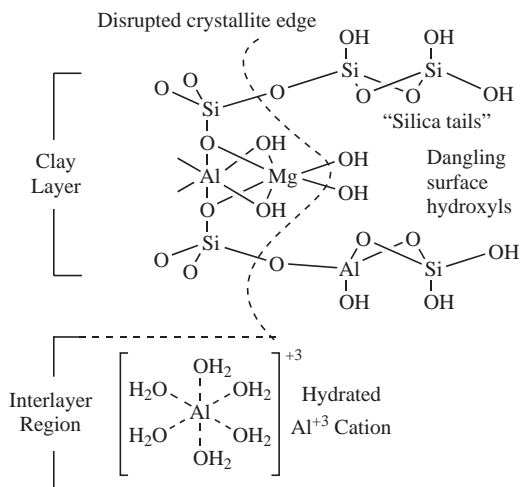


**Figure 8.** Surface-active moieties (adsorption sites) on activated carbon. (Courtesy Norit Nederland B.V.)

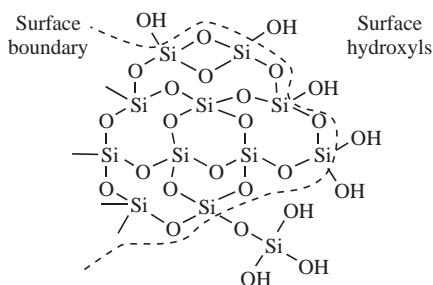


**Figure 9.** Surface hydroxyls at crystallite edges and hydrated interlayer Ca<sup>+2</sup> cation before acid-activation.



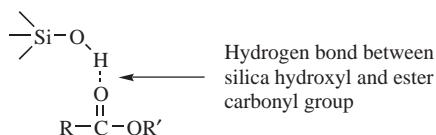


**Figure 10.** Generation of new surface hydroxyls after acid-activation shows replacement of (alkaline) hydrated  $\text{Ca}^{+2}$  cation with (acidic) hydrated  $\text{Al}^{+3}$  cation.



**Figure 11.** Surface hydroxyls on silica hydrogel.

mechanisms. For example, a silica hydroxyl group may hydrogen bond to an organic adsorbate ( $\text{RCOOR}'$ ):



Using diffuse reflectance Fourier transform infrared spectroscopy, Adhikari et al. studied the binding of oleic acid (88), triacylglycerol (89), and phosphatidylcholine (90) on silica gel at room temperature. Their interpretation was that oleic acid and triacylglycerols bind to silica surface hydroxyls via hydrogen bonding interactions between the carboxylate and ester carbonyls of these molecules, respectively. In contrast, phospholipids hydrogen bond to silica surface hydroxyls via the phosphate

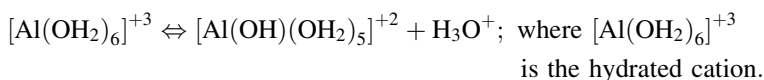
group; in this case, the interaction must be strong because most of the phosphatidylcholine remained bound even after washing with isopropanol in hexane.

These same authors also studied binding of oleic acid, triacylglycerol, and phosphatidylcholine on bleaching clay (91) at room temperature and obtained similar results to those using silica gel. This led them to conclude hydrogen-bonding interactions between surface hydroxyls and carbonyl or phosphate groups were responsible for adsorption as in the case of silica gel. It can be appreciated that aldehydes and ketones could also interact by similar hydrogen-bonding mechanisms via their carbonyl groups. It should be remembered, however, that none of these studies were conducted under actual bleaching conditions where high temperature and reduced pressures remove surface moisture, which would allow direct contact with dehydrated surfaces.

The major focus for maximizing performance of activated carbon is to provide an optimum balance between increasing adsorptive capacity for polyaromatic hydrocarbons and dioxins while decreasing oil retention in the filter cake. It has been found that steam-activated carbon derived from peat yields the best combination of properties for this application.

The situation with respect to binding of color pigments (chlorophylls, pheophytins, carotenoids) to active sites on bleaching clay is somewhat more complex. Many authors have shown that the type of interlayer cation and its associated acidity are fundamentally related to pigment adsorption capacity (11, 12, 92, 93). Table 7 gives results when acid-activated clay was completely exchanged with different interlayer cations. As shown, the presence of interlayer cations possessing higher surface acidity (measured by *n*-butylamine uptake using Hammett indicator) corresponds to higher levels of pigment adsorption.

Interlayer cations can act as Bronsted acids (i.e., proton source) via hydrolysis:



The Bronsted and Lewis acid acidity of surface hydroxyls and interlayer cations can be measured using Hammett indicators in inert hydrocarbon solvents (94–96). Silica surface hydroxyls are weak Bronsted acids possessing very little acidity;

**TABLE 7. Bleaching Properties\* vs. Surface Acidity (12)**

Exchange Cation	Surface Acidity**	Carotene Adsorbed <sup>†</sup>	Chlorophyll Adsorbed <sup>†</sup>
Al <sup>+3</sup>	1.00	12.04	0.511
Mg <sup>+2</sup>	0.56	10.90	0.509
Ca <sup>+2</sup>	0.53	9.64	0.499
Na <sup>+</sup>	0.45	9.33	0.489
K <sup>+</sup>	0.38	7.77	0.474

\* Refined soybean oil. Contact @ 330°F/20 min., vacuum 28.5 inches of mercury.

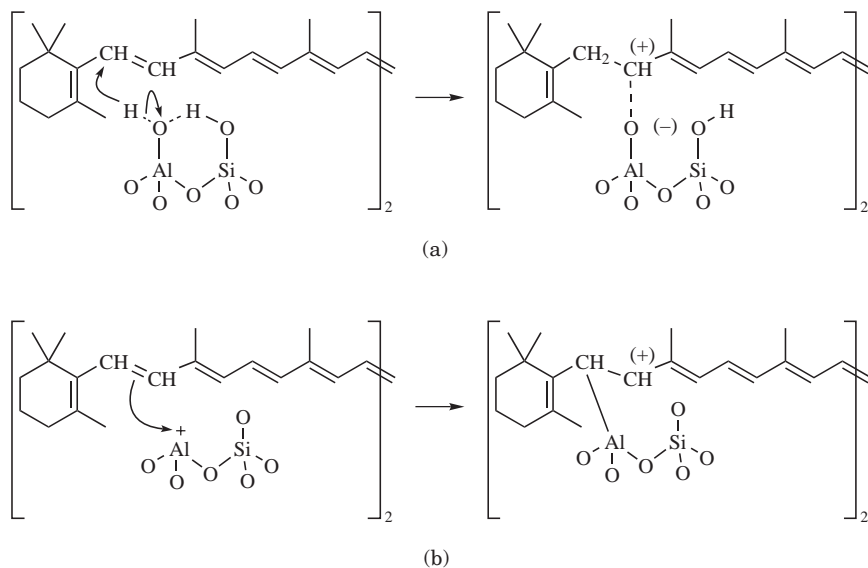
\*\* μmol *n*-butylamine titrated/g clay using Hammett indicator of pK<sub>a</sub> +5.

† μmol/g clay.

however, surface hydroxyls associated with aluminum atoms in tetrahedral coordination and hydrated aluminum cations can be very strong (see Figure 10). It is worth noting, in this regard, that some of the exchangeable interlayer cations in acid-activated clays possess acid strength equaling that of highly concentrated (70–90%) sulfuric acid (95).

The strength (97) and capacity (98) of acid sites on montmorillonite clays increase with increased drying. Replacement of less acidic alkali and alkaline earth cations with more acidic hydronium ( $\text{H}_3\text{O}^+$ ) or transition metal cations (i.e.,  $\text{Al}^{+3}$ ,  $\text{Fe}^{+3}$ ) increases reaction rates (99) and the capacity for adsorbate molecules (12). At the temperatures used during bleaching, water (including water of hydration) is removed from adsorbent surfaces, leaving bare surface hydroxyls or bare interlayer (Lewis acid) cations. At the beginning of the bleaching process, however, the clay initially has moisture and possesses Bronsted type acidity. At this stage, protons generated by hydrolysis of the hydrated interlayer cations can be donated to carotenoid and chlorophyll molecules acting as proton acceptors. The resultant protonated molecules subsequently form carbonium ion–cation complexes that can be held in place by strong electrostatic forces with negatively charged sites in or on the clay surfaces.

Sarier and Guler (100, 101) studied the mechanism of adsorption of  $\beta$ -carotene on acid-activated bleaching clay using infrared and ultraviolet spectrometry and adsorption techniques. Based on their studies, they concluded that protonation of



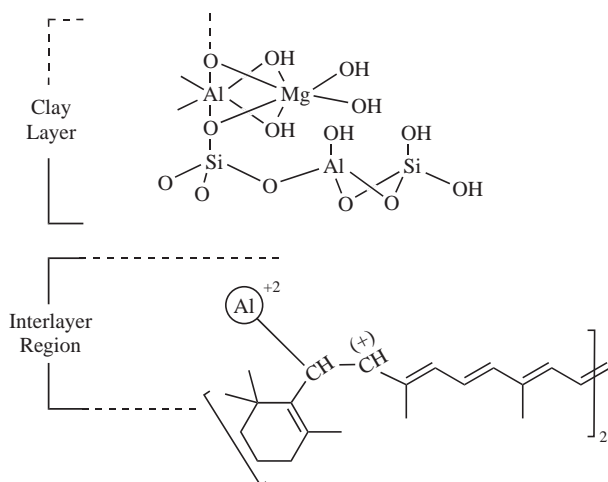
**Figure 12.** (a) Chemisorption of  $\beta$ -carotene via electrostatic attraction of carbonium ion to negatively charged tetrahedral aluminum site in tetrahedral layer of acid-activated clay. (b) Chemisorption of  $\beta$ -carotene via coordination bonding between carbonium ion and Lewis acid aluminum site in tetrahedral layer of acid-activated clay.

the 7–8 double bond of  $\beta$ -carotene yields a carbonium ion, which is subsequently chemisorbed via electrostatic bonding with Bronsted, or coordinative bonding with Lewis acid sites, respectively, in the tetrahedral layer of the clay (Figures 12a, and b). Because the adsorption isotherm had two steps, the authors proposed a second layer of  $\beta$ -carotene molecules might associate with the initial carbonium ion layer via ion-dipole interactions.

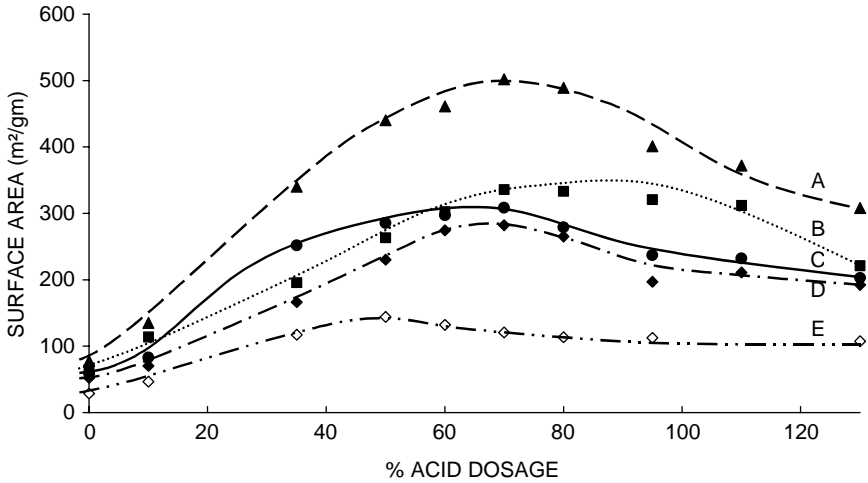
Although carbonium ion formation is reasonable, this author is of the opinion the more likely binding would be with aluminum (Lewis acid) cation sites in the interlayer region (Figure 13). This opinion is based on the knowledge that increasing levels of acid-activation eventually destroys bleaching activity, which is mirrored by loss of exchangeable interlayer cations. Bronsted and Lewis acid sites in the tetrahedral layer, however, continue to increase with increasing acid-activation. If these sites were the only sites responsible for carotene adsorption, it would be expected that carotene adsorption should continue to increase with increasing degree of acid-activation. This does not happen.

Guler and Tunc (102) also studied the adsorption of chlorophylls *a* and *b* (mixture) on acid-activated bleaching clay using techniques similar to those they employed with  $\beta$ -carotene. They concluded that chlorophyll was initially converted to pheophytins, which were subsequently adsorbed on Lewis and Bronsted acid sites on the clay.

**4.4.3. Relationship Between Adsorbent Properties and Adsorptive Performance** Acid-activation: Properties for bleaching clays (surface area, porosity, cation exchange capacity, surface acidity) vary considerably depending on the degree of acid-activation. Taylor and Jenkins (12) showed that surface area



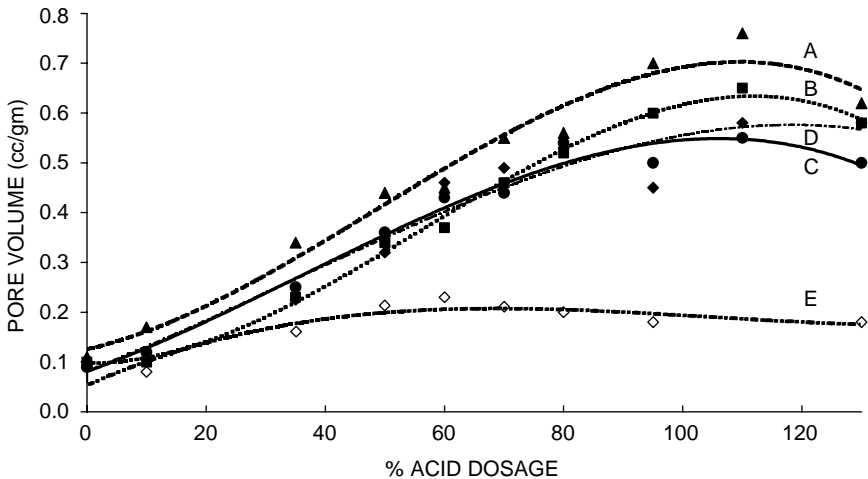
**Figure 13.** Chemisorption of  $\beta$ -carotene via coordination bonding between carbonium ion and Lewis acid aluminum cation site in interlayer region of acid-activated clay.



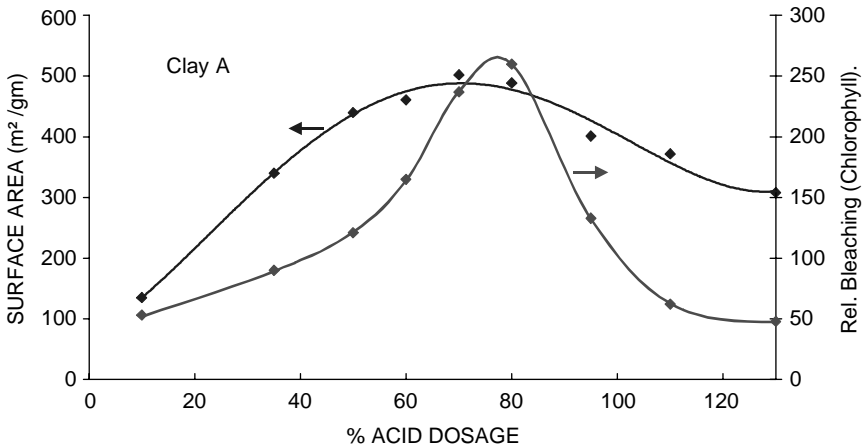
**Figure 14.** Effect of acid dosage on surface area of various calcium (A–D) and sodium (E) montmorillonite clays.

(Fig. 14) and porosity (Fig. 15) increase, sometimes dramatically, for a series of montmorillonite clays from different sources when subjected to increasing dosages of acid. Beyond a certain point, however, it can be seen that both surface area and porosity begin to decrease with increased degree of leaching.

Even though clays in this study were obtained from different sources, all are montmorillonite and each reacts somewhat differently to the acid leaching process, most notably, (1) calcium montmorillonites (A–D) attain considerably higher



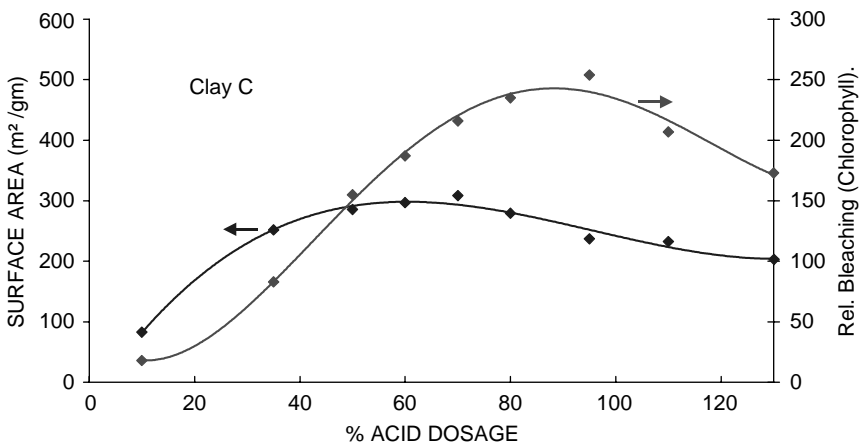
**Figure 15.** Effect of acid dosage on pore volume of various calcium (A–D) and sodium (E) montmorillonite clays.



**Figure 16.** Correlation between surface area and relative bleaching efficiency (chlorophyll) for Clay A. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

surface area/porosity maxima than sodium montmorillonite (E) and (2) acid requirements to achieve surface area maxima vary from one clay to another.

Bleaching efficiency response to acid leaching displays a similar trend (103, 104) as for surface area and porosity—at first increasing—then decreasing with increasing acid dosage. Although it might at first be suspected that surface area and adsorption activity are therefore related, it turns out that increasing surface area, although a consequence of the acid-activation process, is not of major importance in determining bleaching efficiency. The fact that surface area is not fundamentally important can be illustrated by comparing the graphs shown in Figures 16 and 17, which show surface area and relative chlorophyll bleaching efficiency as a



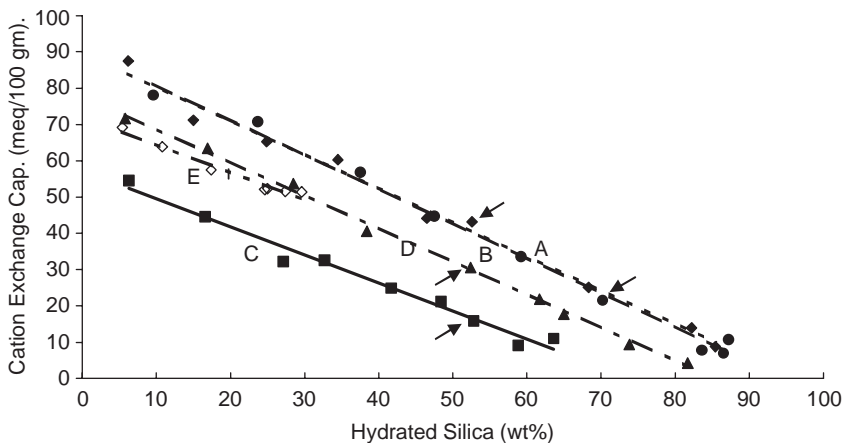
**Figure 17.** Correlation between surface area and relative bleaching efficiency (chlorophyll) for Clay C. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

function of acid dosage for Clays A and C from the above series. Ignoring for a moment the dissimilar shapes for the surface area and relative chlorophyll adsorption curves for Clay A in response to increasing acid dosage (Figure 16), it might be argued there is at least a rough correspondence between surface area and bleaching efficiency.

However, as shown by the curves in Figure 17, bleaching efficiency for Clay C does not attain maximum activity levels until well after the surface area curve has peaked and started to decline. Note also that both clays achieve practically identical maxima in chlorophyll bleaching efficiencies (albeit requiring different acid dosages), but Clay C only achieves a maximum surface area of about 300 m<sup>2</sup>/g, whereas Clay A achieves a maximum surface area of nearly 500 m<sup>2</sup>/g. Clearly, surface area, per se, is not correlated with chlorophyll adsorption capacity. Similar curves (not shown) were obtained for carotene adsorption (12).

On the basis of the activation diagram in Figure 2 (Section 3.1.1), the percentage silica in an acid-activated clay should *increase* and, although perhaps not as intuitively apparent, cation exchange capacity should *decrease* with increasing degree of acid leaching. The reason (see Section 3.1) is because positively charged cations in the interlayer region are required to balance the negative charges associated with the clay layers. A negative charge on the clay layers develops because the (+2) positive charge on Mg<sup>+2</sup> in the octahedral layer is unable to completely balance the net (-3) negative charge of its six nearest-neighbor oxide atoms; thus, a net negative charge of (-1) develops for every Mg<sup>+2</sup> ion present in the octahedral layer. It follows that cation exchange capacity should decrease as Mg<sup>+2</sup> ions are leached from the octahedral layer.

These expectations are supported by the data plotted in Figure 18. Here cation exchange capacity (milliequivalents of exchangeable cation/100 g clay) has been



**Figure 18.** Correlation between cation exchange capacity and hydrated silica (arrows point to coordinates where maximum bleaching efficiency is attained for clays A–D). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

plotted against percentage hydrated silica for the previously described clays. Hydrated silica (a form of silica soluble in aqueous sodium carbonate) corresponds to the amorphous silica left behind after clay leaching (see Figure 10). As expected, cation exchange capacity decreases (linearly) as percentage hydrated silica and degree of acid-leaching are increased.

The arrows in Figure 18 identify the x,y-coordinates at which cation exchange and degree of leaching (as measured by hydrated silica) yield points corresponding to maximum bleaching efficiency for Clays A–D. Notably, these clays (all calcium montmorillonites) attain hydrated silica values in the range 50–70% before achieving maximum bleaching efficiencies. Clay E (sodium montmorillonite) strongly resists acid attack, and it only attains about 30% hydrated silica. It does not yield a suitable product for the adsorptive purification of fats and oils, and in accord with the precepts just discussed, it was found to be only one-half to one-third as active as calcium montmorillonites for removal of carotenes and chlorophyll from refined soybean oil.

The essence of the foregoing discussion is that optimization of bleaching clay performance depends on achieving a balance between the degree to which the montmorillonite structure is “opened up” by acid dissolution and the extent to which acidic (and more active) cations leached from the clay structure replace less acidic (and less active) native cations in the interlayer region. Subtle chemical and structural differences between clays from different sources dictates the particular acid regimen required for any given clay to achieve optimum activity. Too little dissolution yields a structure with less-than-optimum porosity and inadequate degree of replacement of native cations by acidic ions leached from the structure. Too much dissolution yields a structure with good porosity and almost complete exchange of native cations by acidic leach ions, but with too few cation sites left to be effective.

Although other clay minerals can also be acid leached and improved somewhat by this treatment, they do not match activity levels attained by calcium montmorillonite. It has been hypothesized that calcium montmorillonite is superior in this regard because it *simultaneously* develops a high concentration of strong acid sites, and extensive porosity in 50–200-Å diameter pores after acid-activation (85). In any case, only those clays that achieve high hydrated silica values upon acid leaching are found to be good candidates for the acid-activation process.

**Particle Size:** The effect of particle size on the performance of bleaching clays is well known (2, 87). As discussed by Henderson (105), smaller particles (finer grinds) give better bleaching performance, but worse filtration (i.e., reduced cycle times, increased pressure buildup). The improved bleaching performance can be understood as a positive consequence of increased numbers of available cation exchange sites that are exposed in the case of more finely ground particles. However, increased pressure buildup and reduced cycle times are a negative consequence of the tighter interparticle packing that results when filtering smaller particles. In practical reality, manufacturers of bleaching clays supply a range of activity/particle size options that can be matched to the capabilities of the plant equipment and the requirements imposed by the type of oil being processed.



**Moisture Content:** As shown by Table 2, bleaching clays typically possess about 10–17 wt% free moisture. Although this moisture is boiled off during the course of the bleaching process, it is necessary. According to Richardson (2), optimum bleaching clay moisture depends on the source of the clay from which it is prepared. Although complete removal of moisture during manufacturing is possible, bleaching earth containing no moisture has been shown to be ineffective for bleaching (2, 5).

In the case of silica hydrogel, substantial moisture (usually >50 wt%) during the beginning stages of contact is necessary in order for the product to perform well. Phospholipids interact with surface water because of strong hydrogen bonding with P-O and quaternary ammonium nitrogen groups (91). It has been proposed that the phospholipids are at first strongly associated with water molecules in the silica pores. Later, as water molecules boil off under the combined action of temperature and vacuum, they become trapped by collapse of the silica structure.

**Residual Acidity:** Acid-activated bleaching clays are prepared by treatment with strong mineral acids (typically HCl or H<sub>2</sub>SO<sub>4</sub>). After the leaching process is completed, any unused acid plus excess acidic salts (primarily ferric and aluminum salts) are washed from the clay. Depending on the degree of washing, some “residual acidity” in the form of acidic salts and unused acid can be left with the clay. Residual acidity can be quantified (mg KOH/g clay) by extracting a known quantity of clay with boiling water and then titrating to phenolphthalein endpoint. It should be noted that residual acidity (because of the presence of residual acid and acidic salts) is not to be confused with surface acidity (because of surface hydroxyls and exchangeable acidic cations). Although residual acidity helps somewhat by enhancing pigment adsorption, if levels are too high, it can cause a problem by promoting the hydrolysis of triacylglycerols or soaps resulting in the generation of additional free fatty acids (2).

Boki et al. (106) have reported the type of oil being treated can also affect the outcome of the hydrolysis reaction when using acid-activated bleaching clay. In their study, the increase (relative to starting oil) was highest in canola oil (12%) but fairly modest for sunflower and soy oils (4% and 0.2%, respectively). Relative FFA levels actually dropped for cottonseed oil (−3.4%) and even more significantly in the case of corn and safflower oils (−11% and −33.7%, respectively).

**Porosity:** A common feature of all sorbents used for adsorptive purification of fats and oils is the presence of substantial porosity in the mesopore range (20–500 Å diameters) and significant surface area. Optimization requires making sure pores are wide enough to ensure access by large molecules while maintaining sufficient surface area for presentation of as many adsorption sites as possible. If pores are too large, surface area may be negatively affected; if pores are too small, passage into adsorbent interiors may be negatively affected.

**Laboratory Evaluation of Adsorbents:** AOCS Method Cc 8f-91, in conjunction with Methods Cc 8a-52 and Cc 8b-52, details a procedure for contacting bleaching clays and other adsorbents with oils on a laboratory scale using common laboratory glassware that, in essence, attempts to duplicate the actual process employed on a much larger scale at the plant level. Table 8 lists the most common

**TABLE 8. Evaluating Bleaching Clays/Adsorbents: Recommended AOCS Test Methods.**

Test	Description	AOCS method
Active oxygen method (hrs)	Time (in hours) for heated oil to reach peroxide value of 100-meq/kg oil while air is bubbled through the sample	Cd 12-57
<i>p</i> -Anisidine Value	Determines aldehydes (esp. conjugated aldehydes) based on spectrophotometric measurement of colored <i>p</i> -anisidine-aldehyde addition products at 350 nm	Cd 18-90
Chlorophyll (mg/kg)	Determines chlorophyll concentration using spectrophotometric measurements @ 630, 670, 710 nm	Cc 13d-55
Fatty acid composition (by GLC)	Quantitative determination of fatty acid distribution in oil by GLC	Cd 1-62
Free fatty acid (wt% as oleic)	Determines free fatty acids in oil by titration with sodium hydroxide solution	Ca 5a-40
Karl Fischer moisture (%)	Determines the actual water content of fats by titration with Fischer reagent, which reacts quantitatively with water	Ca 2e-94
Lovibond color (unitless)	Determines red/yellow intensity (carotenoids) of oil using Lovibond Tintometer	Cc 13e-92
Metals by atomic absorption	Analysis of Cr, Cu, Fe, Ni in oils dissolved in methyl isobutyl ketone by atomic absorption spectrophotometry	Ca 15-75; Ca 18-79
Metals by ICP	Sample is ashed, dissolved in HNO <sub>3</sub> , and elements determined by inductively coupled plasma spectroscopy	AOAC* 985.01
Oven moisture (%)	Moisture content of oils	Ca 2c-25
Oxidative stability index (hrs)	Time (in hours; induction period) for fats/oils to begin to undergo rapid acceleration in oxidation	Cd 12b-92
Peroxide value (meq / kg)	Measures peroxides (oxidation of KI $\Rightarrow$ I <sub>2</sub> ) in fats/oils; iodine determined by sodium thiosulfate titration	Cd 8-53; Cd 8b-90
Phospholipids in oil (mg P/liter oil)	Measures turbidity (nephelometric method) in oil-acetone mixtures that correlate with level of phospholipids	Ca 19-86
Phosphorus content in oil (%)	Colorimetric method (% transmittance @ 650 nm) for total phosphorus based on conversion to molybdenum blue	Ca 12-55
Soap in oil (ppm)	Measures alkalinity (as sodium oleate) of oil sample by titration to bromophenol blue endpoint with 0.01 N HCl	Cd 17-95

\*Association of Official Analytical Chemists.

AOCS methods that are pertinent with regard to evaluating the effectiveness of these adsorbents. It must be noted, however, that although laboratory adsorption testing using AOCS Method Cc 8f-91 and methods in Table 8 are adequate for *relative* evaluations (i.e., how well one adsorbent performs versus another or the effect of one set of bleaching conditions versus another, etc.), they cannot be used for *predictive* performance at the plant level. The problem, as discussed by Henderson

(105), is that laboratory-bleaching experiments are conducted as a batch process while most plants operate in a continuous mode. As a consequence, the so-called “press effect” or the extra adsorption that takes place when oil is passing through the filter cake in a plant filter press is not adequately duplicated by the one-pass filtration employed in AOCS Method Cc 8f-91. It is not uncommon that clay dosages in laboratory scale bleaches are twice those employed in the actual plant process to achieve similar levels of pigment reduction.

## 5. ADSORPTIVE PURIFICATION PROCESS: GENERAL DESCRIPTION

Adsorptive purification, in its most general sense, involves the use of adsorbents to remove undesirable constituents and contaminants from fats and oils by adsorptive mechanisms. It must be noted, however, that although different adsorbents do exhibit some degree of selectivity for certain adsorbates (see Section 4.3), none exhibit *specific* selectivity for a single compound or chemical. Some trace constituents that are desirable (e.g., tocopherols) will also be removed. According to Boki et al. (106), 20–40% of the tocopherols present in most alkali-refined oils are removed by bleaching with acid-activated bleaching clay; the exception is soybean oil, which only loses 3–5% (71, 105). Buxton has reported (107) that activated carbon removes antioxidants from fish liver oils and renders the vitamin A in the oil unstable.

### 5.1. Function of Preprocessing Steps

From the standpoint of traditional bleaching processes, the major objectives of water degumming and alkali refining/water washing (acid-water degumming in the case of physical refining) are to reduce phosphatides and soaps to low levels; these constituents are easily adsorbed by bleaching clays and interfere with the adsorption of pigments and other bleaching functions. For water degummed/alkali refined oils, the desired levels are as follows: phosphorous <3 ppm, soaps <40 ppm, and free fatty acids <0.05%. Somewhat higher levels, phosphorous <8 ppm and free fatty acids <0.8%, are acceptable in the case of acid-water degummed oils destined for physical refining. With the use of silica as an option, the entire degumming-refining-adsorptive purification process can be modified in ways not heretofore possible (108). The most direct approach is simply to add silica along with bleaching clay into the oil and bleach in the normal fashion. Alternatively, the silica is used to pre-adsorb phosphatides and soaps in the bleacher; the silica-treated oil is then filtered through a filter precoated with bleaching clay. In other variations, which do require some design modifications, silica can be used to eliminate the water wash step (along with the water wash centrifuge) or ultimately even the soap stock (along with the primary centrifuge). In all of these cases, the use of bleaching clay can be reduced; of course, cost of silica must be considered because

**TABLE 9. Recommended Purity Criteria for Oils Before Bleaching and Expected Purity After Bleaching.\***

Before Vacuum Bleaching	Max. P (ppm)	Max. Moisture (%)	Max. Soaps (ppm)
Crude (low phosphorus) oils	30	0.2	N/A
Acid degummed oils <sup>1</sup>	30	0.3	200 <sup>2</sup>
Organically refined oils <sup>3</sup>	10	0.3	N/A
Neutralized oils <sup>4</sup>	10	0.3	300
After Vacuum Bleaching	Max. P (ppm)	Max. Moisture (%)	Max. Soaps (ppm)
Crude (low phosphorus) oils <sup>5</sup>	3	0.05	N/A
Acid degummed oils	2	0.05	0
Organically refined oils	1	0.05	N/A
Neutralized oils	0	0.05	0

\*Courtesy K. Carlson, RBD Technologies, Inc.

<sup>1</sup> Various forms of physical refining, which means FFA can vary.

<sup>2</sup> Applies when using silica before the bleaching earth and adding caustic to the oil to enhance the activity of the silica. In these cases, soap is “good.”

<sup>3</sup> Organic Refining Process (ORP™) by IPH, a citric-acid-based physical refining process.

<sup>4</sup> Oil neutralized (chemically refined) by “long-Mix” process. “Short-Mixed” oils, i.e., as mostly practiced in Europe, require waster washing to get soap below 300 ppm.

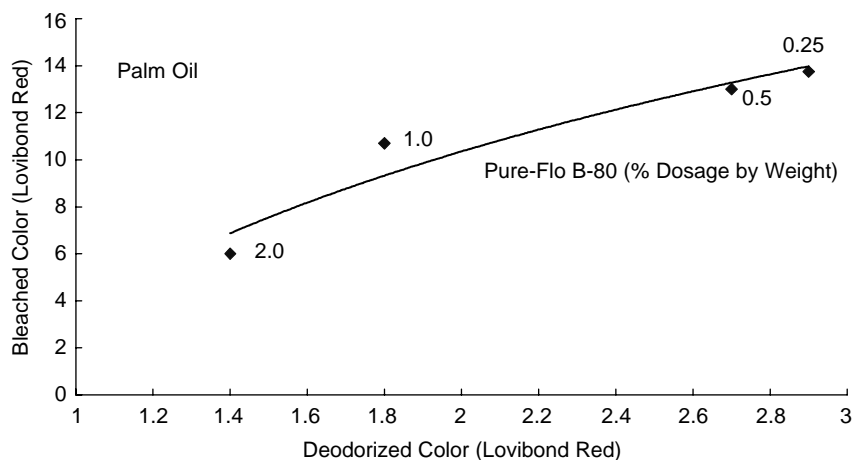
<sup>5</sup> Oils that have been citric acid conditioned and bleached with non acid-activated clays.

it is the more expensive reagent. Regardless of the particular preprocessing steps employed, recommended purity criteria for oils prior to bleaching and expected purity levels after bleaching are shown in Table 9.

## 5.2. Effect of Processing Conditions on Bleaching Efficiency

**Choice of adsorbent type and activity:** Acid-activated bleaching clays, as previously discussed (Section 3.1.2), are manufactured in a number of different activity grades. Because different oils possess significantly different concentrations of trace constituents, the choice of adsorbent (and activity) must be considered. For instance, palm oils, which are characterized by low levels of phospholipids and high levels of carotenoids, lend themselves to physical refining and usually only require the use of low activity or natural bleaching clays. Figure 19 shows the effect of dosage on bleached and deodorized colors when using natural bleaching clay (109) to bleach palm oil. This graph also makes the point that because carotenoids in palm oil are easy to “heat bleach” during deodorization, only moderate red color reduction is necessary in the bleach step to ensure adequate reduction of red color after deodorization.

Another advantage for the use of neutral bleaching clays in processing of palm oil is the absence of delta increase in free fatty acids ( $\Delta$  FFA). Brooks and Shaked (110) examined the bleaching of three palm oils (2.3–2.9 DOBI, 3.72–4.12% FFA)



**Figure 19.** Palm oil bleaching with natural clay: Effect of dosage on bleached and deodorized color (Lovibond red). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

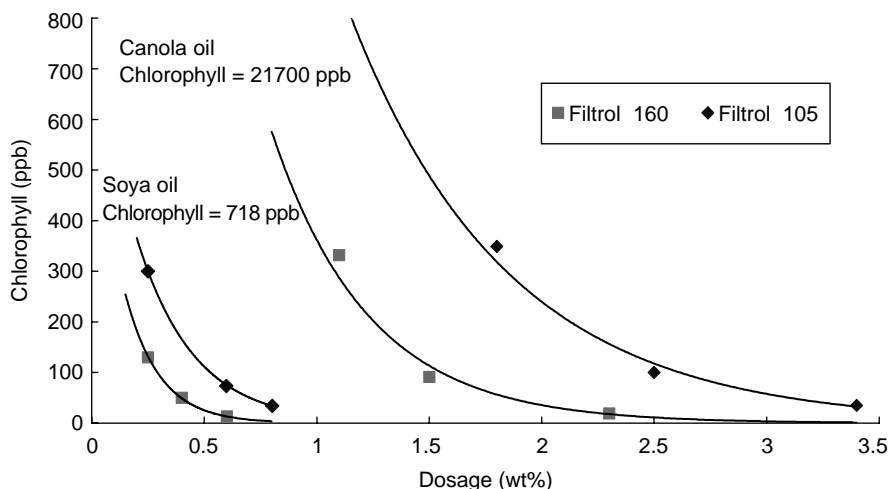
using a number of natural and acid-activated bleaching clays. Their work (Table 10) shows that acid-activated bleaching clays always caused an increase in  $\Delta$  FFA, whereas natural bleaching clays either had no effect or even reduced  $\Delta$  FFA. Based on their work, they calculated that a 1000-MT/day plant using 0.6% sorbent would lose 365 MT/year of oil for every 0.1% increase in  $\Delta$  FFA rise.

Canola oils, which can be high in chlorophyll, usually require high-activity bleaching clays to achieve satisfactory color reduction before deodorization. Figure 20 compares the dosage requirements versus clay activity for soya and canola oils with different starting chlorophyll levels (111). As is clear, the higher activity Filtrol 160 is significantly better for bleaching canola oil containing moderately high levels of chlorophyll as compared with the less-active Filtrol 105. Here, the savings in neutral oil losses and disposal costs that result from using  $\sim 1$  wt %

**TABLE 10.** Comparison of Acidity, pH, and FFA Change (108).

Sorbent	Class	Acidity (mg KOH/g clay)	pH	Ave. $\Delta$ FFA	<i>t</i> -test results*
A	Acid-Act.	0.02	2.8	0.09	+
B	Natural	0.00	6.8	-0.03	-
C	Natural	0.04	5.4	-0.1	NC
D	Natural	0.01	4.4	-0.1	NC
E	Acid-Act.	0.30	3.3	0.06	+
F	Acid-Act.	0.03	6.3	0.08	+
G	Natural	0.04	3.6	-0.01	NC
H	Natural	0.00	8.6	-0.05	-

\* One-column *t*-test, 0.05  $\alpha$ ,  $H_0$  = difference is zero. Incorporating  $\Delta$  FFA data across three oils.



**Figure 20.** Effect of starting chlorophyll level and clay activity on dosage requirements. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

less clay would be expected to offset the increased cost of the higher activity clay. However, for the soya oil, which is relatively low in chlorophyll to start with, there is little incentive for choosing the more active product because the difference in dosage requirements between the two grades is too small. Soybean and canola oils, because they are relatively high in phospholipids may benefit from dual silica/bleaching clay processing.

The primary purpose of silica in fats and oils purification is the preemptive adsorption of phospholipids and soaps so that bleaching clay dosages can be lowered. Adsorption of phospholipids by bleaching clays reduces their capacity for adsorbing color pigments. Because silica hydrogels adsorb 7–10 times as much phospholipid (112) as bleaching clay, they can be used at low levels to partially replace and reduce the overall dosage requirements for bleaching clay. However, given the enormous diversity between plants, their design, and the impact of local conditions on their operation, it is perhaps not surprising that the decision whether to use silica hydrogel can be a difficult one. Careful and detailed economic analysis is required to determine whether overall operating costs will be lowered by the combined use of silica and bleaching clay. Possible economic inducements include lower overall adsorbent cost, lower neutral oil loss, lower disposal cost, reduced city water/sewer bills, and lower operating cost (reduction or elimination of centrifuge maintenance and repairs). Possible negative economic considerations include cost of conversion (more silos, bins, feeders, mix tanks, etc.) in case of older plants, loss of flexibility to handle oils of variable quality or multiple-type feedstocks, and additional material handling. Also, in efficient plants where low phospholipids and soaps are routinely obtained, cost savings may be minimal.

**TABLE 11. Influence of Water on Oil Properties During Bleaching\* of Canola Oil with Tonsil Optimum E (113).**

H <sub>2</sub> O (%)	Lovibond red	Lovibond yellow	Chlorophyll a (ppm)	POV (meq O <sub>2</sub> /kg)	FFA (% Oleic)
0	2.3	34	0.03	0	0.061
0.3	2.2	34	0.03	0	0.061
0.6	2.1	33	0.02	0	0.061
1.2	3.7	47	0.17	0	0.063
Starting oil	8.7	57	9.58	9.4	0.034

\*Pretreatment: T – 80°C, t – 30 min., Press. – Atmospheric; bleaching: T – 90°C, t – 30 min., Press. – 15 mm Hg.

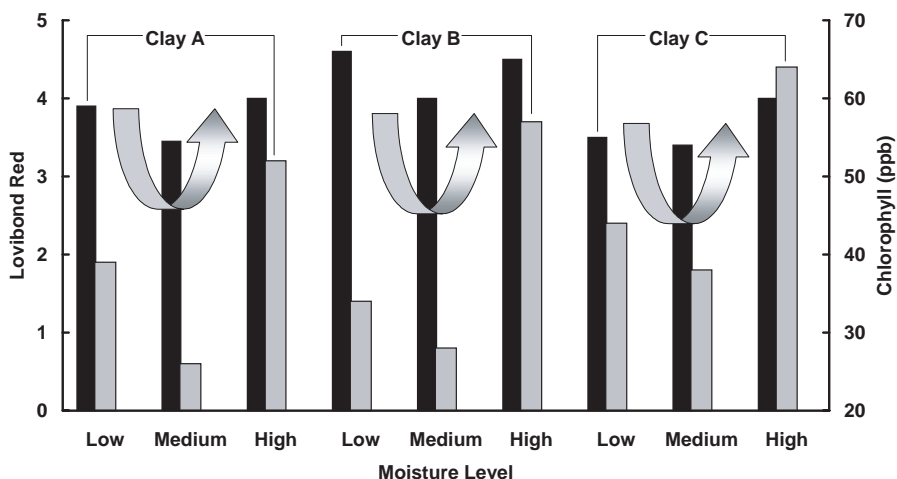
**Bleaching Conditions:** The major factors that affect the bleaching process, aside from the actual quality of incoming oil, are the conditions employed during the contact between oil and adsorbent. Assuming good quality oil (i.e., low in phospholipids, soaps, and free fatty acids) and the proper choice of adsorbent, other important factors include *moisture of oil, absence of air during bleaching and bleaching temperature and time.*

Understanding the effect of moisture on bleaching is somewhat problematic because both bleaching clays and incoming oils can have different levels to begin with. Zschau has reported (113) that the addition of *some* level of moisture can have a beneficial effect in bleaching of canola oil. His work (Table 11) shows Lovibond red and yellow, and chlorophyll levels improve with increasing moisture up to 0.6 %. Beyond that level, however, additional moisture is deleterious from the standpoint of color reduction.

Ball et al. (67) concluded water addition was not beneficial (Table 12; data derived from figure in paper) in the case of soya oil bleaching. Reduced bleaching efficiency (as measured by chlorophyll adsorption) occurred at all levels of moisture addition. More recently, Brooks (114) studied the bleaching of refined soya oil over a more narrow moisture range (0.1–0.45 %) with three different bleaching clays. His results (Figure 21) confirm Zschau's findings and indicate an inflection

**TABLE 12. Relationship of Bleach Performance to Water Content of Soya oil: Chlorophyll a (67).**

H <sub>2</sub> O (%)	Chlorophyll a (ppb)	
	0.5% dosage of clay	1.0% dosage of clay
Dry oil	180	49
0.5	280	100
1.0	345	125
Starting oil	810	



**Figure 21.** Moisture effect on refined soya oil bleaching. Moisture levels: low <math><0.10\%</math>, medium = 0.15–0.17%, high = 0.3–0.45%. Solid bars—Lovibond red; gray bars—chlorophyll (ppb). Bleach conditions:  $T=115^{\circ}\text{C}$ ,  $t=45\text{ min.}$ , Press.—25 in. Hg. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biopf>.)

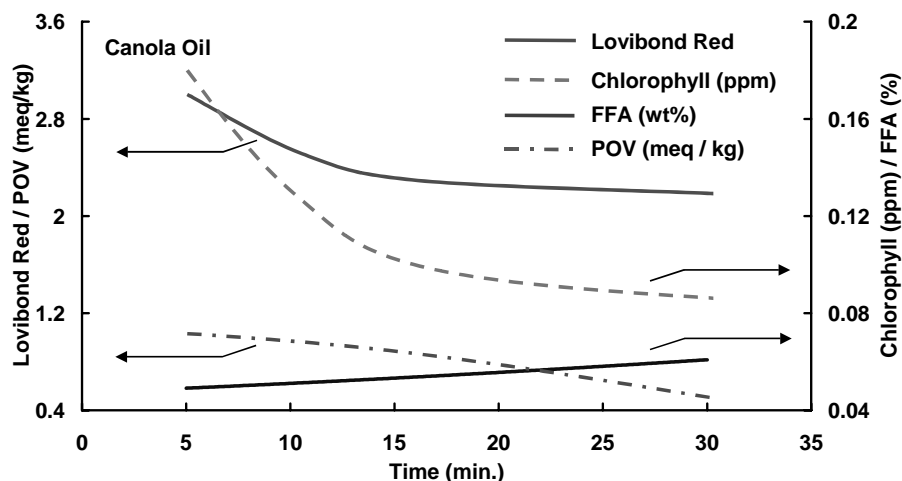
point for optimum Lovibond red and chlorophyll values at moisture levels near 0.15–0.17%. Based on this data, it can be understood that Ball et al. simply missed the inflection point because the lowest moisture level they examined (0.5 %) was already beyond the point of inflection noted by Brooks.

These studies show that although there is an optimum level of moisture in the oil, it can occur over a narrow range; too much moisture is clearly detrimental. Because oils coming from the vacuum dryer will usually have near-optimum moisture content, the best advice is that moisture addition should be used judiciously, and only in cases where special circumstances and experience warrants it. Moisture levels in the range 0.1–0.2% are probably near optimal.

The exclusion of air during the bleaching process is absolutely essential. If oxygen is present during the bleach, it can lead to the formation of red-yellow chroman-5,6-quinones (115). King and Wharton have shown that oxidation during a bleach not only develops new pigments, which are resistant to adsorption, but also that the adsorbent strongly catalyzes the oxidation process (116). In most modern-day bleaching plants, oxygen is removed from the clay/oil system before bleaching by vacuum. Generally speaking, the vacuum during bleach should be below 50 mm Hg, and most references recommend vacuum levels in the range 20–28 mm Hg or even lower.

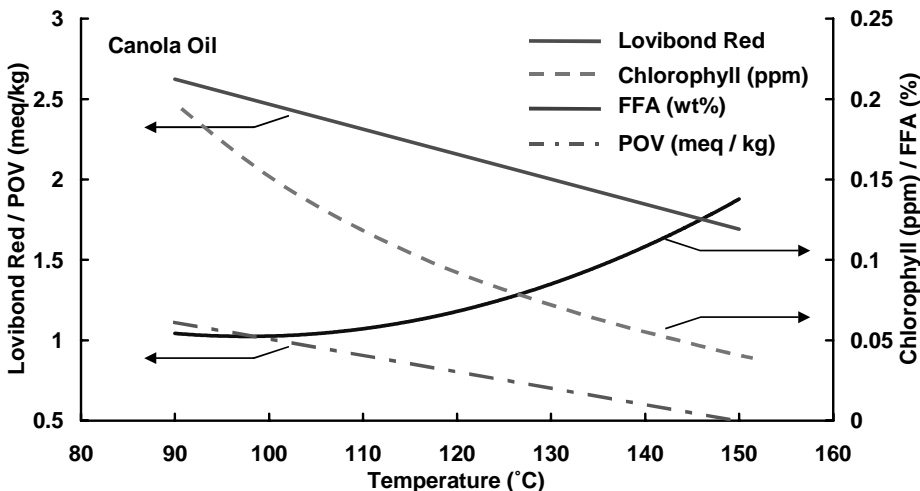
Many workers (8, 85, 105, 117) have reported on the effect of bleaching time and temperature on bleached oil properties. Data presented in Figures 22 and 23, from Taylor (8), are typical. As shown, pigment levels drop quickly (Figure 22) for the first 15–20 minutes of the bleach, during which time they reach equilibrium conditions and then begin to level off.





**Figure 22.** Canola oil: Effect of bleach time. Bleach conditions: Clarion 470 (1.5 wt% dosage/vacuum/110°C bleach temperature). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

Peroxides slowly decrease and free fatty acids slowly increase over the course of the 30-minute bleach. Even though some oils, in particular, palm oil, will take longer to bleach, the pattern noted above will hold. Namely, that adsorption and catalytic processes are initially vigorous, but then begin to level off; by the time moisture from the clay and oil are removed by heat and vacuum, most of these processes will have been completed and longer residence times will yield diminishing returns.



**Figure 23.** Canola oil: Effect of bleach temperature; bleach conditions: Clarion 470 (1.5 wt% dosage/vacuum/30 min. bleach time). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

Higher temperatures clearly promote pigment adsorption and various catalytic reactions (peroxide decomposition, triacylglycerol hydrolysis). As a consequence, pigment and peroxide levels drop with increasing temperatures, whereas free fatty acids levels increase dramatically (Figure 23) because of increased triacylglycerol hydrolysis reactions.

Although the work just described used canola oil, similar increases in free fatty acid levels as a consequence of higher bleach temperatures have been reported for soya (118) and palm (86, 119) oils. The latter workers also reported decreased peroxide values but increased anisidine values as bleach temperature was increased. This conforms to expectation because the anisidine test is diagnostic for shorter chain aldehydes (including alkenals and dienals), which are secondary oxidation products of peroxide decomposition.

Based on the discussions thus far, it might be concluded that higher bleaching temperatures are desirable because increased pigment removal and peroxide decomposition could be achieved with the lesser amounts of bleaching clay. The problem, of course, is the increased levels of free fatty acids generated by higher bleach temperatures. Even though they can be removed during the deodorization, they have relatively low value as compared with the triacylglycerol and represent a serious economic loss. Furthermore, at least in the case of palm oil, there is good evidence (119) that higher temperature bleaching yields oils, which exhibit much poorer color and oxidative stability than oils bleached at more moderate temperatures.

Even if incoming oil quality is good (see Section 5.1), moisture levels are low and good vacuum conditions can be maintained during bleaching, bleaching requirements differ to some extent between one oil and another. Table 13 lists

**TABLE 13. Typical Bleach Conditions (Time/Temperature/Dosage) for Some Common Oils**

Oil	Bleach Time (min.)	Bleach Temp. (°C)	Typical BE Dosage* (wt%)	Comment
Canola	20–30	100–105	1–3	High in chlorophyll; typically requires highly active BE
Corn	20–40	90–110	0.5–1.5	Alkali refined
	20–40	90–110	1–3	Physically refined
Cottonseed	15–30	90–95	1.5–3	Aflatoxin, if present, removed by BE
Palm	30–45	100–120	0.8–2	Stay below 110°C if using highly active BE
Safflower	20–30	95–100	0.2–0.4	May need activated carbon if PAHs present
Soya	20–30	95–110	0.3–1.5	
Sunflower	30–45	90–100	0.3–1.5	Typically requires highly active BE; may need activated carbon if PAHs present

\*In cases where silica can be used, bleaching earth (BE) requirements can be reduced. As a general rule of thumb, one part BE can be replaced by a combination of (1/6–1/4) part silica + (1/2–3/4) part BE.

some typical ranges for bleaching times, temperatures, and bleaching earth (BE) dosages for some of the more common oils.

Summarizing then, some general guidelines for optimizing the bleaching process would be (1) keep oil moisture low (0.1–0.2%) but not necessarily zero; (2) maintain adequate vacuum (<50 mm Hg); (3) use the minimum bleach time necessary to attain equilibrium conditions between adsorbate and adsorbent; and (4) do not bleach at temperature in excess of about 120°C.

### 5.3. Variations: Bleaching Process/Bleaching Systems

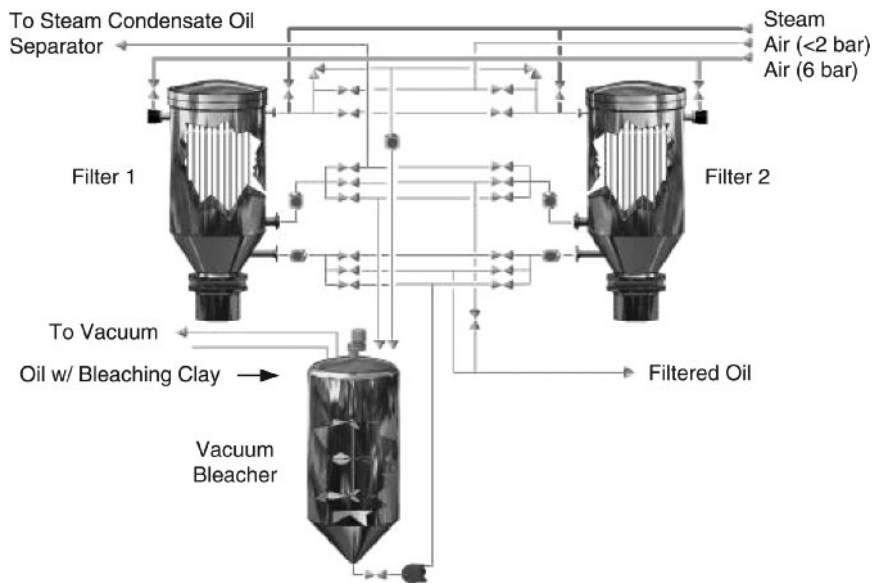
**5.3.1. Batch Bleaching** Batch bleaching refers to those processes where the entire charge of oil, admixed with bleaching earth (and/or other adsorbents), is added to a single vessel. Usually the bleaching earth is added at a temperature around 70–80°C. The temperature is then raised somewhat, and the bleaching earth/oil mixture is mechanically agitated for an appropriate time (Table 13). Upon completion of the contact period, the contents of the vessel are pumped to a filter. Oil is recycled between filter and bleach tank to allow for cake buildup on the filter; during this time, the oil experiences additional bleaching (“press bleach effect,” Section 5.4.3). Once the oil is running clear and color specifications achieved, the bleached oil is then diverted to storage.

**5.3.1.1. Atmospheric Bleaching** Atmospheric batch bleaching is conducted as described above; bleach vessels are open to the atmosphere. Because air can cause oxidation of the oil, mixing systems for carrying out atmospheric bleaches should be designed to minimize splashing or aeration at the surface but still maintain good suspension of the clay in the oil. In such systems, dry steam can be used to good effect for heating, agitation, and deaeration.

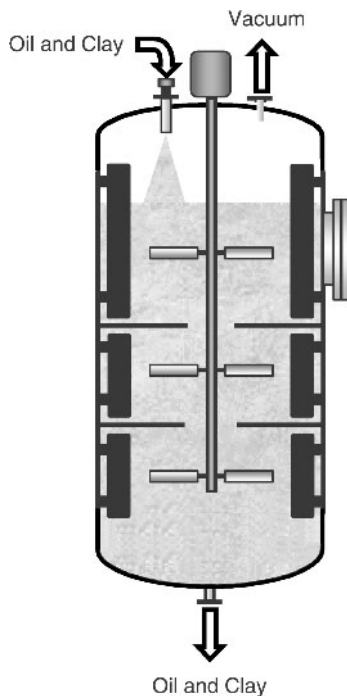
**5.3.1.2. Vacuum Bleaching** In the case of vacuum bleaching, the vessel is closed and maintained under partial vacuum (typically 20–28 mm Hg) while being heated. Vigorous agitation with splashing at the surface is desired to enhance deaeration of the clay/oil mixture. Whether the adsorbent is added before heating the oil or after the oil has reached bleach temperature, care should be taken to ensure that both oil and bleaching earth are fully deaerated before adding the bleaching earth.

**5.3.2. Continuous Vacuum Bleaching** Most modern, large-scale plants today use some form of continuous vacuum bleaching. Figure 24 depicts a standard arrangement for this type of bleaching system. The advantages of the continuous system, aside from the fact that the bleacher is always in operation, are that it allows for reduced energy consumption, simpler process controls, and the elimination of intermediate storage capacity. In these operations, the oil and clay continuously come together in a premix tank before entering the bleaching vessel whose volume determines their retention time in the reactor.

Many different vacuum bleacher designs are available (Figure 25) to the industry, some with single-stage spraying of the premixed clay/oil slurry into the vacuum



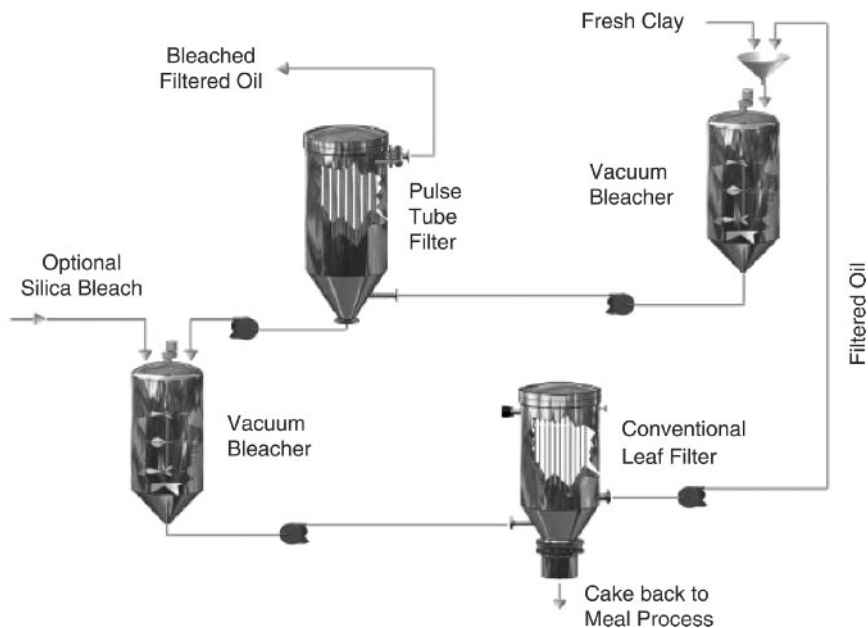
**Figure 24.** Standard continuous vacuum bleaching system. (Diagram Courtesy LFC Lochem B.V.) (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)



**Figure 25.** Model 1 vacuum bleacher. (Courtesy Crown Ironworks.) (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

unit, and others with two stages to allow for more intimate contact and to better control contact time. In any case, the spraying of the clay/oil slurry under vacuum is much more efficient for deaeration and further reduces oxidation during the bleaching process. Upon exiting the reactor, the bleaching clay–oil slurry is separated in a filtration unit equipped with two filters so that one filter is in use while the other is in the cleaning or standby mode.

**5.3.3. Countercurrent Continuous Bleaching** Countercurrent continuous bleaching is perhaps the most advanced bleaching system yet to evolve. Figure 26 depicts such a system. Although similar in some respects to the packed bed continuous system, this design has the advantage that the partially spent clay to be reused can be in contact with the oil much longer in a vacuum bleacher than is the case when the oil is simply passing through a packed bed filter. In this design, a pulse tube filter allows for the return of the once-used clay back to the first bleacher in slurry form, which is optimal. By using dry nitrogen to “pulse” the partially spent cake from the filter tubes, moisture and oxygen are rigorously excluded from entering the first bleacher. Up to 40% reduction in overall clay consumption, spent cake, and retained oil losses have been claimed for this process (120).



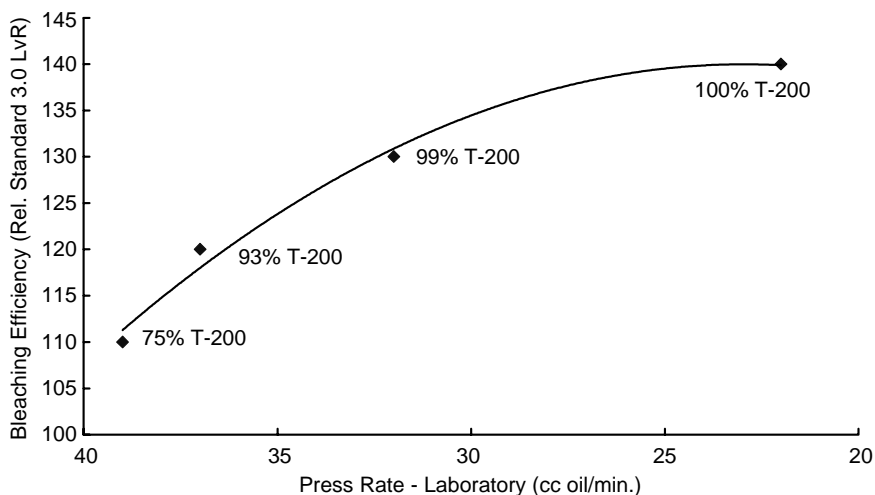
**Figure 26.** Öhmi Bleach® countercurrent continuous bleaching. (Diagram Courtesy LFC Lochem B.V.) (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

## 5.4. Filtration

**5.4.1. Effect of Adsorbent Particle Size** Richardson (2) and others (105, 121) have demonstrated that bleaching efficiency (pigment adsorption) improves, as the average size of bleaching clay particles gets smaller. If there was no penalty for such action, it would make good sense to produce very finely ground materials because this is well within the capabilities of bleaching clay manufacturers and it would allow refiners to use less bleaching clay. Unfortunately, there are at least two negative consequences. The first, as illustrated by Figure 27 (after Richardson<sup>2</sup>), is that press rate (rate of oil flow through the filter cake under the influence of pressure) decreases as the average size of the bleaching clay particles is made smaller. Even though the data presented in the figure refer to a laboratory press, the same basic effect is also noted at the plant level.

A second drawback (121) is that oil retention is increased as average particle size is decreased. However, the negative effect (oil loss) may be partially offset because less clay can be used. The loss of filtration rate is, in any event, a far more serious problem because this immediately impacts the primary measure of plant productivity—the rate at which fats and oils are produced.

It is possible, to some extent, for manufacturers of bleaching clay to simultaneously improve bleaching efficiency and filtration rates by exerting very precise control over particle size distribution during grinding and using higher levels of acid during activation. However, there are limitations to this approach because product yields are lower, and this drives costs up. As a practical reality, highest activity products will always depend, to some degree, on moving the manufacturing process in a direction that favors production of smaller sized particles.



**Figure 27.** Effect of bleaching clay particle size on bleaching efficiency and press rate; particle size measured as percentage particles passing through a Tyler 200 mesh screen. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp.>)

Brooks and Shaked (122) have studied the relationship between the percentage of particles finer than 5  $\mu\text{m}$  in diameter versus filtration rate and permeability. For both, good correlations ( $r^2$  values  $> 0.92$ ) existed between loss of permeability (and filtration rate) and the percentage particles less than 5  $\mu\text{m}$  diameter. Increased levels of these finer particles decreased both filtration rate and permeability. The correlations could be expressed as nonlinear exponential equations of the form:

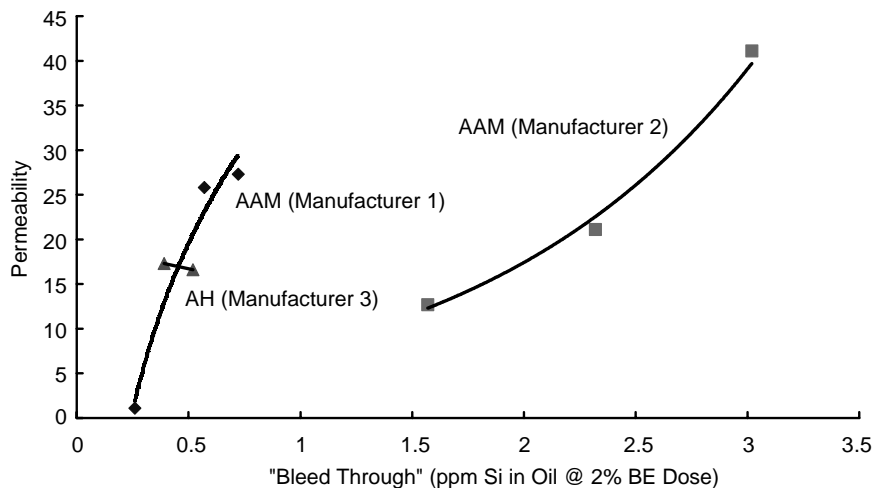
$$y = me^{bx},$$

where:

$y$  = permeability constant (or filtration rate)

$x$  = % particles  $< 5 \mu\text{m}$  diameter

In this study, the authors examined eight different commercial bleaching clays from three different manufacturers and measured the part-per-million of metals (“bleed through”) found in the filtered oil. Two of the manufacturers supplied acid-activated montmorillonite (AAM) and the remaining supplied activated hormite (AH). The authors wanted to determine if clays exhibiting high permeability would be more likely to pass ultrafines (i.e., particles  $< 10 \mu\text{m}$  diameter) through the filter cake into the filtered oil. Figure 28 shows a plot of permeability versus “bleed through” (measured as ppm Si in filtered oil) using data taken from their study. Higher permeability was indeed found to correspond with increased “bleed through” in the case of the acid-activated montmorillonite products; however, this was not the case for the activated hormite products. It was also noted that “bleed through” in the case of AAM produced by manufacturer 2 was more pronounced than AAM produced by manufacturer 1.



**Figure 28.** Relationship between permeability and “bleed through” for acid-activated montmorillonite (AAM) and activated hormite (AH). (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

Based on their data, the authors concluded fast-filtering (high-permeability) clays are more prone to “bleed through” and recommended making sure polish filters are in place and monitored frequently when using such clays. Regarding the difference in “bleed through” characteristics between AAM products, the authors hypothesized that leached silica at the edges of the particles (Figure 10, Section 4.4.1) may be more friable for one clay as compared with the other. Attrition of these silica edges during mixing in the bleacher and subsequent filter packing would produce microparticles small enough to migrate through the filter cakes with high permeability.

**5.4.2. Filtration Equipment** Good filtration equipment and proper operation are essential if the filtration process is to be effective and economical. Modern-day bleaching earths should not, except in rare cases, require the use of filter aids such as diatomaceous earth. Rather, the clay is used as the precoat. According to Veldkamp (123), the two main objectives in precoat filtration are as follows:

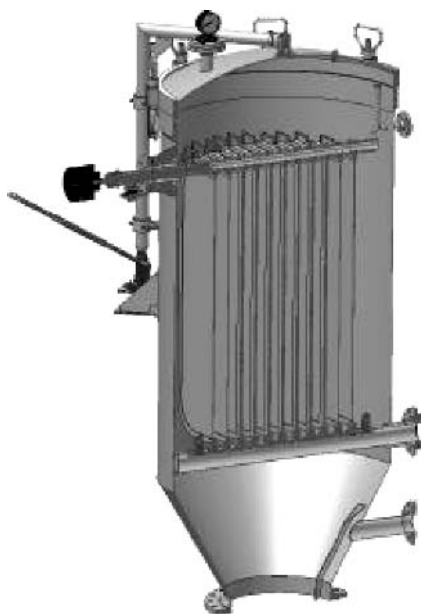
1. To provide a septum or precoat layer, which is “tight” enough to retain all of the suspended solids from the liquid to be filtered.
2. In addition, to provide this same septum with maximum porosity so that the maximum quantity of suspended solids can be retained before this septum becomes blocked. When the septum is blocked, cleaning becomes necessary, which in turn causes an interruption in flow. Obviously, when the suspended solids are smaller in size than the pores of the cake, the solids will pass through. Fortunately, adsorbents are commercially available in many grades, which allow various particle sizes to be retained.

Precoating is accomplished by recirculating clay/oil slurry through the filter unit until the oil becomes clear. A minimum amount of cake is required to form a stable layer through which the subsequent filtration can take place. The minimum amount needed is related to filter area—at least 0.5-kg clay/m<sup>2</sup> filter area (0.1 lb clay/ft<sup>2</sup>). When clay content is in the range 0.5–2%, oil clarity should be achieved after 10–15 minutes of recirculation time.

Various filter designs are available to the industry, including plate and frame filter presses, horizontal tank/vertical leaf pressure filters with retractable bundles or shells, and vertical tank/vertical leaf pressure filters. The latter (Figure 29) has been accepted by the industry as perhaps the best overall choice in terms of price, performance, space requirements, and ease of automation. Filter sizes up to 85-m<sup>2</sup> filter area/filter are available with this model. However, the horizontal tank/vertical leaf model with either retractable bundle or retractable shell also has its proponents. Commonly, a major factor in deciding on this type filter is the fact that these units are available in sizes up to 200-m<sup>2</sup> filter area/filter.

Plate and frame filter presses, once the industry standard, have lost favor over the years because the residual oil content of spent filter cakes from these units typically runs 35–40%. For pressure leaf-type filters, residual oil content of less than 25% in the spent filter cake is possible. This lower oil loss is one of the reasons why they





**Figure 29.** Vertical pressure leaf filter. (Courtesy LFC Lochem B.V.) (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp.>)

have become the preferred type of filter nowadays. As mentioned in Sections 5.3.2–5.3.3, continuous and countercurrent continuous bleaching systems require the use of two or more filters. One filter will always be in service with the remaining in cleaning or standby modes. For those plants with a batch or semicontinuous process that frequently changes the feedstock, the use of leaf or tube type filters is not recommended. In this case, a modern filter press should be considered despite its higher price.

Once the filter cake on the filter leaf reaches design thickness (18–22 mm), the filter tank is emptied and the residual oil is removed by blowing with dry steam or air (or air/inert gas). Recommended conditions and expected results for cake blow-down are given in Table 14.

Conventional filter systems are bound by one immutable constraint, namely, that clay particles must be separated from the clay/oil slurry by passing through a pre-coat of clay that is “tight” enough to retain all the suspended solids from the liquid to be filtered, but still permeable enough to produce economical rates of oil production. If particles become too small, they cause the filter rate to decrease so much

**TABLE 14. Recommended Conditions for Filter Cake Blow Down (123).**

Medium	Time (min)	Consumption	Residual oil
Air	15–20	0.1–0.15-Nm <sup>3</sup> air/m <sup>2</sup> filter area/minute @ 2–3 bar	30–35%
Steam	10–20	0.5-kg dry steam (140–150°C)/kg dry clay @ 2–3 bar	22–25%

that economical rates of oil production cannot be maintained. Transfeld (124) has described a new kind of system that allows the use of fine-grained ( $<10\ \mu\text{m}$ ) bleaching clay. The process, called “electrofiltration,” employs an electric field to agglomerate superfine clay particles before their filtration on conventional filters. Reductions in clay usage up to 40% are claimed for this process.

**5.4.3. Press Bleach Effect** Although there had long been a general awareness in the industry that bleaching clay in the form of a filter cake seemed to impart some additional bleaching to the oil (sometimes called “press bleach effect” or “packed bed bleaching”), it was not until Henderson’s seminal paper and subsequent publication (7, 105) that the effect was systematically studied and explained. To understand this effect, it is necessary to start with a general consideration of adsorption theory as it applies to the bleaching process.

The adsorptive purification process obeys the Freundlich equation, which can be expressed mathematically as

$$x/m = Kc^n, \tag{1}$$

where  $x$  is the amount of pigment adsorbed,  $m$  is the amount of adsorbent, and  $c$  is the amount of residual pigment still in solution ( $K$  and  $n$  are constants related to adsorptive properties of the bleaching clay). Rewriting eq. 1:

$$K = \frac{x/m}{c^n}. \tag{2}$$

As  $n$  is a constant, we can see that the ratio  $x/m$  divided by  $c^n$  must always yield the constant,  $K$ . Assume some reasonable values for  $x, m, c$ , and  $n$ . According to Table 7, a reasonable value for chlorophyll adsorption is  $0.5\ \mu\text{mol/g}$  or  $x = 0.5\ \mu\text{mol}$ ,  $m = 1\ \text{g}$ . Let us arbitrarily assume  $c = 1.75\ \mu\text{mol}$ , and to keep the math simple, let us further assume  $n = 1$  (125). Then

$$K = \frac{0.5/1}{1.75^1} = 0.2857. \tag{3}$$

If we double the amount of adsorbent ( $m = 2\ \text{g}$ ), according to eq. 1, we would expect the amount adsorbed to increase by some amount,  $x'$  and the amount of residual pigment to decrease by the same amount. By substitution into eq. 3, it is then an easy matter to solve for  $x'$ .

$$K = \frac{(0.5 + x')/2}{(1.75 - x')^1} = 0.2857.$$

or

$$x' = 0.318\ \mu\text{mol}.$$

**TABLE 15. Demonstration of Press Effect Using Freundlich Equation.**

	<i>m</i> (amount of bleaching clay)	
	1 g	2 g
<i>x</i> (amount pigment adsorbed)	0.5 $\mu\text{mol}$	0.818 $\mu\text{mol}$
<i>c</i> (amount of residual pigment)	1.75 $\mu\text{mol}$	1.432 $\mu\text{mol}$

Using this result, we can calculate what effect doubling the amount of clay has on the amount of pigment adsorbed, and the amount of residual pigment left behind (Table 15).

The press effect can be understood in terms of these data. In essence, what these data show is that as the amount of clay relative to the amount of pigment is increased, the amount of pigment adsorbed will increase. This is precisely what happens in the filter as oil passes through the filter cake. Here, the amount of clay relative to the amount of pigment is many times greater than when the clay is initially added to the refined oil during bleaching. Under the rule of the Freundlich equation, unused adsorptive capacity that is not available under dilute conditions prevailing in the bleacher becomes available once the clay is concentrated in the filter press.

In practical terms, the press bleach effect is extremely important in terms of some of the more advanced processing schemes being practiced today. A variation on the scheme shown in Figure 24 called “packed bed” continuous bleaching (123) assumes the adsorptive capacity of bleaching clay in the filters is not completely depleted when it has only been used once. By adding a third filter, the clay in one filter can be used (in packed bed mode) to pre-bleach oil on its way to the bleacher while the remaining two filters are used as just described (i.e., one for filtering the vacuum bleached oil and one in cleaning or standby mode). Such a system allows for up to 10% reduction in clay usage.

By combining silica purification with packed bed bleaching, very efficient adsorbent utilization can be achieved. This approach, called the “Tri-Clear TriSyl” refining process by W.R. Grace (126) and the “Combi or Double bleach” by Extraction DeSmet, employs the same basic principles as for packed bed bleaching. However, before the oil passes through the clay layer on the filter, it is treated with silica, and dried. A countercurrent bleaching process called “Öhmi bleach” (120) also takes advantage of the press bleach effect; up to 40% reduction in overall clay consumption is claimed for this process.

## 5.5. Spent Bleaching Earth

**5.5.1. Oil Retention/Treatment and Recovery of Entrained Oil** As discussed above, the extent of oil retention by the spent bleaching earth (SBE) is mostly a function of filtration equipment performance and the particular blow-down procedure employed by the refiner. Certain properties of SBE (particularly, porosity, and

fineness of grind) can also influence oil retention to some extent. Typically, after blow down, the refiner will be left with spent cake containing 25–35% retained oil. If lucky enough to be part of a fully integrated facility (seed crushing and refining), incorporating the spent cake into the seed meal is a straightforward, cost-effective solution. However, for refiners who do not have this option, disposal in landfill is most common, albeit costly. Chung (127) recently analyzed the economy of various disposal methods and found that incorporation of SBE into meal, or sale into animal feed markets, were better options than either landfill or iso-propanol extraction.

Various methods for extracting the spent bleaching earth to recover the adsorbed oil have been evaluated. In all cases, the extracted earth from such processes contains low enough levels of oil that spontaneous combustion is no longer an issue and can be disposed of as a nonhazardous waste. Hexane extraction after steam stripping yields extracted oil comparable in quality with crude oil (128, 129). Extraction by boiling 40–50% SBE slurries in 1.5–3% sodium carbonate or sodium hydroxide solutions yields low-quality oil suitable only for fatty acid production (128, 129). The extracted bleaching earth contains less than 5% oil and, as mentioned above, is suitable for disposal as a nonhazardous landfill or can be used as an ingredient in cement plants. High-temperature water extraction (130) of a 40% SBE slurry in an autoclave at 240°C for two hours directly generates fatty acids suitable for further refining. In this case, the extracted earth contains only 0.5–1% oil.

Although there are no commercially viable methods for regenerating spent bleaching earth at present, it is appropriate to mention here the work that has been done in this area. Kalam and Joshi have reported on the high-temperature aqueous regeneration (131) as well as high-temperature wet oxidative regeneration (132) of SBE. In the former case, 5–10% slurries of hexane-extracted SBE were heated in an autoclave at 235°C for three to six hours. The bleaching earth recovered from this treatment possessed about 80% the activity of fresh earth. In the latter case, 5–10% slurries of hexane-extracted SBE were heated in an autoclave at 200°C for two hours while a 0.5-MPa oxygen partial pressure was maintained. In this case, the recovered earth was reported to possess 100% the activity of fresh bleaching earth. More recently, a multistep, patented process (133) employing high-pressure aqueous extraction followed by oxidative treatment and acid-wash regeneration that restores 98–100% of original bleaching activity has been reported. The process was subsequently scaled up to the semiworks stage (134, 135), but never fully commercialized. Waldmann and Eggers (136) have studied high-pressure, supercritical CO<sub>2</sub>-extraction of spent bleaching clay using (liquid) carbon dioxide. They found good quality oil could be recovered by this process, but that the extracted clay only possesses about 50% as much activity as fresh bleaching clay and still contained about 5% oil. The loss of activity was attributed to the extremely tight binding of carotenoids in the pores of the bleaching clay; the authors noted that total porosity in pores below 800 Å diameter was reduced 35–50% for the extracted clay after bleaching. These carotenoids could not be removed from the clay by using supercritical carbon dioxide extraction.

**5.5.2. Smoldering/Spontaneous Combustion** As is well known, SBE saturated with oil from the refining process is susceptible to rapid oxidation and, in the extreme, may even spontaneously ignite (128, 129). A study examining the key parameters involved in the process of spontaneous combustion of clay/oil masses was published (137), which concluded that clay type, filter cake age and temperature, oil retention, and moisture were the most significant variables. It was determined that two separate and distinct exothermic steps are required if a clay/oil mass is to undergo spontaneous combustion. Furthermore, if the temperature of the clay/oil mass is not too high to begin with, the second exotherm, which leads to combustion, fails to occur.

For the refiner trying to mitigate the problem of spontaneous combustion, probably the two most important parameters to control are as follows: (1) spent filter cake temperature and (2) oil content. Although the actual temperature marking the onset of second-stage charring will vary from plant to plant depending on type of oil and clay activity, the most important point is that spontaneous combustion should not occur if the spent filter cake temperature can be sufficiently lowered before exposure to the atmosphere. Obviously, water addition is one way to lower temperature and this approach is probably the simplest and most reliable method.

Combustibility is also influenced by the amount of oil present; the minimum reaction temperature (i.e., the minimum temperature required to cause onset of spontaneous combustion) is lowest in the region where the clay is saturated with 50–60% oil and rises (i.e., becomes less pyrophoric) as the oil content is further reduced to 40% and then to 30%. What these data suggest is that spent clay should be stripped of as much oil as possible before the resultant filter cakes are exposed to the atmosphere. The incorporation of ethoxyquin (a well-known antioxidant used to control spontaneous combustion in fish meal) into the clay/oil mass at the 100-ppm level did reduce the tendency to undergo spontaneous combustion; however, whether this approach is practical and how it could be accomplished in plant operations is an open question.

**5.5.2.1. Uses and Methods of Disposal** Concerning disposal, there are a number of strategies for dealing with spent bleaching earth, as summarized below:  
Disposal as Generated

- Direct disposal—landfill
- Direct disposal—farmland
- Blend with oil seed/re-extract oil
- Use as animal feed supplement
- Use as low-grade fuel for power/heat generation

Transformation

- Preparation of graphitic sorbents for waste organics
- Biogas generation ⇒ biofertilizer

Which strategy any given plant might adopt will depend on a number of factors related to the location and operations of the plant, local environmental regulations, and economic considerations. For each of these strategies, there are limitations or drawbacks that need to be considered. The following discussion summarizes the various disposal strategies and indicates some of the known advantages as well as disadvantages associated with each.

The most straightforward method of handling spent bleaching earth is simply to have it hauled off for burial in local landfill sites. As just discussed, the oil saturated bleaching earth does possess the potential for spontaneous combustion (128, 129, 137), so care must be taken to keep the spent clay in covered, fireproof dumpsters at the plant site. Note that the addition of water to spent cake is very effective in reducing the possibility of ignition, particularly if the water can be uniformly sprayed onto the moving cake at some point before reaching the dumpster. Aside from the safety issue, many localities are restricting the types of industrial wastes they will accept and almost all are charging higher prices for dumping.

Direct disposal of spent bleaching earth on farmland is another option that has been examined and found to work well. Studies (138) have shown that 60–90% of the oil is decomposed during the course of a normal six-month growing season by soil bacteria; this approach works best in warm climates on sandy soils where some fertilizer has been added. Plants grown in soils treated with spent bleaching earth were normal and may even have benefited from improvements in soil water retention caused by the spent clay addition.

Another approach for directly disposing spent bleaching earth is to blend it back in with seed being processed through an oil extraction plant. However, if too much is blended in, it can adversely affect the quality of the crude oil being extracted and may cause the mineral limits of the seed meal to be exceeded (128, 129). Spent bleaching clay can also be used as animal feed supplement (139, 140), particularly in broiler feed diets. In pelletized form, the spent bleaching clay is noncombustible.

A final strategy for the direct disposal of SBE is to use it as a low-grade fuel for power and heat generation. The heating value for soybean oil (141) is 39,500 kJ/kg (16,987 BTU/lb.). Assuming 30–40% oil retention, it can be estimated that spent bleaching earth should possess heating values of about 11,900 to 15,800 kJ/kg (5100–6800 BTU/lb.). In comparison, the heating value of lignite falls in the range 14,650 to 19,300 kJ/kg (6300–8300 BTU/lb) (142). For boilers equipped to handle high-ash fuels such as coal and lignite, the incorporation of relatively high percentages of spent bleaching earth in the boiler feed should pose no problem.

Before concluding this subject, mention is made here of two more novel approaches for using SBE. Pollard et al. (143) have reported that SBE can be used to prepare a pseudo-graphitic char suitable as a low-cost replacement for activated carbon in the stabilization/solidification of industrial wastes. In their process, they char 2:1 blends of SBE and  $ZnCl_2$  at 450°C/1 hour and then activate the material at 600°C/1 hour. The resultant hybrid material is as effective as activated carbon for fixing toxic organics and, because of its aluminosilicate framework, exhibits additional pozzolanic activity in the cement-based stabilization/solidification reactions in which these materials are used. Very recently (144), Bohling reported on

the case for using SBE as an input in biogas generation plants. In this scheme, a consortium of farmers (supplying liquid manure) and oil refining plants (supplying SBE) would form a holding company, which would run the biogas generation plant. Outputs would be electricity (from cogeneration) or natural gas and biofertilizer derived from the fermentation of the manure/SBE mixture. According to the author, such an operation should be profitable. The beauty of these approaches is that the wastes are actually being converted into salable products.

## 5.6. Future Trends and Considerations

Taken from a global perspective, if the future for the fats and oils industry could be summed up in a single word, that word would probably be *health*. At every turn, be it the latest newspaper article on demonstrations against genetically modified crops in Europe to the increasing number of health-related articles in our own society's journals, the growing focus on health is the beginning of a megatrend destined to engage not only the full spectrum of this industry's activities, but indeed the world and all its peoples.

For our industry, it will mean processing in new ways to preserve the benefits of natural antioxidants (145–147) and mitigate the deleterious health effects (148) of lipid oxidation by the use of chelating agents (149), natural antioxidant/citric acid blends (146), and/or other combinations. Over the past few years, there have been numerous articles indicating the beneficial effects of phyosterols in lowering cholesterol levels (150–152). Phospholipids in the diet are thought to lower cardiovascular disease risk factors (153, 154). These are, of course, the very same compounds removed by refining, bleaching, and deodorization steps. If their beneficial effects are substantiated, there will be challenges and opportunities for those able to enhance the presence of these compounds in the fats and oils we eat.

The whole subject of fatty acid composition as it relates to human health—whether we have too much or not enough linolenic acid in our diet (155), too much saturated fatty acids (156), or whether trans-fatty acids are really a major concern—is an area guaranteed to receive heightened scrutiny and research. And as already discussed, increasingly stringent demands will be made on our industry to further reduce levels of exogenous contaminants like polyaromatic hydrocarbons, polychlorinated biphenyls, dioxins, mycotoxins, and pesticides in the fats and oils we produce. These are difficult areas to work in; the chemistry is complex, and understanding takes concerted, committed effort. It is, nonetheless, extremely important that we be proactively engaged in this effort. In our absence, reasoned debate backed by good science will be lacking; this will leave the regulatory climate to be set by others whose agendas and motives may be unintentionally biased at best, or deliberately antagonistic at worst.

Wastes and their mitigation, already a major concern, will demand our increasing attention. Although the initial impact of wastes falls on the environment, there is growing awareness that human health and the quality of life are inexorably linked to the environment. We must seek new and innovative answers to these challenges; we must do it in a way to ensure we are part of the solution—and not part of the problem.

## REFERENCES

1. A. D. Rich, *J. Amer. Oil Chem. Soc.*, **47**, 564A (1970).
2. L. L. Richardson, *J. Amer. Oli Chem. Soc.*, **55**, 777 (1978).
3. J. C. Cowan, *J. Amer. Oli Chem. Soc.*, **43**, 300A (1966).
4. D. R. Taylor, C. B. Ungermann, and Z. Demidowicz, *J. Amer. Oli Chem. Soc.*, **61**, 1372 (1984).
5. L. H. Weidermann, *J. Amer. Oli Chem. Soc.* **58**, 15 (1981).
6. D. R. Erickson, *Practical Handbook of Soybean Processing & Utilization*, AOCS Press, Champaign, Illinois, 1995, p. 203.
7. J. H. Henderson, in adsorptive bleaching, 83rd Annual AOCS Meeting and Exposition, Toronto, Ontario, Canada, 1992.
8. D. R. Taylor, in AOCS World Conference, "Oilseed Technology & Utilization," Budapest, Hungary, 1992.
9. L. B. Wrenn, *Inform*, **6**, 94 (1995).
10. A. D. Rich, *Industrial Minerals and Rocks*, 3rd ed., Amer. Institute of Mining Engineers, New York, 1960, pp. 93–101.
11. W. Zschau, *Fat Sci. Technol.*, **89**, 184 (1987).
12. D. R. Taylor and D. B. Jenkins, *Soc. Mining Eng. AIME, Annu. Trans.*, **282**, 1901 (1988).
13. P. Crossley, *Indust. Minerals*, **408**, 69 (2001).
14. R. W. Mumford, U. S. Patent 1,377,021, May 3, 1921.
15. J. M. Bogdanor and W. A. Welsh, *J. Amer. Oil Chem. Soc.*, **64**, 669 (1967).
16. W. A. Welsh and Y. O. Parent, U. S. Patent. 4,629,588, December 16, 1986.
17. P. M. Parker and W. A. Welsh, U. S. Patent 4,734,226, March 29, 1988.
18. J. N. Pryor, J. M. Bogdanor, and W. A. Welsh, U. S. Patent 4,781,864, November 1, 1988.
19. J. N. Pryor, J. M. Bogdanor, and W. A. Welsh, U. S. Patent 4,877,765, October 31, 1989.
20. W. A. Welsh and J. M. Bogdanor, in 77th AOCS Annual Meeting, Honolulu, Hawaii, May 14–18, 1986.
21. W. A. Singleton and C. E. McMichael, *J. Amer. Oil Chem. Soc.*, **32**, 1 (1955).
22. P. Transfeld, *Inform*, **7**, 756 (1996).
23. R. E. Grimm, *Clay Mineralogy*, 2nd ed., McGraw-Hill, New York, 1968, pp. 32–34.
24. I. E. Odom, *Transactions R., Philosophical Soc., London*, **A311**, 171 (1984).
25. H. H. Murray in S. J. Lefond, in *Industrial Minerals and Rocks*, 5th ed., Amer. Instit. of Mining, Metallurgical and Petrol., Eng., Inc., Baltimore, MD, 1983, pp. 596–601.
26. W. F. Moll and G. R. Goss, in D. I. B. Vander Hooven and L. D. Spicer, eds., *Pesticide Formulations and Applications Systems*, Vol. 6, *ASTM STP 943.*, American Soc. for Testing & Materials, Phil., 1987, p. 167.
27. H. X. Bay, Bulletin, *Mississippi Geological Survey*, **29**, 1 (1935).
28. G. A. Kiersch and W. D. Keller, *Economic Geology*, **50**, 469 (1955).
29. C. E. Weaver, *Clays & Clay Minerals*, **6**, 154 (1959).
30. R. E. Grimm, *Clay Mineralogy*, 2nd ed., McGraw-Hill, New York, 1968, pp. 174–177.
31. D. R. Taylor and C. B. Ungermann, U. S. Patent 5,008,226, April 16, 1991.
32. A. Proctor et. al., *J. Amer. Oil Chem. Soc.*, **73**, 527 (1996).
33. A. Proctor et al., *J. Amer. Oil Chem. Soc.*, **74**, 685 (1997).



34. A. Proctor et al., *J. Amer. Oil Chem. Soc.*, **74**, 1579 (1997).
35. A. Proctor et al., *J. Amer. Oil Chem. Soc.*, **75**, 615 (1998).
36. A. Proctor et al., *J. Amer. Oil Chem. Soc.*, **77**, 785 (2000).
37. O. E. Vohler, H. von Sturm, M. von Kienle, M. Voll, and P. Kleischmit, in W. Gerhartz, ed., *Ullman's Encyclopedia of Industrial Chemistry*, 5th ed., Berlin: 1986.
38. F. S. Baker, C. E. Miller, A. J. Repik, and E. D. Tolles, *Kirk-Othmer Encyclopedia of Chemical Technology* **4**, 1015–1037, (1992).
39. Norit Technical Bulletin, "Introduction to Porous Structure of Activated Carbon," TB 73/08-95, pp. 3–4.
40. M. T. C. Scholten, Sr. Applic. Spec., Norit Nederland B. V. , private e-mail communication, December 9, 2002.
41. A. Proctor, P. K. Clark, and C. A. Parker, *J. Amer. Oil Chem. Soc.*, **72**, 459 (1995).
42. Y. Y. Chang, C. I. Lin, and H. K. Chen, *J. Amer. Oil Chem. Soc.*, **78**, 657 (2001).
43. M. Kokken, *Inform*, **13**, 721 (2002).
44. T. Swetman and D. Evans, *Inform*, **10**, 706 (1999).
45. S. Moret, A. Dudine, and L. S. Conte, *J. Amer. Oil Chem. Soc.*, **77**, 1289 (2000).
46. J. K. Huwe, *J. Agric. Food Chem.*, **50**, 1739 (2002).
47. Council for Agricultural Science and Technology, Ames, Iowa, Task Force Report 139, "Mycotoxins: Risks in Plant, Animal, and Human Systems," January 2003, p. 4.
48. Communication from the Commission, to the Council, the European Parliament, and the Economic and Social Committee—"Community strategy for dioxins, furans, and polychlorinated biphenyls," March 4, 2002.
49. M. Scholten, Soc. of Chem. Ind. Symposium, Noordwijk, The Netherlands, February 18, 2003.
50. Council Regulation (EC) 2375/2001, "Amending Commission Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs," November 29, 2001.
51. Commission Regulation (EC) No 466/2001, "Setting maximum levels for certain contaminants in foodstuffs," March 8, 2001.
52. Council for Agricultural Science and Technology, Ames, Iowa, Task Force Report 139, "Mycotoxins: Risks in Plant, Animal, and Human Systems," January 2003, p. 109.
53. B. Braae, *Chem. Ind. (London)*, **36**, 1152 (1958).
54. C. D. Evans, P. M. Cooney, C. R. Scholfield, and H. J. Dutton, *J. Amer. Oil Chem. Soc.*, **31**, 295 (1954).
55. M. Y. Jung and D. B. Min, *J. Amer. Oil Chem. Soc.*, **68**, 653 (1991).
56. Y. Endo, R. Usuki, and T. Kaneda, *J. Amer. Oil Chem. Soc.*, **61**, 781 (1984).
57. G. R. Greenbank and G. E. Holm, *Ind. Eng. Chem.*, **25**, 167 (1933).
58. H. J. Dutton, H. A. Moser, and J. C. Cowen, *J. Amer. Oil Chem. Soc.*, **24**, 261 (1947).
59. C. D. Evans, A. W. Schwab, H. A. Moser, J. E. Wawley, and E. H. Melvin, *J. Amer. Oil Chem. Soc.*, **28**, 68 (1951).
60. H. Yoshida, I. Kondo, and G. Kajimoto, *J. Amer. Oil Chem. Soc.*, **67**, 165 (1990).
61. D. A. Morgan, *Oil Soap*, **19**, 193 (1942).
62. B. Minshew, *Soybean Dig.*, **37**(10) (1977).

63. E. N. Frankel, in D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soy Oil Processing & Utilization*, Amer. Soybean Assoc., St. Louis, Missouri, and Amer. Oil Chem. Soc., Champaign, Illinois, 1980, pp. 229–244.
64. F. Ullrich and W. Grosch, *Fat Sci. Technol.*, **90**, 332 (1988).
65. H. Guth and W. Grosch, *Fat Sci. Technol.*, **91**, 225 (1989).
66. H. Gruth and W. Grosch, *Lebensm.-Wiss. U. Technol.*, **23**, 59 (1990).
67. M. G. Ball, D. B. Shaw, M. J. Sidebottom, and F. B. J. Wright, in 76<sup>th</sup> Annual AOCS Meeting, Philadelphia, Pennsylvania, 1985.
68. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, American Oil Chemists' Society, Champaign, Illinois.
69. F. Gunstone, *Inform*, **12**, 895 (2001).
70. W. A. Pons, Jr., C. L. Hoffpauir, and R. T. O'Connor, *J. Amer. Oil Chem. Soc.*, **28**, 8 (1951).
71. M. Y. Jung, S. H. Yoon, and D. B. Min, *J. Amer. Oil Chem. Soc.*, **66**, 118 (1989).
72. Y. Young, *Chem. Ind. (London)*, **18**, 692 (1978).
73. C. B. Ungermann and D. R. Taylor, 79th Annual AOCS Meeting, Phoenix, Arizona, 1988.
74. D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soy Oil Processing and Utilization*, 4th ed., American Soybean Assoc. St. Louis, Missouri and American Oil Chemists' Soc., Champaign, Illinois, 1987, Ch. 9.
75. D. B. Shaw, *Inform*, **4**, 538 (1993).
76. B. K. G. Theng, *The Chemistry of Clay-Organic Reactions*, Wiley, New York, 1974, p. 204.
77. D. M. Chapman, *J. Amer. Oil Chem. Soc.*, **71**, 397 (1994).
78. S. T. Council, G. R. Goss, and D. Shaked, U. S. Patent 6,346,286, February 12, 2002.
79. W. B. Parker and D. Melnick, *J. Amer. Oil Chem. Soc.*, **43**, 635 (1966).
80. M. S. Kuk, R. J. Hron, G. Abraham, and P. J. Wan, *J. Amer. Oil Chem. Soc.*, **69**, 1154 (1992).
81. K. Boki et al., *ibid.*, **69**, 282 (1992).
82. K. Boki, et al., *ibid.*, **69**, 372 (1992).
83. K. Boki et al., *ibid.*, **71**, 595 (1994).
84. W. Jones et al., *Ibid.*, **70**, 241 (1993).
85. D. R. Taylor, D. B. Jenkins, and C. B. Ungermann, *ibid. Soc.*, **66**, 334 (1989).
86. D. A. Morgan, D. B. Shaw, M. J. Sidebottom, T. C. Soon, and R. S. Taylor, *ibid.*, **62**, 292 (1985).
87. W. Zschau in R. D. O'Brien, W. E. Farr, and P. J. Wan, eds., *Introduction to Fats and Oils Technology*, 2nd ed., AOCS Press, Champaign, Illinois, 2000, pp. 160–161.
88. C. Adhikari, A. Proctor, and G. D. Blyholder, *J. Amer. Oil Chem. Soc.*, **71**, 201 (1994).
89. C. Adhikari, A. Proctor, and G. D. Blyholder, *ibid.*, **71**, 589 (1994).
90. C. Adhikari, A. Proctor, and G. D. Blyholder, *ibid.*, **72**, 337 (1995).
91. C. Adhikari, A. Proctor, and G. D. Blyholder, *ibid.*, **74**, 1265 (1997).
92. K. Y. Liew, S. H. Tan, F. Morsingh, and L. E. Khoo, *ibid.*, **59**, 480 (1982).

93. K. Y. Liew, F. Morsingh, S. H. Tan, and L. E. Khoo, Annual PORIM Meeting, Kuala Lumpur, Malaysia, 1983.
94. C. Walling, *J. Amer. Chem. Soc.*, **72**, 1164–1168 (1995).
95. H. A. Benesi, *ibid.*, **78**, 5490 (1956).
96. H. A. Benesi, *J. Phys. Chem.*, **61**, 970 (1967).
97. B. K. G. Theng, *The Chemistry of Clay-Organic Reactions*, Wiley, New York, 1974, p. 157.
98. M. M. Mortland and K. V. Raman, *Clays & Clay Minerals*, **16**, 393 (1968).
99. F. M. Fowker et al., *J. Agr. Food Chem.*, **8**, 203 (1960).
100. N. Sarier and C. Guler, *J. Amer. Chem. Soc.*, **65**, 776 (1988).
101. N. Sarier and C. Guler, *ibid.*, **66**, 917 (1989).
102. C. Guler and F. Tunc, *ibid.*, **69**, 948 (1992).
103. S. C. Kheok and E. E. Lim, *ibid.*, **59**, 129 (1982).
104. E. Srasra, F. Bergaya, H. Van Damme, and N. K. Ariguib, *Applied Clay Sci.*, **4**, 411 (1989).
105. J. H. Henderson, *J. Amer. Oil Chem. Soc.*, **70**, 831 (1993).
106. K. Boki, M. Kubo, T. Wada, and T. Tamura, *ibid.*, **69**, 323 (1992).
107. L. O. Buxton, *Ind. Eng. Chem.*, **34**, 1486 (1942).
108. “TriSyl Silica for Edible Oil Refining,” [www.gracedavison.com/products/fcph/trisyl/overview.htm](http://www.gracedavison.com/products/fcph/trisyl/overview.htm).
109. D. Brooks, “Understanding the Bleaching Operation,” Technical Bulletin, Pure Flo Product Group, Div. Oil-Dri Corp. of America.
110. D. Brooks and D. Shaked, in PORIM International Palm Oil Congress, Kuala Lumpur, Malaysia, 1999.
111. K. A. Delaney, R. C. Hastert, G. M. Lengen, and C. B. Ungermann, “Adsorptive Bleaching of Canola Oil,” Filtrol Clay Products, Technical Presentation 556.
112. J. M. Bogdanor, in 79th Annual AOCS Meeting, Phoenix, Arizona, 1988.
113. W. Zschau, in 76th Annual AOCS Meeting, Philadelphia, Pennsylvania, 1985.
114. D. Brooks, CISCE, 2002.
115. C. Golubic, *J. Amer. Chem. Soc.*, **64**, 2337 (1942).
116. R. R. King, F. W. Wharton, *J. Amer. Oil Chem. Soc.*, **26**, 201 (1949).
117. W. Zschau and J. A. Ortiz, in 83rd Annual AOCS Meeting, Toronto, Canada, 1992.
118. T. K. Mag, in D. R. Erickson, ed., *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Amer. Oil Chem. Soc., Champaign, Illinois, 1990, 107–116.
119. T. C. Soon, D. B. Shaw, and P. K. Stemp, in 88th Annual AOCS Meeting, Seattle, Washington, 1997.
120. [www.oehmi-engineering.de](http://www.oehmi-engineering.de).
121. W. Zschau, AOCS Symposium “Basic Principles of Vegetable Oil Processing,” Newark, New Jersey, April 1988.
122. D. Brooks and D. Shaked, in PORIM International Palm Oil Congress, Kuala Lumpur, Malaysia, 1999.
123. F. Veldkamp, in Soc. of Chem. Ind. Symposium, Noordwijk, The Netherlands, February 18, 2003.

124. P. Transfeld, *Inform*, **9**, 502 (1998).
125. A. S. Hodgson, in Y. H. Hui, ed., Fifth Ed. Bailey's Industrial Oil & Fat Products, Vol. 4, Wiley, New York, 1996, p. 199.
126. M. Jalapoor, Society of Chemical Industry Symposium, Noordwijk, The Netherlands (2003).
127. C. Chung, *Inform*, **8**, 739 (1997).
128. C. Svensson, *J. Am. Oil Chem. Soc.*, **53**, 443 (1976).
129. J. T. L. Ong, *Ibid.*, **60**, 266A (1983).
130. J. M. L. Penninger, *J. Chem. Tech. Biotechnol.*, **29**, 154 (1979).
131. A. Kalam and J. B. Joshi, *Ibid.*, **65**, 1917 (1988).
132. A. Kalam and J. B. Joshi, *Ibid.*, **65**, 1536 (1988).
133. R. S. Nebergall, D. R. Taylor, and C. J. Kucharz, U.S. Patent 5,468,701, November 21, 1995.
134. R. S. Nebergall and T. Jacobson, in 86th Annual AOCS Meeting, San Antonio, TX, 1995.
135. R. S. Nebergall, *Inform*, **7**, 206 (1996).
136. C. Waldmann and R. Eggers, *J. Amer. Oil Chem. Soc.*, **68**, 922 (1991).
137. D. R. Taylor, D. R., and Jenkins, D. B., *ibid.*, **67**, 678 (1990).
138. G. Mucsy, E. Kranicz-Pap, and G. Urganyi, *Amer. Soc. for Testing Materials*, 135 (1976).
139. O. Herstad, *Acta Agric. Scand.*, **26**, 87 (1976).
140. R. Blair, J. Gagnon, R. E. Salmon, and M. D. Pickard, *Poult. Sci.*, **65**, 2281 (1986).
141. A. W. Schwab, G. J. Dykstra, E. Selke, S. C. Sorenson, and E. H. Pryde, *J. Am. Oil Chem. Soc.*, **65**, 1781 (1988).
142. M. Gary, R. McAfee, and C. Wolf, *Glossary of Geology*, Amer. Geol. Institute, 1972.
143. S. J. T. Pollard, C. J. Sollars, and R. Perry, *Environ. Tech.*, **11**, 1113 (1990).
144. C. Bohling, in Society of Chemical Industry Symposium, Noordwijk, The Netherlands, 2003.
145. O. I. Aruoma, *Inform*, **8**, 1236 (1997).
146. E. N. Frankel, *ibid.*, **10**, 889(1999).
147. T. Mag, *ibid.*, **13**, 836 (2002).
148. S. Kubow, *Trends in Food Sci. & Tech.*, **67**, September (1990).
149. E. A. Decker and D. Julian-McClements, *Inform*, **12**, 251 (2001).
150. R. Moreau, R. A. Norton, and K. B. Hicks, *ibid.*, **10**, 572 (1999).
151. C. Wang and R. Wixon, *ibid.*, **10**, 315 (1999).
152. V. Yankah and P. J. H. Jones, *ibid.*, **12**, 808, 899, 1011 (2001).
153. D. Canty, *ibid.*, **11**, 537 (2000).
154. T. Yanagita, *ibid.*, **14**, 65 (2003).
155. W. E. M. Lands, *Fish and Human Health*, Academic Press, Inc., Orlando, Florida, 1986.
156. J. B. Allred, *J. Nutr.*, **123**, 1453 (1993).

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# *Deodorization*

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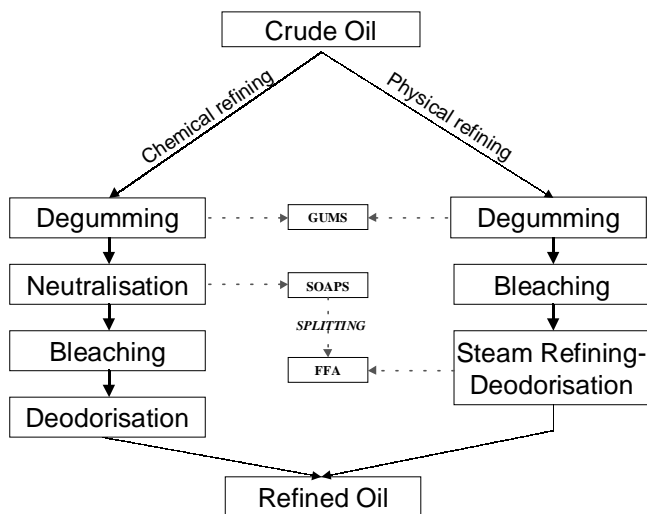
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Brussels, Belgium*

## **1. INTRODUCTION**

All crude oils and fats, obtained after rendering, crushing, or solvent extraction, inevitably contain variable amounts of nonacylglycerol constituents such as free fatty acids, sterols, tocopherols, hydrocarbons, pigments (gossypol, chlorophylls), vitamins, contaminants (pesticides, polycyclic aromatic hydrocarbons, etc.), heavy metals, glycolipids, protein fragments, as well as resinous and mucilaginous materials.

The objective of the refining process is to remove the unwanted constituents from the oil with the least possible negative effect on triacylglycerols and minimal loss of the desirable constituents. To be able to meet the ever-increasing quality requirements (mainly regarding the nutritional quality) and to further reduce the processing costs, equipment manufacturers are obliged to continuously improve their technology. Industrial deodorization technology and operating conditions have been adapted to meet the required organoleptic and nutritional quality standards.

Two major processing alternatives exist for the processing of edible oils and fats, known as “chemical” and “physical” refining (Figure 1). As the term “refining” is sometimes used for different processes and in a different context, it seems useful to introduce some definitions for the terms used further in this chapter.



**Figure 1.** General overview of a physical and chemical refining line.

*Chemical refining:* Sequence of process stages, which definitively includes alkali refining (also referred to as caustic refining or neutralization) to remove most of the FFA and, in most cases, also deodorization in the last stage.

*Physical refining:* Sequence of process stages that starts with a degumming process, may include a bleaching stage, and definitively includes a steam-refining stage.

*Deodorization:* Vacuum-stripping process in which neutral oil is treated to remove malodorous compounds to obtain fully refined oil.

*Steam refining:* Vacuum-stripping process in which acid oil is treated to remove both free fatty acids and malodorous compounds to obtain fully refined oil.

In literature, the distinction between *deodorization* and *steam refining* is rarely and certainly not consistently made. As the process is best known as *deodorization*, it is used as general term in this chapter as well.

The physical refining route can offer important advantages to the refiner, including a higher overall yield, the use of fewer chemicals (e.g., phosphoric acid, sulfuric acid, or caustic soda) and, above all, a much lower effluent (byproduct) production. This last advantage has become very important because of the increasingly stringent limits on the quality and quantity of waste water discharged from the refineries. Physical refining, however, is a delicate process that is less adapted than chemical refining to crude oils of low quality. Most undesirable constituents are more easily removed by an alkali treatment than by an advanced degumming.

Some oils, like cottonseed oil, cannot be physically refined, as it requires an alkali treatment to remove the gossypol. For lauric oils and palm oil, on the other hand, physical refining is preferred in terms of both operating cost and refining loss. In the case of soybean and rapeseed oils, physical refining is suitable only for crude oils of a high quality, i.e., with a low degree of oxidation and a sufficiently low

phosphatide content after degumming (<15 ppm). If not, chemical refining will yield better results.

Another important factor is the free fatty acid content of the crude oil. In general, physical refining only becomes advantageous when the acidity of the crude oil is sufficiently high. For relatively cheap oils, like soybean oil, the higher oil yield with the physical refining is less important than the higher bleaching earth consumption, making chemical refining more attractive. For other unsaturated oils with a higher value, such as peanut oil and sunflower seed oil, physical refining will be more attractive.

## 2. DEODORIZATION PRINCIPLE

Although the process is commonly named deodorization, it is actually a combination of three different effects on the oil: (1) stripping: Stripping of volatile components (free fatty acids, odorous compounds, tocopherols, sterols, and contaminants such as pesticides and light polycyclic aromatic hydrocarbons, etc.), (2) actual deodorization: Removal of different off-flavors, and (3) temperature effect: Thermal destruction of pigments and unwanted side reactions such as *cis-trans*-isomerization, polymerization, conjugation, and so on.

Optimal stripping parameters (temperature, time, operating pressure, and amount of stripping gas) are governed by the properties of the ingoing product, the specifications of the outgoing product, equipment limitations, and the need to minimize costs. In Table 1, some typical deodorization conditions for edible oils are given. As observed, steam refining applied during physical refining requires more severe conditions than deodorization in case of chemical refining. This is mainly because of the removal of FFA by distillation, which is more significant in physical refining, as the initial FFA levels are considerably higher.

To obtain the required final FFA content of 0.03–0.05% by physical refining, it is necessary to adjust the operating conditions. The easiest way is to increase the

**TABLE 1. Typical Operating Conditions for Deodorization of Vegetable Oils.**

Conditions	Chemical		Physical Europe
	U.S.	Europe	
Temperature (°C)	250–260	220–240	230–250
Pressure (mbar)	3–4	2–3	2
Spurge steam (%)	0.5–2 <sup>(a)</sup>	0.5–1.5	1–2
Deodorization time (min)	20–40	40–60	60–90
Final acidity (% FFA)	← 0.03–0.05 →		
<i>Trans</i> fatty acids (%)	← 0.5–1 →		
Tocopherol loss (%) <sup>(b)</sup>	up to 60	max 25	max 25

<sup>a</sup>To remove tocopherols, a higher amount of steam is required.

<sup>b</sup>For example, for soybean oil in the United States, the minimum is 500 ppm; in Europe, it is 900 ppm.

**TABLE 2. Effect of Pressure and Temperature on Sparge Steam Volume.<sup>a</sup>**

Pressure (mbar)	Volume in m <sup>3</sup> /kg Steam		
	230°C	240°C	250°C
4	581	592	604
3	774	790	805
2	1162	1185	1208

<sup>a</sup>Volume steam (m<sup>3</sup>/kg) = (4.6189 \* (273.15 + X))/Y (from equation PV = nRT), where X = Temperature (°C) and Y = Pressure (mbar).

steam refining temperature. A high temperature is beneficial for a good stripping of FFA and heat bleaching, but it also results in a higher *trans*-fatty acid content as well as a higher loss of tocopherols. Alternatively, the deodorizer pressure can be lowered or the amount of stripping steam increased, but this, in turn, raises the overall cost of production. Shortening the overall residence time can be considered for heat sensitive oils (e.g., fish oils, cocoa butter, high PUFA oils, etc.). However, experience has shown that certain reactions within the oil, unrelated to FFA removal, are necessary to obtain a stable oil. These reactions, as well as heat bleaching, are time- and temperature-dependent. To allow these reactions to occur, commercial deodorizers have to provide a certain holding period at the deodorization temperature.

Today, most deodorizers operate at a temperature between 230°C and 260°C, a pressure of 3 mbar or lower, and with a stripping steam consumption of around 10 kg per ton of processed oil. All of these parameters have a direct impact on the design of the vacuum unit. For a given amount of stripping steam, the volume of the gas phase to be removed by the vacuum production unit increases considerably when the system pressure is reduced (Table 2). The gas volume also increases with increasing temperature, but the effect is less considerable. In a conventional vacuum production unit with steam-jet ejectors (boosters), more motive steam per kilogram stripping steam is therefore required to remove the gas phase (Table 3). Consequently, steam refining is more expensive than deodorization.

**TABLE 3. Effect of Deodorizer Pressure on Steam and Fuel Consumption.<sup>a</sup>**

Pressure		Stripping Steam (kg/ton oil)	Booster Steam (kg/ton oil)	Fuel (kg)
Booster	Deodorizer			
(Chemical refining)				
2.5	3 mbar	10	45	4.23
(Physical refining)				
1.5	2 mbar	15	93	8.31

<sup>a</sup>Conditions: Barometric condenser water inlet temperature: 24°C; outlet temperature: 30°C; assumed total pressure drop of 0.5 mbar in fatty acid scrubber; assumed fuel consumption of 1 kg for each 13 kg of steam produced.



## 2.1. Theoretical Considerations of Stripping

**2.1.1. Vapor Pressure** The volatility of a given component is expressed by its vapor pressure, which increases with increasing temperature (Figure 2). The lower the vapor pressure, the lower the volatility and, thus, the more difficult to remove the component from the oil. For each specific component, the vapor pressure-temperature relationship can be expressed by the Equation of Antoine:

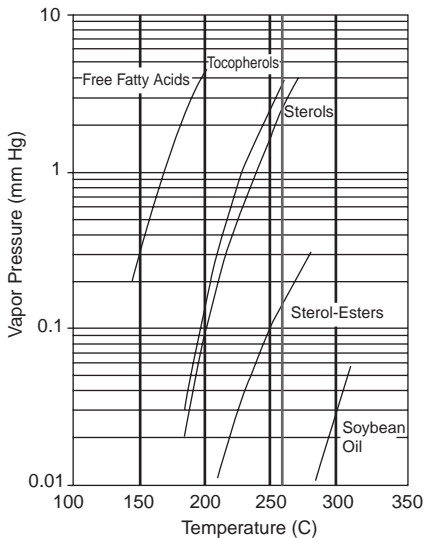
$$\ln P_i^0 = A - \frac{B}{T + C}, \quad (1)$$

with  $P_i^0$ -vapor pressure of a given component  $i$ ,  $T$ -absolute temperature (K), and  $A, B, C$ -chemical constants, typical for each component.

For fatty acids, the vapor pressure-temperature relationship can also be calculated according to the empirical formula of LEDERER (1):

$$\ln P_i^0 = \frac{-\lambda_0}{R \cdot T} + 1.75 \ln T - \frac{\gamma}{R} T + C, \quad (2)$$

with  $P_i^0$  = vapor pressure (mbar) of a given fatty acid  $i$  at a given absolute temperature  $T$  (K),  $\lambda_0$  = molar heat of vaporization at absolute zero (kJ/mol),  $R = 8.314$ , gas-constant (kJ/mol · K),  $\gamma$  = temperature coefficient of the difference between the specific heat in the liquid and vapor phase (kJ/mol · K), and  $C$  = so-called “conventional chemical constant.”



— 260°C

Component	Mol. Weight	Relat. volatility
Fatty acid	280	2.5
Squalene	411	5
Tocopherol	415	1
Sterol	410	0.6
Sterol ester	675	0.04
Oil	885	<small>

Figure 2. Vapor pressure-temperature relationship for different components in edible oils.

TABLE 4. Values of  $\lambda_0$ ,  $\gamma$ , and C for Different Fatty Acids According to LEDERER (1).

Fatty Acid	$\lambda_0$	$\gamma$	C
Caprylic acid	96240	0.1398	26.33
Lauric acid	98750	0.1461	26.54
Myristic acid	104480	0.1398	27.33
Palmitic acid	109050	0.1365	26.91
Stearic acid	114200	0.1315	27.00
Oleic acid	111600	0.1275	26.40
Erucic acid	122692	0.1105	26.67

The values of  $\lambda_0$ ,  $\gamma$ , and C are available for different fatty acids (Table 4). These constants allow one to calculate the vapor pressure or the boiling point of a fatty acid over a given temperature interval.

**2.1.2. Activity Coefficient  $\alpha$**  The efficient removal of volatile substances during edible oil deodorization depends not only on their vapor pressure but also on their concentration in the oil. The theoretical principle of the deodorization process has already been described in many publications (2–4). All calculations start from the basic laws of Raoult and Dalton, which are valid for ideal mixtures. However, in practice, the mixture of a fatty acid and a vegetable oil has a “nonideal” behavior. Consequently, a so-called activity coefficient  $\alpha$  was introduced and Raoult’s law may be written as follows:

$$P_{L,i} = \alpha \cdot x_i \cdot P_i^0, \quad (3)$$

with  $P_{L,i}$  = partial pressure of a given fatty acid in the liquid phase,  $\alpha$  = activity coefficient,  $x_i$  = molar fraction of the same fatty acid in the liquid phase, and  $P_i^0$  = vapor pressure of the same fatty acid.

Activity coefficients were determined for solutions of stearic acid in peanut oil (5). At low FFA concentration (<1%), positive deviations from theory resulting in activity coefficients around 1.5 were observed. At FFA concentration > 30%, the activity coefficients are close to one. This observation was explained by the presence of an association in the form of a fatty acid/triacylglycerol complex that is in equilibrium with the free fatty acids and the triacylglycerol molecules. A low fatty acid concentration favors this equilibrium to shift toward dissociation, which means that higher vapor pressures are measured at lower fatty acid concentrations.

**2.1.3. Vaporization Efficiency E** According to Dalton’s law,

$$P_{V,i} = y_i \cdot P_t, \quad (4)$$

in this,  $P_{V,i}$  = partial pressure of the volatile component in the gas phase,  $P_t$  = total pressure of the gas phase, and  $y_i$  = molar fraction of the volatile component in the gas phase.

In the case of an ideal mixing between the stripping steam and the oil,  $P_{L,i} = P_{V,i}$ . However, in practice, an ideal mixing cannot be achieved and therefore:

$$P_{V,i} = E \cdot P_{L,i}, \quad (5)$$

with  $E$  = vaporization efficiency.

The vaporization efficiency  $E$  has to be considered a measure of the completeness with which the steam bubbles become saturated with volatile substances during their passage through the oil (2).

Mathematically,

$$\frac{dP_{V,i}}{dt} = k \cdot A \cdot (P_{L,i} - P_{V,i}), \quad (6)$$

where,  $t$  = contact time between the steam bubble and the oil,  $A$  = surface area of the steam bubble, and  $k$  = gas-diffusion coefficient.

From Equation 5 and integration of Equation 6, Equation 7 can be deduced:

$$A \cdot k \cdot t = \ln \frac{P_{L,i}}{(P_{L,i} - P_{V,i})} = \ln \frac{1}{(1 - E)}, \quad (7)$$

and as a consequence,

$$E = 1 - e^{-Akt}. \quad (8)$$

From Equation 8, it can be derived that the vaporization efficiency increases with an extension of the total surface area and with the time of contact between steam bubble and oil. In practice, the vaporization efficiency can be influenced by the depth of the oil layer and the steam injection geometry. The shallower the oil layer, the higher the risk that the steam leaves the oil surface without being saturated. At the other extreme, for deep oil layers, the contact time is longer but then problems can occur with the agitation, leading to an insufficient renewal of the upper oil layer and a nonuniformly treated oil mass.

Lab-scale trials indicated that elimination of reflux and radiation losses combined with an optimization of the steam distributor could reduce the necessary steam consumption significantly (6). As a result, a final overall vaporization efficiency of 0.93 could be achieved (7). Industrial deodorizers, however, have a lower efficiency, as they usually operate under conditions that are less ideal than those of a laboratory deodorizer.

**2.1.4. Principles of Deodorization/Steam Refining** The stripping medium requirements during deodorization/steam refining are described by the following mathematical equation:

$$S = \frac{P_t}{E \cdot P_i^0} \cdot \ln \frac{V_a}{V_0} + \left( \frac{P_t}{E \cdot P_i^0} - 1 \right) (V_a - V_0), \quad (9)$$

where  $S$  = total moles of steam or any other stripping agent,  $P_t$  = total pressure of the gas phase,  $P_i^0$  = vapor pressure of a given fatty acid  $i$ ,  $E$  = vaporization efficiency,  $V_a$  = initial molar concentration of the volatile component in the oil, and  $V_0$  = final molar concentration of the volatile component in the oil.

When the initial FFA content is low, as in the case of a classical deodorization,  $(V_a - V_0)$  becomes so small that Equation 9 can be simplified to:

$$S = \frac{P_t}{E \cdot p_i^0} \cdot \ln \frac{V_a}{V_0} \quad (10)$$

In case of steam refining, the partial pressure of the fatty acids must be taken into account and omitting the term  $(V_a - V_0)$  would lead to a considerable overestimation of the stripping medium requirements.

The simplified Equation 10 is also known as the "Bailey Equation." It states that the amount of steam required for deodorization or steam refining is directly proportional to the amount of oil and the absolute pressure in the deodorizer and inversely proportional to the vapor pressure of the pure volatile component at the process temperature and the overall vaporization efficiency  $E$ .

The last factor in Equation 10 ( $\ln V_a/V_0$ ) indicates that it is impossible to eliminate all volatile components from the oil, as this would require an infinite amount of stripping medium. This factor also indicates that, e.g., halving the concentration of a given volatile requires the same amount of stripping medium irrespective of its absolute level.

## 2.2. Actual Deodorization

Another main objective of the deodorization process, besides FFA stripping, is the removal of the odoriferous compounds. Different opinions exist about the time-dependent character of this process.

It is important to make a distinction between odoriferous compounds already present in the crude oil and odoriferous compounds formed by thermal degradation of flavor precursors. Removal of the first group of compounds is similar to FFA stripping and can be considered as nearly not time dependent (as in the "Bailey" equation). However, perfect deodorization is a more complex and longer process than the stripping of volatile components. This difference is mainly because of the presence of a wide range of "nonvolatile" flavor precursors. A certain deodorization time is required to convert these compounds into more volatile off-flavors that can be stripped from the oil. If this minimum time is not respected, some flavor precursors will stay in the deodorized oil resulting in the development of off-flavors during high-temperature usage (e.g., deep frying).

The higher the concentration of these thermally labile flavor precursors, the longer the deodorization time to arrive at a stable fully refined oil. On the other hand, oils with a very low content of such flavor precursors will need a much shorter deodorization.

## 2.3. Temperature Effect

Besides a bland odor and taste, (light) color is also a quality parameter for most refined oils. In the chemical and physical refining process, the color components can be removed during the bleaching process by adsorption onto a suitable bleaching earth or by thermal degradation during deodorization. This latter phenomenon, also known as “heat bleaching,” is well recognized but, at the same time, poorly understood. From the few studies on heat bleaching, it can be concluded that it is a purely time-temperature-dependent reaction, not affected by the deodorization pressure (8).

Literature data on heat bleaching of palm oil indicate that the rate of carotene breakdown doubles per 20°C increase in temperature (9). For illustration, heat degradation of carotene takes a few hours at 210°C but only a few minutes at 270°C (10).

However, if the oil is not properly pretreated (too high residual phosphatides, iron, other “impurities”) or if traces of bleaching earth are still present, the oil can become darker during deodorization. This phenomenon is also known as “color fixation” because it is almost impossible to remove these color pigments once they are formed.

Other undesired effects of high deodorization temperature (*trans*-fatty acid formation, polymerization, etc.) will be discussed further in this chapter.

## 3. REFINED OIL QUALITY

The quality of a refined oil is usually evaluated by traditional quality parameters such as a low residual FFA content, a high oxidative stability, a light color, and a neutral odor and taste. In addition, high-quality food oils should contain low *trans*-fatty acid (TFA) levels, high amounts of natural antioxidants and vitamins, low levels of polymeric and oxidized triacylglycerols, and no contaminants (pesticides, polycyclic aromatic hydrocarbons, dioxins and polychlorinated biphenyls, etc.) (Tables 5 and 6).

### 3.1. Free Fatty Acids

The efficiency of deodorization/steam refining is usually quantified by the stripping of free fatty acids (FFA). As a result of their relatively low volatility, an efficient

**TABLE 5. Overview of the Different Minor Components in Food Oils and Fats.**

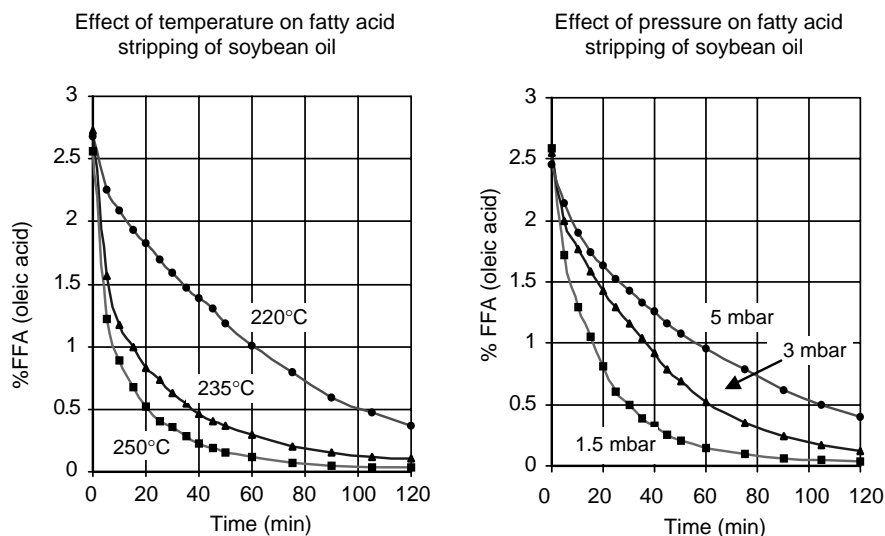
Group	Minor Components
Valuable minor components	Tocopherols, sterols, squalene, oryzanol
Fat degradation products	<i>Trans</i> -fatty acids, polymeric and oxidized triacylglycerols, cyclic fatty acids
Contaminants	Pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, dioxins, furans, . . . .

**TABLE 6. Characteristics of Fully Refined Soft Oils and Hard Fats (Target Figures).**

	Vegetable Oils	Rape/Soybean	Corn/Sun	Palm	Lauric Oils
FFA <sup>a</sup> (%)	max	0.05	0.05	0.05	0.05
<i>Trans</i> (%)	max	1	0.5	0.5	/
Moisture (%)	max	0.05	0.05	0.05	0.05
Impurities (%)	max	0	0	0	0
POV <sup>b</sup> (meq O <sub>2</sub> /kg)	max	0.5	0.5	0.5	0.5
Phosphorus (ppm)	max	1	1	1	1
Tocopherols (ppm)	min	500/1000 <sup>d</sup>	1000/750 <sup>e</sup>	/	/
Total metal (ppm)	max	0.1	0.1	0.1	0.1
PAH <sup>c</sup> (ppb)	max	25	25	25	25
Color (5 <sup>1/4</sup> " cell)	max	1.5R-15Y	2.0R-20Y	2.5R-30Y	1.5R-15Y
Cold test (0°C)	min	48h	48h	/	/
Smoke point (°C)	min	220	220	220	/

<sup>a</sup>FFA = free fatty acids; <sup>b</sup>POV = Peroxide value; <sup>c</sup>PAH = polycyclic-aromatic hydrocarbons; <sup>d</sup>target figure for soybean oil; <sup>e</sup>target figures valid for max. tocopherol retention.

FFA removal usually indicates good removal of other more volatile components. Experience has shown that flavor and odor removal correlate well with the FFA reduction. Nevertheless, some differences exist among different oils. Soybean oil, for example, requires less steam than rapeseed oil (0.6–0.8% vs. 0.8–1.2%). This difference is mainly because of the typical rapeseed odor, which is more difficult to remove. In general, oils are easier to deodorize during chemical refining, not only



**Figure 3.** Effect of (a) temperature and (b) pressure on FFA removal during deodorization/steam refining of soybean oil. Conditions (a) 3 mbar, 0.5% sparge steam/h, varying temperature; (b) 230°C, 0.5% sparge steam/h, varying pressure.

because of the lower acidity before deodorization but also because a whole range of polar minor components, oxidation products, and pigments has already been removed during alkali refining.

For most oils, the targeted residual FFA content is 0.03–0.05%. Figure 3 shows the effect of pressure and temperature on FFA stripping from  $C_{18}$ -rich oils in a cross-flow-type deodorizer. With packed columns, stripping steam consumption is about 20–30% lower because of the countercurrent stripping effect.

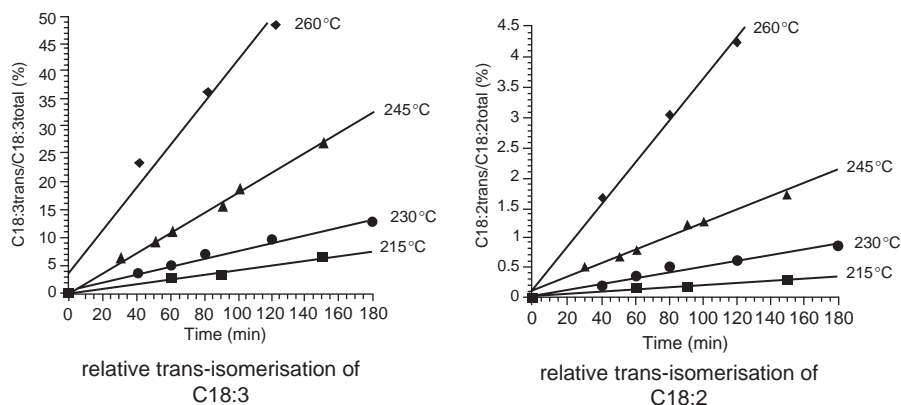
## 3.2. Fat Isomerization and Degradation Products

### 3.2.1. *Trans*-Fatty Acids

*Trans*-fatty acids (TFAs) are either monounsaturated fatty acids or polyunsaturated fatty acids with one or more double bonds in the *trans*-configuration. TFAs have a shape comparable with that of saturated fatty acids. Consequently, they have a higher melting point than the corresponding *cis*-isomers.

Reported activation energy values for the thermal *cis-trans*-isomerization of linoleic and linolenic acid are rather low (178 kJ/mole vs. 144–148 kJ/mole, respectively) (8). This is an indication that TFAs are relatively easily formed at elevated deodorization temperature. Different studies show that the relative isomerization rate can be expressed as follows:  $C_{18:3}$  (100)  $\gg$   $C_{18:2}$  (10)  $\gg$   $C_{18:1}$  (1) (11–13). Consequently, oils with a high linolenic acid content, such as soybean and rapeseed oils, are most sensitive to *cis-trans*-isomerization during deodorization.

*Cis-trans*-isomerization during deodorization is considered to be a first-order reaction, requiring the presence of a methylene-interrupted diene and only influenced by time and temperature. Generally, *trans*-formation is negligible below 220°C, whereas it becomes significant between 220°C and 240°C, and exponential above 240°C (Figure 4).



**Figure 4.** Effect of temperature and time on *trans*-isomerization of linolenic acid ( $C_{18:3}$ ) and linoleic acid ( $C_{18:2}$ ) (10).

**TABLE 7. *Trans*-Fatty Acid and Dimeric Triacylglycerols in Commercial Fully Refined Vegetable Oil Samples (10).**

Oil Type	<i>Trans</i> -Fatty Acids (%)	Dimeric Triacylglycerols (%)
Soybean oil	0.9–3.5	0.6–1.3
Sunflower seed oil	0.3–1.3	0.7–1.2
Rapeseed oil	0.9–1.5	1.2–1.5
Corn oil	0.6–4.1	1.0–1.5
Groundnut oil	0.1–0.3	0.5–1.3
Used Deep frying oil	/	> 10

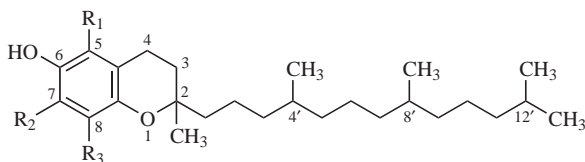
Today, *trans*-fatty acid levels below 1% for rapeseed and soybean oils and below 0.5% for other liquid oils (e.g., sunflower seed oil and corn oil) are required.

**3.2.2. Polymeric and Oxidized Triacylglycerols** Other substances, such as polymeric and oxidized triacylglycerols, are also formed during deodorization. The increasing evidence that these compounds may be detrimental to health implies that their presence needs to be restricted to the lowest possible levels (Table 7) (14).

Polymerization and, more specifically, dimerization may be of thermal or oxidative origin. Contrary to *cis-trans*-isomerization, polymerization already takes place during storage and crushing of the oilseeds and pretreatment of the crude oil. Oxidized triacylglycerols act hereby as catalysts (15). The level of oxidized triacylglycerols should, therefore, be kept to a minimum by limiting the oil-oxygen contact as much as possible in the different refining stages.

### 3.3. Tocopherols and Sterols

**3.3.1. Tocopherols** Tocopherols are widely distributed in nearly all vegetable oils but not in animal fats. Their basic structure consists of the hypothetical tocol molecule, a chromanol structure with a farnesyl side chain, substituted by one, two, or three methyl groups in the positions 5, 7, or 8. (Figure 5). A homologous



Trivial name	Chemical name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
α-Tocopherol	5,7,8-Trimethyltolcol	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>
β-Tocopherol	5,8-Dimethyltolcol	CH <sub>3</sub>	H	CH <sub>3</sub>
γ-Tocopherol	7,8-Dimethyltolcol	H	CH <sub>3</sub>	CH <sub>3</sub>
δ-Tocopherol	8-Methyltolcol	H	H	CH <sub>3</sub>

**Figure 5.** Structure formula and nomenclature for the different tocopherols.



TABLE 8. Tocopherol Content and Composition of Some Oils and Fats.

Oil	Total Tocopherols (ppm)	% of Total Tocopherols			
		$\alpha$ -T	$\beta$ -T	$\gamma$ -T	$\delta$ -T
Cocoa butter	275–290	6	—	91	3
Coconut <sup>1</sup>	30–80	7	—	29	—
Corn	900–1600	16	1	80	3
Cottonseed	250–900	54	—	46	—
Olive	45–215	93	—	7	—
Palm <sup>2</sup>	360–560	18	2	—	2
Peanut	250–500	56	1	42	1
Rapeseed	550–900	35	—	63	2
Soybean	900–1400	10	1	65	24
Sunflower	500–900	96	2	2	—

<sup>1</sup>64%  $\alpha$ -Tocotrienol.

<sup>2</sup>33%  $\alpha$ -Tocotrienol, 36%  $\gamma$ -Tocotrienol, 9%  $\delta$ -Tocotrienol.

series of tocotrienols is also known (e.g., in palm oil), which differs only in the presence of three double bonds in the side chain (in positions 3'-4', 7'-8', and 11'-12') (Figure 5).

Tocopherols and tocotrienols are important compounds of the unsaponifiable fraction of vegetable oils (Table 8). The tocopherol composition is specific for every type of oil and is therefore sometimes used for their identification.

$\gamma$ -Tocopherol is the most abundant homologue in nearly all vegetable oils, with relative percentages up to 65% in soybean oil and 80% in corn oil. The tocopherol fraction of sunflower seed oil consists almost exclusively of  $\alpha$ -tocopherol (96%). The detection of  $\delta$ -tocopherol is usually an indication of the presence of soybean oil, whereas  $\beta$ -tocopherol is rarely found in vegetable oils (Table 8).

Tocopherols are the most important natural antioxidants of a phenolic nature, present in vegetable oils. Different studies have shown that the least substituted isomers form stable radicals more easily and are thus the most potent antioxidants (16, 17).

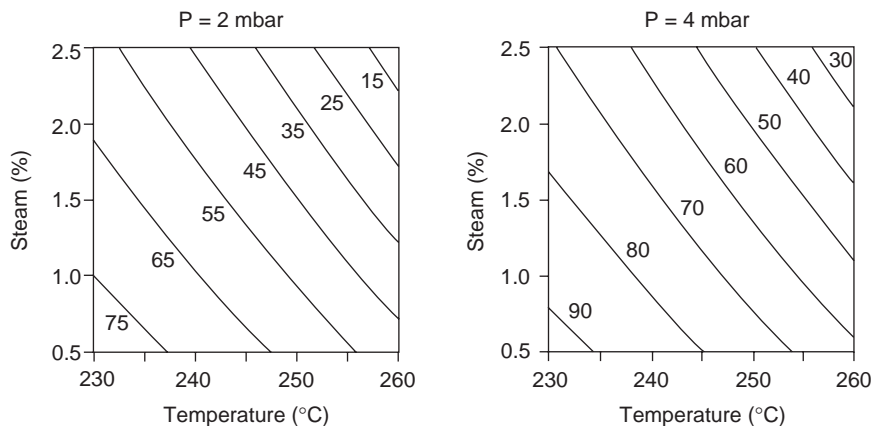
Recent research has shown unequivocally that tocopherols are always present in vegetable oils in the free form and not as tocopheryl esters (18). From a biochemical point of view, this could be expected because only tocopherols with a free (and not esterified) hydroxyl group can act as an antioxidant.

Aside from their antioxidant activity, tocopherols also have an important Vitamin E activity, generally expressed as:

$$\text{Vitamin E} = \alpha\text{-toco} + 0.25 \beta/\gamma\text{-toco} + 0.01 \delta\text{-toco}. \quad (11)$$

Consequently, oils rich in  $\alpha$ -tocopherol (sunflower seed, cottonseed oil) in particular have a high Vitamin E content.

During deodorization, some of the tocopherols are lost during stripping and thermal and oxidative degradation. The major factors affecting stripping losses



**Figure 6.** Relative total tocopherol retention during the deodorization of soybean oil (10).

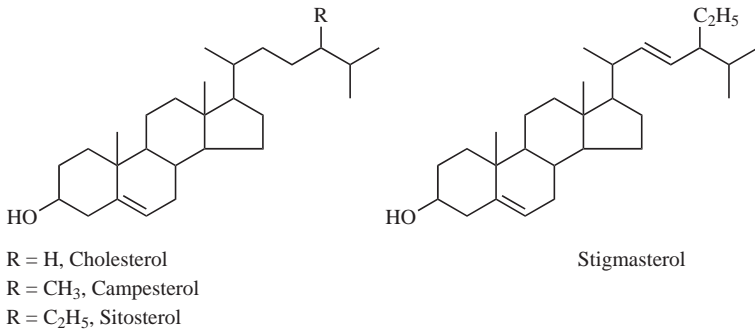
are deodorization temperature, pressure, and stripping steam. In general, more tocopherols will be stripped from the oil with decreasing pressure and increasing sparge steam and temperature. The specific effect of the different process parameters on tocopherol retention during deodorization of soybean oil is shown in Figure 6 (12). The established mathematical models allow an accurate selection of the deodorization parameters resulting in a controlled stripping of tocopherols and, hence, the desired tocopherol level in the deodorized oil (12).

Tocopherol stripping or retention during deodorization is approached differently in Europe and the United States. In the United States, most soft oils are chemically refined and tocopherol stripping during deodorization is maximized. A level of 500 ppm for soybean oil, for example, is considered sufficient to protect the oil from oxidation. The excess tocopherols are collected in the deodorizer distillate (FAD), which is valorized as a high-value-added byproduct. Tocopherol levels in the FAD may range from a few percent to up to 20%.

Especially for soybean oil deodorizer distillate, prices are primarily determined by the tocopherol concentration: The higher the level, the higher the price per kilogram of tocopherol.

In Europe, where physical refining is more widespread, tocopherol retention in the refined oil is maximized. A good quality deodorized corn oil should have a tocopherol content of at least 800 ppm, and preferably 1000 ppm.

When drawing up the tocopherol balance during deodorization, it seems that a certain amount is lost. Some factors that are involved in this tocopherol loss are incomplete vapor condensation and thermal and oxidative degradation. Pure thermal degradation depends on time and temperature and only becomes significant at elevated temperatures ( $>260^{\circ}\text{C}$ ). Tocopherol degradation in vegetable oils mainly occurs due to oxidation because of small, inevitable air leakages in industrial deodorizers. It is generally accepted that tocopherols, while protecting the oil, are first oxidized to quinones and tocopherol dimers (16). More specifically,



**Figure 7.** Overview of the most common phytosterols.

4a, 5-epoxy- $\alpha$ -tocopherolquinone, 7,8-epoxy- $\alpha$ -tocopherolquinone, and  $\alpha$ -tocopherolquinone were identified as the most important oxidation products (18).

**3.3.2. Sterols** Sterols are quantitatively the most important components of the unsaponifiable fraction of vegetable oils and animal fats (Figure 7).

Animal fats, such as milkfat, tallow, and fish oils, are characterized by the exclusive presence of cholesterol, whereas the sterol fraction in vegetable oils consist of a wide group of compounds also known as phytosterols.

The majority of the crude oils contain 1000–5000 ppm sterols. Exceptions are corn and rice bran oil that may contain up to 1–2% of phytosterols (Table 9).

$\beta$ -Sitosterol is quantitatively the most important phytosterol, followed by campesterol and stigmasterol. Other phytosterols, like  $\Delta^5$ -avenasterol,  $\Delta^7$ -avenasterol,  $\Delta^7$ -stigmasterol, and brassicasterol, are only present in minor concentrations or in specific oils (e.g., brassicasterol in rapeseed oil).

Contrary to tocopherols, sterols can be partially present in the esterified form. Esterified phytosterols are a heterogenous group of components in which the

**TABLE 9. Sterol Content and Composition of Some Oils and Fats.**

Oil or Fat	Total Sterols (ppm)	Sterol Composition (%)			
		Campesterol	Stigmasterol	$\beta$ -Sitosterol	Other
Soybean	2000–4000	15–21	10–24	57–72	—
Palm	2000–2500	25	14	65	—
Sunflower	2500–4500	7–12	8–12	62–75	—
Rapeseed	900–1000	30–33	<1	50–55	12–14 <sup>a</sup>
Cottonseed	2700–5500	6–14	3–6	75–90	2–5 <sup>b</sup>
Coconut	500–1000	6–9	18–19	69–75	13–25 <sup>b</sup>
Rice Bran	10000	20–28	8–15	49–54	5–11 <sup>b</sup>
Corn	8000–20000	18–24	4–8	55–67	4–8 <sup>b</sup>
Olive	100	<1	<1	75–80	4–14 <sup>b</sup>

<sup>a</sup>brassicasterol; <sup>b</sup> $\Delta$ -5 avenasterol.

3-hydroxyl group of the phytosterol part is esterified to a fatty acid or a phenolic acid. The relative proportion of free and esterified phytosterols varies widely among different oils. Analyses of more than 90 crude and refined vegetable oils resulted in an average esterified/free sterol ratio of 3/5 (19).

Free sterols are slightly less volatile than tocopherols (Figure 2). Deodorization of soybean oil under varying process conditions (temperature: 220–260°C, low pressure: 1.5 mbar and 1.5% steam) resulted in a 10–35% reduction of the total sterol content (20). This sterol reduction is totally attributed to the free sterol fraction because esterified sterols are not volatile under the conditions prevailing inside the deodorizer. In case of steam refining, an increase of the steryl ester content can sometimes be observed, probably because of a heat-promoted esterification reaction between free sterols and fatty acids (20). This phenomenon will not take place during deodorization of chemically neutralized oil because the initial FFA concentration is much lower in that case.

Although severe process conditions are required (low pressure: 1–2 mbar; high temperature: up to 250°C), steam refining is the most suitable and economical process to lower the cholesterol content of animal fats (e.g., milkfat and tallow).

The positive nutritional aspects of phytosterols have recently gained much interest, especially after the successful commercial introduction of margarines and spreads enriched in phytosterols and phytosterols. Phytosterols can be isolated from deodorizer distillates through a whole series of purification processes. Depending on the type of oil, the refining technique applied (chemical vs. physical refining), deodorization conditions, and the design of the vapor scrubber, the sterol concentration (including sterol esters) in the deodorizer distillate can vary between 5% and 25%.

### 3.4. Contaminants

Most, if not all, crude food oils contain some contaminants. The most hazardous contaminants in edible oils and fats are the so-called “persistent organic pollutants” (POP). In 1995, the United Nations urged for immediate action on a group of 12 POP, including 8 chlorinated pesticides, polychlorinated biphenyl (PCB), hexachlorobenzene (HCB), polychlorinated dibenzo-p-dioxin (PCDD), and polychlorinated dibenzofuran (PCDF). Polycyclic aromatic hydrocarbons (PAH) are not on the shortlist, but their presence in edible oils is also a matter of concern.

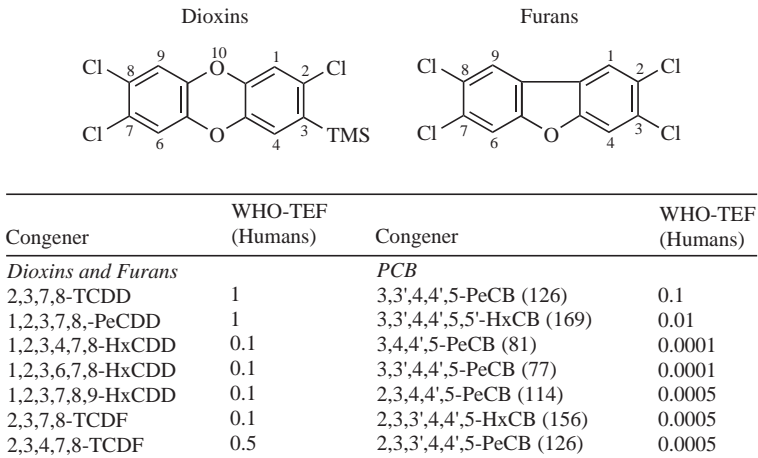
Contaminants need to be removed during the refining process in order to achieve residual levels that are as low as reasonably achievable (ALARA-concept). In practice, this can be achieved by selective adsorption onto a suitable adsorbent or during the deodorization process.

**3.4.1. Pesticides** Many studies have been published regarding the removal of pesticides from oils and fats (21–23). All concluded that organo-chlorine and organo-phosphorus pesticides are fully removed during deodorization, provided that the process temperature is high enough (>230°C) and the applied pressure sufficiently low (<4 mbar). As a result of their relatively high volatility, most pesticides are

removed by stripping. Some pesticides like captan and dichlorvos are thermally decomposed into more volatile products that are subsequently stripped from the oil (23). When appropriate deodorization conditions are applied, the residual pesticide content in refined food oils is generally below the limit of detection (10–50 ppb) (21,22). Pesticides can present a problem in so-called “mild-refined” oils that are usually deodorized at a lower temperature (<220°C). Unfortunately, “mild” deodorization will not always guarantee a complete removal of pesticides.

**3.4.2. Polycyclic Aromatic Hydrocarbons** Only the light polycyclic aromatic hydrocarbons (PAH) with four or less benzene rings in their chemical structure (e.g., anthracene, chrysene, etc.) are volatile enough to be removed during deodorization. Commonly applied deodorization conditions for coconut oil (210–235°C) are sufficient to strip light PAH and reduce the concentration to below 20–25 ppb, a maximum level that is frequently set in refined oil specifications. However, literature data show that light PAH can still be present in significantly higher amounts in refined coconut oil (25). Problems seem to be mainly related to crude coconut oil from Southeast Asia, which are heavily contaminated with PAH during the “smoke drying” of the copra. Analyses of the PAH levels in ten different refined coconut oil samples of Southeast Asian origin showed “light” PAH levels ranging from 220 to 420 ppb, with a mean residual contamination of 337 ppb (25).

**3.4.3. Polychlorinated Biphenyls and Dioxins** “Dioxin” is the systematic name for organic compounds consisting of two benzene rings linked by two oxygen atoms and with at least one chlorine atom. The 2,3,7,8-tetrachlorodibenzo-*p*-dioxins (2,3,7,8-TCDD) are the most important isomers. Dioxins are often linked to the group of the polychlorinated dibenzofurans, which have similar physical, chemical, and biological properties (Figure 8).



**Figure 8.** Structural formula of 2,3,7,8-TCDD (dioxins) and 2,3,7,8-TCDF (furans) and overview of the WHO Toxicity Equivalency Factors (WHO-TEF) for humans and mammals.

Polychlorinated biphenyls (PCBs) are a group of 209 discrete synthetic chemical compounds, called congeners, in which one to ten chlorine atoms are attached to a biphenyl. The empirical formula for PCB is thus  $C_{12}H_{10-n}Cl_n$ , where  $n$  is one to ten. The 209 congeners are systematically numbered from one to 209.

The toxic potential of pure dioxins and PCBs is indicated in toxic equivalence factors (TEF) giving the relative toxicity of the relevant dioxin or PCB relative to the toxic effect of 2,3,7,8-TCDD. TEF for different dioxins and furans vary from 0.01 to 1, whereas TEF for the different PCB congeners is much lower (0.0001–0.01) (26, 27). (Figure 8).

Toxic equivalencies (TEQ) are calculated by multiplying the TEF by the concentration of each of the different congeners present in a sample and adding them up. The TEQ represents the relative toxicity of a dioxin/PCB mixture in terms of the toxic effect of 2,3,7,8-TCDD.

Food contamination with dioxins and PCB seems to be comparable in terms of TEQ and is usually below 5 ppt-TEQ (= 5 picogram-TEQ/g fat) (26, 27). A higher degree of contamination (>10 ppt-TEQ) is commonly detected in fish and fish products.

The vapor pressure of PCB can vary within a wide range. The more volatile PCBs have a vapor pressure around 40–75 mbar, which is similar to the vapor pressure of some organo-chlorine pesticides. Our own lab deodorization trials showed that PCBs and dioxins can be stripped from fish oil without degradation of the  $\omega$ -3 fatty acids (eicosapentaenoic acid, EPA, and docosahexaenoic acid, DHA) provided that the deodorization pressure is very low (>2 mbar) (Table 10). Other studies showed that deodorization at 230°C and 5 mbar was insufficient to remove a PCB heat-transfer agent from contaminated rice bran oil (28).

### 3.5. Deodorizer Distillate

Volatile components removed during deodorization are collected in the deodorizer distillate. The overall composition of the deodorizer distillate depends on the

**TABLE 10. PCB and Dioxin Stripping from Fish Oil.**

	Fish Oil	
	Crude	Deodorized <sup>a</sup>
Dioxins (ppt <sup>b</sup> )	5.3	1.8
Non-ortho PCB (ppt <sup>b</sup> )	17.9	5.5
Mono-ortho PCB (ppt <sup>b</sup> )	7.2	1.3
Free fatty acids (%)	0.66	0.11
EPA <sup>c</sup> (%)	8.7	8.6
DHA <sup>d</sup> (%)	12.7	12.6

<sup>a</sup>Lab deodorization: 190°C-1 mbar-2% steam; <sup>b</sup>WHO-TEQ ppt;

<sup>c</sup>Eicosapentenoic acid; <sup>d</sup>Docosahexenoic acid.

**TABLE 11. Detailed Composition of Deodorizer Distillate Obtained During Chemical or Physical Refining of Different Soft Oils [Concentrations Expressed as % (w/w)].**

	Soybean		Corn	Sunflower Seed		Rapeseed
	Chemical	Physical	Physical	Chemical	Physical	Chemical
Squalene	1.3–2.1	0.6	0.2–1.0	0.7	1.0	0.1–0.4
δ-Tocopherol	4.4–5.6	2.0	0.1	n.d.	n.d.	0.2–0.3
β-Tocopherol	0.4–0.5	n.d.	0.1	n.d.	n.d.	0.1–0.2
γ-Tocopherol	10.7–11.3	5.0	1.1–2.8	0.3	0.1	2.3–2.5
α-Tocopherol	0.8	0.5	0.2–0.4	4.8	1.2	0.9–1.4
<b>Total tocopherols</b>	<b>16.3–18.2</b>	<b>7.5</b>	<b>1.5–3.4</b>	<b>5.1</b>	<b>1.3</b>	<b>3.5–4.4</b>
Brassicasterol	n.d. <sup>3</sup>	n.d.	n.d.	n.d.	n.d.	1.6–2.8
Campesterol	5.1–5.7	1.9	0.8–1.7	1.6	0.5	2.9–4.4
Stigmasterol	4.1–4.8	1.4	0.2–0.4	2.0	0.6	n.d.
β-Sitosterol	7.9–8.3	3.0	1.7–3.4	8.6	2.6	4.1–6.2
Other sterols <sup>1</sup>	n.d.	n.d.	n.d.	1.7	0.6	n.d.
Steryl esters	2.3–2.6	4.5	0.6	0.3	0.1	1.4–5.3
<b>Total sterols<sup>2</sup></b>	<b>19.4–21.4</b>	<b>10.8</b>	<b>3.3–6.1</b>	<b>14.2</b>	<b>4.4</b>	<b>10.0–18.7</b>
Monoacylglycerols	1.2–1.9	1.9	0.1	0.9	n.d.	1.4–2.1
Diacylglycerols	2.7–3.8	8.1	0.5–1.3	1.9	0.7	3.8–3.9
Triacylglycerols	5.1–5.9	3.8	0.1–0.8	2.6	2.7	3.0–7.5
FFA (as C18:1)	33	73.8	77–81	39.2	70.8	39–42

<sup>1</sup>Sum of Δ5-avenasterol; Δ7-avenasterol and Δ5-stigmasterol.

<sup>2</sup>Sum of free and esterified sterols.

<sup>3</sup>Not detectable.

processed oil characteristics, the applied refining mode (chemical or physical refining), the operating conditions during deodorization, and the design of the scrubber. Aside from desired components (fatty acids, tocopherols, sterols, etc.), volatile contaminants (pesticides, light PAH, etc.) will also be concentrated in the deodorizer distillate.

Deodorizer distillates from physical refining consist mainly of free fatty acids (>80%) (Table 11) (29). This byproduct can have some value for use in feed products provided that it contains (very) low levels of contaminants. Where a higher degree of contamination exists, it can only be sold as a source of technical-grade fatty acids.

Deodorizer distillate flow in physical refining can be 5% or more of the oil flow to the deodorizer, depending on the initial FFA content of the oil. Consequently, the theoretical concentration factor of the volatile contaminants in the deodorizer distillate will be around 20. Knowing that the concentration of light PAH in crude coconut oil can be high, levels of up to 10 ppm can be expected in coconut oil deodorizer distillate (Table 12).

Deodorizer distillates obtained during the deodorization of chemical refined soybean oil usually have a significantly higher added value as a result of the high concentration of valuable minor components such as tocopherols and sterols (Table 11). A complex downstream processing of these deodorizer distillates, consisting of a combination of chemical and physical separation processes, finally results in the production of purified tocopherols and sterols.

Deodorizer distillate flow is much lower in the case of chemical refining (0.2–0.5% of the oil flow to the deodorizer). Consequently, contaminant concentration in the distillate can theoretically become 200–500 times higher than in the crude oil. For pesticides, the observed concentration factor is significantly lower, mainly because of thermal decomposition of some pesticides and incomplete condensation of volatile pesticides in the vapor scrubber. The limited amount of data available in the literature, combined with our own research figures, indicate that pesticide concentration in soybean, sunflower seed, and rapeseed deodorizer distillate is usually

**TABLE 12. Polycyclic Aromatic Hydrocarbon Content (PAH) of Refined Coconut Oil and the Corresponding Deodorizer Distillate (Data in ppb).**

PAH	Refined Coconut Oil	Deodorizer Distillate
Naphtalene	3.3	1670
Phenantrene	62.9	10,968
Pyrene	48.5	8142
Benzo(b)fluoranthene	1.5	8.9
Dibenz(a,h)anthracene	0.1	0.5
Benzo(a)pyrene	0.7	1.3
<i>Sum of light PAH</i>	168.9	38,690
<i>Sum of heavy PAH</i>	3.8	21.3

Own research data; coconut oil from Southeast Asian origin.



below 1 ppm. Only in very exceptional cases, when the degree of contamination in the crude oil is unacceptably high ( $>1$  ppm), the pesticide concentration in the corresponding deodorizer distillate can increase to 50 ppm.

Although contaminants can be removed from the deodorizer distillate during the downstream processing, their presence is certainly unwanted and may affect the commercial value of the distillate in a negative way. For this reason, edible oil refiners are becoming more and more interested in (new) technologies that can either remove the contaminants from the deodorizer distillate prior to sales or avoid their presence in (part of) the distillate. The first option seems to be the most straightforward choice because known technology, either adsorption or stripping, can be used. The overall volatility of the contaminants will be higher in the deodorizer distillate as a result of the higher initial concentration (cfr. Law of Raoult). Therefore, contaminants can be removed to a certain extent from the deodorizer distillate by stripping under appropriate processing conditions. Process technology and conditions have to be optimized to maximize the amount of the low-contaminant distillate stream. On the other hand, this process will always result in a certain contaminated residue fraction that has to be considered as a waste stream with no value. Alternatively, improved design of the scrubber (e.g., dual condensation) offers the possibility of collecting two different distillate fractions, one enriched in FFA and the other in unsaponifiable components (sterols, tocopherols, etc.).

### 3.6. Oil Loss During Deodorization

Aside from volatile components (e.g., free fatty acids, secondary oxidation products, tocopherols, sterols, etc.), the deodorizer distillate also contains some neutral oil (tri-, di-, and mono-acylglycerols). With the exception of the more volatile monoacylglycerols, this neutral oil is present mainly as a result of mechanical entrainment by the stripping steam and is therefore considered as a direct refining loss.

Neutral oil loss (NOL) mainly depends on the deodorization conditions. In general, NOL increases with higher deodorization temperature, lower pressure, and a larger amount of stripping steam. At the same time, NOL during steam refining is higher than during deodorization. This is because mechanical entrainment causes NOL to be proportional to the distillate flow or the amount of stripping steam, which are both higher in the case of steam refining.

Improvement of the deodorizer design by the installation of baffles and demisters in the vapor chimneys has significantly reduced entrainment losses to 0.1–0.2% in chemical refining. For steam refining, an additional loss directly proportional to the FFA content has to be taken into account. For most oils (soybean oil, palm oil, etc.), NOL is exclusively due to mechanical carry-over. However, in lauric oils, part of the NOL is a consequence of effective evaporation of volatile short-chain mono- and diacylglycerols (30). (Table 13). This distillation loss of NOL is inherently due to the deodorization conditions, but is not affected by the deodorizer design.

**TABLE 13. Melting and Boiling Points of Some Fatty Acids and Glyceridic Components.**

Component Chain Length	Fatty Acid	Monoacylglycerol Melting Point (°C)	Diacylglycerol	Triacylglycerol
C6	-3.4	19.4	/	-25
C8	16.7	/	/	8.3
C10	31.6	53	44.5	31.5
C12	44.2	63	57.8	46.4
C14	54.4	70.5	66.8	57.0
C16	62.9	77	76.3	63.5
C18	69.6	81.5	79.4	73.1
C18:1	16.3	35.2	21.5	5.5

Component Chain Length	Fatty Acid	Monoacylglycerol Boiling Point (°C) at 1 mm Hg	Diacylglycerol	Triacylglycerol 0.05 mm Hg
C6	61.7	/	/	135
C8	87.5	/	/	179
C10	110.3	175	/	213
C12	130.2	186	/	244
C14	149.2	199	/	275
C16	167.4	211	/	298
C18	183.6	190 <sup>a</sup>		313
C18:1	/	186 <sup>a</sup>		308 <sup>b</sup>

<sup>a</sup>At 0.2 mm Hg; <sup>b</sup>olive oil.

Expected NOL can be estimated from the initial and final FFA content of the oil and the FFA content of the deodorizer distillate (FAD) by the following formulas:

$$\text{NOL}(\%) = \text{FAD flow} * (100 - \text{FFA}_{\text{FAD}} - \text{Unsaps}_{\text{FAD}}) / 100, \quad (12)$$

$$\text{FAD}(\%) = (\text{FFA}_{\text{OIL IN}} - \text{FFA}_{\text{OIL OUT}}) / (\text{FFA}_{\text{FAD}} - \text{FFA}_{\text{OIL OUT}}) * 100. \quad (13)$$

In practice, NOL can be slightly higher as a result of hydrolysis of the refined oil during deodorization. Our own research showed that short-chain oils (e.g., coconut oil) are more prone to hydrolysis than long-chain oils (e.g., soybean oil). Hydrolysis during deodorization of coconut oil resulted in the production of 0.01–0.03% additional FFA (30).

### 3.7. Handling and Storage of Deodorized Oil

Deodorized oils require particular handling and storage conditions to avoid oxidation or other degradation reactions that may affect the quality. Flavor deterioration, in particular, and color reversion, to a lesser extent, may occur if the oil is not properly protected. Saturation of the oil with nitrogen after deodorization and low-temperature storage in stainless steel tanks protect the oil against oxidation when stored in bulk for a longer time. Modern processing plants are usually equipped with an inert gas blanketing system through the different refining stages. All parts in contact

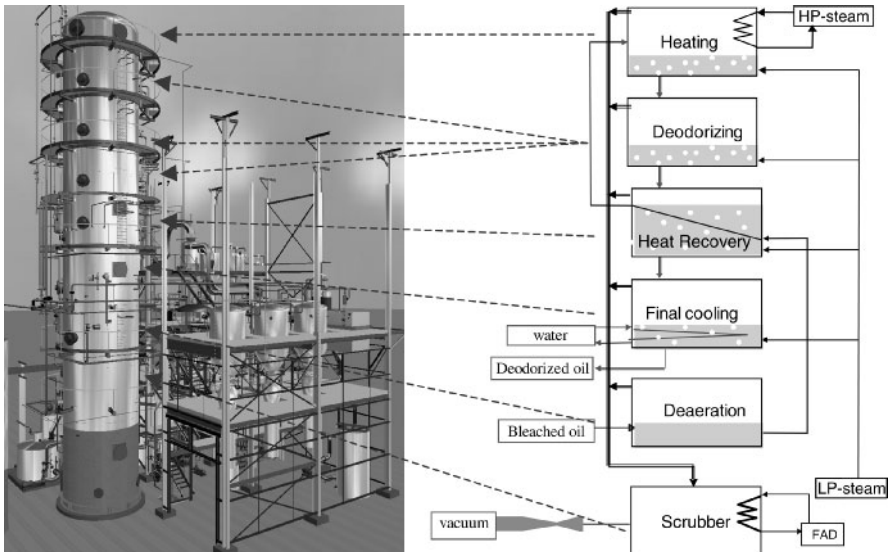
with the oil are made best of stainless steel (minimum SS 304) to avoid migration of Fe ions into the oil. Furthermore, the storage temperature is best kept as low as possible as the autoxidation rate increases as the temperature rises. For example, the rate of oxidation doubles with each 10°C increase in temperature.

It has become common practice to add a small amount of citric acid (20–50 ppm) to the oil after deodorization, because it improves the flavor stability and, at the same time, acts as a metal chelator. Some refiners even add natural antioxidants (e.g., tocopherols), although it seems more logical to prevent the oil from losing too many natural antioxidants during deodorization by using less-severe conditions.

In a modern refining operation, end-product storage is minimized. Refined oil is shipped in bulk or bottled as soon as possible. Furthermore, there is an increasing tendency to integrate the refinery with the finishing lines in the crushing plant as this strongly reduces intermediate and final oil storage. Despite careful measures taken during bulk handling and shipment to industrial customers, a large part of the refined oil is redeodorized prior to its final use. This “brush” deodorization serves to remove small amounts of off-flavors formed during transport and storage. Redeodorization normally requires less-severe process conditions.

#### 4. DEODORIZER TECHNOLOGY

Deodorization is a multi-step process comprising de-aeration, heating, deodorization-deacidification, and cooling of the oil (Figure 9).



**Figure 9.** General overview of the different stages in deodorization.

## 4.1. De-Aeration

As a first step, the oil is de-aerated prior to heating in order to avoid excessive oxidation and, hence, risk of polymerization.

Information of solubility of gases in oils is rather limited. Vegetable oils readily dissolve between 4% and 10% of their own volume of air and other gases at ambient temperature. All gases, with exception of carbon dioxide, increase in solubility with increasing temperature. The relation between solubility (S) and temperature (t) can be expressed by following linear equations (30):

$$\text{For Nitrogen} \quad S(N_2) = [0.0590 + 0.000400 \bullet t] \bullet 100, \quad (14)$$

$$\text{For Oxygen} \quad S(O_2) = [0.1157 + 0.000443 \bullet t] \bullet 100. \quad (15)$$

With S: Solubility, gas in oil (% v/v) at atmospheric pressure; t: Temperature (°C).

To achieve a proper de-aeration, the bleached oil is sprayed into a vessel under reduced pressure, before entering the heating section. The lower the pressure applied, the lower the residual oxygen level in the oil. Usually, the oil is heated to at least 80°C and sprayed in a tank, which is kept at a pressure below 50 mbar. Some refiners even use the low pressure of the deodorizer or add some sparge steam in the spraying vessel to improve de-aeration.

## 4.2. Heating and Cooling

The subsequent heating of the oil is usually accomplished in two stages. In the first stage, the incoming oil is heated countercurrently in an oil-oil heat exchanger (economizer), with the finished oil leaving the deodorizer. Finally, the oil is heated under reduced pressure to the final deodorization temperature with a high-temperature source. Nowadays, nearly all deodorizers operate with high-pressure steam boilers (Figure 10) (Table 14). Thermal oil heaters were quite commonly used in the past to

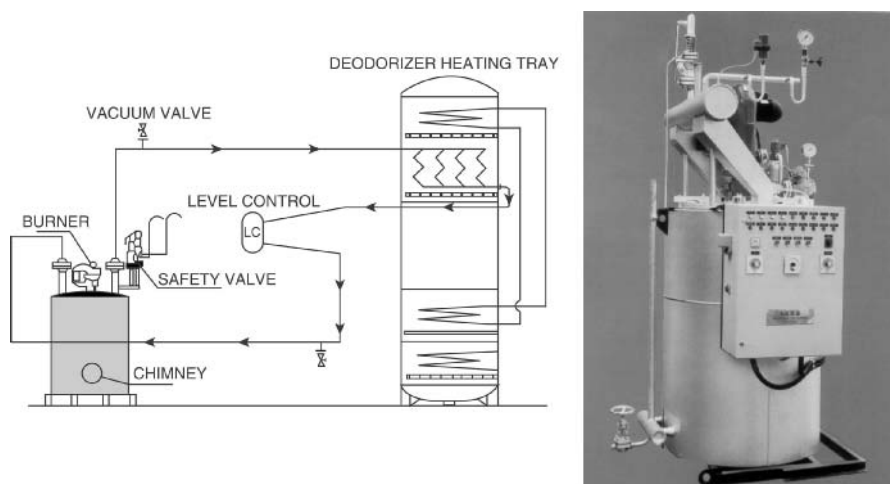


Figure 10. High-pressure steam boiler used in deodorization (Geka).

**TABLE 14. Correlation Among Steam Pressure, Temperature, Latent Heat, and Specific Volume**

Pressure (bar)	Steam Temperature (°C)	Latent Heat (kJ/kg)	Specific Volume (m <sup>3</sup> /kg)
1	99.6	2258	1.694
2	120.2	2202	0.8853
3	133.5	2163	0.6056
5	151.8	2108	0.3747
7	164.9	2065	0.2762
10	179.9	2014	0.1943
15	198.3	1945	0.1316
20	212.4	1889	0.09952
30	233.8	1794	0.06663
40	250.3	1713	0.04975
50	263.9	1640	0.03943

heat edible oils, but, due to the potential risk of contamination, the use of thermal heating fluids has mostly been abandoned. The use of diphenyl/diphenyloxide (e.g., Downtherm<sup>®</sup> A from Dow Chemical Co.) is still allowed, but only in exceptional cases and if no other alternative is available. In that case, a control and loss-detection system has to be installed and the deodorized oil needs a certificate of noncontamination.

The net heating energy required for a deodorization system can be calculated as:

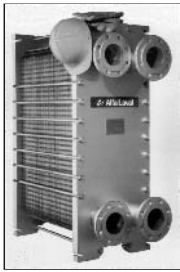
$$H = [O \cdot c \cdot (T_2 - T_1)] \cdot f_L \cdot f_R, \quad (16)$$

where  $O$  is the amount of oil (kg),  $T_1/T_2$  is the incoming and final temperature of the oil (°C),  $c$  is the average specific heat capacity of vegetable oils (typically 2.2–2.4 kJ/kg°C),  $f_L$  is the heat loss factor from radiation (typically 1.05–1.15), and  $f_R$  is the heat recovery factor [1-(%heat recovery/100)].

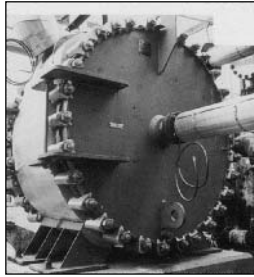
In industrial practice, heat recovery has become an important factor because it minimizes the cost of additional heating of the oil to the deodorization temperature. In recent years, there has been a very fast evolution in the manufacture of heat exchangers for heat recovery. Generally speaking, they can be divided into external and internal heat exchangers (Figure 11).

External heat exchangers usually result in high recovery and provide easier access for cleaning. On the other hand, internal heat exchangers allow energy recovery under vacuum and ensure less cross-contamination and less risk of fouling. The final choice for a heat-exchange system is based not only on its thermal performance but also on other criteria such as easy maintenance, low risk of fouling, low level of cross-contamination, and an acceptable installation cost compared with the expected energy recovery.

Heat recovery can be achieved directly by exchange of heat between two oil streams at different temperatures (e.g., bleached vs. deodorized oil), flowing in a countercurrent direction through the exchangers, or indirectly by steam production.



plate



spiral



oil-steam heat exchanger

Internal heat exchangers

shell & tube



oil-oil heat exchanger



External heat exchanger

**Figure 11.** Examples of external and internal heat exchangers used in edible oil deodorization (Alfa Laval, Ciat, De Smet).

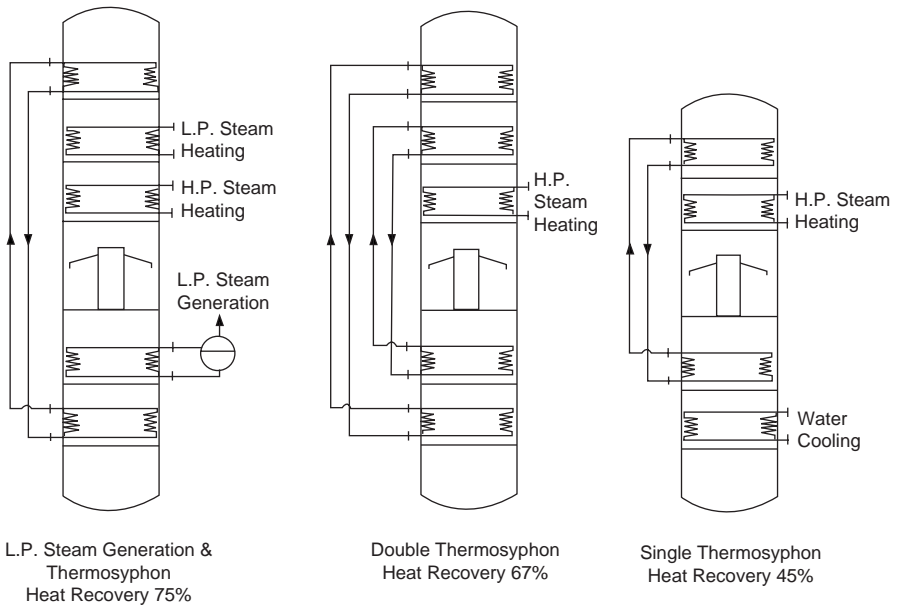
Direct heat recovery is the most efficient, with up to 85% of the heat recoverable. It is usually applied in continuous deodorizers, whereas the indirect heat recovery system is used preferentially in semi-continuous deodorizers with frequent feedstock changes. The efficiency of the indirect heat recovery depends largely on the type and design of the system (Figure 12).

A special indirect heat-recovery device is the themosiphon system. The steam produced in the oil cooling section is sent in a closed loop to the oil heating section. The steam will condense there, and the water is returned to the cooling section.

Final cooling of the oil is usually conducted under reduced pressure to prevent the possible production of degradation byproducts. The necessity of conducting cooling under vacuum while maintaining steam injection has always been a matter of discussion. As a result of the technological complexity and for cost reasons, cooling under vacuum is usually applied only in a large capacity deodorizer. Small capacity plants often make use of external oil-oil heat-exchanging devices.

### 4.3. Steam Stripping

The necessary amount of stripping agent is directly proportional to its molecular weight. Therefore, stripping agents with the lowest possible molecular weights are selected. For economic reasons, steam is generally used, but the use of nitrogen has been studied extensively. Nitrogen has the advantage of being an inert and



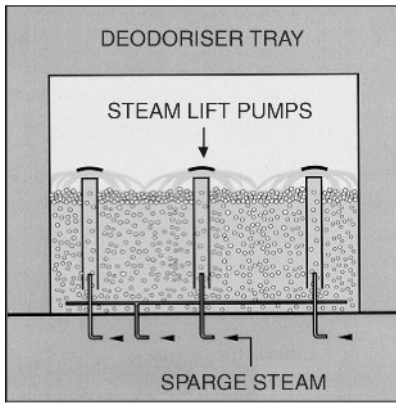
**Figure 12.** Indirect heat recovery systems used in semicontinuous deodorizers (thermosiphon systems) (De Smet). LP: low-pressure steam ; HP: high-pressure steam.

noncondensable gas. Theoretically, its use will result in lower loss (no hydrolysis) and a more pure deodorizer distillate. Although it is possible to work with nitrogen under the commonly applied process conditions, experiments have shown that the profitability is very uncertain, depending on the existing installations in the factory and the nitrogen supply (31, 33). Further studies have indicated that color, residual FFA, oxidative stability, as well as the formation of *trans*-fatty acids and the stripping of tocopherols are not affected by the nature of the stripping agent (34, 35).

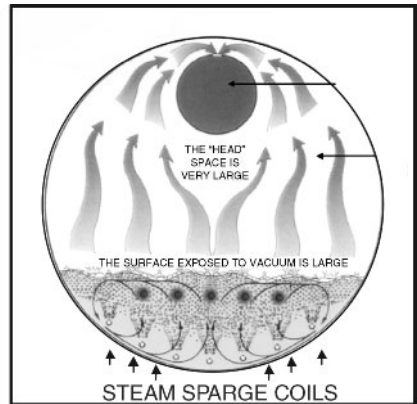
In any case, the stripping agent must be “dry” and free of oxygen. Superheating will ensure that the stripping agent is “dry” and that no cooling of the oil occurs.

Apart from the stripping agent, different deodorizer designs attempt to provide the best contact between the gas phase and the oil phase by creating a large contact surface, together with an optimal sparge steam distribution. In this way, a maximum vaporization efficiency can be reached.

Deodorization only occurs at the vapor-liquid contact zone where the lowest operating pressure exists. It is therefore essential to expose all parts of the oil to surface conditions. In most deodorizers, the stripping agent is introduced into the oil through special sparge coils with very fine holes (with a diameter between 0.5 and 2.5 mm) or by steam lift pumps. However, the main function of steam lift pumps is to improve agitation and enhance overall deodorization efficiency by continuously refreshing the oil in the top layer (Figure 13). A minimum oil layer height (more than 0.8 m) is required to allow good operation of a steam lift pump.



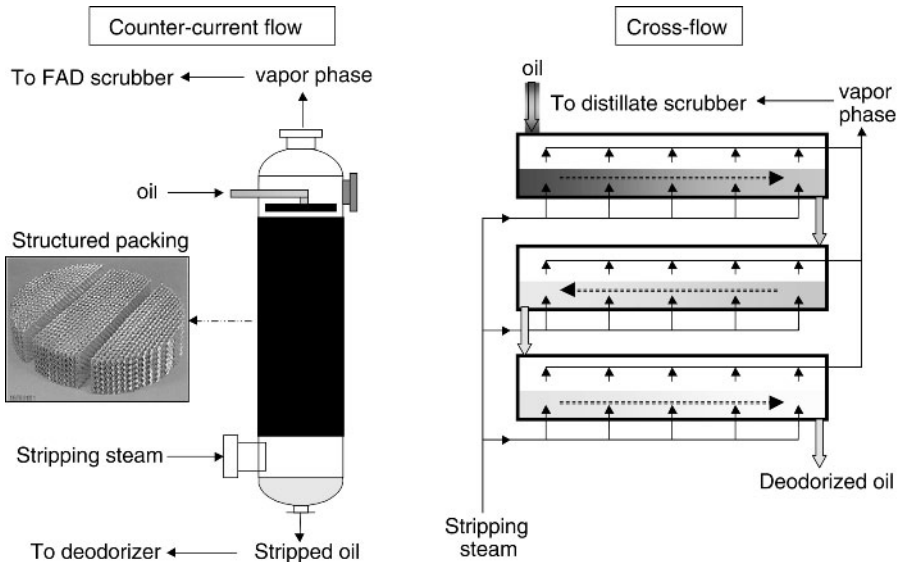
Deep bed deodorizer



Shallow bed deodorizer

**Figure 13.** Sparge steam injection systems used in deodorizers (De Smet, Tirtiaux).

Another way to improve the stripping is to increase the contact surface between steam and oil. In edible oil deodorization, this is accomplished in so-called packed columns that can be filled with various types of surface-extending devices. Packed columns have already been applied in edible oil deodorization for decades. A very good contact between the vapor and the oil at low pressure is created by a continuous thin film of oil flowing over the packing material. Both random and structured packings are used, but the structured packing is most preferred for its lower pressure drop and higher vaporization efficiency. As a result of the fact



**Figure 14.** Principle of countercurrent and cross-flow stripping.



**TABLE 15. Calculation of the Residual Free Fatty Acid Content in a C-18 Oil Under Ideal Conditions (Efficiency E = 1).**

Conditions	Cross Flow Deodorizer			Packed Column		
Theoretical trays	5			5		
Steam (kg/ton)	6			6		
FFA-in (%)	0.3			0.3		
Temperature	230	245	260	230	245	260
Pressure (mbar)	3			3-4		
FFA-out (%)	0.14	0.08	0.032	0.09	0.023	0.003
Pressure (mbar)	2					
FFA-out (%)	0.097	0.043	0.014			
Pressure (mbar)	1.5					
FFA-out (%)	0.07	0.026	0.008			

that stripping steam is introduced into the column in a countercurrent way, packed columns require less steam than tray deodorizers, which work according to the cross-flow principle (4) (Figure 14). For chemically refined oils, for example, a stripping steam consumption of 0.5–0.7% is reported as being sufficient for packed columns, compared with 1.0–1.2% for tray deodorizers. However, modern tray deodorizers today operate with even less steam, as low as 0.7–0.9%.

The stripping efficiency of a deodorizer can be improved either by incorporating a packed column or by reducing the operating pressure of the deodorizer (Table 15). The best solution, of course, is a combination of both, but this results in an expensive deodorization technology.

A convenient way of controlling the stripping steam flow through the steam distributors is to maintain a fixed pressure upstream of an orifice plate of known size. As the pressure always falls to a low value beyond the orifice, the flow of steam will be proportional to the absolute pressure on the upstream side of the orifice and the orifice surface (Table 16).

Orifice plates are usually on each steam sparge coil to allow an independent adjustment and control of the steam flow rates. Steam from the main low-pressure

**TABLE 16. Steam Flow Rates for Orifices of Different Size at Different Steam Pressure.**

Pressure (bar)	0.5	0.7	0.9	1.1	1.3	1.5	1.7	1.9
Orifice Size (mm)	Steam Flow Rate (kg/hr)							
1	0.15	0.21	0.27	0.33	0.39	0.45	0.51	0.67
1.5	0.34	0.48	0.61	0.75	0.88	1.02	1.16	1.29
2	0.61	0.86	1.09	1.33	1.37	1.82	2.06	2.30
2.5	0.95	1.32	1.70	2.08	2.46	2.84	3.21	3.59
3	1.36	1.90	2.45	2.99	3.54	4.08	4.62	5.17
4	2.42	3.38	4.35	5.31	6.28	7.25	8.21	9.18
5	2.78	5.29	6.80	8.31	9.82	11.5	12.8	14.3

sparge steam line (3–5 bar) is distributed to different deodorizer compartments. Before entering into the sparge steam coils, the steam pressure is reduced to the required pressure by means of a pressure reducing valve. Usually, there is one pressure reducer per compartment to allow different steam injection rates over different deodorizer trays.

Aside from a higher stripping efficiency, a packed column is also characterized by a very short holdup time. This may be sufficient for the stripping of certain volatile components (e.g., FFA, tocopherols, etc.) but not enough for a complete deodorization. Therefore, a holding vessel is usually placed after a packed column to properly deodorize the oil. The steam introduced in the retention vessel can be reused as stripping vapor for the packed column, which reduces overall steam consumption. The reuse of this “dirty” steam, however, may have a negative effect on the final oil quality.

### 4.4. Vapor-Scrubbing Systems

The volatile components, stripped during deodorization, are condensed and usually recovered in a direct condenser or vapor scrubber (Figure 15).

The vapor from the deodorizer consists mainly of steam, volatile fatty substances, and some noncondensables (e.g., air). The volatile substances are condensed by creating an intimate contact between the vapor and the fatty acid distillate circulating in the scrubber. This is done either by a series of sprayers

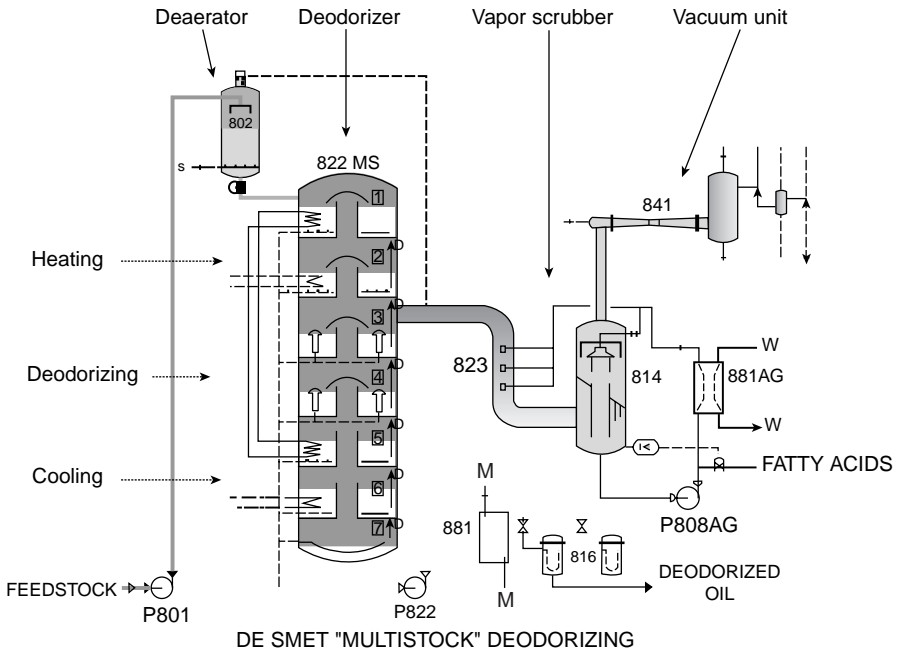


Figure 15. Flowsheet of a stock change deodorizer (De Smet).

**TABLE 17. Composition of Deodorizer Distillates from Single- and Dual-Temperature Condensation.**

Vegetable Oil <sup>1</sup>	Dual-Temperature Condensation				Single Temperature	
	Distillate 1		Distillate 2		Condensation	
	FFA (%)	Tocos (%)	FFA (%)	Tocos (%)	FFA (%)	Tocos (%)
Soybean oil	84.0	1.10	62.0	7.4	76.0	3.5
Sunflower oil	86.0	0.65	61.0	5.3	77.0	2.4
Palm oil	93.0	0.25	40.0	1.7	84.5	0.8

<sup>1</sup>Initial FFA:  $\pm 1\%$ .

mounted in the ducts or through a packed bed (random or structured packing) in the scrubber vessel. The distillate is usually circulated at the lowest possible temperature (just above the melting point) to obtain the best possible condensation of the fatty matter present in the vapor phase that leaves the deodorizer.

A demister is sometimes installed at the top of the scrubber ahead of the vacuum unit, to reduce liquid carryover of small oil droplets, which would otherwise end up in the water from the barometric condenser or in the condensate from the cold (or dry) condensers.

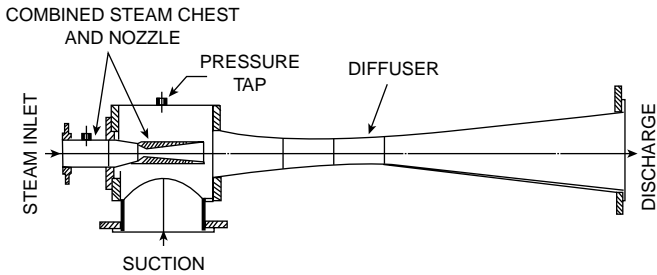
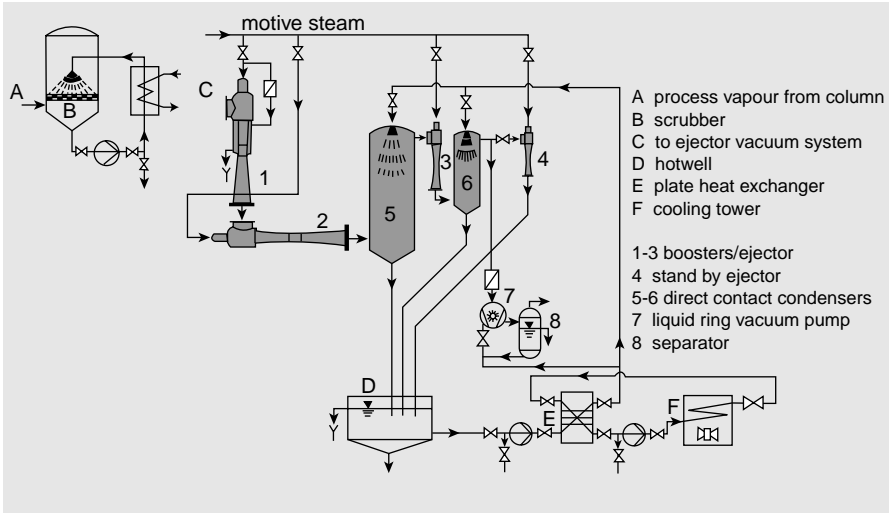
Apart from efficient cooling of the vapor and condensing of the fatty matters, the pressure drop in the scrubber should be kept as low as possible because it directly affects the operating pressure of the main deodorizer. The pressure drop should be below 1 mbar, and preferably below 0.5 mbar.

The conventional vapor-scrubber design results in one single deodorizer distillate. The main factors determining the overall composition of the distillate have been discussed earlier in this chapter. Recently, improved scrubbers operating at two different temperatures (so-called dual condensation principle) have been introduced. Especially in case of physical refining, this design can result in higher value-added distillates because it allows the collection of a first distillate enriched in FFA and a second distillate with a higher concentration of unsaponifiable components (sterols, tocopherols, etc.) (Table 17).

## 4.5. Vacuum Systems

**4.5.1. Conventional Vacuum Systems** The low absolute pressure required in a deodorizer, usually between 2 and 4 mbar, is commonly generated by vacuum systems consisting of a combination of steam ejectors (boosters), vapor condensers, and mechanical (liquid ring) vacuum pumps (Figure 16). Liquid ring pumps are used in the final stage of the vacuum system to remove the noncondensable gases. As a result of the large volume of vapor to be removed, motive steam consumption in such steam ejectors is quite high and may account for up to 85% of the steam consumed in a deodorizer.

A way to reduce motive steam consumption in a steam-ejector system with barometric condensers is to lower the temperature of the water recirculating in the



**Figure 16.** Vacuum steam ejector system with barometric condensers used in edible oil deodorization (Körting).

barometric condensers (Table 18). The benefit of the lower motive steam consumption, however, must be weighed against the extra chilling capacity required and, thus, the electrical energy needed to cool the barometric condenser water. Another benefit from using a lower barometric condenser water temperature is a better condensation of volatile odoriferous material, which, in turn, reduces the odor emission problem. Together with the condensed steam and highly volatile material, a small

**TABLE 18.** Effect of Barometric Condenser Water Temperature on Motive Steam Consumption in Steam Ejector System.

Booster	Pressure		kg Motive Steam per kg Stripping Steam	
	Deodorizer		30°C (1)	10°C (2)
2.5	3 mbar		4.5	1.6
1.5	2 mbar		6.2	2.5

Note: (1) Barometric condenser water inlet temperature: 24°C; outlet temperature: 30°C.

(2) Barometric condenser water inlet temperature: 5°C; outlet temperature: 10°C.

amount of fatty matter is usually found in the condenser water,  $\pm 1\%$  of the stripping steam. This fatty matter may decant partially and separate from the water. The waste water is usually sent to a water effluent treatment plant where it is mixed with other effluent streams from the refinery.

**4.5.2. Dry Condensing Systems** Special vacuum production units have been developed to obtain lower pressures and operating costs and, at the same time, to reduce emissions by more efficient condensation of the volatiles. The dry condensing system is becoming more and more standard in new refining plants. With this system, the sparge steam is condensed on surface condensers working alternately at a very low temperature (around  $-30^{\circ}\text{C}$ ). The remaining noncondensables are removed either by mechanical pumps or roots blowers in series with a liquid ring pump or by a vacuum steam-ejector system (booster). The dry condensing system reduces the motive steam consumption but requires extra electrical energy.

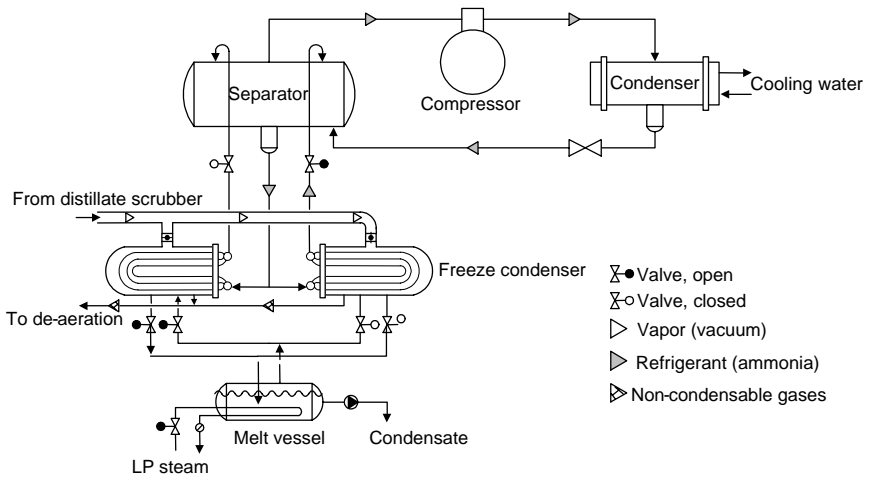
As a result of the relatively high capital cost, the return on investment (ROI) for a dry condensing system may take several years and depends largely on the ratio between the cost of steam and electricity. In Europe, with higher fuel costs, the production cost of steam is higher, which improves the ROI of a dry condensing system compared with a classic vacuum system. As an additional benefit, much lower waste water quantities are produced by dry condensing, which significantly reduces the cost of effluent treatment, thereby also improving ROI.

The pressure in the deodorizer is always slightly higher (0.5–1.5 mbar) than on the suction side of the vacuum unit, because of pressure losses caused by the oil demisters, the fatty matter scrubbers, and other equipment. Consequently, to reach an effective deodorization pressure of 2 mbar, a pressure of not more than 1.5 mbar at the suction side is required. To obtain an efficient steam sublimation at this low pressure, special stripping steam condensers operating at extremely low temperatures ( $-30^{\circ}\text{C}$ ) are required. (Table 19).

The commercially available dry condensing systems consist of two or more freeze condensers with horizontally or vertically orientated straight tubes, a refrigeration plant for the generation of cold refrigerant, which is evaporated in the tubes, and a vessel with relatively warm water for defrosting and cleaning of the tubes after a certain period of freezing.

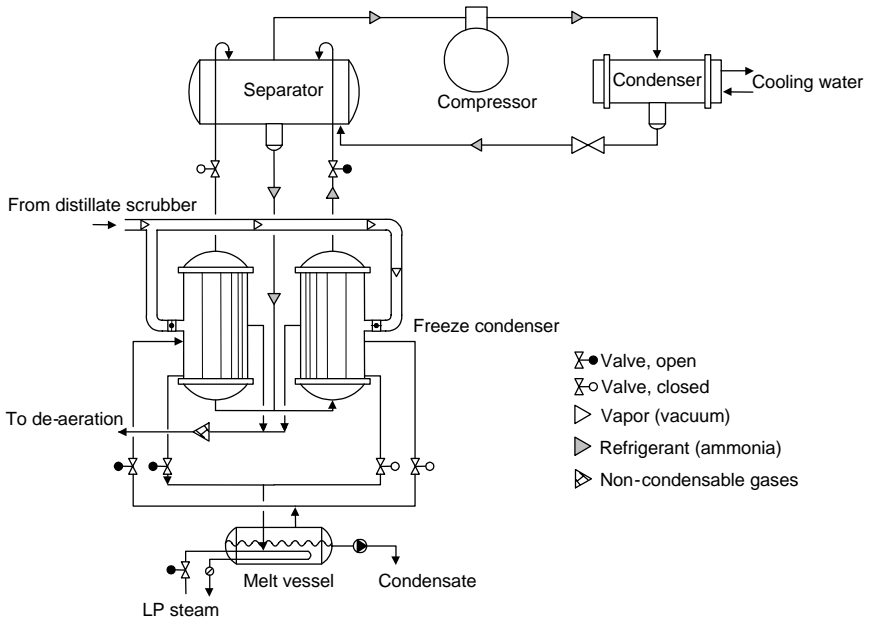
**TABLE 19. Effect of Suction Pressure on Sublimation Point of Stripping Steam.**

Pressure at Condenser Side (mbar)	Sublimation Point of Water ( $^{\circ}\text{C}$ )
0.5	-27.3
1	-20.3
2	-12.9
3	-8.4
5	-2.4
10	7.0
20	17.5
30	24.1



**Figure 17.** Schematic diagram of a dry condensing system with horizontal freeze condensers (e.g., Niro-Gea, Körtling).

Dry condensing equipment can be equipped with either with horizontal or vertical freeze condensers (Figures 17 and 18). Advantages of “horizontal” dry condensing systems are the relatively simple and compact construction of the freeze condensers. On the other hand, the mass of the refrigerant in the gravity system is typically high.



**Figure 18.** Schematic diagram of a dry condensing system with vertical freeze condensers (e.g., Graham Corporation).

Compared with horizontal systems, “vertical” freeze condensers have a more efficient removal of ice and fatty matter from the tubes, which can drain freely by gravity. A disadvantage of the vertical orientation is the static pressure of the refrigerant column in the vertical tubes, which causes higher evaporating temperatures at the bottom. In order to guarantee sufficient sublimation of sparge steam over the entire height of the tubes, this evaporating temperature increase should be compensated by a similar reduction of the refrigerant temperature in the separator, reducing the energy efficiency of the refrigeration plant.

The most essential feature of the recently developed SUBLIMAX system is the vertical orientation of the freeze condensers, combined with individual refrigerant injection at the top of the tubes to produce a falling film (Figure 19). This design results in high-heat-transfer coefficients and constant evaporating temperatures along the entire tube length.

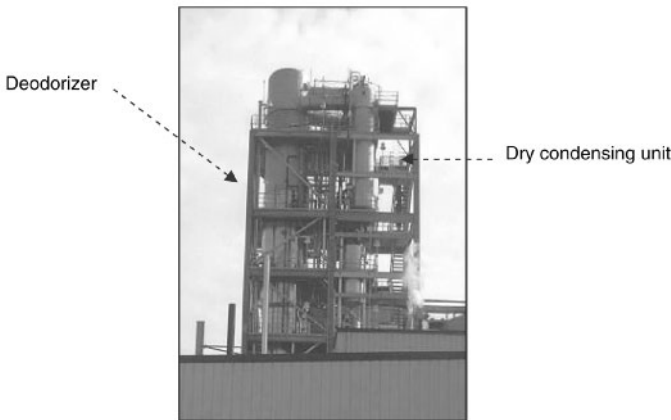
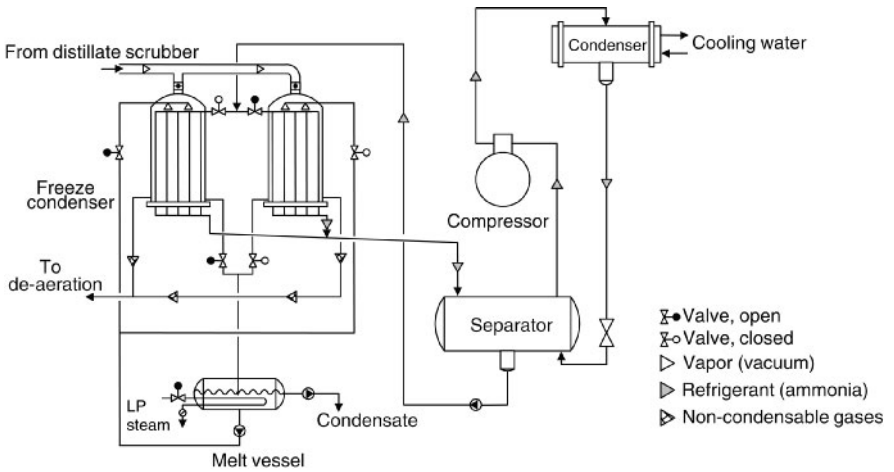


Figure 19. Schematic diagram of the SUBLIMAX Dry Condensing system (De Smet-Solutherm).

## 5. COMMERCIAL DEODORIZER SYSTEMS

Deodorization can be performed in different ways (continuous, semicontinuous, or batch). The selection of most appropriate deodorizer technology depends on many factors, such as the number of feedstock changes, heat recovery, investment, and operating costs.

### 5.1. Batch Deodorization

Batch deodorization is especially suitable for small capacities (<50 ton/day), irregular production, or in processing small batches of different oils that demand minimum cross-contamination. Batch deodorizers mainly consist of a single-shell welded vertical cylindrical vessel (Figure 20). Their major advantages are very simple construction and low capital cost. Low capacities, high operating costs (high steam consumption, very low heat recovery), and relatively long processing times (sometimes up to 8 h) have made batch deodorization less attractive in today's oil-refining industry.

### 5.2. Semicontinuous Deodorization

Semicontinuous deodorizers are basically batch systems designed for larger capacities. Their main application is in plants with frequent feedstock changes of oils sensitive to cross contamination. In most designs, a batch of oil is transferred into

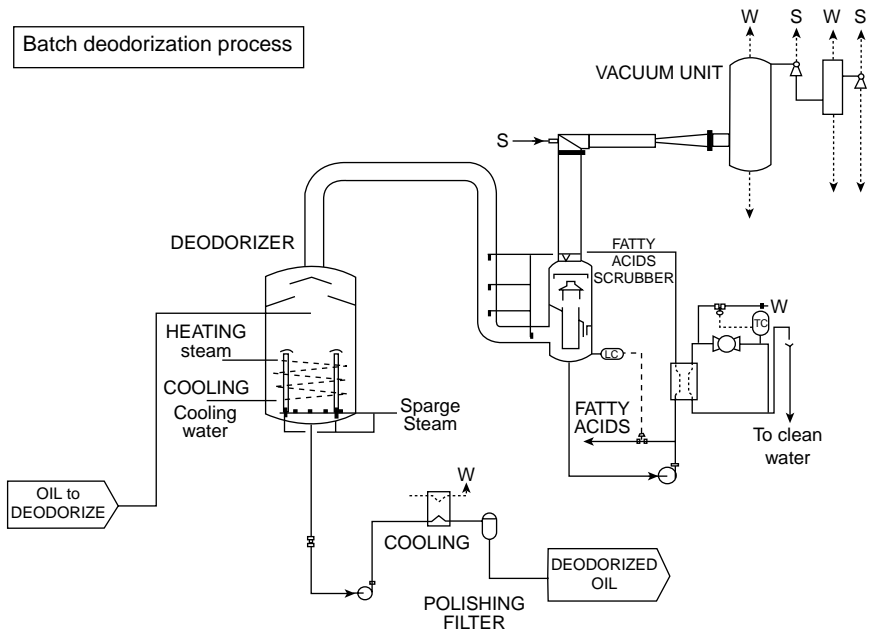
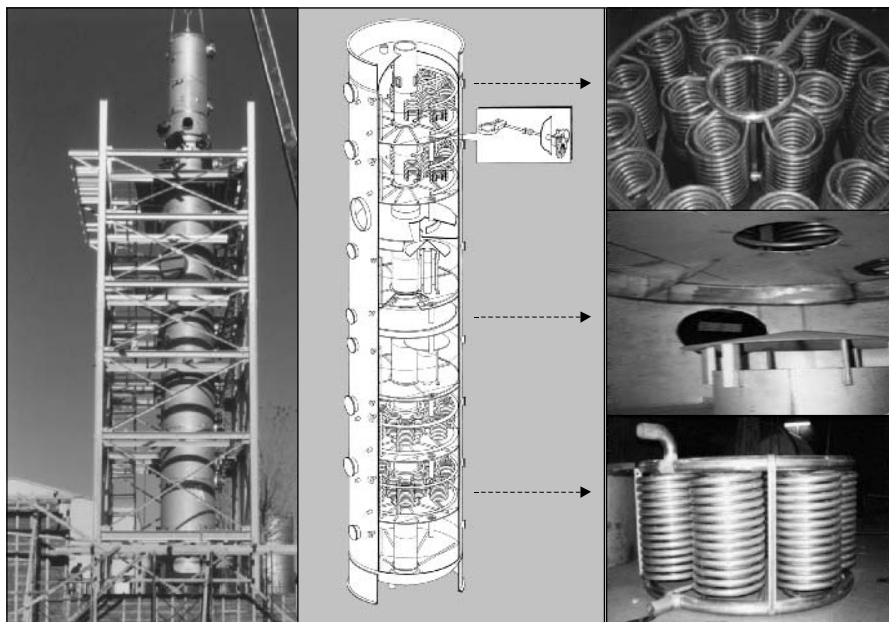


Figure 20. Flowsheet of a batch deodorization process (De Smet).





**Figure 21.** Semicontinuous deodorizer for frequent stock changes (Multistock-De Smet).

the system and then flows by gravity in a time sequence through a number of vertically stacked compartments or trays (Figures 15 and 21).

The semicontinuous design allows a more efficient heat recovery than a batch system. Heat recovery is performed by means of indirect economizers (Figure 12). Steam produced in the bottom deodorized oil-cooling section is sent in a closed thermosiphon loop to the top bleached oil-heating section to heat the incoming oil. A single thermosiphon system has a recovery efficiency of  $\sim 50\%$ . With a double system, coupled with a low-pressure steam-production device, up to 75% of heat can be recovered.

Today, even for small capacity plants (10–50 TPD), semicontinuous deodorizers are clearly more suitable than conventional batch deodorizers. The shorter hold-up time in a semicontinuous deodorizer is a good argument in its favor, even in cases when small capacities with very frequent feedstock changes are required. Compared with continuous systems, the main advantages of semicontinuous deodorization are its shorter time for feedstock change as well as its much lower cross contamination. Heat recovery is, however, less efficient than in continuous operation.

### 5.3. Continuous Deodorization

Continuous deodorizers are generally preferred for high-capacity plants with few stock changes. The main advantages are the moderate investment costs, the possibility of high heat recovery and the easy maintenance.

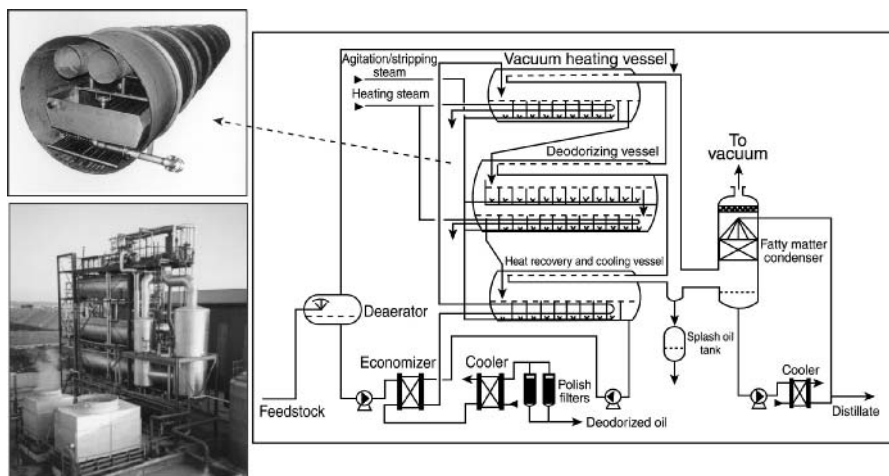


Figure 22. Continuous multivessel horizontal deodorizer (Tirtiaux).

There are several configurations of continuous deodorizers: horizontal vessels, vertical tray-type deodorizers, and packed columns.

Horizontal deodorizers consist of a series of horizontal single-shell cylindrical vessels, with agitation steam injected by multiple pipe distributors running the length of the vessel (e.g., Tirtiaux—Figure 22). Heating, deodorization, and heat recovery are performed in separate horizontal vessels. The deodorization vessels have an upper and lower deck with oil flowing from one side to the other in a shallow bed. Collection of the vapors from the different vessels is done in a separate vertical manifold. Scrubbing is performed in a combined spray and packed-tower-type fatty acid condenser.

Vertical tray-type deodorizers are probably the most commonly used type of continuous deodorizers. Their design is based on a series of trays or compartments stacked vertically in a cylindrical shell, with each tray designed for a specific task (e.g., De Smet—Figure 23). All operations, heating, deodorization, and heat

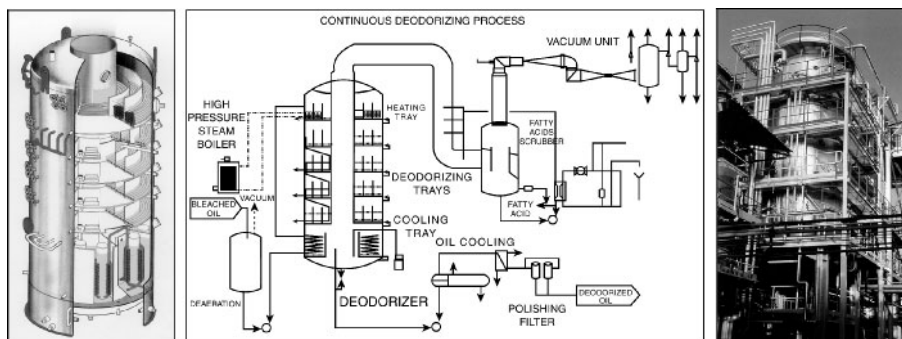


Figure 23. Continuous vertical deodorizer (Unistock-De Smet).

recovery are combined in a single vessel. Steam is injected through multiple coils, sometimes in combination with gas lift pumps to ensure perfect agitation. The oil flows in a concentric path from the inner to the outer ring, ensuring a nearly perfect plug flow. The vapors from the different compartments are collected in a central chimney and sent to a separate vapor scrubber with sprayer system. In some cases, an extra structured packing is installed in the scrubber to improve condensation and to reduce fatty matter carryover to the barometric water-condenser system.

The single vessel concept allows easy installation and maintenance as well as reducing the risk of air leakages.

Although not a real deodorizer, packed columns are also used in edible oil deodorization. A packed column is basically a single-shell vertical vessel in which a structured packing has been installed. Deaerated and fully heated oil flows over the packing from the top to the bottom while stripping steam is injected counter-currently (e.g., Alfa Laval—Figure 24). A structured-type packing with a surface of  $\sim 250 \text{ m}^2/\text{m}^3$  is most commonly used. Packed columns typically have a packing height of 2–4 m and a capacity of  $\sim 10 \text{ ton/h}\cdot\text{m}^2$  cross section at a pressure drop of 0.2–0.5 mbar per meter packing.

Packed columns are often installed to increase the capacity of existing deodorizers. Their main function is to reduce the vapor load of the deodorizer vessel

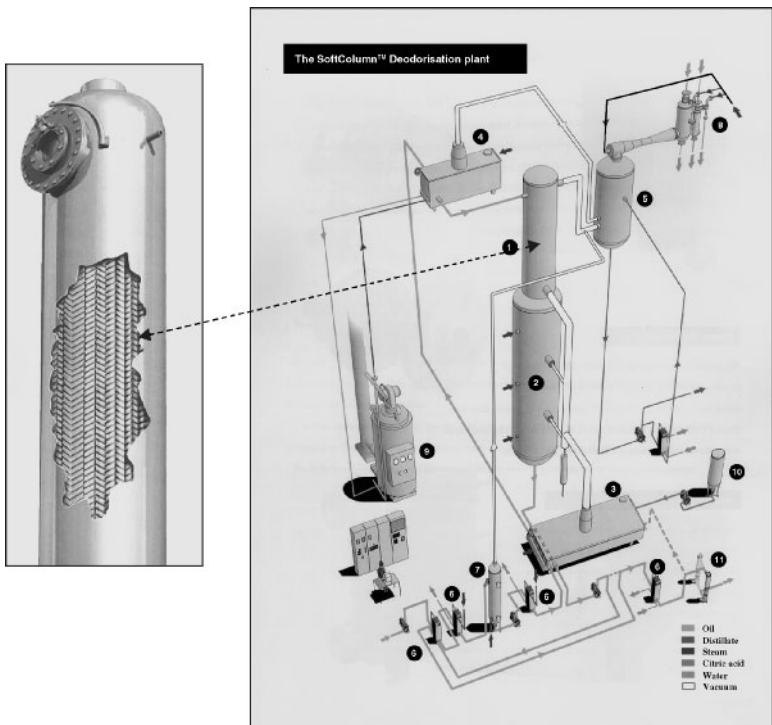


Figure 24. Packed column stripper (Soft Column–Alfa-Laval).

by prestripping most of the volatile material. The deacidified oil is then sent to the deodorizer for the final deodorization. In palm oil deodorization, for example, the FFA level is first reduced from 3–5% to < 0.5 % in a packed column before the oil enters the deodorizer vessel. In this way, the capacity of a deodorization plant can be increased significantly.

The main advantages of the packed column are the higher stripping efficiency and the short residence time (only a few minutes).

The higher stripping efficiency is important because it will result in a lower overall steam consumption to achieve a given final FFA content. However, part of this advantage is lost because of the pressure drop over the structured packing.

The combination of high stripping efficiency and short residence time make packed columns a suitable solution for the stripping of valuable minor components (tocopherols, sterols, etc.) or contaminants (pesticides, light PAH, PCB, etc.) from heat-sensitive oils. Stripping of these minor components in a classical tray-type deodorizer usually requires a higher stripping-steam consumption and longer residence time. The latter, in particular, is not desirable because it may induce unwanted side reactions such as the formation of *trans*-fatty acids, degradation of polyunsaturated fatty acids (e.g., in fish oil) or a less favorable crystallization (e.g., cocoa butter).

At the same time, however, the short residence time does not provide a great deal of heat bleaching or effective deodorization. To overcome this, a holding vessel is provided as part of the system, before or after the packed column.

Packed columns entail a higher risk of fouling due to the large oil-metal contact surface. Consequently, more frequent cleaning is required to secure consistent oil quality and, at the same time, prolong the lifetime of the structured packing. The frequency of cleaning depends largely on the type of oil processed, the frequency of plant shutdowns and feedstock changes, the purity of the feedstock, and the air tightness of the deodorizer equipment.

Risk of fouling is highest during the start-up and shut-down of the plant. Packed columns are therefore usually not recommended for semicontinuous plants. Furthermore, packed columns running on physically refined oils require more frequent cleaning (once every 6–10 months), and the structured packing needs to be replaced more often (every 2–3 years) than when processing chemically refined oils (cleaning once every year and a lifetime of 3–4 years).

In recent years, new developments in deodorization technology were mainly determined by the strong demand from edible oil refiners to reduce the capital and operating cost of deodorizers and the increased importance of the nutritional aspects of food oils and fats.

A fully modular single vessel (continuous) stand-alone deodorizer has been designed that requires no building and minimum piping. In this way, the erection costs can be significantly reduced. (Figure 25—Qualistock, De Smet). This concept allows the integration of different processing options with specific functions to improve the overall quality of the deodorized oil (deodorization at two different temperatures: so-called dual-temperature concept, deep-or shallow-bed deodorization, inclusion of a packed column section, etc.).



*Figure 25. Modular single-vessel stand-alone deodorizer (Qualistock-De Smet).*

#### 5.4. Suppliers for Oil Deodorization Processes

In addition to equipment suppliers already mentioned in this chapter (Alfa-Laval, De Smet, Tirtiaux), there are a number of other suppliers of commercial-deodorizer systems for oils and fats (e.g., Andreotti, CMB, Crown, Kirchfeld, Krupp, Lipico, Oiltek, etc.).

A detailed description of the features of every commercial deodorizer design available today is beyond the scope of this chapter.

In addition to the deodorizer types described here, there are also other designs including deodorizers custom designed for a specific company. Deodorizers are usually purchased as a unit based on performance guarantees, utility requirements, and quality of finished oil. Furthermore, the engineering competence and technical support of the supplier, as well as the after-sales service and financial credibility, are becoming more and more important factors in the final decision

### 6. FUTURE CHALLENGES

New developments in deodorization technology are driven mainly by the continuous need for more efficient processes and increased attention to the nutritional quality of food oils and fats.

Profit margins in edible oil deodorization are low. In that respect, further reduction of the operating cost is a critical factor. New heat exchangers are being developed to improve heat recovery. More efficient sparge-steam-distributor systems have been designed and, for some specific applications, packed columns have been integrated to improve stripping efficiency and, hence, reduce sparging steam consumption. Maximum valorization of the side streams (e.g., deodorizer distillate) becomes more important, to improve the overall profitability of the refining process.

For this purpose, new types of scrubbers operating at two different temperatures (“dual condensation” concept) have been developed.

Optimizing deodorization technology and process conditions for the removal of specific contaminants (pesticides, PAH, dioxins, PCB, mycotoxins, . . .) and maximum retention of the natural characteristics will be an important challenge for the future as well. In that respect, the development of new (dry condensing) vacuum systems capable of reaching a very low operating pressure in the deodorizer (1 mbar) is very important because it allows a reduction of the deodorization temperature without affecting the stripping efficiency in a negative way. Negative temperature effects (geometrical *cis-trans* isomerization, positional isomerization, and polymerization, etc.) can be minimized further by use of dual-temperature deodorizers. These deodorizers operate at different temperatures to reach the best compromise between required residence time for deodorization (at a low temperature) and heat bleaching and final stripping at a high temperature (for a short period).

Furthermore, the increasing pressure to limit environmental pollution from refineries and crushing plants as much as possible makes further improvement of the air-cleaning systems and effluent treatment plants necessary.

There remain some exciting challenges in the oil-refining industry for the future. The introduction of new technologies (e.g., use of enzymes, ultrafiltration, etc.) may require the development of specific refining and deodorization methods.

## REFERENCES

1. K. S. Markley, in A. E. Bailey, ed., *Fatty Acids: Their Chemistry and Chemical Properties*, Interscience Publishers, New York, 1947, pp. 161–162.
2. A. E. Bailey, in *Ind. and Eng. Chem.*, **33**, 404–408 (1941).
3. A. Athanassiadis, *Fat Sci. Technol.*, **90**, 522–526 (1988).
4. A. J. Dijkstra, *J. Amer. Oil Chem. Soc.*, **76**, 989–993 (1999).
5. S. Szabó Sarkadi, *J. Amer. Oil Chem. Soc.*, **35**, 479–481 (1958).
6. S. Szabó Sarkadi, *J. Am. Oil Chem. Soc.*, **35**, 472–475 (1958).
7. E. Deffense, in Proceedings of PIPOC Porim International Palm Oil Conference, Kuala Lumpur, 1993.
8. A. J. Dijkstra, Personal communication, not published.
9. M. Loncin, B. Jacobsberg, and G. Evrard, *Rev. Franç. Corps Gras*, **18**(2), 69–82 (1971).
10. Arbeitskreis ‘Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten’, *Fat Sci. Technol.*, **94**(1), 1–8 (1992).
11. G. Hénon, Zs. Kemény, K. Recseg, F. Zwoboda, and K. Kövári, *J. Amer. Oil Chem. Soc.*, **76**, 73–81 (1999).
12. W. F. J. De Greyt, Ph.D. dissertation, University of Ghent, Belgium, 1998.
13. M. León-Camacho, M. V. Ruiz-Mendéz, M. Graciani-Constante, and E. Graciani-Constante, *Eur. J. Lipid Sci. Technol.*, **103**, 85–92 (2001).
14. G. Marquez-Ruiz, M. Tasioula-Margari, and M. C. Dobarganes, *J. Amer. Oil Chem. Soc.*, **72**, 1171–1176 (1995).

15. M. C. Dobarganes and M. C. Perez-Camino, *J. Amer. Oil Chem. Soc.*, **65**, 101 (1988).
16. G. Hoffmann, *The Chemistry and Technology of Edible Oils and Fats and Their High Fat Products*, Academic Press, New York, 384 1989.
17. H. Yoshida, G. Kajimoto, and S. Emura, *J. Amer. Oil Chem. Soc.*, **70**, 989–995 (1993).
18. T. Verleyen, Ph.D. dissertation, University of Ghent, Belgium, 2002.
19. T. Verleyen, M. Forcades, R. Verhé, K. Dewettinck, A. Huyghebaert, and W. De Greyt, *J. Amer. Oil Chem. Soc.*, **79**, 117–122 (2002).
20. T. Verleyen, U. Sosinska, S. Ioannidou, R. Verhé, K. Dewettinck, A. Huyghebaert, and W. De Greyt, *J. Amer. Oil Chem. Soc.*, **79**, 947–953 (2002).
21. H. B. ten Brink, N. F. Polder, and G. A. Jongenotter, Poster presented on the 2nd EuroFedLipid Conference, Strasbourg, France, Nov. 2002.
22. J. Nilsson, Paper presented at the Nordic Lipid Symposium, Kolding, June 1999.
23. M. Miyahara and Y. Saito, *J. Agric. Food Chem.*, **41**, 731–734 (1993).
24. A. Thomas, *Fette Seifen Anstrichmittel*, **84**, 133–136 (1982).
25. D. Evans, Ph.D. dissertation, University of Greenwich, United Kingdom (1997).
26. A. Huyghebaert, Paper presented on the Symposium on 'New Trends in Edible Oil Processing, Ghent, Jan. 2000.
27. D. Buckler-Golder et al., *AEA Technology* (1999).
28. R. F. Addison and R. G. Ackman, *J. Amer. Oil Chem. Soc.*, **51**, 192–194 (1974).
29. T. Verleyen, R. Verhé, L. García, A. Huyghebaert, K. Dewettinck, and W. De Greyt, *J. Chromatogr. A*, **921**, 277–285 (2001).
30. V. Petrauskaitė, W. F. De Greyt, and M. J. Kellens, *J. Amer. Oil Chem. Soc.*, **77**, 581–586 (2000).
31. M. W. Formo, in D. Swern, ed., *Bailey's Industrial Oil and Fats Products*, 4th ed., John Wiley & Sons, New York, Vol. 1, pp. 177–232 1979.
32. E. Graciani-Constante, J. C. Bada Gancedo, F. Rodriguez Berbel, and M. V. Ruiz-Méndez, *Gracas Aceites*, **45**, 132–146 (1994).
33. M. V. Ruiz-Méndez, G. Márquez-Ruiz, and M. C. Dobarganes, *J. Amer. Oil Chem. Soc.*, **73**, 1641–1645 (1996).
34. A. V. Tsiadi, E. Stavridis, and A. Handa-Corrigan, *J. Amer. Oil Chem. Soc.*, **78**, 381–385 (2001).
35. B. Vanbrabant, Student thesis, University of Gembloux, Belgium, 1999.

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## *Hydrogenation: Processing Technologies*

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### **1. INTRODUCTION**

Hydrogenation is a means of converting liquid oils to semisolid plastic fats suitable for margarine, shortening, heavy-duty frying fats, and other specialty products (1). Liquid oils containing an undesirable component can be selectively hydrogenated to modify the component, followed by a physical separation of the undesirable component, as in the manufacture of lightly hydrogenated, winterized soybean salad oil (2–4). Hydrogenation consists of direct addition of hydrogen at the double bonds in the fatty acid chains of the triacylglycerol, or oils. For hydrogenation to take place, gaseous hydrogen, liquid oil, and nickel catalyst are placed in a specially designed reaction vessel under controlled temperature and pressure (5).

By definition, a catalyst is a substance or a compound that alters the speed of a chemical reaction without becoming a part of the reaction. The catalyst is not changed in composition or chemical structure. Nickel catalyst is the most common catalyst currently used in the hydrogenation of fats and oils. Commercially available nickel catalyst contains 22–25% active catalyst supported by a totally saturated or completely hydrogenated fat. Other catalyst supports include alumina, kieselghur, silica, as well as proprietary supports (5). The oil, catalyst, and gaseous hydrogen mixture is agitated to promote the introduction of hydrogen into the oil



**TABLE 1. Effect of Hydrogenation on Melting Point.**

Ester		Configuration	Melting Point
Linolenic	C <sub>18:3</sub>	Three double bonds	-13°C (9°F)
Linoleic	C <sub>18:2</sub>	Two double bonds	-7°C (19°F)
Oleic	C <sub>18:1</sub>	One double bond	16°C (61°F)
Stearic	C <sub>18:0</sub>	No double bonds	70°C (158°F)

and to renew the oil continuously at the catalyst surface. The rate or speed at which the reaction takes place and the type of product produced to give a particular solid fat index (SFI) curve, iodine value, and melting point depends on the following process variables: (1) starting temperature of the oil, (2) activity of the catalyst, (3) concentration of the catalyst, (4) hydrogen uptake rate, (5) reaction temperature, (6) oil quality, (7) hydrogen purity, and (8) degree of agitation. When the reaction is complete and the end point is confirmed, the batch is cooled and filtered to remove all the catalyst and other impurities. The oil may also be post bleached with filter-aid and bleaching clay.

Although one may not need to be a chemist or chemical engineer to supervise or operate the hydrogenation process, some knowledge of the chemistry of fats and oils is helpful to explain the reaction process in the oxidation and hydrogenation of oils.

The number of double bonds in a fatty ester radical significantly affects both physical and chemical properties of the triacylglycerol. The highly unsaturated (three double bonds) linolenic acid (18:3) is unstable to oxidation, and undesirable odors and flavors can develop. The rate of oxidation 18:3 is 15-fold greater than that of oleic acid (18:1).

If the linolenic ester is hydrogenated to linoleic (18:2), the relative oxidation rate is ten-fold greater than that of 18:1. The oxidation rate of 18:1 is ten-fold greater than that of stearic acid (18:0), and the oxidation potential is completely eliminated if hydrogenation proceeds to 18:0 (totally saturated, or all double bonds removed). The relative rates of 18:3 and 18:2 hydrogenation are, respectively, 40-fold and 20-fold greater than that of 18:1. As the degree of hydrogenation (or saturation) increases, the melting point of the fat increases, as shown in Table 1.

A more stable soybean salad oil (more resistant to oxidation) is manufactured by selectively hydrogenating the 18:3 ester content from 8% to less than 3%. To maximize winterizing yields and winterizing performance, it is necessary to minimize formation of 18:1 and 18:0 esters. Thus, preferential selectivity is needed to ensure that most of the 18:3 is converted to 18:2, with little conversion of 18:2 to 18:1, and very little 18:1, converted to 18:0 (2).

Lightly hydrogenated, winterized soybean salad oil became popular in the United States in the early 1960s (6), and all retail salad oil was of this type until the mid-1980s, when a new Wesson Oil was introduced. This oil, processed by the Wesson patented process, was stable and had a long shelf life without the need for hydrogenation. All other manufacturers soon changed to RBD (refined, bleached, deodorized) salad oil.

**TABLE 2. Factors Influencing *cis-trans*-isomerization.**

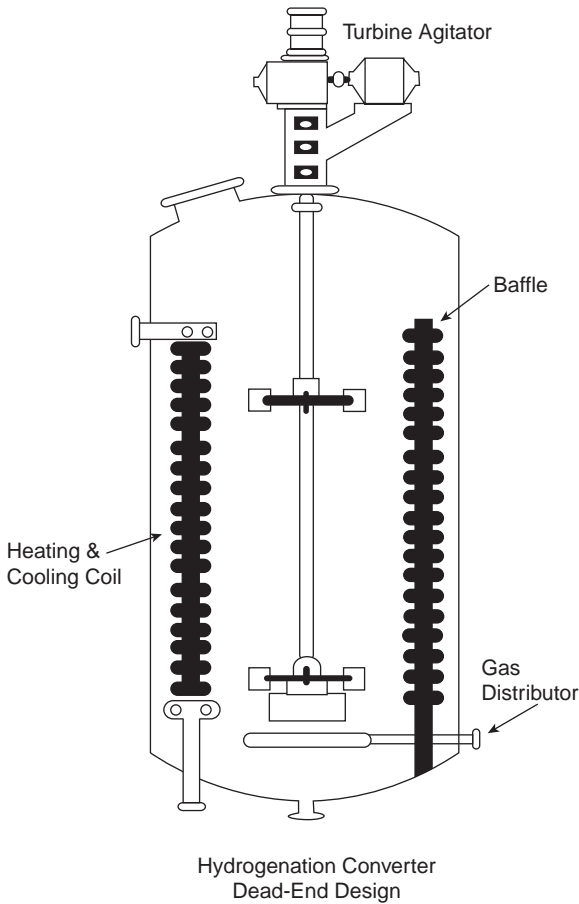
	High <i>trans</i>	Low <i>trans</i>
Temperature	High	Low
H <sub>2</sub> pressure	Low	High
Catalyst dosage	Low	High
Agitation	Slow	Fast
Catalyst	Ni-S	Ni

The manufacture of lightly hydrogenated, winterized soybean oil led to the new terms “selective hydrogenation” and “selectivity catalyst.” “Selective hydrogenation” technically defines the preferential conversion of 18:3  $\gg$  18:2 relative to 18:1 > 18:0. In practical terms, this process reflects the selective removal of double bonds via hydrogen addition such that saturated fatty acid (stearic) formation is minimized (7).

“Catalyst selectivity” is somewhat meaningless unless the term is defined. There also are selective catalysts that do not meet the technical or practical definition of hydrogen selectivity. Such catalysts are sulfur-poisoned catalyst. Sulfided nickel catalyst produces high *trans*-isomers, has lower activity than conventional nickel, exhibits longer reaction times, and is used for specialty applications (e.g., coating fats and hard butters).

Most unsaturated bonds in vegetable oils naturally occur in the *cis*-form. During partial hydrogenation, part of the *cis*-isomers is changed to *trans*-isomers. *Trans*-isomers have a dramatically higher melting point (42°C) as compared with *cis*-isomers (6°C). The creation of *trans*-isomers is desirable in margarine oil in that a higher melting point can be achieved without developing a higher level of nutritionally undesirable saturated compounds. Altering hydrogenation conditions to produce higher (or lower) *trans*-isomers is termed “*trans*-isomer selectivity.” Factors influencing *cis-trans*-isomerization are shown in Table 2.

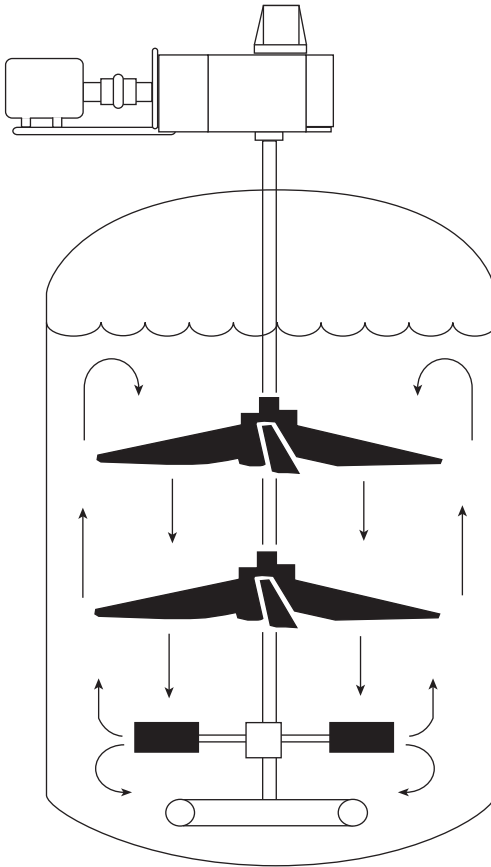
A typical hydrogenation converter is shown in Figure 1. The converter is the heart of the complete hydrogenation system. Proper design and maintenance of the hydrogen gas distributor, the agitator, and the heating cooling coils are mandatory for optimum productivity and consistency of basestocks produced. Most converters are 30,000-pound, 40,000-pound, or 60,000-pound batch sizes with some now as large as 90,000 pounds. The common agitator design provides approximately 100 rpm, and radial flow impellers are used. The lower impeller is positioned slightly above the hydrogen gas distributor; therefore, the diameter of the gas distributor and the tip-to-tip dimension of the lower impeller are critical. Originally, the middle and top impellers were of the radial flow type also. Some converters have now been operating for many years with an axial flow impeller at the top position. Although the lower and middle radial flow impellers are ideally suited for gas dispersion, the top impeller pumps the oil downward, and if positioned properly, hydrogen gas in the headspace re-enters the oil. This design has enhanced the success of dead-end hydrogenation, dramatically reducing the amount



**Figure 1.** Hydrogenation converter.

of purge or vent gas. These improvements are demonstrated in Figure 2. Other special agitation and hydrogen distribution systems have been developed, such as the Buss reactor, and the AGR (Advanced Gas Reactor), but these systems are falling out of favor because the added maintenance offsets any advantages these systems were supposed to provide.

Proper hydrogen gas distribution and agitator design is important. Stratification of reacted and unreacted areas in the converter, as a result of improper agitation or hydrogen distribution, will add to unpredictability in basestocks from batch to batch. A complete semicontinuous hydrogenation plant is depicted in Figure 3. The main features of this system are: (1) a preheating and measuring tank, (2) a reactor or converter, (3) a drop tank, (4) a heat-recovery system, (5) steam generation via reactor cooling, and (6) single-step filtration.

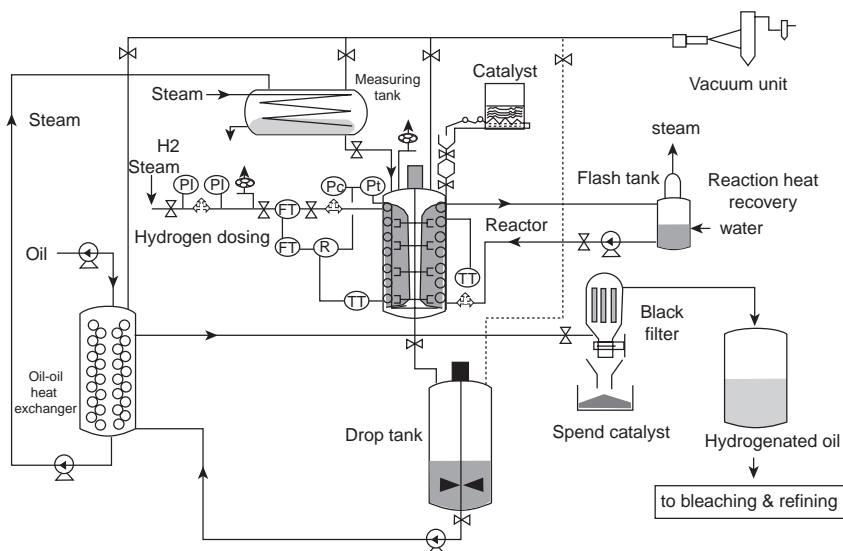


**Figure 2.** Improved hydrogenation design.

By arranging all the vessels for gravity drop, very rapid turnover of batches in the reactor results. In this manner, the reactor is used for reaction only; all heating, cooling, and filtration is accomplished external to the reactor. For example, if the average iodine value (IV) drop for all basestocks produced can be achieved in one hour, then a single system can deliver 24 batches per day (a 24-hour period).

This system also demonstrates the latest technologies in heat recovery by heat exchange of the hot oil in the drop tank with the incoming cold oil and by steam generation for reactor cooling. The hydrogenation department becomes a net exporter of steam, the ultimate form of energy conservation.

The system depicts improved reactor agitator design and improved automation. The complete process is controlled by programmable logic controllers (PLCs), giving precise in-point control that leads to extreme consistency from batch to batch.



**Figure 3.** Semicontinuous hydrogenation processing plant.

## 2. IS THERE A FUTURE FOR HYDROGENATION?

There will always be a need for hydrogenated fats to provide functionality and improved stability. Hydrogenation, as practiced today, is the only way to produce commodity-priced, hardened oils. Any alternatives are going to have higher capital and operating costs.

Although *trans*-fat in hydrogenated products is a consumer concern, the magnitude of consumer concern is unknown. If consumers believe that *trans*-fat is bad for their health, then hydrogenation (increasing saturated fat) is bad also. Products such as household shortening and heavy-duty frying fats are so high in saturated fat that any concern about using these products will be based on concerns about saturated fat as much as concerns about *trans*-fat.

In summary, there is a continued need for hydrogenation of many products, but the volume of oils hydrogenated is certain to decrease, even with a population increase. All consumers worldwide are going to develop more and more concern for improving their health and longevity. Thus, the consumption of hydrogenated oils, and oils naturally high in saturated fat, must decrease. The first step will be the elimination of *trans*-fat in food products, because we now have the technology to do that. Removal of *trans*-fat will allow us time to find ways to reduce saturated fat, and remove hydrogenation from many products.

## 3. RESEARCH ON *TRANS*-REDUCTION BY HYDROGENATION

Over the past decade, *trans*-acid reduction via hydrogenation has been of interest to catalyst producers (8) and research workers (9–11). An early study (13) clearly

showed that, with other factors being equal, i.e., pressure, agitation, and catalyst concentration, temperature has a marked effect on *trans*-acid formation in stirred batch reactors. The lower the temperature, the lower the extent of *trans*-acid formation. Although the reasons for this effect is not clearly understood, it would appear that, once triacylglycerols are adsorbed onto the catalyst surface, lower temperature favors saturation of double bonds rather than isomerization of *cis*-double bonds to the *trans*-form. Bailey (13) stated that at higher temperatures, where hydrogen lean conditions on the catalyst surface exist, more competition for the more unsaturated triacylglycerol molecules occurs. However, at higher temperatures, desorption with isomerization from *cis*- to *trans*-is more likely to occur than at lower temperatures.

*Trans*-suppression in hydrogenated oils is the subject of a paper published in 1995 in which conditions necessary for *trans*-acid reductions in soybean and canola oils are reported (8). *Trans*-suppression in hydrogenated soybean oil was observed at lower temperatures, higher pressures, and higher catalyst concentrations compared with conditions where *trans*-acid formation was maximized. For example, reactions carried out at a temperature of 204°C, a catalyst concentration of 0.02% and 15 psi hydrogen pressure, normally used for selective hydrogenation, produced a maximum *trans*-acid (44%) at an iodine value of about 70. By comparison, reducing the temperature to 77°C and increasing the catalyst concentration to 0.11% at a pressure of 250 psi resulted in a 50% reduction in *trans*-acids. The author concluded that the rate of the reaction under conditions where *trans*-acids are suppressed are sufficiently fast for large scale adaptation. However, our results suggest that, at high pressures and at a temperature of 120°C with normal catalysts concentrations, 0.02% nickel, the rate of hydrogenation is much slower than normally observed (9).

Another approach to reducing *trans*-acids involves electrochemical hydrogenation with a palladium catalyst in a solid-state electrolyte reactor (10) where about 50% reduction in *trans*-was observed compared with nickel at iodine values of about 90.

#### 4. THE TRANS-FAT ISSUE

Most unsaturated fatty acids in naturally occurring edible oils and fats contain carbon chains with double bonds in the *cis*-configuration. The *trans*-geometric isomers in unsaturated oils, and the fats produced from them, are a result of industrial processing at elevated temperatures such as physical refining, deodorization, and, particularly partial hydrogenation of the unsaturated oils. The amount of *trans*-fatty acids (TFA) formed is influenced by the duration and the temperature of processing and the initial degree of unsaturation. As a result of the presence of double bonds, unsaturated oils are more chemically reactive than saturated fats, and the reactivity increases with the number of double bonds. Thus, unsaturated oils, and especially polyunsaturated oils, are extremely vulnerable to heat, oxygen, and light. Consequently, they are not suitable for deep-frying and for the preparation of foods that are stored, such as snack foods.

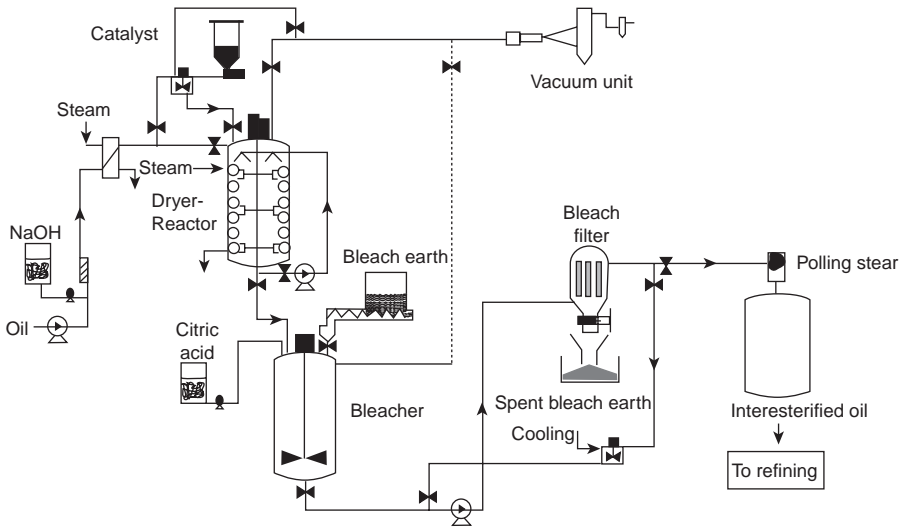


Figure 4. The chemical interesterification process.

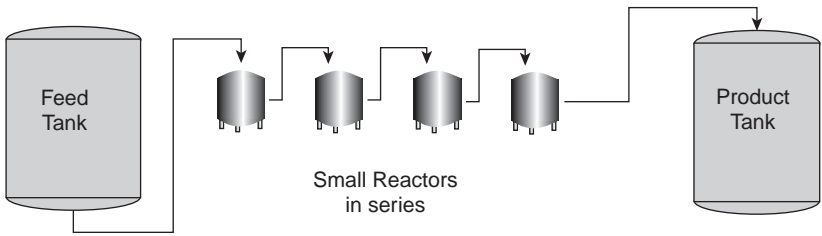
When these oils are partially hydrogenated (i.e., when hydrogen atoms are added to some of the unsaturated sites on the carbon chain in the presence of heat and a metal catalyst), the number of double bonds is reduced. The melting point and oxidative stability increase and the liquid oil is converted to a semisolid or solid resistant to oxidation and rancidity. In addition, some of the double bonds that are normally in the *cis*-configuration change into the *trans*-configuration.

The consumption of foods high in TFA has been shown to raise low-density lipoprotein cholesterol (LDL or “bad” cholesterol), which increases the risk of developing coronary heart disease (CHD). This prompted the Food and Drug Administration (FDA) to require mandatory labeling of the *trans*-fat content in foods. Food manufacturers have to comply by January 1, 2006. The FDA’s chemical definition of TFA or *trans*-fats (TF) is “unsaturated fatty acids that contain one or more isolated (i.e., nonconjugated) double bonds in the *trans*-configuration.”

The one food product that may be influenced the most by *trans*-fat concerns is margarine. Obviously, margarine must be a solid product, and this product cannot be replaced by liquid oils with improved stability. The most probable replacement for hydrogenation for margarine oils is interesterification. A chemical interesterification system is depicted in Figure 4. Although the chemical interesterification process is over 50 years old, it is coming back into popularity to process low *trans*-solid products.

A new technology, an alternative to chemical interesterification, namely enzymatic interesterification is emerging. The process was introduced in the United States in 2003. This process is patented by Novozymes. Figure 5 depicts this process in a simplified form.

Again, an interesterification system will have higher capital and operating costs, and a lower productivity than the hydrogenation process. Will food processors pay



**Figure 5.** The enzymatic interesterification process.

the higher price for interesterified margarine oils? It remains to be seen, and again, it will be driven by consumer concerns.

Another good alternative for making margarine oils will be by the use of high-stearate (high stearic-acid) soybean oil (14, 15). High-stearate soybean oil is genetically altered to have a higher (approximately 22%) stearic acid content. With a small amount (approximately 3%) of near-zero-IV soybean oil (zero *trans*-) added, a good tub margarine can be made. To make a stick margarine, the high-stearate soybean oil can be fractionated, with a high percentage of the stearine portion going into the stick margarine formulation.

The author is developing (mid-2004) zero *trans*-, no hydrogenation, no inter-esterification, no modification of any kind, tub and stick margarines. The formula will include specially processed soybean oil, corn oil, cottonseed oil, or mid-oleic sunflower seed oil, and double-fractionated palm oil, or double-fractionated cottonseed oil. The products are proprietary for now.

The leading manufacturer of prepared poultry products is changing (mid-2004) to a blend of lightly hydrogenated soybean oil and corn or cottonseed oil. This offers good fry-life while being zero *trans*-per serving size. The hydrogenated soybean oil lowers the price of the blend as a result of the rising cost of the highly stable liquid oils.

The genetically altered oils, 3% linolenic acid, and now 1% linolenic acid soybean oil, may become popular as zero *trans*-frying fats, as the size of the crop for these seeds advances. These oils would be zero *trans*- and no hydrogenation. As touted by the "Better Bean Initiative," the 3% linolenic variety could become the mainstream variety, requiring no identity preservation. (Note: If 3% linolenic acid soybean oil is lightly hydrogenated, less *trans*-would be produced because of the lower initial linolenic acid content).

One must understand that the inter-esterification process, chemical or enzymatic, is of no value in making heavy-duty frying fats. The high percentage of unsaturated oil, even with inter-esterification and addition of near-zero IV fat to provide plasticity, is still unstable under heavy-stress frying conditions.

The most logical zero *trans*-frying fats are the naturally stable oils, such as cottonseed oil, corn oil, peanut oil, and mid-oleic sunflower seed oil. The use of these oils increased dramatically in 2003 and early 2004 for this purpose. Already in limited supply, this heavy demand will dramatically increase the price of these oils.



The leading manufacturer of household shortening (and frying fat) is introducing (mid-2004) a new formula made up of lightly hydrogenated soybean oil, high palmitic refracted cottonseed oil (45% palmitic), and a low percentage of zero IV soybean or cottonseed oil (near-zero IV is zero *trans*-). This product provides zero *trans*-per serving size of a food product, and no interesterification is needed.

A new zero *trans*-, no hydrogenation, no modification of any kind, rich in omega-3 fatty acids, RBD soybean oil frying fat has been developed (patented) by Carolina Soy Products (Warsaw, North Carolina). Extensive fry tests prove that this oil has a fry life equal to, and often better, than the heavy-duty hydrogenated (high *trans*-) frying fats. The secret to this process is in the processing, not modification. This soybean oil (regular variety) is extruded and expeller-pressed, no solvents, no harsh chemicals, and is physically refined.

## 5. HYDROGEN SUPPLY FOR HYDROGENATION

For laboratory and pilot plant hydrogenation, high-pressure hydrogen gas cylinders can be used. For large industrial hydrogenation systems, either liquid hydrogen is



**Figure 6.** Steam/Methane Reforming Plant HYDRO-CHEM Processing, Subsidiary of Linde AG. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

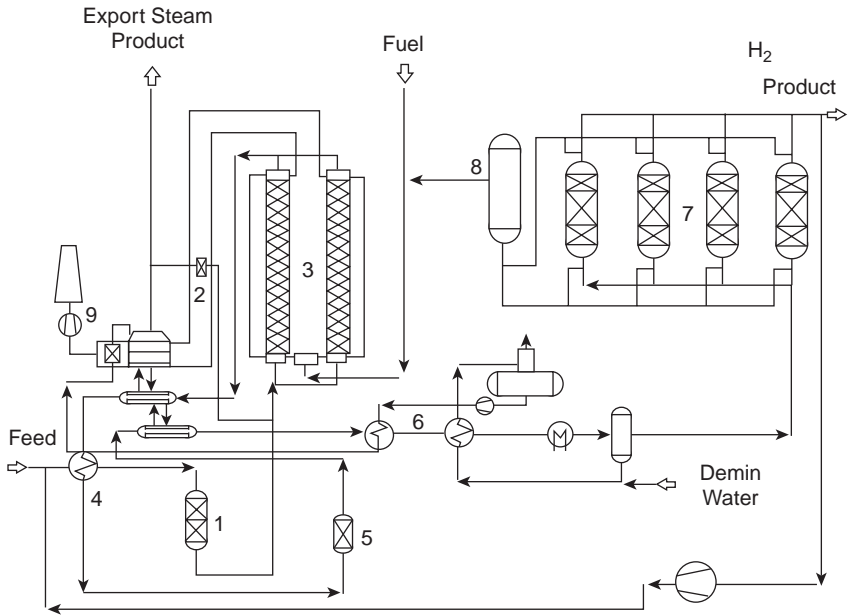


Figure 7.

stored on site, or there is an on-site hydrogen gas-generating plant. Justification on using liquid hydrogen is dependent on the proximity of the liquid hydrogen manufacturing facility, and on the contract the hydrogen manufacturer is willing to negotiate. Often, particularly for a new plant, installation of an on-site hydrogen-generating plant can be justified. A typical high-capacity hydrogen-generating plant is shown in Figure 6.

Needless to say, there will continue to be a need for hydrogenation for some products, certainly for coating fats, cocoa butter substitutes, cake icing, and cookies and crackers. These products, often blended with sweeteners, will probably become indulgence foods, to be used sparingly. The main fats and oils in the diet must be low *trans*-, lower saturated fat, and with a minimization of hydrogenation. Although offering challenges to the fats and oils manufacturer and food processor/food service, these challenges should be welcomed and embraced to improve the health of mankind.

## REFERENCES

1. D. R. Erickson and M. D. Erickson, in D. R. Erickson, E. H. Pryde, T. L. Mounts, O. L. Brekke, and R. A. Falb, eds., *American Soybean Association Monograph, Handbook of Soy Oil Processing and Utilization*, AOCS Press, Champaign, Illinois, 1980, pp. 218–238.

2. G. R. List and T. L. Mounts, in D. R. Erickson, E. H. Pryde, T. L. Mounts, O. L. Brekke, and R. A. Falb, eds., *American Soybean Association Monograph, Handbook of Soy Oil Processing and Utilization*, AOCS Press, Champaign, Illinois, 1980, pp. 193–214.
3. G. E. Hammerstrand and G. R. List, in D. R. Erickson, E. H. Pryde, T. L. Mounts, O. L. Brekke, and R. A. Falb, eds., *Handbook of Soy Oil Processing and Utilization*, AOCS Press, Champaign, Illinois, 1980, pp. 214–216.
4. R. D. O'Brien, in D. R. Erickson, E. H. Pryde, T. L. Mounts, O. L. Brekke, and R. A. Falb, eds., *American Soybean Association Monograph, Practical Handbook of Soy Oil Processing and Utilization*, AOCS Press, Champaign, Illinois, 1995, pp. 258–276.
5. H. B. W. Patterson, *Hydrogenation of Fats and Oils: Theory and Practice*, AOCS Press, Champaign, Illinois, 1994.
6. J. C. Cowan, *Soybean Digest*, **26**(12); 48–53 (1966).
7. R. R. Allen, *J. Amer. Oil Chem. Soc.*, **45**; 312A (1968).
8. J. Hasman, *Inform*, **8**; 1150–1158 (1995).
9. J. W. King, R. L. Holliday, G. R. List, and J. M. Snyder, *J. Amer. Oil Chem. Soc.*, **78**; 107–113 (2001).
10. K. Warner, W. E. Neff, G. R. List, and P. Pintauro, *J. Amer. Oil Chem. Soc.*, **77**; 1113–1117 (2000).
11. F. Eller, J. Teel, K. R. Steidley, and G. R. List, *Effects of Pressure and Temperature on Oil Properties*, Abstracts, World Conference on Oilseeds, Istanbul, Turkey, 2002.
12. Stingley and R. J. Wrobel, *J. Amer. Oil Chem. Soc.*, **38**; 201–205 (1961).
13. A. E. Bailey, *Industrial Oil and Fat Products*, 2nd ed., Interscience Publishers, New York, 1951, pp. 690–708.
14. G. R. List, T. L. Mounts, F. Orthoefer, and W. E. Neff, *J. Amer. Oil Chem. Soc.*, **73**; 729–732 (1996).
15. G. R. List, T. Pelloso, F. Orthoefer, K. Warner, and W. E. Neff, *J. Amer. Oil Chem. Soc.*, **78**; 103–104 (2001).
16. W. E. Farr, in R. F. Wilson, ed., *Hydrogenation: Proceedings of the World Conference on Oilseed Processing/Utilization*, AOCS Press, Champaign, Illinois, 2001.
17. W. E. Farr and G. R. List, in N. T. Dunford, ed., *Hydrogenation Techniques, Nutritionally Enhanced Processing*, AOCS Press, Champaign, Illinois.

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## *Supercritical Technologies for Further Processing of Edible Oils*

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### 1. INTRODUCTION

Supercritical CO<sub>2</sub> (SCCO<sub>2</sub>) technology has been widely investigated for the processing of fats and oils because SCCO<sub>2</sub> offers an environmentally friendly alternative compared with the conventional processes involving organic solvents, resulting in solvent-free extracts and residues obtained under moderate operating conditions. The ability to modify solvent properties by changing operating conditions (temperature and pressure) or by the addition of cosolvents makes the SCCO<sub>2</sub> process versatile, giving a unique advantage. This operational flexibility enables the processor to fine tune solvent properties for the specific separation problem at hand.

Health benefits of minor lipid components, such as tocopherols, sterols, and certain fatty acids ( $\omega$ -3 fatty acids [ $\alpha$ -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)],  $\gamma$ -linolenic acid, and conjugated linoleic acid) have been widely investigated in recent years (1, 2). Increasing evidence of health benefits of these lipid components coupled with changing consumer attitudes (increased awareness of diet-health link and increased tendency to self-medicate),

which is reflected in the considerable growth in the functional foods and nutraceutical market (3) have led to the re-evaluation of conventional fats and oils processing for the recovery/concentration of these bioactive components. Supercritical fluid (SCF) technology has been increasingly used for the processing of nutraceuticals, including bioactive lipids, on a commercial level as it provides a solvent-free, “natural” product, which has a wide consumer appeal.

With such major developments in the field of SCF technology, the objective of this chapter is to provide a critical overview of the solubility behavior of lipid components in  $\text{SCCO}_2$ , which is fundamental for optimal process design as well as the unit operations of extraction, fractionation, and reactions using  $\text{SCCO}_2$  as applied to fats and oils processing.

## 2. DEFINITION AND PROPERTIES OF SUPERCRITICAL FLUIDS

A fluid is in its “supercritical state” at temperatures and pressures higher than its critical values (Figure 1). Critical temperatures and pressures ( $T_c$  and  $P_c$ , respectively) of selected solvents are listed in Table 1. The critical point defines the highest pressure and temperature at which gas and liquid phases can coexist. As the critical point is approached, the distinction between the gaseous and liquid phases diminishes such that their properties are identical at the critical point.

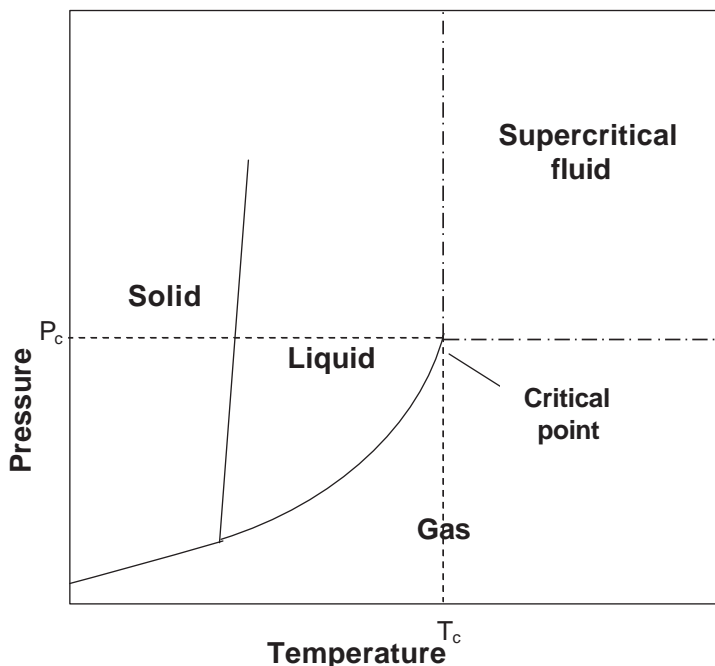


Figure 1. A typical phase diagram.

**TABLE 1. Critical Points of Selected Solvents (4).**

Solvent	T <sub>c</sub> (K)	P <sub>c</sub> (MPa)
Carbon Dioxide	304	7.38
Ethane	305	4.88
Ethylene	282	5.03
Propane	370	4.24
Propylene	365	4.62
Methanol	513	8.09
Acetone	508	4.70
Benzene	562	4.89
Toluene	592	4.11
Ammonia	406	11.3
Water	647	22.0

**TABLE 2. Properties of Gases, Liquids, and Supercritical Fluids (5).**

	Density (g/mL)	Viscosity (g/cm.s)	Diffusivity <sup>a</sup> (cm <sup>2</sup> /s)
Gas, 101.3 kPa, ~15–30°C	(0.6–2)*10 <sup>-3</sup>	(1–3)*10 <sup>-4</sup>	0.1–0.4
Supercritical fluid, T <sub>c</sub> , P <sub>c</sub>	0.2–0.5	(1–3)*10 <sup>-4</sup>	0.7*10 <sup>-3</sup>
~T <sub>c</sub> , 4P <sub>c</sub>	0.4–0.9	(3–9)*10 <sup>-4</sup>	0.2*10 <sup>-3</sup>
Liquid, ~15–30°C	0.6–1.6	(0.2–3)*10 <sup>-2</sup>	(0.2–2)*10 <sup>-5</sup>

<sup>a</sup> Self-diffusion for gas and SCF, binary mixture for liquid.

Supercritical fluids are attractive solvents as they exhibit physicochemical properties intermediate between those of liquids and gases (Table 2). The density, thus the solvating power, of a SCF approaches that of a liquid, whereas the diffusivity and viscosity are intermediate between gas-like and liquid-like values, resulting in faster mass transport capacity (5). As a result of the large compressibility near their critical points, SCFs' densities/solvent power can be varied by changing operating conditions (temperature and pressure), resulting in operational flexibility, which can be exploited to achieve the required separation.

Carbon dioxide is the solvent of choice for food applications. It is an inert, non-toxic, nonflammable, environmentally friendly solvent with a moderate critical temperature (31°C) and pressure (7.4 MPa), which is readily available in high purity and low cost (6).

### 3. HISTORIC DEVELOPMENT AND COMMERCIAL APPLICATIONS

The discovery of the critical point of substances dates back to the 1820s when Cagniard de la Tour observed the disappearance of the gas-liquid meniscus at temperatures higher than critical values under pressure (7). The solvent power of SCFs has

been reported as early as 1879 (8). Although potential applications of near-critical fluids (SCFs and liquefied gases) had been proposed since the 1930s for various extraction and separation processes, such as the separation of high-molecular-weight mixtures (9), de-asphalting of petroleum (10), and purification of fatty oils (11), it was the work of Zosel (12) at the Max-Planck Institute in Mannheim, Germany, that brought this technology into commercial focus in the 1960s, which eventually led to its commercialization for coffee decaffeination and hops extraction purposes in Germany in the late 70s and early 80s.

Since then, a number of supercritical fluid extraction (SFE) plants have been built around the world in varying sizes (ranging from 4 L to 6,500 L) for the processing of natural products such as hops, tobacco, spices and herbs, aromas, and nutraceuticals (13–17). In addition to extraction processes, applications of SCF technology have widened in recent years to include fractionation, particle design, coating, aerosols, impregnation, cleaning, supercritical water oxidation, analytical extraction and chromatography, production scale chromatography, extrusion, nucleation, infiltration of materials into polymers, and chemical reactions (15, 17).

Motivations for commercialization of SCF technology included concerns over the use of organic solvents, which was reflected in tightening government regulations (for example, prohibition of the use of methylene chloride for coffee decaffeination in Germany was the driving force behind the commercialization of supercritical coffee decaffeination technology), changing consumer attitudes, improved product quality, increased demands on product performance, and development of innovative products or processes (14, 16). SFE has been increasingly used in recent years around the world for the processing of nutraceuticals as a “natural” alternative to traditional solvent-extraction processes. The ability to claim “natural extracts” in marketing these products is a significant advantage in today’s marketplace. SFE also offers the advantage of mild operating conditions for heat-sensitive compounds (compared with distillation), and a solvent-free extract and residue (compared with solvent extraction). In addition, it provides an oxygen-free environment and, thus, limits oxidative degradation of the product.

#### 4. SOLUBILITY BEHAVIOR OF LIPID COMPONENTS

Fats and oils are complex mixtures containing lipid components belonging to major lipid classes, such as fatty acids, acylglycerols, and esters of fatty acids, and minor lipid components, such as sterols, tocopherols, hydrocarbons (e.g., squalene), and pigments ( $\beta$ -carotene and others). Successful application of SCF technology to any process requires information on the solubility behavior of the solutes of interest as affected by operating conditions and solute properties. Totally predictive modeling of multicomponent phase behavior in SCFs has not been realized yet. Therefore, experimental solubility measurements play an essential role in both development of thermodynamic models and process design. The accuracy/reliability of the experimental data also determines the success of thermodynamic modeling studies. Although solubility behavior of binary systems of lipids and  $\text{SCCO}_2$  has been

widely investigated (18–20), data on ternary and multicomponent systems are quite scarce. Multicomponent data are available for a limited number of systems, such as fatty acid ester mixtures, deodorizer distillates, and vegetable oils (21–25). A systematic in-depth analysis of the available solubility data ranging from binary to ternary and multicomponent systems has been carried out by the authors to establish the general solubility trends of minor and major lipid components as affected by temperature, pressure, and mixture composition, to study the deviation from binary behavior and assess implications for process development targeting fractionation of complex lipid mixtures (26–29).

#### 4.1. Binary Systems

Available literature solubility data of pure lipids belonging to major (fatty acids, mono-, di- and triacylglycerols, and fatty acid esters) and minor lipid classes (pigments, sterols, vitamins, and hydrocarbons) in  $\text{SCCO}_2$  were compiled (26, 27). These references (26, 27) contain exhaustive bibliography on lipid +  $\text{SCCO}_2$  binary systems. Literature data were correlated using Chrastil's equation, which is an empirical model used quite commonly to correlate the solubility of lipid components (30). This model is based on the formation of a solute-solvent complex on association of the solute and solvent molecules and establishes a linear relationship between  $\ln(\text{solubility})$  and  $\ln(\text{density})$  as follows:

$$\ln c = k \ln d + a/T + b, \quad (1)$$

where  $c$  is the solubility of the solute in the supercritical solvent (g/L),  $d$  is the density of the pure solvent (g/L), and  $k$  (association number) is the number of molecules in the solute-solvent complex. Parameter  $a$  is dependent on the total heat of the reaction (heat of solvation + heat of vaporization), and  $b$  is dependent on the molecular weights of the solute and solvent and the association constant. Parameter  $k$ , which is the slope of the solubility isotherm, reflects the density dependence of solubility. Parameter  $a$ , which is the slope of  $\ln(\text{solubility})$  versus  $1/T$  plot, is a measure of the temperature dependence of solubility at constant density. At constant temperature, Equation 1 simplifies to

$$\ln c = k' \ln d + b'. \quad (2)$$

Chrastil's equation was adopted in the systematic study of binary solubility behavior because it is easy to use and it does not require information on the properties of lipid components. Its parameters can then be used to interpret the effect of operating conditions on solubility. Its value, however, is limited for predictive modeling of solubility data, which should involve in-depth thermodynamic models (e.g., using an Equation of State (EOS) approach), describing all the phases present at equilibrium.

The study of the binary component solubility database revealed wide discrepancies between experimental data reported by different researchers, primarily due to



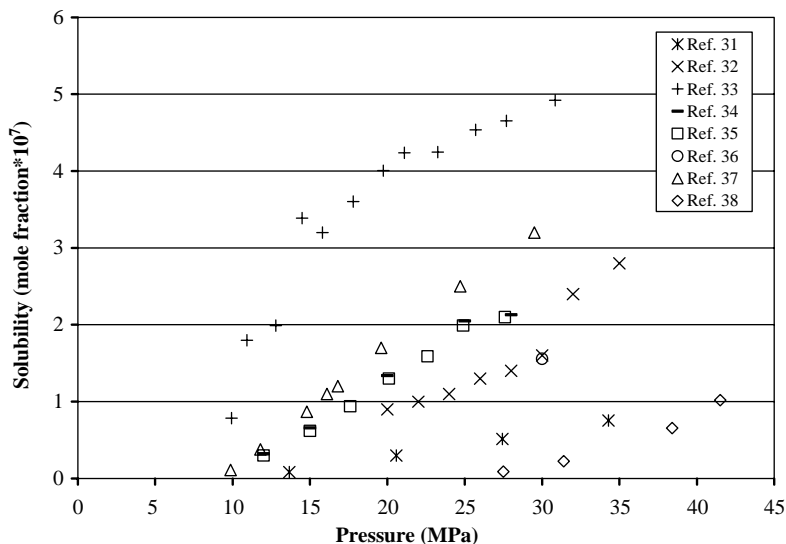


Figure 2. Solubility isotherms of  $\beta$ -carotene at 313 K.

limitations of the experimental methods used and purity of samples. Although discrepancies were observed for a number of solutes, they were most apparent for sensitive solutes of low solubility, such as  $\beta$ -carotene (Figure 2). Impurities present in the samples as well as those deriving from sample degradation and isomeric purity of the solutes contributed to this variation. This finding highlights the need to exercise extreme caution not only when making solubility measurements, but also when interpreting the data.

Solute solubility behavior in  $\text{SCCO}_2$  is determined by solute vapor pressure and intermolecular interactions between the solute and  $\text{SCCO}_2$  and, hence, is affected by operating conditions and solute properties. In binary systems of a homologous series (such as fatty acids), where intermolecular interactions are similar, solute solubilities are determined by molecular weight/vapor pressure such that solubility increased with decreasing molecular weight of the solute (Figure 3). Unsaturation in a compound affected solubility mainly through its effect on the physical state of the solute, as the melting point of fatty acids decreases with the introduction of double bonds. For example, the solubility of liquid oleic acid was substantially higher than that of stearic acid, which is a solid under the temperature and pressure conditions examined (Figure 3). As the differences between solute properties such as polarity increase, their molecular weight does not correlate with solubility in  $\text{SCCO}_2$ , hence, other factors, such as specific molecular interactions, should also be considered. For example, in the acylglycerol series of oleic acid, (Figure 4, solubility isotherms constructed using Equation 2), oleic acid was the most soluble solute followed by mono- and diolein (for which relative solubilities were density dependent, such that more polar but lower molecular weight mono-olein was more soluble at low fluid densities). Triolein was the least soluble solute in this series.

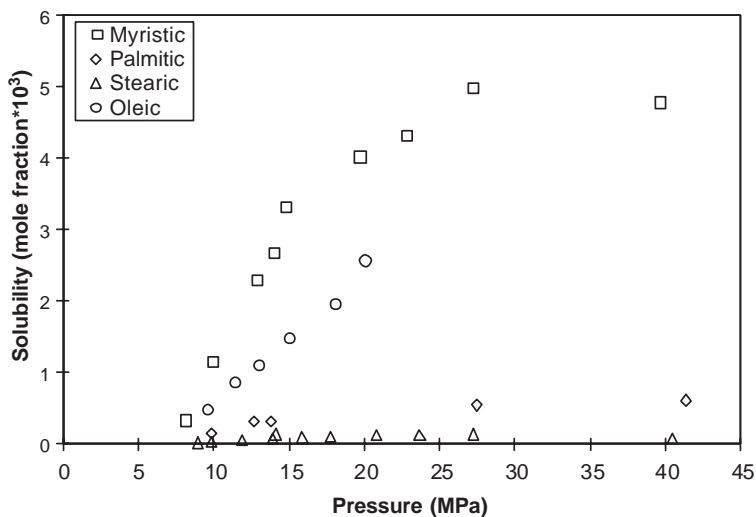


Figure 3. Solubility isotherms of myristic, palmitic, stearic, and oleic acids in  $\text{SCCO}_2$  at 308 K (26).

When the solubility of various minor lipid components were compared with that of selected components of other major lipid classes, it was found that  $\alpha$ -tocopherol, oleic acid (a liquid fatty acid), and squalene were the most soluble solutes and  $\beta$ -carotene had the lowest solubility in  $\text{SCCO}_2$  (Figure 5, solubility isotherms constructed using Equation 2).

Although the physical state of the solute had a significant impact on lipid solubility behavior, information on melting behavior of lipids in  $\text{SCCO}_2$  (e.g., melting

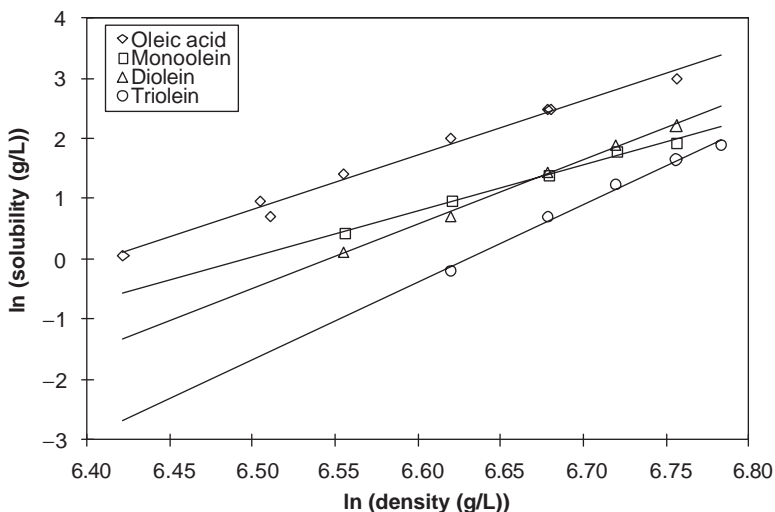
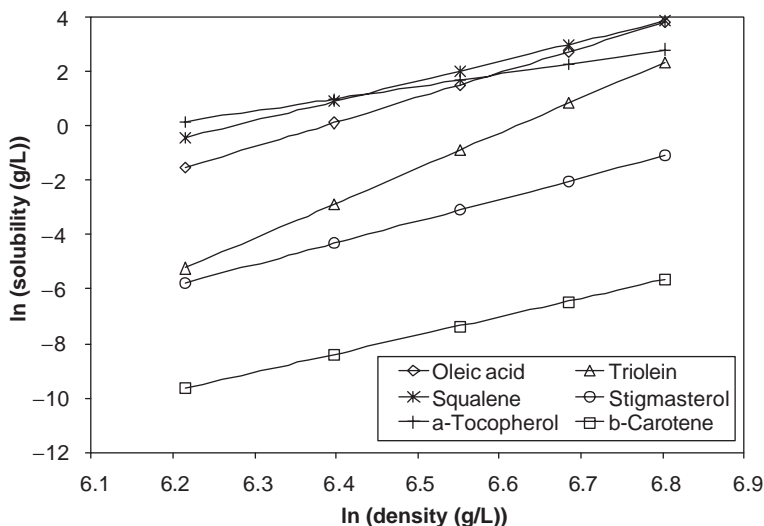


Figure 4. Solubility of isotherms for acylglycerol series of oleic acid in  $\text{SCCO}_2$  at 323 K. (Solid lines represent regression results obtained using Equation 2).



**Figure 5.** Solubility isotherms of lipid components at 323 K plotted using model parameters estimated using Equation 2.

point depression in  $\text{SCCO}_2$ ) was rather limited. Further research is required to provide information on the melting behavior of lipids in binary and multicomponent systems. Melting behavior of solutes should be noted during solubility measurements directly (using a view cell) or indirectly by studying the solute after the measurements for any evidence of melting.

Lipid solubility increased with pressure for all the studied solutes, whereas a solubility maximum was observed in the solubility isotherms of solid solutes such as stearic acid,  $\beta$ -carotene, and  $\beta$ -sitosterol at pressures higher than 30 MPa (26, 27). Although the investigated liquid systems did not exhibit similar behavior, solubility maxima have been well established for vegetable oils at high pressures. Increasing the temperature at constant  $\text{CO}_2$  density increases the solubility because of an exponential increase in the solute vapor pressure. A temperature effect at constant  $\text{CO}_2$  density on solubility was observed for all compounds, although the magnitude of the effect varied. An isobaric increase in temperature decreases the solvent density and increases the vapor pressure of the solute. The overall impact of these two opposing effects of temperature on solubility is dependent on the pressure, resulting in a crossover of solute solubility isotherms. Below the crossover pressure, the density effect predominates and the solubility decreases with increasing temperature, which is referred to as retrograde behavior. Above this crossover point, the solubility increases with temperature because of the vapor pressure effect. In general, solid solutes (such as stigmasterol,  $\beta$ -carotene, and solid fatty acids) had a low crossover pressure and, therefore, showed nonretrograde behavior in the range of operating conditions commonly employed. Although the presence of a crossover point in solid systems has been well established previously, liquid systems and the effect of melting on the crossover behavior have not been addressed

adequately. Although a crossover pressure was observed for some liquid lipid components, solubility of liquid solutes, such as fatty acid esters, decreased with temperature in the investigated experimental range.

Binary data can be used to determine the optimum fractionation conditions in the design of fractionation processes for mixtures such as fatty acid esters. For more complex mixtures containing different lipid classes, such as deodorizer distillates, or for mixtures/mixture components that undergo melting, the effect of operating conditions on multicomponent solubility behavior can vary greatly, depending on mixture composition. Regardless, binary solubility data can still provide invaluable information for the fractionation of lipid components and estimation of the degree of separation. For example, fractionation of solutes with similar binary solubilities, such as fatty acids, tocopherols, and squalene, would be very difficult to achieve by varying the operating conditions and may warrant additional processing steps; whereas solutes with different solubilities, such as triacylglycerols/sterols and fatty acids, can be separated with ease in a fractionation column.

## 4.2. Cosolvent Systems

It is well known that the phase behavior of solutes in  $\text{SCCO}_2$  can be modified by the addition of a small amount of cosolvent, such as ethanol. The main effect of a cosolvent is the solubility enhancement that results from an increase in the density of  $\text{SCCO}_2$  + cosolvent mixture or intermolecular interactions between the cosolvent and particular solutes. Selectivity of a separation can be improved by cosolvent addition only if there are specific intermolecular interactions between the cosolvent and one or more of the mixture components, as solubility of all mixture components is enhanced due to the density effect.

Literature equilibrium solubility data of ternary systems of major and minor lipid components, cosolvents, and  $\text{SCCO}_2$  have been compiled (28), and the effect of cosolvent addition on the solubility behavior of fatty acids (stearic, palmitic, and behenic acids), squalene, and  $\beta$ -carotene studied. Cosolvent effect is quantified as solubility enhancement, which is the ratio of solubility obtained with cosolvent addition to that without a cosolvent (28). This reference (28) contains an exhaustive bibliography on cosolvent +  $\text{SCCO}_2$  + lipid systems.

Solubility enhancements observed for lipid components in  $\text{SCCO}_2$  + ethanol are summarized in Table 3. The high solubility enhancement observed in the presence of ethanol for fatty acid systems were attributed to H-bonding interactions. Such specific intermolecular interactions between a solute and a cosolvent can be exploited for fractionation of lipid mixtures.

In the literature, the thermodynamic advantages of cosolvent addition have been emphasized; however, the effect of cosolvents on other aspects of the process, such as mass transfer, overall cost, and product/residue properties, has not been considered in depth. Benefits of cosolvent addition must be balanced against its disadvantages for a specific application. Cosolvent introduction and solvent recovery (separation of the cosolvent from the extract, SCF, and solids residue) increase the complexity of process design. As well, an increase in solvent loading may result

**TABLE 3. Cosolvent Effect (Solubility Enhancement) of Ethanol in Lipid Systems.**

Solute	Solubility Enhancement <sup>a</sup>	Ethanol Concentration	T (K)	P (MPa)	Data from ref.
<i>Fatty acids</i>					
palmitic acid	1.5–63.7 <sup>b</sup>	1.0–8.8 <sup>e</sup>	308	9.9, 19.7	39
stearic acid	1.2–63.2 <sup>b</sup>	0.5–8.8 <sup>e</sup>	308, 318	8–19.7	39–41
behenic acid	2.0–29.2 <sup>b</sup>	1.21–6.7 <sup>e</sup>	308, 318	8–16	42
<i>Minor lipid components</i>					
β-carotene	2.2–9.8 <sup>b</sup>	0.3–2.4 <sup>f</sup>	313–333	15–28	43
stigmasterol	4.0 <sup>b</sup>	3.5 <sup>g</sup>	308	15.2	44
squalene	1.8–5.9 <sup>c</sup>	4.1–12.0 <sup>f</sup>	333	20–27.5	45
<i>Vegetable oils</i>					
palm oil	20 <sup>d</sup>	10 <sup>f</sup>	343	20	46
pistachio oil	4.8 <sup>d</sup>	10 <sup>f</sup>	333	34.5	47

<sup>a</sup>Solubility enhancement = the ratio of solubility obtained with cosolvent addition to that without a cosolvent.

<sup>b</sup>Calculations based on solubility in mole fraction.

<sup>c</sup>Solubility in w/w.

<sup>d</sup>Solubility in wt %.

<sup>e</sup>mol % in the supercritical phase (solute inclusive).

<sup>f</sup>wt % (solute free).

<sup>g</sup>mol % (solute free).

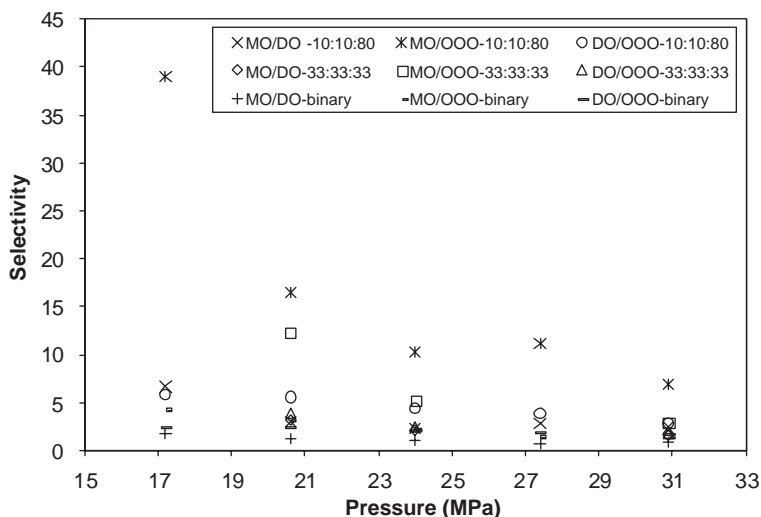
in the coextraction of undesirable compounds. The effect of cosolvent addition on the sample matrix and solutes of interest, such as alteration of functional properties of extraction residue and degradation of the extract by the cosolvent, should also be considered. Cosolvents should be used with caution as one of the major advantages offered by SCCO<sub>2</sub>, namely the ability to produce “natural” products with no organic solvent residue, may be negated. Ethanol is the cosolvent of choice for food applications because of its GRAS (Generally Recognized As Safe) status; but the removal of ethanol from the extract and residue requires the application of heat via evaporation, which may be detrimental to the quality of the extract and residue. This step also undermines another advantage of SCCO<sub>2</sub> extraction that it can be carried out at low temperatures.

### 4.3. Multicomponent Systems

Literature phase equilibrium data of ternary and higher (quaternary and quinary) systems of lipids and SCCO<sub>2</sub> have been compiled (29). This reference (29) contains exhaustive bibliography of multicomponent lipid + SCCO<sub>2</sub> systems. For systems where an adequate number of data points were available, partition coefficients and selectivities were calculated, and the data analyzed by plotting vapor phase concentration (solubility in SCCO<sub>2</sub>), liquid phase concentration, partition coefficients, and selectivities as a function of pressure to determine the effect of operating conditions and feed composition on solubility behavior (29). The ternary systems studied included SCCO<sub>2</sub> and two triacylglycerols (trilaurin (LLL)/tripalmitin

(PPP), trimyristin (MMM)/PPP, LLL/MMM), two fatty acids (oleic acid (OA)/linoleic acid (LA)), two fatty acid methyl esters (methyl myristate (MeM)/methyl palmitate (MeP) and methyl oleate (MeO)/methyl linoleate (MeL)), fatty acid/triacylglycerol (OA/triolein (OOO)), and fatty acid methyl ester/fatty acid (MeO/OA). The quaternary and quinary systems analyzed contained three triacylglycerols (LLL/MMM/PPP), three acylglycerols (mono-olein (MO)/diolein (DO)/OOO), and four triacylglycerols (PPP/palmitoyl-dioleoylglycerol (POO)/oleoyl-dipalmitoylglycerol (PPO)/OOO), respectively, in SCCO<sub>2</sub>. Binary solubility data were also included in the analysis to determine any deviation from expected behavior based on binary data.

The value of binary data was limited in predicting solubility behavior in ternary and higher lipid systems as multicomponent solubilities deviated from binary behavior. Although solubility of the less soluble component increased in ternary systems of solid triacylglycerols in SCCO<sub>2</sub>, that of the more soluble component stayed the same, or was not affected. Solubility diminution was observed for both solutes in some liquid mixtures, such as fatty acid (oleic acid/linoleic acid) and fatty acid ester (methyl oleate/methyl linoleate) mixtures. The extent of this diminution was dependent on the initial feed concentration of the solute. However, in other liquid mixtures, solubility enhancement for one of the mixture components was also observed (for example, for oleic acid in the presence of methyl oleate). These deviations, in turn, affected the separation efficiency (assessed in terms of partition coefficient and selectivity) of the solutes of interest. Separation efficiency was lower than that predicted by binary data when the solubility of the less soluble solute was enhanced, in the mixture. On the other hand, separation efficiency was improved if the solubility of the more soluble component was enhanced, as observed in the quaternary mixture of SCCO<sub>2</sub> and acylglycerols (MO/DO/OOO). Figure 6 shows that



**Figure 6.** Selectivities (MO/DO, DO/OOO, MO/OOO) in quaternary acylglycerol mixture (data from Ref. 20).

the presence of a less polar component in the mixture (OOO, 33% or 80% of feed mixture) leads to solubility enhancement of the polar mixture components (MO and DO). This “dilution” effect can have important implications for fractionation processes, as it may improve the separation efficiency significantly if the more polar components are more soluble than the diluting component.

## 5. SUPERCRITICAL FLUID PROCESSING OF FATS AND OILS

The ability to fine tune solvent properties of SCFs through changes in operating conditions can be exploited in a wide range of applications, such as extraction, fractionation, and reaction processes, where flexibility in process implementation offers the researcher/processor a gamut of possibilities.

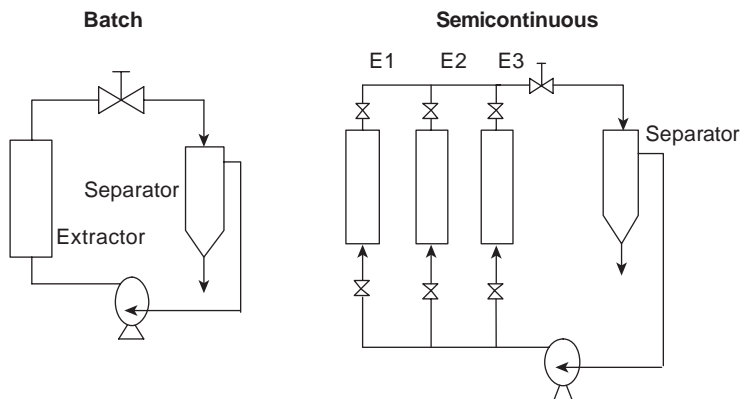
Supercritical fluid processing of fats and oils has been widely investigated over the last three decades. Earlier work on SCF processing of fats and oils as reviewed by Brunner and Peter (48) included extraction of oil from oilseeds, extraction of fat from egg yolk and starch-containing vegetable matter, extraction of lanolin from wool grease, refining of natural oils, purification of monoacylglycerols, and fractionation of cod liver oil, using a variety of solvents such as propane, propylene, ethylene, CO<sub>2</sub>, and ethane. Although some of the earlier studies focused on the extraction of commodity oils, such as canola and soybean (49, 50), extraction of specialty oils (oils high in bioactive components) (51–55) and fractionation of fats and oil mixtures (56, 57) have been the subject of more recent studies.

### 5.1. Supercritical Fluid Extraction

#### 5.1.1. Fundamentals

**5.1.1.1. Mode of Operation** The SFE process consists of two basic steps: extraction and separation. During the extraction step, the soluble material is extracted under high pressure from the solid feed material and transported away by the SCF. Separation of the supercritical solvent from the extract (regeneration of the supercritical solvent and recovery of the solute) can be achieved by reducing the density/solvent power of the SCF solvent by decreasing the pressure or by elevating the temperature or both. The process must be adapted to the separation problem at hand, considering the target material (58).

The process can be operated on batch, semicontinuous, or continuous mode (59). Batch processing (Figure 7) involves contacting a batch of solid feed material with a continuous solvent stream. However, the necessity to depressurize the vessel for the introduction of a new batch of material limits the efficiency of the process. Therefore, most production plants are operated semicontinuously, where three or four vessels are operated in a cyclic fashion, such that as one vessel is being extracted, another is being loaded, and a third vessel is being pressurized/depressurized (Figure 7). Continuous introduction of the solid feed material into the



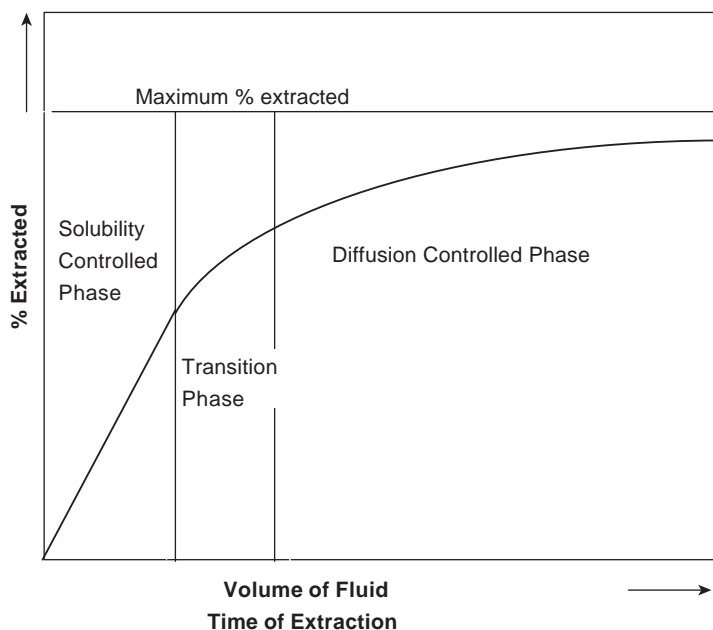
**Figure 7.** Typical supercritical fluid extraction systems.

extraction vessel under high pressure poses an equipment design challenge, especially for the handling of large volumes of oilseeds. Lock hopper vessels and screw conveyors are some alternatives that are in operation or under development for the continuous processing of solid feed material under high pressure (7, 60).

**5.1.1.2. Extraction Kinetics** As in any extraction process, SFE kinetics consists of three periods; solubility-controlled or a period of constant extraction rate, a transition period of falling extraction rate, and diffusion-controlled or asymptotic period with respect to solute extraction rate (Figure 8). During the initial period, the solute's accessibility results in an extraction rate that is constant. Hence, the slope of the extraction curve, weight of extract versus volume or weight of  $\text{CO}_2$ , can be used to determine the loading of the solvent. This value corresponds to solubility only if equilibrium is attained during extraction by operating at low enough solvent flow rates. The extraction rate starts to decline in the transition period and the mechanism of extraction kinetics is switched to diffusion of the solvent and solute into and out of the sample matrix. Therefore, efficiency of SFE process is affected by operating conditions (i.e., temperature, pressure, flow rate, solvent-to-feed ratio) and feed material properties (i.e., particle size, particle density, and porosity) and these parameters should be optimized for an efficient process. Flaking has been shown to be the most efficient pretreatment for the SFE of many oilseeds (49, 61–63). Mass transfer modeling of SFE of oilseeds has been studied by various researchers (49, 64, 65).

**5.1.2. Applications in Fats and Oils Processing** Supercritical  $\text{CO}_2$  extraction of oilseeds has been the focus of extensive research activity in the 1980s when a variety of oilseeds, such as soybean, cottonseed, corn germ, rapeseed, and sunflower, were extracted using  $\text{SCCO}_2$  (66). Although  $\text{SCCO}_2$  extracted oils were shown to have similar quality compared with hexane extracts, they also had lighter color and lower iron and phospholipid content, resulting in a lower refining loss and reduction of subsequent refining steps (67–70). However, the oxidative stability of





**Figure 8.** A typical supercritical fluid extraction curve.

CO<sub>2</sub> extracts was lower than that of hexane-extracted oils, which was attributed to the lower phospholipid content of the CO<sub>2</sub> extracts (71, 72). Protein quality of the CO<sub>2</sub>-extracted meal was comparable with that of hexane-extracted meal (73). In spite of the high volume of research carried out on extraction of oilseeds, commercial-scale SCCO<sub>2</sub> extraction of oilseeds was not readily accepted. Even though the overall process based on SCCO<sub>2</sub> extraction would be simpler compared with conventional hexane extraction in terms of eliminating the need for hexane evaporators and meal desolventizer, the high equipment costs associated with the SCCO<sub>2</sub> process and the inability to achieve continuous processing of high volumes of oilseeds under SCF conditions have been cited as the major impediments to commercialization of the SCCO<sub>2</sub> process (60). However, recent developments in equipment design (i.e., the coupling of CO<sub>2</sub> with expeller technology) and stricter government regulations on the use of hexane may make this a reality in the near future.

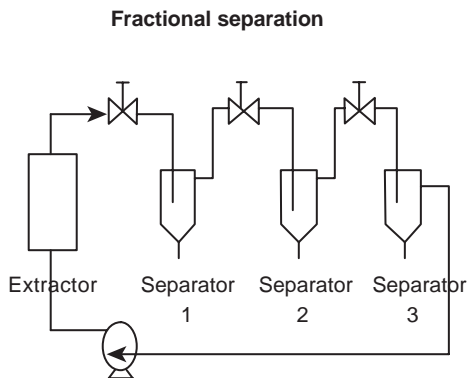
In the case of specialty oils, the cost of SFE can be balanced by the high value of the product and the added advantage of “natural” processing. Rice bran (74–77) and corn fiber oils (78) are currently receiving attention because of their high sterol content. Cereals such as wheat germ (79, 80) and barley (81) in addition to plants such as *Silybum marianum* (milk thistle) (82) have also been investigated as sources for the production of oils rich in tocopherols. SCCO<sub>2</sub> extraction of oils rich in carotenes from sources such as palm fruit and oil fibers (83, 84) have also been

reported. In the case of highly unsaturated oils, such as evening primrose (51–53), flaxseed (54), and poppy seed oils (55), which are good sources of  $\alpha$ - and  $\gamma$ -linolenic and linoleic acids, the ability to extract at mild operating conditions and in an oxygen-free environment offers a significant advantage of SCCO<sub>2</sub> processing for these labile oils.

## 5.2. Supercritical Fluid Fractionation

**5.2.1. Fundamentals** In SCF fractionation, separation is based on differences in the solubility behavior of mixture components deriving from both the differences in component volatilities (as in distillation) and the differences in intermolecular interactions between the mixture components and the SCF solvent (as in solvent extraction), as noted previously (12). SCF fractionation of extracts can be achieved by fractional extraction or fractional separation. During SFE, fractionation occurs as a function of time, which is reflected in the compositional differences of the fractions collected throughout the process. Fractional extraction, thus, relies on compositional differences between fractions obtained throughout the extraction. It is largely governed by the differences in the solubility behavior of extract components, but may also be affected by the location of the solutes in the solid sample matrix. During the extraction of vegetable oils, fractionation of minor components, such as phospholipids, sterols, and tocopherols, occurs as evidenced by their higher concentrations in specific fractions (50). Concentration of free fatty acids (FFA) has also been observed during SCCO<sub>2</sub> extraction of oilseeds (50, 85). Free fatty acids are usually concentrated in the earlier fractions as a result of their higher solubility compared with that of triacylglycerols (85); however, Friedrich et al. (50) observed increasing concentrations of FFA in the later fractions during soybean oil extraction. Eggers and Sievers (63) observed that a higher amount of water was extracted compared with oil toward the end of SCCO<sub>2</sub> extraction for rapeseed press-cake, and they attributed this to the higher transport resistance of water relative to oil in the cake. The compositional differences between fractions can be accentuated by using a density gradient, which can be achieved by increasing the pressure or adding a cosolvent at certain time intervals during extraction. In fractional separation (Figure 9), where the differences in the solubility behavior of extract components are exploited, fractionation is achieved after the extraction step using a series of separators operated at conditions adjusted to yield a stepwise decrease in solvent power/density.

Supercritical fractionation of a liquid lipid feed material is usually carried out in a packed column. Standard columns are not available commercially and have to be custom built either in-house or by manufacturers of extraction units. Lab-scale and pilot-scale supercritical columns, 0.6–13.6 m high with internal diameters of 14.3–68 mm are available in research labs around the world and have been used for the processing of deodorizer distillates (56, 57, 86–90), vegetable and fish oils (91–105), cocoa butter, and milkfat (106–109). A schematic diagram of a typical SCCO<sub>2</sub> fractionation column (2.8 m, 2.54 cm o.d.), which was designed and built



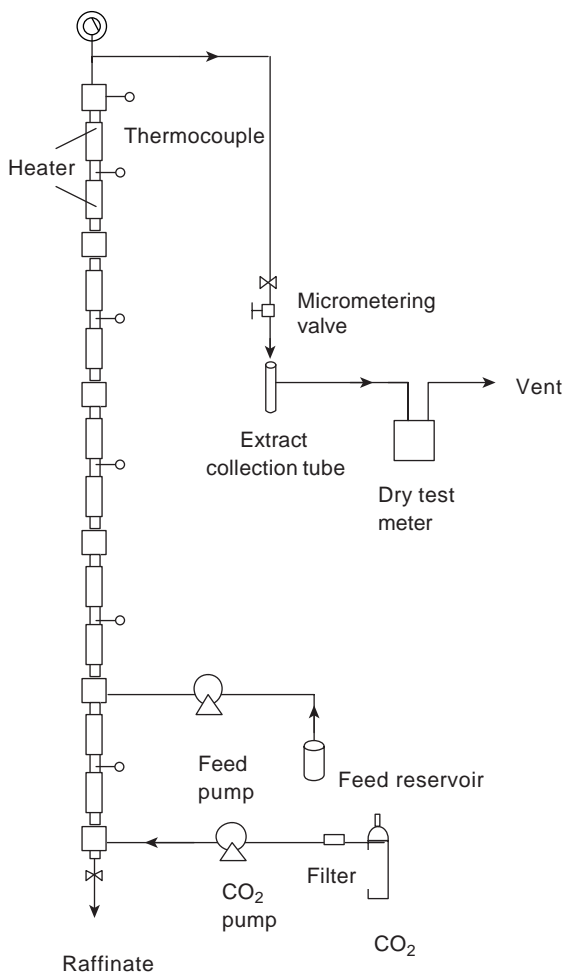
**Figure 9.** A typical supercritical fluid fractional separation system.

in our lab and others is shown in Figure 10. Packed columns can be operated in a continuous mode by introducing the liquid feed material into the column using a feed pump to achieve a cocurrent or countercurrent operation. A thermal gradient along the column can be applied to generate an internal reflux such that feed components are subjected to higher temperatures (i.e., lower solvent densities) as they move up the column, resulting in enhanced separation efficiency. In earlier fractionation column designs, the thermal gradient was achieved using a hot finger mounted at the top of the column (12, 110), whereas in recent designs, the thermal gradient is achieved by independent temperature control of column zones (96). An external reflux can also be generated by adding an external reflux pump (111).

**5.2.2. Applications in Fats and Oils Processing** Supercritical CO<sub>2</sub> fractionation of fats and oils has been investigated by various researchers for the refining (deacidification and degumming) of oils, for the concentration of bioactive components of fats and oils and byproducts and for the fractionation of milkfat.

Supercritical CO<sub>2</sub> processing has been investigated as an alternative to traditional oil refining processes for various oils, such as palm (93, 112), rice bran (75, 92, 96), olive (91, 103), black cumin seed (113), peanut (94), and soybean oils (114). Dunford and King (92) achieved deacidification of rice bran oil to a level of <1% FFA while retaining free sterol and oryzanol contents at 0.35% and 1.8%, respectively, using a SCCO<sub>2</sub> fractionation column. Ooi et al. (93) studied refining of palm oil using continuous column fractionation and fractional separation with three separators and reported that the addition of a cosolvent (ethanol) improved the refining process. List et al. (114) used the low solubility of phospholipids in SCCO<sub>2</sub> to achieve degumming of soybean oil.

Byproducts of conventional oil extraction and refining have been investigated as raw materials for the concentration of bioactive components. Birtigh et al. (115) investigated SFE of carotenes and tocopherols from waste products of palm oil production (i.e., the residue of mechanical processing and palm leaves). Ibanez et al. (116) studied the separation of tocopherols from olive byproducts using fractional



**Figure 10.** Schematic diagram of  $\text{SCCO}_2$  fractionation column.

separation with two separators. Fractions from the first separator contained triacylglycerols, waxes, and sterols, whereas the tocopherols were concentrated in the second separator.

Supercritical fluid extraction, fractional extraction, fractional separation, and column fractionation have all been used to concentrate the bioactive components of deodorizer distillates. During the SFE of deodorizer distillates (57, 117, 118), FFA were preferentially extracted, whereas tocopherols and sterols were enriched in the raffinate. Lee et al. (117) concentrated the tocopherols in the sterol-free, esterified soybean distillate (containing 18 wt% tocopherols) up to 40 wt% using  $\text{SCCO}_2$  extraction, and concluded that a countercurrent multistage column was required to achieve a higher tocopherol concentration. Chang et al. (119) studied

SCCO<sub>2</sub> fractional extraction of soybean deodorizer distillate using an extractor with a reboiler at the bottom and obtained 83.6% tocopherol recovery at the optimal conditions of 31.03 MPa, 363 K top, and 343 K bottom temperature. Free fatty acids and squalene were concentrated in the extract together with tocopherols, whereas sterols were concentrated in the raffinate.

Supercritical CO<sub>2</sub> column fractionation of soybean (57, 86, 88, 89) and rice bran oil (57) deodorizer distillates has been investigated to enrich their sterol and tocopherol contents. Brunner et al. (89) reported that the FFA were enriched in the top (in the extract), whereas the monoacylglycerols, tocopherols, and diacylglycerols were enriched in the bottom (in the raffinate) fraction during the fractionation of soybean oil deodorizer distillate. Saure and Brunner (88) achieved a tocopherol concentration of more than 70% (w/w) using continuous column fractionation of soybean deodorizer distillate where squalene was almost completely found in the top product, whereas sterols and tocopherols were enriched in the bottom product. King and Dunford (57) developed a two-step column fractionation scheme (13.6 MPa and 27.2 MPa at 313 K) for the enrichment of phyosterols from soybean and rice bran oil deodorizer distillates such that the FFA were removed in the first step and sterols were enriched in the oil fraction in the second step.

Supercritical CO<sub>2</sub> column fractionation has also been investigated for the production of squalene concentrates from olive oil deodorizer distillates (56, 87). Bondioli et al. (87) used saponification and esterification steps to convert the FFA and fatty acid esters to triacylglycerols in order to improve squalene separation prior to countercurrent continuous fractionation. The highest squalene purity and extraction yield was achieved at 15 MPa and 313 K, using a temperature gradient of 303–323 K along the column to improve the squalene purity and yield. Ruivo et al. (120) studied countercurrent packed column fractionation of model lipid mixtures containing 40% and 70% squalene and methyl oleate, and achieved squalene yields as high as 90%.

Supercritical fluid technology has also been investigated for the enrichment of minor oil components. SFE coupled with supercritical fluid chromatography has been investigated for the enrichment of ferulate phytosterol esters from corn bran (121) and corn fiber oils (122), as well as tocopherols from soybean (123), wheat germ (123–125), and rice bran oils (123). Enrichment of squalene by SCCO<sub>2</sub> extraction of olive husk oil (126) and column fractionation of shark liver oil (98, 99), as well as column fractionation of cod liver oil for the recovery of Vitamin A (98), have also been studied. Ibanez et al. (105) investigated the use of column fractionation for the concentration of sterols and tocopherols from olive oil and concluded that SCCO<sub>2</sub> processing offers an alternative for the value-added processing of low-quality olive oil.

The use of fish oils as raw materials for the production of EPA and DHA concentrates has been widely investigated for nearly two decades (97, 101, 102, 104, 110). The fractionation of fish oil triacylglycerols is a rather complex process, where an esterification step usually precedes the fractionation where the triacylglycerols are converted into more soluble fatty acid esters. Although earlier studies used batch column fractionation to attain the concentration of EPA and

DHA (97, 102), recent research is focused on the continuous column operation (101, 104).

Milkfat is a mixture of triacylglycerols, which vary greatly in their physical properties such as melting point. Milkfat fractionation offers the potential of tailoring milkfat fractions for specific food applications, which would, in turn, increase milkfat use. SCCO<sub>2</sub> fractionation of milkfat has been investigated using fractional extraction with a density gradient (127, 128) and packed columns (106, 107), resulting in the concentration of triacylglycerols according to carbon number and yielding fractions with varying melting behavior. Although earlier work targeted the removal of cholesterol from milkfat (106), recent efforts on column fractionation of milkfat focus on the concentration of conjugated linoleic acid for its nutraceutical properties (109).

### 5.3. Lipid Reactions in Supercritical Fluid Media

Although the use of a SCF as a reaction medium has been investigated as early as 1947 (129), the earliest reports of enzymatic reactions in SCFs date back to the studies of Hammond et al. (130), Randolph et al. (131), and Nakamura et al. (132) in 1985/86, who investigated the activity of polyphenol oxidase, alkaline phosphatase, and lipase in SCFs, respectively. Since then there has been increasing research activity on the use of SCFs as reaction media, which has been motivated by their unique physicochemical and solvent properties (133–137). Some of the advantages associated with the use of SCFs as reaction media include higher conversion rates that can be achieved compared with those in conventional media; the ability to control phase behavior of the reaction mixture and, hence, the reaction by varying temperature and pressure; eliminating the use of organic solvents; ease of product recovery; and potential of coupling the reaction process with extraction and fractionation steps carried out under supercritical conditions.

#### 5.3.1. Fundamentals

**5.3.1.1. Mode of Operation** Reactions in SCFs can be carried out in batch or continuous mode. In batch processing, after loading the reactor with the reagents and the catalyst (possibly an enzyme) in the desired ratios, the reactor is heated and pressurized by pumping in the SCF. Molecular sieves can be used to dry the SCF prior to entering the reactor if the presence of moisture is detrimental to the desired conversion. Batch reactors are also stirred to decrease the external mass transfer resistance. In enzymatic reactions, the mixture components are not mixed prior to pressurization and the reaction is initiated by stirring the reactor contents. Alternatively, the reaction can be initiated by introducing the enzyme to the system after pressurization using a catalyst addition device (138, 139). Noncatalytic reactions usually require high temperatures (200–250°C for the case of glycerolysis of soybean oil in SCCO<sub>2</sub>) (140). In this case, the reaction is terminated by rapidly cooling the reactor. Samples can be withdrawn from the reaction mixture at various time intervals using sampling valves/loops to follow the progress of the reaction.

Reactions can also be carried out under continuous solvent flow conditions. Substrates can be introduced directly into the pressurized solvent stream or, alternatively, SCF can be saturated with the substrate(s) by passing it through a saturation column(s) containing the substrate(s) prior to their introduction into the reactor. Sampling can be carried out prior to depressurization using sampling loops/valves, and online analysis is possible by introducing a sample directly to a chromatographic system. Samples can also be collected on depressurization of the solvent stream. External loops can be used to recirculate the solvent or reaction mixture as well as for sampling purposes. The use of sapphire windows on a reactor cell enables visual observation of the reaction mixture. Continuous enzymatic reactions can be carried out in packed bed (141–148) or supported membrane reactors (149).

**5.3.1.2. Control of Phase Behavior** One of the main advantages of using SCFs as a medium for conducting chemical reactions is the ability to control the phase behavior of the reaction mixture and, hence, the reaction (rate, selectivity, product recovery) by varying temperature and pressure (133–137). As in the case of extraction and fractionation processes, the use of SCFs as reaction media gives the processor the ability to modify the phase behavior of the reaction system through changes in operating conditions. A good understanding of the phase equilibria is thus required to control the reaction and fully exploit the advantages of supercritical solvents (136).

Visual observation of the reaction provides valuable information on phase behavior. Yan and Nagahama (150) observed the phase behavior of the reaction system containing tuna oil + free lipase + water (or buffer solution) during the hydrolysis of tuna oil in SCCO<sub>2</sub> using a high-pressure view cell at 313 K. They observed two liquid phases before the addition of CO<sub>2</sub>, a lower aqueous phase containing the enzyme and an upper oil phase. An additional CO<sub>2</sub> phase was formed on pressurization to 7.8 MPa. The volume of the oil phase increased significantly as a result of CO<sub>2</sub> solubilization and numerous small water droplets were distributed throughout the oil phase. They postulated that the hydrolysis reaction took place at the interface between the dispersed water phase containing the lipase and the continuous lipid phase.

Gunnlaugsdottir et al. (151) studied the phase behavior of the reaction system cod liver oil + ethanol + immobilized lipase in SCCO<sub>2</sub> using a sapphire view cell. They concluded that at 9–24 MPa and 313 K, the reaction system was comprised of three phases: solid (enzyme)-liquid-vapor throughout the reaction (151).

Reaction mixtures are complex multicomponent systems, and their phase behavior is dictated by the composition of the mixture and operating conditions. Organic solvents present in the reaction medium as reagents may act as cosolvents and result in solute solubility enhancement (as discussed in Section 4.2). For example, the decrease in reaction rate observed at high ethanol concentrations for the lipase-catalyzed esterification of myristic acid + ethanol in SCCO<sub>2</sub> has been, in part, attributed to the solubility enhancement of water, resulting in drying of the enzyme

support (138). Marty et al. (152) also observed a decrease in enzyme activity with an increase in substrate ethanol concentration during the esterification of oleic acid with ethanol. However, they attributed the decrease in enzyme activity to substrate inhibition rather than the cosolvent effect of ethanol causing drying of the enzyme support as this situation persisted even at higher water concentration. Also, organic solvents have also been shown to affect the reaction rate through their effect on the extent of aggregation of the substrate. For example, cholesterol aggregation with the addition of cosolvents like iso-butanol and tert-butanol led to an increase in cholesterol oxidation rate because of an increase in local cholesterol concentration and stronger binding of the cholesterol oxidase enzyme to cholesterol aggregates (153). Special consideration should also be given to the solubility behavior of water in  $\text{SCCO}_2$  (154–157), as it can be of great consequence in enzymatic reactions (see Section 5.3.1.5).

By using SCFs, the phase behavior of the reaction system can be modified to yield a single homogeneous reaction phase, thereby enhancing the solubility and accessibility of reactants, resulting in higher reaction rates. For example, the problems resulting from the low solubility of hydrogen in the liquid oil phase in hydrogenation reactions of fats and oils can be overcome by carrying out the reaction in a single homogeneous phase in either supercritical  $\text{CO}_2$  or propane (158–161).

It is also possible to shift the equilibrium of the reaction by removing the formed reaction products (according to the Le Chatelier's principle) (162). For example, the ability to remove water in continuous esterification reactions in  $\text{SCCO}_2$  has been cited as one of the advantages of using  $\text{SCCO}_2$  versus traditional solvents, such as hexane (163). In their study on the interesterification of tricaprylin and methyl oleate in  $\text{SCCO}_2$ , Adschiri et al. (164) concluded that the selective extraction of the reaction product, methyl caprylate shifted the equilibrium of the reaction forward.

One of the main advantages of using SCFs as reaction media derives from their unique solvent properties, which can be exploited to simplify product recovery steps (162). Extractive (continuous) reactions involve the removal of the reaction products in the SCF stream. The reaction products can be further fractionated using fractional extraction or fractional separation. If fractional extraction is carried out to concentrate the reaction product, then the reaction and separation parameters cannot be optimized individually. Fractional extraction of a single homogeneous reaction phase results in lower selectivity, as the product will be contaminated with unreacted reagents (151). Gunslaugsdottir et al. (151, 165–168) investigated the concentration of ethyl esters produced by alcoholysis of cod liver oil in  $\text{SCCO}_2$  using fractional extraction. Marty et al. (169) studied post reactional separation of an esterification system (oleic acid + ethanol) in  $\text{SCCO}_2$  using a series of four separation vessels and reported good selectivities and product recovery.

**5.3.1.3. Mass Transfer Enhancement** Low viscosity, high diffusivity, and very low surface tension of SCFs can improve the rates of diffusion-limited reactions (such as enzymatic reactions) by reducing mass transfer resistances (170). Mass



transfer limitations play a significant role in enzymatic reactions, where the internal mass transfer limitation (i.e., diffusion resistance) present in the pores of enzyme support should also be considered in addition to the transfer of the substrate from the bulk to the surface of the enzyme support (139). In batch reactors, external (bulk) mass transfer limitations can be eliminated by agitation. No external diffusion limitations were observed in the lipase-catalyzed esterification of oleic acid and ethanol (152) and myristic acid and ethanol (138) in  $\text{SCCO}_2$ ; however, Knez et al. (144) observed external limitations in the batch esterification of oleic acid with oleyl alcohol, as evidenced by the effect of rotational speed and mixture viscosity on the reaction rate.

Internal mass transfer limitations can be characterized by calculating the Thiele modulus (139), or by determining the effect of support particle size on reaction rate (142, 144). Internal limitations were present in the esterification reaction of oleic acid with oleyl alcohol in  $\text{SCCO}_2$ , as evidenced by increasing enzyme activity with decreasing size of the support (diameter of enzyme beads) (144). During the esterification of ethanol and myristic acid, internal limitations were present in the hexane system to a higher degree than in the  $\text{CO}_2$  system (such that hexane reaction was diffusion-controlled, whereas an intermediate rate, somewhere between kinetic- and diffusion-controlled, was observed for the  $\text{SCCO}_2$  reaction) (139). However, Steytler et al. (142) observed that reaction rates were independent of support bead diameter in the esterification of butanol and lauric acid in  $\text{SCCO}_2$ . No external and internal mass transfer limitations were observed in the lipase-catalyzed interesterification reaction of trilaurin and myristic acid, in a continuous flow packed bed reactor as the flow rate was changed (171).

**5.3.1.4. Effect of Pressure on Reaction Rate Constant** Pressure can have a direct impact on the reaction rate through its effect on the reaction rate constant. The pressure dependence of the reaction rate constant and unusual partial molar behavior of a solute in a SCF can result in enhancement of the reaction rate in the critical region of the mixture (136). According to the transition state theory (172, 173), pressure enhances the rate of a reaction if the activation volume (difference in the partial molar volumes of the activated complex and the reactants) is negative, whereas the reaction is hindered by pressure if the activation volume is positive.

**5.3.1.5. Use of Enzymes** Lipases are widely used in the processing of fats and oils as catalysts of a number of important lipid reactions, such as hydrolysis, esterification, and transesterification reactions (174). There are a wide number of lipases obtained from different sources, which are available commercially in their free/crude or immobilized form. However, enzymes with a higher tolerance of pressure would be welcomed, and more research is needed to hopefully develop such enzymes (i.e., genetic engineering or marine sources of the deep ocean).

The activity and stability of lipases have been widely investigated to ensure their compatibility with  $\text{SCCO}_2$  as a reaction medium (132, 144, 147, 149, 152, 175–181). Stability tests involve exposing the enzyme to  $\text{SCCO}_2$  for an extended period

of time (for example at 30 MPa and 313 K for 24 h) and using it as a catalyst in the reaction of interest under atmospheric conditions. The activity of the used enzyme is then compared with that of a fresh enzyme. In the presence of  $\text{SCCO}_2$ , most lipases maintained their activity and had good stability (132, 144, 147, 149, 152, 175–181).

The effect of pressure and the pressurization-depressurization steps on enzyme structure/conformation, interactions between  $\text{SCCO}_2$  and the enzyme, pH, and water content of the enzyme are factors that influence enzyme activity in  $\text{SCCO}_2$ . Penninston (182) observed that multimeric enzymes were inhibited by application of pressure, whereas activities of monomeric enzymes were enhanced or not affected by the application of hydrostatic pressure in aqueous solutions. He attributed the observed inactivation to the dissociation of protein multimers held together by noncovalent interactions. Protein/enzyme denaturation has been reported during the pressurization and depressurization steps in  $\text{SCCO}_2$  (183, 184). Enzymes with disulfide bridges were inactivated to a lesser extent in  $\text{SCCO}_2$  (176, 183), whereas the extent of enzyme inactivation increased with the number of pressurization/depressurization steps (183). Minimal conformational changes in cholesterol oxidase were reported in  $\text{SCCO}_2$  and  $\text{SCCO}_2$ /cosolvent mixtures at 10.4 MPa (153). Weder (185, 186) attributed the unfolding and partial fragmentation of lysozyme and ribonuclease observed at 30 MPa and 80°C to the presence of water and effect of temperature.

Lower lipase activity observed in the transesterification of methylmethacrylate with 2-ethylhexanol in  $\text{SCCO}_2$  was attributed to the formation of carbamates between  $\text{CO}_2$  and free amino groups on the enzyme surface (187, 188). The decrease in pH as a result of  $\text{CO}_2$  solubilization in the microaqueous environment surrounding the enzyme is also thought to contribute to a decrease in enzyme activity (188).

Water affects the reaction rate through its effect on reaction kinetics and protein hydration, which is required for optimal enzyme conformation and activity. Enzymes need a small amount of water to maintain their activity; however, increasing the water content can decrease the reaction rate as a result of hydrophilic hindrance/barrier to the hydrophobic substrate, or because of denaturation of the enzyme (189). These opposite effects result in an optimum water content for each enzyme. In SCFs, both the water content of the enzyme support and water solubilized in the supercritical phase determine the enzyme activity. Water content of the enzyme support is, in turn, determined by the distribution/partition of water between the enzyme and solvent, which can be estimated from water adsorption isotherms (141, 152). The solubility of water in the supercritical phase, operating conditions, and composition of the system (i.e., ethanol content) can affect the water distribution and, hence, determine the total amount of water that needs to be introduced into the system to attain the optimum water content of the support. The optimum water content of the enzyme is not affected by the reaction media, as demonstrated by Marty et al. (152), for esterification reaction using immobilized lipase in *n*-hexane and  $\text{SCCO}_2$ . Enzyme activity in different solvents should, thus, be compared at similar water content of the enzyme support.

In continuous enzymatic reactions, the optimum water content of the enzyme should be maintained throughout the reaction either by saturation of the solvent stream prior to entering the reactor or by direct addition into the reactants, the level of which is adjusted according to its solubility in SCCO<sub>2</sub> (143). The drying of the enzyme support as a result of continuous removal by the SCCO<sub>2</sub> stream results in a reversible loss of enzyme activity, which can be recovered by restoring the optimum water content (143, 146). However, denaturation of the enzyme as a result of excess water, which occurs at high water flow rates, is frequently not reversible (143, 152). The amount of water loss from the enzyme support is a function of the total amount of CO<sub>2</sub> passing through the enzyme and the solubility of water in SCCO<sub>2</sub> under the reaction conditions. Operating conditions and composition of the reaction system can, thus, affect the reaction rate through their effect on water solubility in SCF. The decrease in conversion with pressure observed in the continuous esterification of oleic acid and oleoyl alcohol was attributed to the effect of pressure on water solubility and, hence, removal from the support (144), as no such pressure effect was observed in batch mode for the same reaction.

The significant drop in the activity of non-immobilized *Candida rugosa* lipase in the hydrolysis of soybean oil at 10 MPa and 40°C was attributed to the high water content of the reaction system (181). A decrease in lipase stability with increasing water content has also been demonstrated in SCCO<sub>2</sub> at 13 MPa and 40°C (152).

**5.3.2. Applications in Fats and Oils Processing** Chemical reactions have been important tools in the processing of fats and oils as a means to produce oleochemicals, such as fatty acids, fatty acid esters, and monoacylglycerols, which find widespread use in pharmaceutical, food, and cosmetics industries, to change the composition of lipid mixtures for fractionation or analytical purposes and to modify the physical properties of lipids. SCCO<sub>2</sub> has been the solvent of choice as reaction media, owing to its previously stated advantages. The study of other SCFs as reaction media has largely been limited to nonenzymatic reactions, such as hydrogenation of oils in supercritical propane (158–161), the production of biodiesel (fatty acid esters) in supercritical alcohols (190–192), or the hydrolysis of vegetable oils in sub- and supercritical water (193, 194).

**5.3.2.1. Production of Oleochemicals** Fatty acid esters, which have traditionally been used as flavors, plasticizers, food preservatives, emulsifiers, and lubricants, are receiving renewed interest as an alternative energy source as biodiesel. They can be produced by the esterification of fatty acids and alcohols or by the alcoholysis of vegetable oils/triacylglycerols.

Lipase-catalyzed esterification of fatty acids with alcohols [oleic acid + ethanol (141, 152, 163, 169, 178, 195–197), oleic acid + oleyl alcohol (144, 179, 198–200), lauric acid + butanol (142), myristic acid + ethanol (138, 139, 143, 201), stearic acid + ethanol (202), anhydrous milkfat fatty acids + ethanol (197)] in SCCO<sub>2</sub> has been widely studied to understand the kinetics/mechanism of the reaction and to determine the effect of operating conditions, substrate concentration, and water content on enzyme activity. Alternative catalysts such as *p*-toluenesulfonic

acid and cation-exchange resins have also been investigated for the esterification of oleic acid with methanol in SCCO<sub>2</sub> (203). Ethanolysis of palm (204) and canola oils (205) in SCCO<sub>2</sub> have also been studied for the production of fatty acid esters.

A recent approach for the production of biodiesel (fatty acid esters) involves the catalyst-free transesterification of vegetable oils in supercritical alcohols (190–192). In this process, supercritical alcohol acts both as a solvent and as a reactant resulting in shorter reaction times and simpler product recovery compared with traditional processes.

Lipase-catalyzed (206, 207) and nonenzymatic glycerolysis of soybean oil (140) in SCCO<sub>2</sub> have been used for the production of monoacylglycerols, which are important emulsifiers in food, pharmaceutical, and cosmetic industries. Nonenzymatic glycerolysis was carried out at 150–250°C and 20.7–62.1 MPa with varying glycerol/oil ratios and water contents to determine the effect of these parameters on product composition (140). The optimum conditions for the lipase-catalyzed reaction were 27.6 MPa and 70°C (206). The lower temperatures used in the lipase-catalyzed reaction reduces the production of undesired side products and increases the reaction efficiency (207).

Hydrolysis has traditionally been used for the production of fatty acids and glycerols, which find widespread application in soaps and detergents, cosmetics, pharmaceuticals, and food products (174). Hydrolysis of soybean (181), canola (147, 208, 209), sunflower (149, 181, 210), tuna (150), and blackcurrant oils (145), tripalmitin (146), triolein (211), and ethyl stearate (202) in SCCO<sub>2</sub> has been reported. These investigations employed a variety of lipases, including immobilized lipase from porcine pancreas (211), Novozyme 435 (146, 181), Lipozyme (147, 150, 208, 209), non-immobilized *Candida rugosa* (150, 181), Lipase OF (150), and Lipolase 100T (149, 181, 210). The effects of water content, enzyme load, operating conditions (temperature and pressure), pH, enzyme/substrate ratio, oil/buffer ratio, and CO<sub>2</sub> flow rate (for continuous reactions) on the hydrolysis reaction were reported.

Batch and continuous hydrolysis of vegetable oils in sub- and supercritical water have also been investigated (193, 194). Water, in this case, acts both as a solvent and a reactant in the hydrolysis reaction. Although the reaction could be carried out effectively in subcritical water, thermal degradation of products and reactants were observed under supercritical conditions (193).

Fatty acid alcohols, which are important raw materials for the production of surfactants and lubricants, have been produced by hydrogenation of fatty acid methyl esters in supercritical propane (158, 159, 212) and SCCO<sub>2</sub> (212). Although the highest potential conversions were achieved using SCCO<sub>2</sub>, supercritical propane resulted in higher throughputs (212). Coupling of the hydrogenation reaction with a lipase-catalyzed transesterification reaction enabled the production of fatty alcohols directly from vegetable oils (212).

Reactions in SCCO<sub>2</sub> have also been used for the production of minor lipid components, such as tocopherols and sterol esters. The synthesis of D, L,  $\alpha$ -tocopherol in SCCO<sub>2</sub> and nitrous oxide by condensation of trimethylhydroquinone with isophytol in the presence of various Bronsted or Lewis acids as catalysts resulted in

high tocopherol yields (213). In their study on the lipase-catalyzed esterification of sterols in SCCO<sub>2</sub>, King et al. (214) obtained continuous production of sterol esters with 99% yield under optimal conditions of 27.6 MPa and 50°C.

**5.3.2.2. Pretreatment for Fractionation and Analytical Purposes** Chemical reactions can be used to modify the composition of a lipid mixture to facilitate its fractionation. As mentioned previously, such approaches have been employed in the fractionation of soybean (117) and olive oil deodorizer distillates (87) and fish oils (97). However, although the fractionation steps in these studies were carried out under supercritical conditions, the use of SCCO<sub>2</sub> as a reaction medium in the pretreatment reactions has not been explored. Gunnlaugsdottir et al. (151, 165–168) investigated the alcoholysis of cod liver oil in SCCO<sub>2</sub> for the concentration of fatty acids such as EPA and DHA. A process for the purification of polyunsaturated fatty acids from biomass on an analytical scale using *in situ* SFE/SCF reaction and chromatography has been patented (215).

Lipase-catalyzed hydrolysis of the vitamin esters in food samples in SCCO<sub>2</sub> has been investigated as a pretreatment step in the analytical determination of vitamins in food samples (216, 217). Lipase-catalyzed transesterification of oils with methanol have been used for the determination of total fat content in lipid-containing samples such as oilseeds and meat samples (218, 219) and for the determination of fatty or resin acid content of tall oil products (220). Esterification of fatty acids with methanol in SCCO<sub>2</sub> has been reported for total fatty acid analysis of soapstock (221).

**5.3.2.3. Modification of the Properties of Fats and Oils** Transesterification reactions (interesterification and acidolysis) of triacylglycerols have been an important tool in fats and oils processing for the modification of their physical and rheological properties by changing the composition and distribution of their fatty acids.

Interesterification of tristearin and palm oil (222) and acidolysis of palm oil by stearic acid (223) were carried out for the production of cocoa butter equivalent. Yoon et al. (224, 225) investigated lipase-catalyzed interesterification between triolein and behenic acid or ethyl behenate in SCCO<sub>2</sub> for the production of 1,3-dibehenoyl-2-oleoyl glycerol (BOB) triacylglycerol, which is a chocolate antiblocking agent. They observed that the higher solubility of ethyl behenate in SCCO<sub>2</sub> resulted in higher reaction rates (225). Lipase-catalyzed randomization of fats and oils in SCCO<sub>2</sub> is affected by pressure, enzyme quantity, and CO<sub>2</sub> flow rate (226). A patented procedure has been reported for the production of margarine with a low *trans*-fatty acid content, involving lipase-catalyzed transesterification of vegetable oil with stearic acid (227). Acidolysis reactions involving OOO and stearic acid (228–233), LLL and myristic acid (171), LLL and palmitic-acid (180), and medium chain triacylglycerols (with acyl carbon numbers 8, 10, 12) and long-chain fatty acids (oleic acid, EPA) (234), and interesterification of AMF and canola oil (197) in SCCO<sub>2</sub> have also been studied. Hydrogenation reactions in supercritical propane and CO<sub>2</sub> have been investigated for the saturation of double bonds in vegetable oils (160, 161, 235–237).

## 6. NOVEL PROCESS DEVELOPMENT: AN INTEGRATED APPROACH

Supercritical fluid technology is a “natural” and environmentally clean alternative for the processing of fats and oils, which offers the processor operational flexibility that can be exploited to meet the required objectives. Although the use of SCF solvents offers a flexible process with no solvent residue and low temperature operation for heat-sensitive materials, commercial applications for fats and oils processing have been limited. Despite the common belief that commercial applications of SCF technology is restricted to high-value products because of the high investment cost, recent studies suggest that economics of the process become favorable at large capacities for oilseed processing (15, 16). Although some specialty oils are being extracted commercially, SFE of commodity oils has not been commercialized yet. Supercritical fluid extraction suffers from a direct comparison with hexane extraction, as this approach does not take into consideration the added advantages of SCF processing: simplification of the refining process and elimination of meal desolventization (60). At a time when the conventional refining processes are being re-examined to maximize the retention of minor lipid components and the search to find an alternative to hexane is ongoing,  $\text{SCCO}_2$  offers a versatile technology with the added advantage of “natural” processing. These factors, coupled with the technological developments in equipment design that would enable continuous processing of large volumes of oilseeds, may make  $\text{SCCO}_2$  extraction of commodity oils a commercial reality in the near future.

Supercritical fluid technology has to be considered as an alternative integrated processing approach in fats and oils processing rather than an alternative to a single extraction step (238). The full promise of SCF technology in fats and oils processing can be realized through the development of novel processing schemes by proper integration of the unit operations involving supercritical extraction, fractionation, reaction, and chromatography.

The integrated approach has successfully been used in analytical applications, where extraction and reaction steps were carried out in a single cell or using two cells connected in series. Sequential extraction/reaction in a single cell has been investigated for the analysis of Vitamin A and Vitamin E in meat and dairy samples (216, 217), for the determination of total nutritional fat content (218, 219), total fatty acid analysis (221), and for the fatty and resin acid analysis of tall oil products (220). The sample was placed in the cell upstream from the enzyme from which it was partitioned by glass wool/hydromatrix. Jackson and King (148) used an extraction vessel connected in series to a reactor containing the enzyme bed placed in an oven for the extraction and methanolysis of oil from oilseeds. It should be possible to connect the reactor/extractor system to a chromatography system (GC/HPLC) to enable online analysis of the extracted and reacted samples.

Rezaei and Temelli (209) developed an online extraction/reaction system with improved control of feed streams and operating conditions by coupling an extraction cell to a reactor cell. Online extraction/hydrolysis (208, 209) and extraction/alcoholysis of canola flakes (205) were studied using this system by determining

the effects of enzyme load, CO<sub>2</sub> flow, canola flake load, size of reaction cell, and moisture content on product yield and composition. In the online extraction/alcoholysis process, the opposite effects of temperature on reaction and extraction performance could be exploited by controlling the temperature of the extractor and reactor cells independently (205).

Zhao et al. (86) used an extractor coupled to a fractionation column for the isolation of tocopherols and sterols from esterified soybean distillate. Sterols were left in the extractor, whereas tocopherols were collected from the bottom of the column and fatty acid methyl esters were collected in the separator.

These are just a few examples of the numerous process development opportunities possible through the use of SCF technology. Novel ways of combining the operations of extraction + fractionation or extraction + reaction + fractionation or extraction + fractionation + reaction + fractionation gives the versatility to start with a lipid-rich feed material and produce a variety of products targeting food, nutraceutical, and industrial applications. Over three decades of research focusing mainly on individual unit operations has enhanced the supercritical technology and know-how to the point where we can develop such novel integrated approaches for fats and oils processing.

## REFERENCES

1. N. T. Dunford, *Food Technol.*, **54** (11), 38 (2001).
2. L. M. Ohr, *Food Technol.*, **57**(7), 91 (2003).
3. A. E. Sloan, *Food Technol.*, **56**(4), 32 (2002).
4. D. F. Williams, *Chem. Eng. Sci.*, **36**, 1769 (1981).
5. L. G. Randall, *Sep. Sci. Technol.*, **17**, 118 (1982).
6. P. Hubert and O. G. Vitzthum, *Angew. Chem. Int. Ed. Engl.*, **17**, 710 (1978).
7. M. A. McHugh and V. J. Krukonic, *Supercritical Fluid Extraction: Principles and Practice*, 2nd ed., Butterworth-Heinemann, Boston, Massachusetts, 1994, p. 17.
8. J. B. Hannay and J. Hogarth, *Proc. R. Soc. London*, **29**, 324 (1879).
9. S. Pilat and M. Godlewicz (to Shell Development Company). U.S. Patent 2,188,013, January 23, 1940.
10. T. P. Zhuze, *Petroleum (London)*, **23**, 298 (1960).
11. G. H. Palmer and N. J. Fanwood (to the M. W. Kellogg Company), U.S. Patent 2,658,907, November 10, 1953.
12. K. Zosel, *Angew. Chem. Int. Ed. Engl.*, **17**, 702 (1978).
13. V. J. Krukonic, in M. Perrut, ed., *Proceedings of the International Symposium on Supercritical Fluids*, vol. 1, 1988, I.S.A.S.F., Nice, France, p. 541.
14. V. Krukonic, in M. Perrut and G. Brunner, eds., *Proceedings of the 3rd International Symposium on Supercritical Fluids*, vol. 1, 1994, Institut National Polytechnique de Lorraine, Nancy Cedex, France, p. 1.
15. L. Chordia, in D. Bush, ed., *Proceedings of the 5th International Symposium on Supercritical Fluids*, 2000, Paper 5416516.

16. M. Perrut, *Ind. Eng. Chem. Res.*, **39**, 4531 (2000).
17. R. Fukuzato, in G. Brunner, I. Kikic, and M. Perrut, eds., *Proceedings of the 6th International Symposium on Supercritical Fluids*, vol. 1, 2003, Institut National Polytechnique de Lorraine, Vandœuvre Cedex, France, p. 1.
18. A. Staby and J. Mollerup, *Fluid Phase Equilib.*, **91**, 349 (1993).
19. T. Bamberger, J. C. Erickson, C. L. Cooney, and S. K. Kumar, *J. Chem. Eng. Data*, **33**, 327 (1988).
20. W. B. Nilsson, E. J. Gauglitz, Jr., and J. K. Hudson, *J. Amer. Oil. Chem. Soc.*, **68**, 87 (1991).
21. V. Riha and G. Brunner, *J. Supercrit. Fluids*, **15**, 33 (1999).
22. J. Stoldt and G. Brunner, *Fluid Phase Equilib.*, **146**, 269 (1998).
23. P. C. Simoes and G. Brunner, *J. Supercrit. Fluids*, **9**, 75 (1996).
24. J. Stoldt and G. Brunner, *J. Supercrit. Fluids*, **14**, 181 (1999).
25. J. Stoldt, C. Saure, and G. Brunner, *Fluid Phase Equilib.*, **116**, 399 (1996).
26. O. Guclu-Ustundag and F. Temelli, *Ind. Eng. Chem. Res.*, **39**, 4756 (2000).
27. O. Guclu-Ustundag and F. Temelli, *J. Supercrit. Fluids*, **31**, 235 (2004).
28. O. Guclu-Ustundag and F. Temelli, *J. Supercrit. Fluids*, submitted for publication.
29. O. Guclu-Ustundag and F. Temelli, *J. Supercrit. Fluids*, submitted for publication.
30. J. Chrastil, *J. Phys. Chem.*, **86**, 3016 (1982).
31. B. N. Hansen, A. H. Harvey, J. A. P. Coelho, A. M. F. Palavra, and T. J. Bruno, *J. Chem. Eng. Data*, **46**, 1054 (2001).
32. M. Johannsen and G. Brunner, *J. Chem. Eng. Data*, **42**, 106 (1997).
33. M. Skerget, Ž. Knez, and M. Habulin, *Fluid Phase Equilib.*, **109**, 131 (1995).
34. H. Sovová, R. P. Stateva, and A. A. Galushko, *J. Supercrit. Fluids*, **21**, 195 (2001).
35. R. L. Mendes, B. P. Nobre, J. P. Coelho, and A. F. Palavra, *J. Supercrit. Fluids*, **16**, 99 (1999).
36. M. J. Cocero, S. Gonzales, S. Perez, and E. Alonso, *J. Supercrit. Fluids*, **19**, 39 (2000).
37. K. Sakaki, *J. Chem. Eng. Data*, **37**, 249 (1992).
38. M. L. Cygnarowicz, R. J. Maxwell, and W. D. Seider, *Fluid Phase Equilib.*, **59**, 57 (1990).
39. Y. Koga, Y. Iwai, Y. Hata, M. Yamamoto, and Y. Arai, *Fluid Phase Equilib.*, **125**, 115 (1996).
40. M. Zhong, B. Han, H. Yan, and D. Y. Deng, *Fluid Phase Equilib.*, **134**, 175 (1997).
41. B. Guan, J. Lu, B. Han, and H. Yan, *Sci. China, Ser. B*, **41**, 410 (1998).
42. B. Guan, Z. Liu, B. Han, and H. Yan, *J. Supercrit. Fluids*, **14**, 213 (1999).
43. H. Sovová, R. P. Stateva, and A. A. Galushko, *J. Supercrit. Fluids*, **21**, 195 (2001).
44. J. M. Wong and K. P. Johnston, *Biotechnol. Prog.*, **2**, 29 (1986).
45. O. J. Catchpole, J. B. Grey, and K. A. Noermark, *J. Chem. Eng. Data*, **43**, 1091 (1998).
46. G. Brunner and S. Peter, *Sep. Sci. Technol.*, **17**, 199 (1982).
47. T. K. Palazoğlu and M. O. Balaban, *Trans. ASAE*, **41**, 679 (1998).
48. G. Brunner and S. Peter, *Ger. Chem. Eng.*, **5**, 181 (1982).
49. M. Fattori, N. R. Bulley, and A. Meisen, *J. Amer. Oil Chem. Soc.*, **65**, 968 (1988).



50. J. P. Friedrich, G. R. List, and A. J. Heakin, *J. Amer. Oil Chem. Soc.*, **59**, 288 (1982).
51. F. Favati, J. W. King, and M. Mazzanti, *J. Amer. Oil Chem. Soc.*, **68**, 422 (1991).
52. J. Gawdzik, Z. Supryniewicz, M. Mardarowicz, J. Poszytek, R. Lodkowski, and K. Pilorz, *Chem. Anal.*, **43**, 695 (1998).
53. J. W. King, M. L. Cygnarowicz, and F. Favati, *Ital. J. Food Sci.*, **9**, 193 (1997).
54. B. Bozan and F. Temelli, *J. Amer. Oil Chem. Soc.*, **79**, 231 (2002).
55. B. Bozan and F. Temelli, *J. Food Sci.*, **68**, 422 (2003).
56. O. J. Catchpole, P. Simoes, J. B. Grey, E. M. M. Nogueiro, P. J. Carmelo, and M. N. da Ponte, *Ind. Eng. Chem. Res.*, **39**, 4820 (2000).
57. J. W. King and N. T. Dunford, *Sep. Sci. Technol.*, **37**, 451 (2002).
58. S. Peter, *Ber. Bunsen-Ges. Phys. Chem.*, **88**, 875 (1984).
59. J. W. King, in T. M. Kuo and H. W. Gardner, eds., *Lipid Biotechnology*, Marcel Dekker, Inc, New York, 2002, p. 663.
60. J. W. King, in P. J. Wan and P. J. Wakelyn, eds., *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*, AOCS Press, Champaign, Illinois, 1997, p. 283.
61. J. M. Snyder, J. P. Friedrich, and D. D. Christianson, *J. Amer. Oil Chem. Soc.*, **61**, 1851 (1984).
62. R. Eggers, U. Sievers, and W. Stein, *J. Amer. Oil Chem. Soc.*, **62**, 1222 (1985).
63. R. Eggers and U. Sievers, *J. Chem. Eng. Jpn.*, **22**, 641 (1989).
64. M. B. King, T. R. Bott, M. J. Barr, and R. S. Mahmud, *Sep. Sci. Technol.*, **22**, 1103 (1987).
65. A. K. K. Lee, N. R. Bulley, M. Fattori, and A. Meisen, *J. Amer. Oil Chem. Soc.*, **63**, 921 (1986).
66. R. Eggers, in J. W. King and G. R. List, eds., *Supercritical Fluid Technology in Oil and Lipid Chemistry*, AOCS Press, Champaign, Illinois, 1990, p. 35.
67. J. P. Friedrich and E. H. Pryde, *J. Amer. Oil Chem. Soc.*, **61**, 223 (1984).
68. J. P. Friedrich and G. R. List, *J. Agric. Food Chem.*, **30**, 192 (1982).
69. G. R. List, J. P. Friedrich, and J. Pominski, *J. Amer. Oil Chem. Soc.*, **61**, 1847 (1984).
70. G. R. List, J. P. Friedrich, and D. D. Christianson, *J. Amer. Oil Chem. Soc.*, **61**, 1849 (1984).
71. G. R. List and J. P. Friedrich, *J. Amer. Oil Chem. Soc.*, **66**, 98 (1989).
72. G. R. List and J. P. Friedrich, *J. Amer. Oil Chem. Soc.*, **62**, 82 (1985).
73. E. Stahl, K-W. Quirin, and R. J. Blagrove, *J. Agric. Food Chem.*, **32**, 938 (1984).
74. W. Zhao, A. Shishikura, K. Fujimoto, K. Arai, and S. Saito, *Agric. Biol. Chem.*, **51**, 1773 (1987).
75. Z. Shen, M. V. Palmer, S. S. T. Ting, and R. J. Fairclough, *J. Agric. Food Chem.*, **45**, 4540 (1997).
76. Z. Shen, M. V. Palmer, S. S. T. Ting, and R. J. Fairclough, *J. Agric. Food Chem.*, **44**, 3033 (1996).
77. M. E. Ramsay, J. T. Hsu, R. A. Novak, and W. J. Reightler, *Food Technol.*, **45**(11), 98 (1991).
78. R. A. Moreau, M. J. Powell, and K. B. Hicks, *J. Agric. Food Chem.*, **44**, 2149 (1996).

79. M. Taniguchi, T. Tsuji, M. Shibata, and T. Kobayashi, *Agric. Biol. Chem.*, **49**, 2367 (1985).
80. Y. Ge, H. Yan, B. Hui, Y. Ni, S. Wang, and T. Cai, *J. Agric. Food Chem.*, **50**, 685 (2002).
81. M. L. Colombo, A. Corsini, A. Mossa, L. Sala, and M. Stanca, *Phytochem. Anal.*, **9**, 192 (1998).
82. M. Hadolin, M. Škerget, Ž. Knez, and D. Bauman, *Food Chem.*, **74**, 355 (2001).
83. L. F. de Franca and M. A. A. Meireles, *J. Supercrit. Fluids*, **18**, 35 (2000).
84. L. F. de Franca, G. Reber, M. A. A. Meireles, N. T. Machado, and G. Brunner, *J. Supercrit. Fluids*, **14**, 247 (1999).
85. M. M. Esquivel and G. Bernardo-Gil, *J. Supercrit. Fluids* **6**, 91 (1993).
86. Y. Zhao, G. Sheng, and D. Wang, in D. Bush, ed., *Proceedings of the 5th International Symposium on Supercritical Fluids*, 2000, Paper 4965768.
87. P. Bondioli, C. Mariani, A. Lanzani, E. Fedeli, and A. Muller, *J. Amer. Oil Chem. Soc.*, **70**, 763 (1993).
88. C. Saure and G. Brunner, in M. Perrut and G. Brunner, eds., *Proceedings of the 3rd International Symposium on Supercritical Fluids*, vol. 2, 1994, Institut National Polytechnique de Lorraine, Nancy Cedex, France, p. 211.
89. G. Brunner, Th. Malchow, K. Sturken, and Th. Gottschau, *J. Supercrit. Fluids*, **4**, 72 (1991).
90. N. T. Machado and G. Brunner, in D. Bush, ed., *Proceedings of the 5th International Symposium on Supercritical Fluids*, 2000, Paper 2296903.
91. P. Bondioli, C. Mariani, A. Lanzani, E. Fedeli, A. Mossa, and A. Muller, *J. Amer. Oil Chem. Soc.*, **69**, 477 (1992).
92. N. T. Dunford and J. W. King, *J. Food Sci.*, **65**, 139 (2000).
93. C. K. Ooi, A. Bhaskar, M. S. Yener, D. Q. Tuan, J. Hsu, and S. S. H. Rizvi, *J. Amer. Oil Chem. Soc.*, **73**, 233 (1996).
94. G. R. Ziegler and Y-J. Liaw, *J. Amer. Oil Chem. Soc.*, **70**, 947 (1993).
95. E. Reverchon, M. Poletto, L. S. Osseo, and M. Somma, *J. Amer. Oil Chem. Soc.*, **77**, 9 (2000).
96. N. T. Dunford and J. W. King, *J. Amer. Oil Chem. Soc.*, **78**, 121 (2001).
97. W. B. Nilsson, E. J. Gauglitz, Jr., J. K. Hudson, V. F. Stout, and J. Spinelli, *J. Amer. Oil Chem. Soc.*, **65**, 109 (1988).
98. O. J. Catchpole, J. B. Grey, and K. A. Noemark, *J. Supercrit. Fluids*, **19**, 25 (2000).
99. O. J. Catchpole, J-C. von Kamp, and J. B. Grey, *Ind. Eng. Chem. Res.*, **36**, 4318 (1997).
100. P. J. Carmelo, P. C. Simoes, and M. N. da Ponte, in M. Perrut and G. Brunner, eds., *Proceedings of the 3rd International Symposium on Supercritical Fluids*, vol. 2, 1994, Institut National Polytechnique de Lorraine, Nancy Cedex, France, p. 107.
101. V. Riha and G. Brunner, *J. Supercrit. Fluids*, **17**, 55 (2000).
102. W. B. Nilsson, E. J. Gauglitz, Jr., and J. K. Hudson, *J. Amer. Oil Chem. Soc.*, **66**, 1596 (1989).
103. P. C. Simoes, M. N. da Ponte, and G. Brunner, in M. Perrut and G. Brunner, eds., *Proceedings of the 3rd International Symposium on Supercritical Fluids*, vol. 2, 1994, Institut National Polytechnique de Lorraine, Nancy Cedex, France, p. 481.
104. U. Fleck, C. Tiegs, and G. Brunner, *J. Supercrit. Fluids*, **14**, 67 (2003).

105. E. Ibanez, A. M. H. Benavides, F. J. Senorans, and G. Reglero, *J. Amer. Oil Chem. Soc.*, **79**, 1255 (2002).
106. A. R. Bhaskar, S. S. H. Rizvi, and J. W. Sherbon, *J. Food Sci.*, **58**, 748 (1993).
107. A. R. Bhaskar, S. S. H. Rizvi, and P. Harriott, *Biotechnol. Prog.*, **9**, 70 (1993).
108. A. R. Bhaskar, S. S. H. Rizvi, and C. Bertoli, in Ph.R. von Rohr and Ch. Trepp, eds., *High Pressure Chemical Engineering*, Elsevier Science, Amsterdam, 1996, p. 297.
109. A. R. Bhaskar, S. S. H. Rizvi, and P. Harriott, *J. Dairy Sci.*, **83**, 20 (2000).
110. W. Eisenbach, *Ber. Bunsen-Ges. Phys. Chem.*, **88**, 882 (1984).
111. G. Brunner, *Eur. J. Lipid Sci. Technol.*, **102**, 240 (2000).
112. M. Markom, H. Singh, and M. Hasan, *J. Supercrit. Fluids*, **20**, 45 (2001).
113. S. Turkay, M. D. Burford, M. K. Sangun, E. Ekinci, K. D. Bartle, and A. A. Clifford, *J. Amer. Oil Chem. Soc.*, **73**, 1265 (1996).
114. G. R. List, J. W. King, J. H. Johnson, K. Warner, and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **70**, 473 (1993).
115. A. Birtigh, M. Johannsen, and G. Brunner, *J. Supercrit. Fluids*, **8**, 46 (1995).
116. E. Ibanez, J. Palacios, F. J. Senorans, G. Santa-Maria, J. Tabera, and G. Reglero, *J. Amer. Oil Chem. Soc.*, **77**, 187 (2000).
117. H. Lee, B. H. Chung, and Y. H. Park, *J. Amer. Oil Chem. Soc.*, **68**, 571 (1991).
118. M. F. Mendes, F. L. P. Pessoa, and A. M. C. Uller, *J. Supercrit. Fluids*, **23**, 257 (2002).
119. C. J. Chang, Y-F. Chang, H. Lee, J. Lin, and P-W. Yang, *Ind. Eng. Chem. Res.*, **39**, 4521 (2000).
120. R. Ruivo, M. J. Cebola, P. C. Simoes, and M. N. da Ponte, *Ind. Eng. Chem. Res.*, **40**, 1706 (2001).
121. S. L. Taylor and J. W. King, *J. Chromatogr. Sci.*, **38**, 91 (2000).
122. S. L. Taylor and J. W. King, *J. Amer. Oil Chem. Soc.*, **77**, 687 (2000).
123. J. W. King, F. Favati, and S. L. Taylor, *Sep. Sci. Technol.*, **31**, 1843 (1996).
124. M. Saito and Y. Yamauchi, *J. Chromatogr.*, **505**, 257 (1990).
125. M. Saito and Y. Yamauchi, *J. Chromatogr. Sci.*, **27**, 79 (1989).
126. R. De Giorgi, B. Marongiu, S. Porcedda, M. A. Dessi, A. Rosa, and D. Sicbaldi, in E. Reverchon, ed., *Proceedings of the 4th Italian Conference on Supercritical Fluids and their Applications*, 1997, CUES s.c.a.r.l. di Fisciano, Salerno, Italy, p. 185.
127. J. Arul, A. Boudreau, J. Makhlouf, R. Tardif, and M. R. Sahasrabudhe, *J. Food Sci.*, **52**, 1231 (1987).
128. H. Chen, J. Schwartz, and G. A. Spanos, *J. Dairy Sci.*, **75**, 2659 (1992).
129. F. Patat, *Monatsh. Chemie.*, **47**, 352 (1947).
130. D. A. Hammond, M. Karel, A. M. Klibanov, and V. J. Krukonis, *Appl. Biochem. Biotechnol.*, **11**, 393 (1985).
131. T. W. Randolph, H. W. Blanch, J. M. Prausnitz, and C. R. Wilke, *Biotechnol. Lett.*, **7**, 325 (1985).
132. K. Nakamura, Y. M. Chi, Y. Yamada, and T. Yano, *Chem. Eng. Commun.*, **45**, 207 (1986).
133. A. A. Clifford, in E. Kiran and J. M. H. Levelt Sengers, eds., *Supercritical Fluids: Fundamentals for Application*, Kluwer Academic Publishers, Dordrecht, the Netherlands, 1994, p. 449.

134. T. W. Randolph, H. W. Blanch, and D. S. Clark, in J. S. Dordick, ed., *Biocatalysts for Industry*, Plenum Press, New York, 1991, p. 219.
135. P. E. Savage, S. Gopalan, T. I. Mizan, C. J. Martino, and E. E. Brock, *AIChE J.*, **41**, 1723 (1995).
136. B. Subramaniam and M. A. McHugh, *Ind. Eng. Chem. Process Des. Dev.*, **25**, 1 (1986).
137. S. V. Kamat, E. J. Beckman, and A. J. Russell, *Crit. Rev. Biotechnol.*, **15**, 41 (1995).
138. T. Dumont, D. Barth, C. Corbier, G. Branlant, and M. Perrut, *Biotechnol. Bioeng.*, **39**, 329 (1992).
139. P. Bernard and D. Barth, *Biocatalysis and Biotransformation*, **12**, 299 (1995).
140. F. Temelli, J. W. King, and G. R. List, *J. Amer. Oil Chem. Soc.*, **73**, 699 (1996).
141. R. Goddard, J. Bosley, and B. Al-Duri, *J. Supercrit. Fluids*, **18**, 121 (2000).
142. D. C. Steytler, P. S. Moulson, and J. Reynolds, *Enzyme Microb. Technol.*, **13**, 221 (1991).
143. T. Dumont, D. Barth, and M. Perrut, *J. Supercrit. Fluids*, **6**, 85 (1993).
144. Ž. Knez, M. Habulin, and V. Krmelj, *J. Supercrit. Fluids*, **14**, 17 (1998).
145. H. Sovová and M. Zarevúcka, *Chem. Eng. Sci.*, **58**, 2339 (2003).
146. J. W. Hampson and T. A. Foglia, *J. Amer. Oil Chem. Soc.*, **76**, 777 (1999).
147. K. Rezaei and F. Temelli, *J. Amer. Oil Chem. Soc.*, **77**, 903 (2000).
148. M. A. Jackson and J. W. King, *J. Amer. Oil Chem. Soc.*, **73**, 353 (1996).
149. Ž. Knez, M. Habulin, and M. Primožič, *Bioprocess Biosyst. Eng.*, **25**, 279 (2003).
150. H. Yan and K. Nagahama, *J. Chem. Eng. Jpn.*, **36**, 557 (2003).
151. H. Gunnlaugsdottir, A. Å. Karlsson, and B. Sivik, *J. Amer. Oil Chem. Soc.*, **74**, 1491 (1997).
152. A. Marty, W. Chulalaksananukul, R. M. Willemot, and J-S. Condoret, *Biotechnol. Bioeng.*, **39**, 273 (1992).
153. T. W. Randolph, H. W. Blanch, and J. M. Prausnitz, *AIChE J.*, **34**, 1354 (1988).
154. M. B. King, A. Mubarak, J. D. Kim, and T. R. Bott, *J. Supercrit. Fluids*, **5**, 296 (1992).
155. R. Weibe and V. L. Gaddy, *J. Amer. Chem. Soc.*, **62**, 815 (1940).
156. R. Weibe, *Chem. Rev.*, **29**, 475 (1941).
157. R. Weibe and V. L. Gaddy, *J. Amer. Chem. Soc.*, **63**, 475 (1941).
158. S. van der Hark, M. Härröd, and P. Møller, *J. Amer. Oil Chem. Soc.*, **76**, 1363 (1999).
159. S. van der Hark and M. Härröd, *Ind. Eng. Chem. Res.*, **40**, 5052 (2001).
160. M-B. Macher and A. Holmqvist, *Eur. J. Lipid Sci. Technol.*, **103**, 81 (2001).
161. M-B. Macher, J. Högberg, P. Møller, and M. Härröd, *Fett/Lipid*, **101**, 301 (1999).
162. A. Marty, S. Manon, D. P. Ju, D. Combes, and J-S. Condoret, *Ann. NY Acad. Sci.*, **750**, 408 (1995).
163. A. Marty, V. Dossat, and J-S. Condoret, *Biotechnol. Bioeng.*, **56**, 232 (1997).
164. T. Adschiri, H. Akiya, L. C. Chin, K. Arai, and K. Fujimoto, *J. Chem. Eng. Jpn.*, **25**, 104 (1992).
165. H. Gunnlaugsdottir and B. Sivik, *J. Amer. Oil Chem. Soc.*, **72**, 399 (1995).
166. H. Gunnlaugsdottir and B. Sivik, *J. Amer. Oil Chem. Soc.*, **74**, 1483 (1997).
167. H. Gunnlaugsdottir, M. Järemo, and B. Sivik, *J. Supercrit. Fluids*, **12**, 85 (1998).

168. H. Gunnlaugsdottir, K. Wannerberger, and B. Sivik, *Enzyme Microb. Technol.*, **22**, 360 (1998).
169. A. Marty, D. Combes, and J-S. Condoret, *Biotechnol. Bioeng.*, **43**, 497 (1994).
170. S. Kamat, E. J. Beckman, and A. J. Russell, *Enzyme Microb. Technol.*, **14**, 265 (1992).
171. D. A. Miller, H. W. Blanch, and J. M. Prausnitz, *Ind. Eng. Chem. Res.*, **30**, 939 (1991).
172. E. Morild, *Adv. Protein Chem.*, **34**, 91 (1981).
173. C. A. Eckert, *Annu. Rev. Phys. Chem.*, **23**, 236 (1972).
174. N. N. Gandhi, *J. Amer. Oil Chem. Soc.*, **74**, 621 (1997).
175. M. Habulin and Ž. Knez, *J. Chem. Technol. Biotechnol.*, **76**, 1260 (2001).
176. A. Gießauf, W. Magor, D. J. Steinberger, and R. Marr, *Enzyme Microb. Technol.*, **24**, 577 (1999).
177. M. Taniguchi, M. Kamihira, and T. Kobayashi, *Agric. Biol. Chem.*, **51**, 593 (1987).
178. A. Marty, W. Chulalaksananukul, J-S. Condoret, R. M. Willemot, and G. Durand, *Biotechnol. Lett.*, **12**, 11 (1990).
179. I. Krmelj, M. Habulin, Ž. Knez, and D. Bauman, *Fett/Lipid*, **101**, 34 (1999).
180. J. C. Erickson, P. Schyns, and C. L. Cooney, *AIChE J.*, **36**, 299 (1990).
181. M. Habulin and Ž. Knez, *Eur. J. Lipid Sci. Technol.*, **104**, 381 (2002).
182. J. T. Penniston, *Arch. Biochem. Biophys.*, **142**, 322 (1971).
183. V. Kasche, R. Schlothauer, and G. Brunner, *Biotechnol. Lett.*, **10**, 569 (1988).
184. J. Zagrobelny and F. V. Bright, *Biotechnol. Prog.*, **8**, 421 (1992).
185. J. K. P. Weder, *Food Chem.*, **15**, 175 (1984).
186. J. K. P. Weder, *Z. Lebensm. Unters. Forsch.*, **171**, 95 (1980).
187. S. Kamat, J. Barrera, E. J. Beckman, and A. J. Russell, *Biotechnol. Bioeng.*, **40**, 158 (1992).
188. S. Kamat, G. Critchley, E. J. Beckman, and A. J. Russell, *Biotechnol. Bioeng.*, **46**, 610 (1995).
189. A. Zaks and A. M. Klibanov, *J. Biol. Chem.*, **263**, 8017 (1988).
190. S. Saka and D. Kusdiana, *Fuel*, **80**, 225 (2001).
191. D. Kusdiana and S. Saka, *Bioresour. Technol.*, **91**, 289 (2004).
192. A. Demirbas, *Energy Convers. Manage.*, **43**, 2349 (2002).
193. R. L. Holliday, J. W. King, and G. R. List, *Ind. Eng. Chem. Res.*, **36**, 932 (1997).
194. J. W. King, R. L. Holliday, and G. R. List, *Green Chemistry*, **1**, 261 (1999).
195. A. Marty, D. Combes, and J-S. Condoret, in J. Tramper, M. H. Vermue, H. H. von Stockar, and U. Beffink, eds., *Biocatalysis in Non-Conventional Media*, Elsevier Science Publishers, Amsterdam, 1992, p. 425.
196. R. Goddard, J. Bosley, and B. Al-Duri, *J. Chem. Technol. Biotechnol.*, **75**, 715 (2000).
197. Z-R. Yu, S. S. H. Rizvi, and J. A. Zollweg, *Biotechnol. Prog.*, **8**, 508 (1992).
198. Ž. Knez and M. Habulin, in J. Tramper, M. H. Vermue, H. H. von Stockar, and U. Beffink, eds., *Biocatalysis in Non-Conventional Media*, Elsevier Science Publishers, Amsterdam, 1992, p. 401.
199. Ž. Knez, V. Rižner, M. Habulin, and D. Bauman, *J. Amer. Oil Chem. Soc.*, **72**, 1345 (1995).
200. Ž. Knez and M. Habulin, *Biocatalysis*, **9**, 115, (1994).

201. S. Srivastava, G. Madras, and J. Modak, *J. Supercrit. Fluids*, **27**, 55 (2003).
202. H. Nakaya, K. Nakamura, and O. Miyawaki, *J. Amer. Oil Chem. Soc.*, **79**, 23 (2002).
203. C. Vieville, Z. Mouloungui, and A. Gaset, *Ind. Eng. Chem. Res.*, **32**, 2065 (1993).
204. J. V. Oliveira and D. Oliveira, *Ind. Eng. Chem. Res.*, **39**, 4450 (2000).
205. M. Kondo, K. Rezaei, F. Temelli, and M. Goto, *Ind. Eng. Chem. Res.*, **41**, 5770 (2002).
206. M. A. Jackson and J. W. King, *J. Amer. Oil Chem. Soc.*, **74**, 103 (1997).
207. M. A. Jackson (to the United States of America), U.S. Patent 5,747,305, May 5, 1998.
208. J. L. Martinez, K. Rezaei, and F. Temelli, *Ind. Eng. Chem. Res.*, **41**, 6475 (2002).
209. K. Rezaei and F. Temelli, *J. Supercrit. Fluids*, **19**, 263 (2001).
210. M. Primožič, M. Habulin, and Ž. Knez, *J. Amer. Oil Chem. Soc.*, **80**, 643 (2003).
211. G. Glowacz, M. Bariszlovich, M. Linke, P. Richter, C. Fuchs, and J-T. Mörsel, *Chem. Phys. Lipids*, **79**, 101 (1996).
212. M. B. O. Andersson, J. W. King, and L. G. Blomberg, *Green Chemistry*, **2**, 230 (2000).
213. S. Wang, W. Bonrath, H. Pauling, and F. Kienzle, *J. Supercrit. Fluids*, **17**, 135 (2000).
214. J. W. King, J. M. Snyder, H. Frykman, and A. Neese, *Eur. Food Res. Technol.*, **212**, 566 (2001).
215. T. Kiy, C. Siffrin, H. Engelhard, and D. Fabritius (to AXIVA GmbH), U.S. Patent 6,350,890, February 26, 2002.
216. C. Turner, J. W. King, and L. Mathiasson, *J. Agric. Food Chem.*, **49**, 553 (2001).
217. C. Turner, M. Persson, L. Mathiasson, P. Adlercreutz, and J. W. King, *Enzyme Microb. Technol.*, **29**, 111 (2001).
218. J. M. Snyder, J. W. King, and M. A. Jackson, *J. Chromatogr. A*, **750**, 201 (1996).
219. J. M. Snyder, J. W. King, and M. A. Jackson, *J. Amer. Oil Chem. Soc.*, **74**, 585 (1997).
220. S. L. Taylor and J. W. King, *J. Chromatogr. Sci.*, **39**, 269 (2001).
221. J. W. King, S. L. Taylor, J. M. Snyder, and R. L. Holliday, *J. Amer. Oil Chem. Soc.*, **75**, 1291 (1998).
222. K-J. Liu, H-M. Cheng, R-C. Chang, and J-F. Shaw, *J. Amer. Oil Chem. Soc.*, **74**, 1477 (1997).
223. M-T. Liang, C-H. Chen, and R-C. Liang, *J. Supercrit. Fluids*, **13**, 211 (1998).
224. S-H. Yoon, O. Miyawaki, K-H. Park, and K. Nakamura, *J. Ferment. Bioeng.*, **82**, 334 (1996).
225. S-H. Yoon, H. Nakaya, O. Ito, O. Miyawaki, K-A. Park, and K. Nakamura, *Biosci. Biotechnol. Biochem.*, **62**, 170 (1998).
226. M. A. Jackson, J. W. King, G. R. List, and W. E. Neff, *J. Amer. Oil Chem. Soc.*, **74**, 635 (1997).
227. P. H. Brown, F. D. Carvallo, R. C. Dinwoodie, M. T. Dueber, D. K. Hayashi, R. G. Krishnamurthy, Z. M. Merchant, J. J. Myrick, R. S. Silver, and C. Thomas (to Kraft General Foods, Inc), U.S. Patent 5,288,619, February 22, 1994.
228. K. Nakamura, Y. M. Chi, and T. Yano, in M. Perrut, ed., *Proceedings of International Symposium on Supercritical Fluids*, vol. 2, 1988, I.S.A.S.F., Nice, France, p. 925.
229. K. Nakamura, in S. S. H. Rizvi, ed., *Supercritical Fluid Processing of Food and Biomaterials*, Blackie Academic Professional, London, 1994, p. 54.

230. K. Nakamura, in A. Fiechter, H. Okada, and R. D. Tanner, eds., *Bioproducts and Bioprocesses*, Springer-Verlag, Berlin, 1989, p. 257.
231. M. Hakoda, N. Shiragami, A. Enomoto, and K. Nakamura, *Bioprocess Biosyst. Eng.*, **24**, 355 (2002).
232. Y. M. Chi, K. Nakamura, and T. Yano, *Agric. Biol. Chem.*, **52**, 1541 (1988).
233. H. Nakaya, O. Miyawaki, and K. Nakamura, *Biotechnol. Tech.*, **12**, 881 (1998).
234. A. Shishikura, K. Fujimoto, T. Suzuki, and K. Arai, *J. Amer. Oil Chem. Soc.*, **71**, 961 (1994).
235. M. Härröd and P. Møller (to Poul Møller Ledelses-OG Ingeniorradgivning APS), U.S. Patent 5,962,711, October 5, 1999.
236. T. Tacke, S. Wieland, P. Panster, M. Blankmann, R. Brand, and H. Magerlein (to Degussa Aktiengesellschaft), U.S. Patent 5,734,070, March 31, 1998.
237. J. W. King, R. L. Holliday, G. R. List, and J. M. Snyder, *J. Amer. Oil Chem. Soc.*, **78**, 107 (2001).
238. F. Temelli and N. Dunford, in S. S. Koseoglu, K. C. Rhee, and R. F. Wilson, eds., *The Proceedings of the World Conference on Oilseed and Edible Oils Processing*, vol. 1, 1996, AOCS Press, Champaign, Illinois, p. 59.

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## *Membrane Processing of Fats and Oils*

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### **1. INTRODUCTION**

According to the Food Agriculture Organization of the United Nations (FAO), food producers and processors face major new challenges as the world population is projected to increase from the present level of 5.8 billion people to about 8.3 billion in the year 2025. Therefore, it is crucial that the new scientific information lead to new technologies, processing methods, and agricultural techniques to produce food from Earth to meet the demands of a growing population. Crude oils typically are degummed, refined, bleached, and deodorized to remove undesirable compounds, including free fatty acids (FFAs), phosphatides, particulates, coloring materials such as chlorophylls and xanthophylls, and miscellaneous unsaponifiable matter. In processing crude vegetable oils, considerable amounts of energy in the form of steam or electricity are required and each step of the edible oil process only removes one or two undesirable components. If crude oil is not properly processed, treatments during the following steps will be more difficult, and time and labor will be consuming. In addition to the energy costs, caustic refining, water washing, and bleaching steps produce various waste streams such as high biological oxygen



demand (BOD) acidic wastewater and used bleaching clay that either need to be treated or recovered because of economical or environmental reasons.

Membrane technology is a mature industry and has been successfully applied in various food industries for separation of undesirable fractions from the valuable components of the feed streams. The industrial membranes are classified into various categories such as microfiltration, ultrafiltration, nanofiltration, reverse osmosis, and pervaporation.

The commercial membrane separation processes are offered in the areas of nitrogen production and waste treatment applications (1). Developing membrane applications in oil milling and edible oil processing are (1) solvent recovery, (2) degumming, (3) free fatty acid removal, (4) catalyst recovery, (5) recovery of wash water from second centrifuge, (6) cooling tower water recovery, (7) protein purification, and (8) tocopherol separation.

The greatest potential for energy saving exists in replacing or supplementing conventional degumming, refining, and bleaching processes with a single-step membrane separation system. Therefore, this contribution will concentrate on membrane degumming applications and represents data on future implications of this technology for energy savings and waste treatment costs.

## 2. COMPOSITION OF CRUDE VEGETABLE OILS

Triacylglycerols (acyl esters of glycerols) constitute nearly 99% of crude vegetable oils. The remaining components include phospholipids, FFAs, pigments, sterols, carbohydrates, proteins, and their degradation products (2).

Fatty acids are the building blocks of triacylglycerols. The fatty acid profiles for different vegetable oils differ greatly. Free fatty acids may undergo reactions with oxygen, hydrogen, acids, metals, heat, and radiation. The free fatty acid content of crude vegetable oils depends on the conditions of harvesting and the moisture content of the seed (3). If present in high amounts, FFA content is the first and most important indication of below-standard quality for a given oil.

Phospholipids are coextracted with triacylglycerols during oil milling as colloidal aggregates called micelles. They are soluble in both aqueous and oil environments and act as important emulsifiers when assessing quality of oils. The most abundant phospholipids in vegetable oils are phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine.

The pigments present in crude vegetable oils include carotenes, xanthophylls, chlorophylls, gossypol, chroman-quinone, diketones, and browning products. Sterols, hydrocarbons, tocopherols, tocotrienols, protein fragments, as well as resinous and mucilaginous materials have also been identified in crude vegetable oils.

## 3. CRUDE VEGETABLE OIL REFINING

The conventional crude oil refining process consists of four steps (2,4–6). The first step is degumming to remove the phospholipids. Crude vegetable oils can either be

degummed or not before caustic refining, depending on whether lecithin is saved and the facilities are available.

Next, FFAs are neutralized with a sufficient amount of sodium hydroxide (NaOH) solution to convert FFAs into soaps. An excess of NaOH is required to reduce the color of the refined oil and ensure the removal of trace elements. Phospholipids also react with water, if they are not removed previously. Soaps are then removed with phospholipids by centrifugation and hot water washing. In this step, water is added at a 10–15% level at 80–90°C.

Third, during the bleaching process, primary oxidation products are destroyed or adsorbed, and pigments and trace metals are removed by adsorbents such as activated bleaching clays. The process usually takes 15–30 min at 90–95°C under vacuum.

Finally, oil is steam distilled under a high vacuum to strip out trace amounts of FFAs, aldehydes, ketones, and other volatile compounds, among others, thus producing a bland oil. During deodorization, the oil is heated to 230–250°C under a vacuum of 1–4 mm Hg.

The replacement of chemical refining with a physical refining process is desirable by the oil industry to reduce energy consumption and environmental problems. In physical refining, the phospholipids and pigments are removed by adsorbents, and the free fatty acids by steam stripping. Physical refining provides a shortcut in the process sequence and results in less oil loss. However, only oils with a low phospholipid content are suitable for physical refining (5).

Miscella refining is another alternative to conventional refining, and it is used almost exclusively with cottonseed oil. The 40–60% miscella is heated to 40°C, and a conditioning agent (phosphoric or citric acid) is added, if necessary. A NaOH solution with 30% excess is also added. The soap formed is melted at 50–65°C, and the slurry is centrifuged after rapid cooling to 45°C. The separated miscella and the soap stock are both evaporated to remove the solvent. This miscella refining process results in less oil loss and a lighter oil color. It is critical that the miscella be refined no later than 7 hours after extracting oil from the seeds; otherwise undesirable color will appear (5).

As a result of the multistep refining process, large amounts of energy are consumed to heat and cool the oil as well as to provide power to pumps, centrifuges, and other equipment. The energy usage can range from 2000 to 4000 BTU/lb, depending on the age of the facilities (7). About 1% of neutral oil is lost in the soap-stock and phospholipid gums (8). In addition to solid waste, high organic-content aqueous effluents are produced. Although improvements have been made in process engineering and equipment design, the basic principles of edible oil refining have not changed in the last 70 years (9).

#### 4. VEGETABLE OIL DEGUMMING

The purpose of degumming is to remove almost all phospholipids, which could settle out during storage or cause flavor reversion of bottled oil or darkening during

frying. Traces of phosphorus in refined oils are blamed for darkening the oil during deodorization (5) and for interfering with the catalyst during hydrogenation (10). The recovered phospholipids can produce lecithin.

#### 4.1. Conventional Degumming Methods

*Water degumming.* This is the traditional degumming method. When water is added to crude oil, most phospholipids in the oil are hydrated and made insoluble in the oil. Water, at about 1–3% of the oil volume, is combined with the crude oil by agitation for 30 min at 50–70°C. Then, the hydrated gums can be separated from the oil by settling, filtering, or centrifuging. Usually 80–95% of the gums are removed by conventional water degumming (11). When oilseeds of high quality are extracted under optimum conditions, the phospholipids can be removed by water degumming to a residual content of 0.002% (12).

Most seed oils contain 0.2–0.8% nonhydratable phospholipids (5), specifically the magnesium (Mg) and calcium (Ca) salts of phospholipids, which cannot be removed by water degumming. For many years, a common way for nonlecithin producers to degum edible oil was to treat the oil with 0.02–1% of concentrated phosphoric acid at 70–90°C, after water degumming. Then, without the removal of any precipitated solids, the oil is caustically refined. Phosphoric acid chelates the Ca and Mg in the oil so that the nonhydratable phospholipids are converted into the hydratable form. The phosphoric acid pretreatment produces a darker lecithin with lower purity (5).

The effectiveness of the conventional degumming process is very much dependent on the quality of the crude oil; inferior quality oils show incomplete degumming with a single process pass. Moreover, about 20% or more of the phospholipids are lost by destructive treatments and as much as 1% of the neutral oil may be lost. Considerable effluent disposal problems arise as well (8).

#### 4.2. Other Developing Degumming Methods

*Degumming with acetic anhydride.* The process is referred to as the Staley 50 process. Acetic anhydride (0.1%) is mixed with preheated oil (60–70°C) for 15 min, and the mixture is stirred for 30 min after the addition of 1.5% water. The degummed oil obtained is claimed to require no caustic refining. However, the Staley process does not work well with highly colored vegetable oils like cottonseed oil (5).

*Superdegumming.* Citric acid is used as a reagent to degum oil at a temperature above 50 °C. Phospholipid levels as low as 7–30 ppm can be achieved in refined oil. Various other degumming agents have also been proposed, including sodium chloride solutions, ammonium hydroxide, and acetic, oxalic, boric, and nitric acids (13).

*Continuous ultrasonic degumming.* Ultrasonic action can generate extremely localized heat and pressure and produce intimate contact of reactants without substantially raising the temperature of the final products. Moulton and Mounts (14) proposed a continuous ultrasonic degumming process in 1990. Compared with a

batch conventional degumming process performed in the laboratory, ultrasonic degumming was five times faster, the percent phosphorus removed was 8% higher (ranging from 90% to 99%), and the percent Mg removed was up to 10% higher. More ultrasonic power seemed to increase the weight of the gums recovered. Water washing of the degummed oil is not required, and potential environmental pollution can be reduced.

*Supercritical CO<sub>2</sub> degumming.* Use of supercritical fluid to extract oilseeds has been shown to possess certain advantages, such as the absence of phospholipids in crude oils, lighter oil color, and less tendency to undergo color fixation. In List et al.'s research (11), a hexane-extracted crude soybean oil was degummed in a reactor by counter-currently contacting the oil with supercritical CO<sub>2</sub> at 55 MPa and 70°C, and it took approximately 4 hr/run for completion. Typically the supercritical CO<sub>2</sub> refined oils have a phosphorus content below 5 ppm, a Lovibond color rating of 10R-70Y, and a free fatty acid content below 0.1%. Most gums (99.2%) are removed.

*Membrane degumming.* Membrane separation has also been evaluated as an alternative process to conventional oil refining processing. Ultrafiltration (UF) and nanofiltration (NF) membranes separate phospholipids almost completely, and FFAs, pigments, and other components can also be removed with the phospholipids to a certain extent. Less effort is required in the later processing steps.

## 5. MEMBRANE PROCESSING OF OILS AND FATS

*Membrane Technology:* The expression “membrane separation” covers a wide range of product separation techniques. They involve the separation of components mostly in fluid or even, sometimes, in gaseous state, through the application of the physical properties of ionic charges, diffusivity, and difference in molecular size of the compounds to be separated. It uses a wide range of inorganic and polymeric membranes, the selection of which depends on the material to be processed.

Membrane technology is a mature industry and has been successfully applied in various food industries for separation of undesirable fractions from the valuable components of the feed streams. The industrial membranes are classified into various categories. They include particle filtration, microfiltration, ultrafiltration, nanofiltration, reverse osmosis, and pervaporation. As shown in Figure 1, particle filtration is concerned with separation of particles that are greater than 2.0 micrometers ( $2 \times 10^4$  angstrom units); microfiltration with macromolecules from 200,000 to 1 million molecular weight (MW) (500–2 million angstrom units); ultrafiltration with molecules 10,000–300,000 MW (40–2,000 angstrom units); nanofiltration with molecules 15,000–150 MW (8–80 angstrom units); and reverse osmosis (also called “hyperfiltration”) with ions and molecules up to 600 MW (20 angstrom units). These processes are used to meet various separation needs as shown in Figure 1.

Reverse osmosis retains all components except water, whereas ultrafiltration is primarily a size-exclusion-based pressure-driven membrane separation process. The

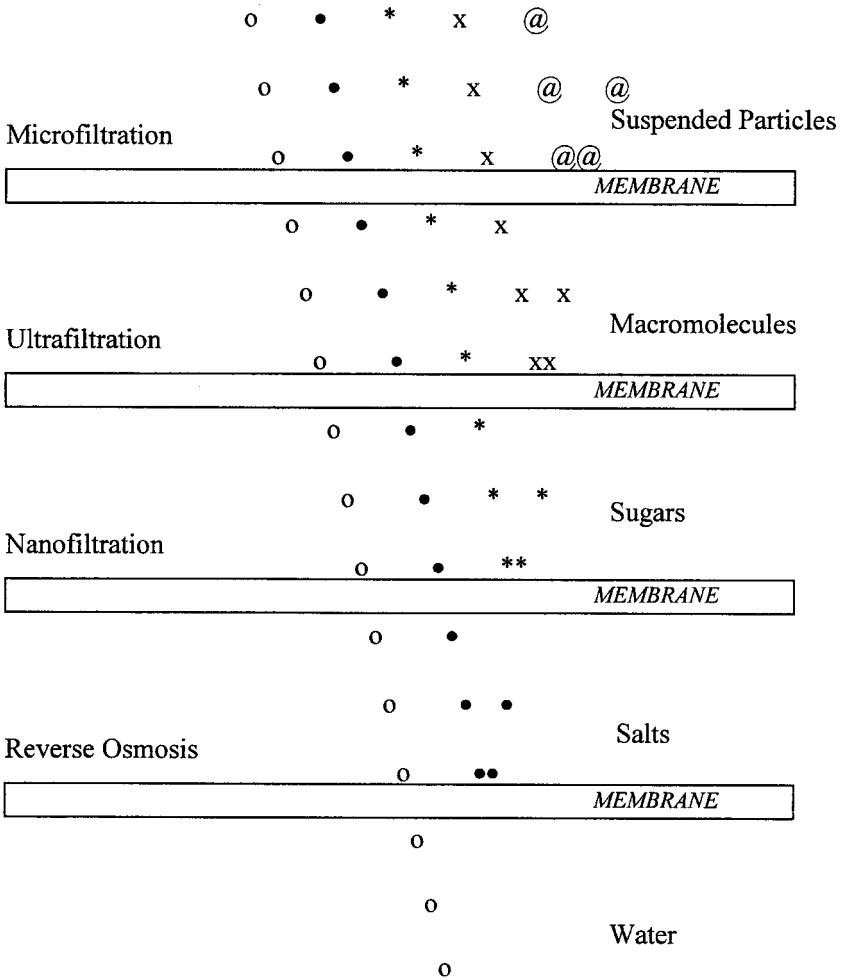


Figure 1. Principles of membrane separation.

UF membranes can retain macromolecules such as polysaccharides, proteins, bio-molecules, polymers, and colloidal particles. Generally, ultrafiltration membranes are classified by the type of material and their nominal molecular weight cutoff (MWCO), which is usually defined as the smallest molecular weight species for which the membrane has more than 90% rejection.

Advantages of membrane separation technology in food processing include gentle treatment, quality improvement, low energy consumption, simultaneous fractionation and concentration, demineralization, increased yield, and simple plant layout. The limiting factors of the technology include concentration, polarization, fouling, osmotic pressure, and viscosity.

The critical parameters for a successful application in oils and fats industries may require special attention to feed characteristics, aging, pretreatment, process conditions, process mode, hygienic design, membrane cleaning, and disinfection.

The low energy requirement of membrane-based separation processes makes them an attractive alternative to thermal technologies. The processing is carried out at low temperatures near ambient by avoiding thermal degradation of valuable components of the final products, which is of great interest to the processors aiming for the premium market, which will pay higher prices for the processed products.

Membrane technology compared with conventional processors is a simple enclosed unit operation that can be clearly controlled and monitored to reduce costs. Each membrane technique and its economic impact to overall processing costs must be evaluated individually.

Worldwide membrane market can be characterized as perhaps the most dynamic among all process technologies. It is, however, difficult to present exact data on its volume. The United States, Western Europe, and Japan are presently the key markets. Despite that all three economies have been going through a prolonged recession for the last few years, growth in the membrane sector has been of the order of 10% p.a. The worldwide membrane/equipment market is approximately \$4.5 billion annually and is expected to have a rapid growth for the next decade. For the installed membrane area in the food sector, the dairy industry remains the major segment representing about 70% of the total market, followed by the fruit and vegetable sector, where about 20% of the installed membranes have to be found.

All commercially available membranes are developed for aqueous applications. However, nonaqueous solvents could be used if appropriate membranes, spacers, and seals are developed. The development of polymeric membranes during the last several years has been rapid, and ceramic membranes made or coated with alumina are now available in the UF range. The membrane modules are available in various configurations such as flat sheet, hollow fiber, spiral-wound, tubular, and disc tube. Desirable membrane properties required, regardless of the separation process, are high resistance to solvent, durability under high temperature and pressure, low fouling ability, selectivity toward the desirable components, and high permeate flux.

*Basic Concepts in Membrane Separation:* The separation efficiency of a specific membrane is characterized by two main concepts: permeate flux and rejection rate.

Permeate flux is defined as the permeation rate per unit of membrane area. Thus, maximizing the flux will reduce system size and cost. Permeate flux can be calculated as follows (15):

$$\text{Flux} = \text{Total quantity passed through membrane} / (\text{Membrane area} \times \text{Time}).$$

Rejection rate measures the ability of the membrane to retain a certain molecule. The observed solute rejection rate  $R_i$  for a given specie  $i$  is given by:

$$R_i = 1 - C_{ip}/C_{ir},$$

where  $C_{ip}$  is the concentration in the permeate and  $C_{ir}$  is the corresponding value in the retentate (16).

The volumetric concentration factor (VCF) indicates how many fold the feed has been concentrated, and it is defined as:

$$VCF = V_f/V_r,$$

where  $V_f$  and  $V_r$  are the volumes of feed and retentate, respectively (16).

*Membrane and Membrane Design:* Most membranes are polymers in nature, but some inorganic membranes have become available. The most common membranes are based on polysulfone, cellulose acetate, polyamide, fluoropolymers, and other compounds. Formation of a symmetric membrane structure is an important element in the success of UF/NF membrane separation (16). The other considerations for membrane separation are as follows: (1) separation capabilities (retention or selectivity), (2) separation rate (flux), (3) chemical and mechanical stabilities, and (4) membrane material cost.

The objective in membrane design is to pack as much permeation surface area into as small a space as possible to minimize operation requirements. Depending on the application, various membrane designs are used, such as flat sheet, disc tube, hollow fiber, spiral wound, and ceramic (17). Module design has a measurable effect on the hydrodynamic performance of the cross-flow membrane device. The advantages and disadvantages of different membrane modules are summarized in Table 1.

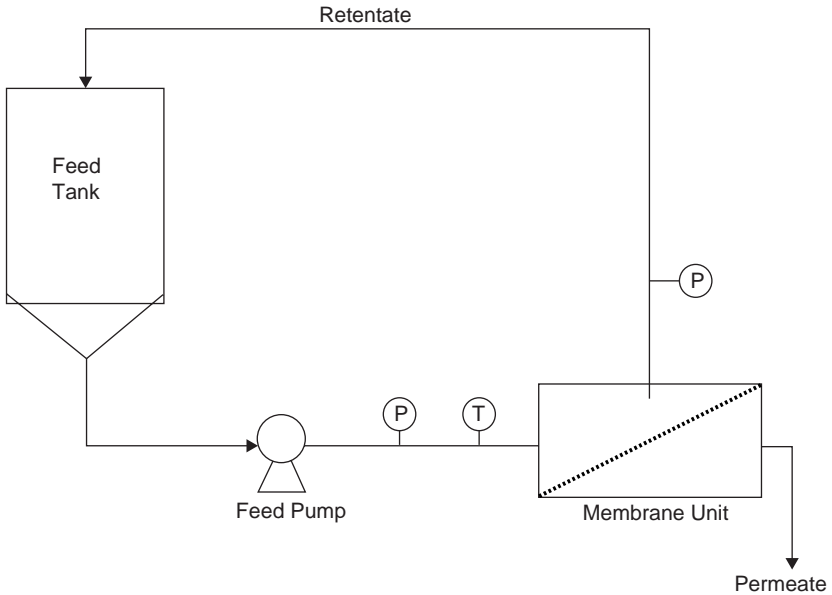
Although most UF/NF applications are aqueous, researchers have also indicated the feasibility of newer organic phase separations. The compatibility of membranes with different inorganic and organic solvents and feedstocks varies with the membrane materials.

*Process Configurations:* The most common UF/NF process configurations are (16) as follows:

1. Batch concentration
2. Feed and bleed
3. Diafiltration

**TABLE 1. Characteristics of Various Membrane Module Configurations.**

Module Type	Membrane Surface Per Module (m <sup>2</sup> /m <sup>3</sup> )	Relative Capital Cost	Relative Operating Cost	Ease of Cleaning in Place
Flat sheet	30–200	High	Low	Poor
Plate	400–600	High	Low	Poor
Spiral wound	800–1000	Very low	Low	Poor
Hollow fiber	600–1200	Low	Low	Fair
Tubular	25–50	High	High	Good



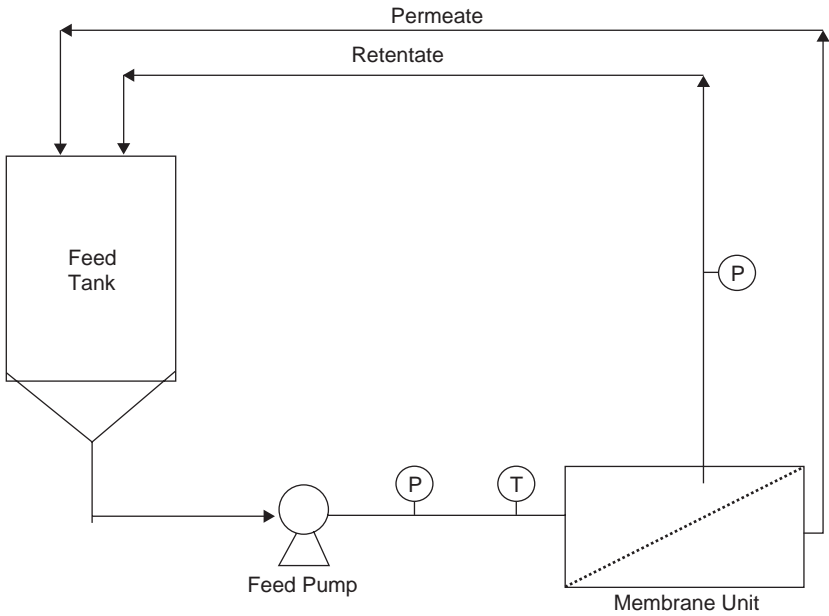
**Figure 2.** Schematic flow diagram for a cross-flow batch concentration membrane system. P: pressure gauge; T: thermometer.

The simplest batch concentration (Figure 2) configuration is most commonly used in laboratory-scale and pilot-scale units. The retentate is returned to the feed tank through the module. It is the fastest method of concentrating a specific amount of material. Another process design with total recycling of both retentate and permeate (Figure 3) is often used in the study of membrane fouling, in which the flux decrease resulting from the increase of feed concentration can be ignored.

*Applications of Membrane Processing to Edible Oils:* The commercial membrane separation processes and developing applications in oilseed processing are given in Tables 2 and 3. Currently, various companies are offering membrane systems for nitrogen production and waste treatment applications that are inexpensive and require minimal labor. Developing membrane applications in oil milling and edible oil processing, as given under the introduction sections are solvent recovery, degumming, free fatty acid removal, catalyst recovery, cooling tower water recovery, protein purification, and tocopherol separation. A thorough discussion of all applications will make this contribution undesirably long. Therefore, this article will only cover membrane degumming applications and represent data about future implications of this technology for energy saving and waste treatment costs.

*Membrane Degumming of Oils and Fats:* The greatest potential for energy saving exists in replacing or supplementing conventional degumming, refining, and bleaching processes with a single-step membrane separation system. In this relation, various groups (18–41) have pursued development of nonaqueous separation technologies. Most membrane degumming of crude vegetable oils has focused on





**Figure 3.** Schematic flow diagram of a cross-flow total recycle membrane system. P: pressure gauge; T: thermometer.

**TABLE 2. Potential Applications of Membrane Technology in Vegetable Oil Processing.<sup>a</sup>**

Application	Membrane Technique
<b>Commercial applications</b>	
Nitrogen production for packaging	Gas separation membrane
Wastewater treatment	UF/RO
Miscella filtration	MF
<b>Developing applications</b>	
Solvent recovery from miscella	NF/RO
Degumming	UF/NF
Deacidification and recovery of FFA	UF/NF
Bleaching	UF/NF
Dewaxing	UF
Vent gas recovery	NF/PV
Removal of trace metals	UF
Recovery of hydrogen catalyst	MF
Boiler condensate clean-up	RO
Fractionation of lecithin	UF
Vitamin E recovery from deodorizer distillate	MF/UF/NF/RO

<sup>a</sup>Abbreviations: UF, ultrafiltrate; RO, reverse osmosis; MF, miscella filtration; UF, ultrafiltration; NF, nanofiltration; and PV, pervaporation.

TABLE 3. Oilseed Processing and Vegetable Oils.

Application	Membrane Type	Ready to Use	Some Testing Required	Technical Development Required
Nitrogen production	GS	X		
Catalyst removal	MF	X	X	X
Degumming	UF, NF		X	X
Protein isolate purification and concentration	UF	X	X	
Hexane vapor recovery	VP	X	X	X
Oily wastewater	UF, MF	X	X	
Evaporator condensate recovery	RO	X	X	
Brine recovery and reuse	NF, UF, MF	X	X	
Wash water recovery and reuse	RO, NF, UF, MF	x	x	
BOD/COD reduction	RO, NF, UF, MF	X	X	
Cleaning chemical recovery	NF, UF, MF	X	X	
Bottle/canning wash water recovery and reuse	RO, NF	X	X	

ultrafiltration and nanofiltration. Most oils have been evaluated on a laboratory scale, and only a few have reached the pilot scale. Comparison of membrane degumming conditions and results from most publications are listed in Table (4). The literature does not contain sufficient experimental data on membrane degumming. The newly available hexane-resistant membranes have provided great opportunities for membrane degumming. However, typical data with modified, hexane resistant, nonaqueous membranes are not available. Seeking a membrane with reasonable flux, high selectivity, and less fouling is still necessary for commercialization of this technology.

Theoretically, triacylglycerols and phospholipids have similar molecular weights (about 900 Da), which makes them difficult to separate by membranes. However, phospholipids are surfactants in nature, with both hydrophilic and hydrophobic ends, and they can form reverse micelles in nonaqueous environments with globular structures (Figure 4). The micelles formed have a molecular weight of 20,000 Da or more (12). Accordingly, phospholipids can be separated in the miscella stage with appropriate membranes. The membrane-based crude oil degumming procedures produce permeate and retentate fractions containing triacylglycerols and phospholipids, respectively (Figure 5). Most coloring materials and some FFAs and other impurities are embedded in the phospholipid micelles and are removed as well.

Processes for refining acylglycerol oils using semipermeable membranes have been disclosed in literature by Gupta (19–22). U.S. Patents 4,062,882 (19) and 4,093,540 (20) of A.K. Sen Gupta describe a process for refining a crude acylglycerol oil composition by passing a solution of this oil in an organic solvent under pressure over a semipermeable membrane. In this process, the membrane retains the

**TABLE 4. Comparisons of Reported Membrane Degumming Conditions and Results.**

Ref.	Degumming <sup>a</sup> Conditions	Membrane Materials	Membrane Module	Permeate Flux (LMH)	Residual Phosphorus Content(ppm)
(19)	20°C, 3 l/hr 20°C	DPE <sup>b</sup> Polysulfone	Tubular Plate Plate	8 60	<2 <sup>c</sup> 16 <sup>c</sup>
(20)	33%, 2 Kg/cm <sup>2</sup> , 50 l/hr	Polyacrylonitrile	N/A	30	10 <sup>c</sup>
(21)	50%, 4bar, 20°C, ammonia 50%, 4bar, 20°C, KOH	Polysulfone Polyamide	Plate Plate	N/A N/A	7 <sup>d</sup> 2 <sup>d</sup>
(25) <sup>f</sup>	30%, 40°C, 0.8 Kg/cm <sup>2</sup> , 150 l/min 30%, 40°C, 3 Kg/cm <sup>2</sup> , 14 l/min 30%, 55°C, 3 Kg/cm <sup>2</sup> , 14 l/min	Polyamide Polyamide Polyimide	Capillary Tubular Tubular	N/A N/A 17.8	221 <sup>c</sup> 219 <sup>c</sup> 24 <sup>c</sup>
(40)	Ultrafiltration	N/A	N/A	33.08	0 <sup>d</sup>
(31)	Dewaxed at 5°C and UF at 15°C, 0.9 Kg/cm <sup>2</sup> , 3.1 l/hr with H <sub>3</sub> PO <sub>4</sub> and NaOH	Polyethylene	Hollow Fiber	3.1	<10 <sup>e</sup>
(32)	25%, 150 psi, 40°C	Polyether imide	Disc tube	7.2	9 <sup>d</sup>
(30)	27%, 3 Kg/cm <sup>2</sup> , 40°C, 14 l/min	Polyimide	Tubular	N/A	25.4 <sup>c</sup>

<sup>a</sup>Listed in sequence are miscella concentration, pressure, temperature, and feed velocity.

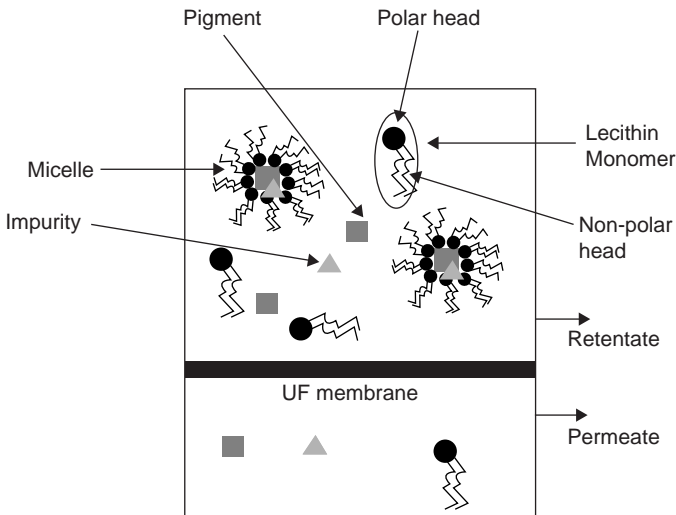
<sup>b</sup>DPE, dimethyl polysiloxane elastomers.

<sup>c</sup>Soybean oil.

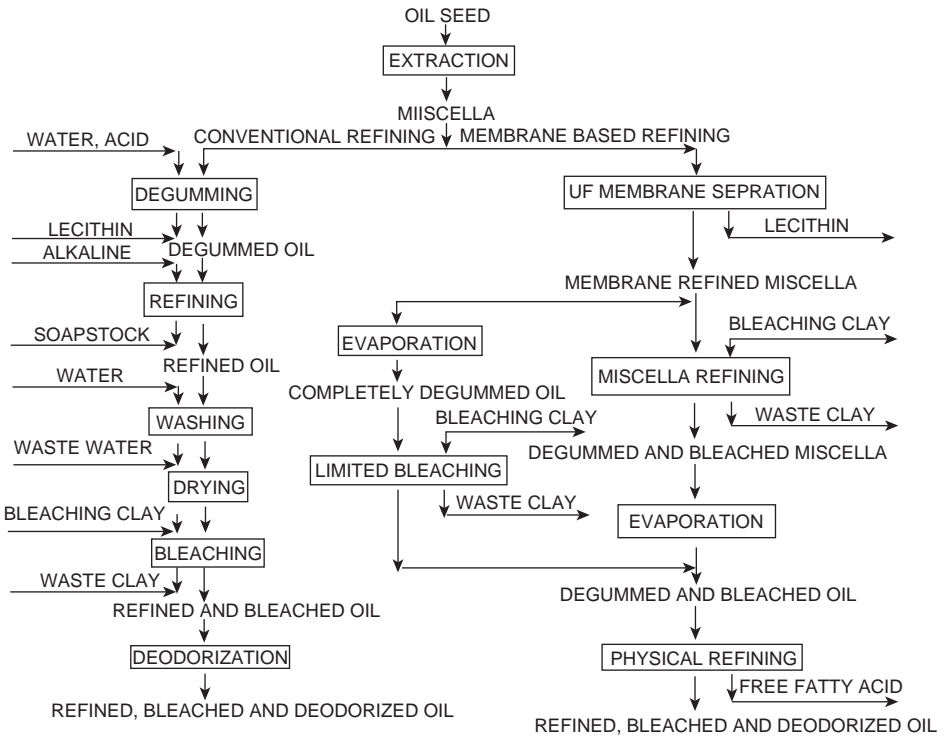
<sup>d</sup>Cottonseed oil.

<sup>e</sup>Sunflower oil.

<sup>f</sup>Pilot-scale.



**Figure 4. Miscella formation and separation mechanism.**



**Figure 5.** Diagrams for the existing chemical refining process and the proposed membrane degumming process (32).

phosphatides present in the oil solution; as a result of which, the oil solution passing through the membrane shows a reduced phosphatide content. The membranes described in U.S. Patent 4,093,540 are made of polyacrylonitrile. The membranes described in U.S. Patent 4,062,882 are made of polyacrylonitrile, a polysulfone, and a polyamide. In U.S. Patent 4,553,501 to Sen Gupta, a membrane filtration process is described that not only removes the phosphatides present in the crude triacylglycerol oil but also the free fatty acids present therein by the addition of a base to the miscella. In addition to the polyacrylonitrile, polysulfone, and polyamide membranes mentioned above, polyimide anisotropic membranes are also used for this purpose. Membranes made of these polymers are also described in U.S. Patent 6,207,209 of Jirjis et al. (41).

The process developed by Gupta (19–22) processed crude oils with a combination of UF membrane separation and silica gel column percolation. The soybean and rapeseed-hexane miscellas were passed through various UF membranes. The solvent was removed from the permeate, and the degummed oil was then treated with bleaching clay, silica, and/or acid and finally was steam deodorized to produce refined, bleached, and deodorized oil. The color readings of the oils obtained

**TABLE 5. Composition of Products Obtained from Membrane Processing of Soybean and Rapeseed Oils.**

Treatment	Chlorophylls%	Color	Phospholipids ppm	FFA%
<b>Soybean Oil</b>				
Crude oil	—	3.5R + 35Y	662	1.82
Ultrafiltered	0.412	3.4R + 34Y	25	0.95
Ultrafiltered acid treated <sup>a</sup> and bleached <sup>b</sup>	0.001 0.000	2.6R + 27Y 0.5R + 4Y	23 21	1.05 0.03
Ultrafiltered, bleached, acid treated <sup>a</sup> , and deodorized <sup>c</sup>	0.000	0.4R + 4Y	24	0.03
Conventional treatment <sup>d</sup> (refining, bleaching, and deodorization)	0.008 0.002	3.3R + 32Y 0.4R + 4Y	20 20	0.98 0.04
Ultrafiltered and bleached				
Ultrafiltered, bleached, and deodorized				
<b>Rapeseed Oil</b>				
Crude oil	19.800	5.9R + 58Y	722	2.84
Ultrafiltered	15.800	5.8R + 47Y	31	1.21
Ultrafiltered, acid treated, and bleached	0.003 0.000	2.9R + 28Y 0.4R + 3Y	28 25	1.32 0.03
Ultrafiltered, acid treated, bleached, and deodorized	0.000	0.4R + 4Y	23	0.03
Conventional treatment (refining, bleaching, and deodorization)				

by conventional treatment (refining, bleaching, and deodorization) and ultrafiltration (ultrafiltration, acid treatment, bleaching, and deodorization) were identical (0.4R-4Y). The FFA content of the ultrafiltrated oils was reduced from their initial amounts of 1.82% and 2.84% to 0.95% and 1.21% for soybean oil and rapeseed oils, respectively (Table 5). The phospholipid contents of the refined, bleached, and deodorized oils from both methods were in the range of 20–25 ppm. In later work (20), the FFA content of soybean, cottonseed, rapeseed, and rice bran oils were reduced from 2.8% to 16.0% to 0.09% to 1.3% by use of additives, gums, soaps, ammonia, and polyvalent compounds (Table 6). Chlorophylls and gossypol contents of the rapeseed and cottonseed oils were reduced by a combination of UF and ammonia treatments from 58 ppm and 3800 ppm to 12 ppm and 100 ppm, respectively. The PM10 membrane (Amicon, Beverly, MA) selectively retained phospholipids in the concentrate fraction, and the permeates contained only 4–10-ppm phosphorus.

Various comparisons of the conventional refining technique versus the ultrafiltration based method using soybean and rapeseed oils are reported, and the results show that there is no difference in the quality of the oils obtained from these two methods. Table 7 compares composition of crude, degummed, superdegummed, and ultrafiltrated oils. The lowest phospholipid, Ca, Mg, and Fe contents were obtained by the ultrafiltration method. Both UF and superdegummed oils were

**TABLE 6. Comparison of Ammonia-KOH-Treated and Membrane-Processed<sup>a</sup> Products and Crude Soybean, Cottonseed, Grapeseed and Rice Bran Oils.**

	Chlorophylls ppm	Gossypol ppm	Color <sup>b</sup>	P %	FFA %
<b>Soybean Oil</b>					
Crude oil	—	—	6.5R + 70Y	900	2.80
Ultrafiltered	—	—	5.6R + 70Y	6	2.80
Ultrafiltered ammonia treated	—	—	4.0R + 40Y	4	0.09
<b>Cottonseed oil</b>					
Crude oil	—	3800	—	666	6.20
Ultrafiltered	—	1100	—	7	6.00
Ultrafiltered ammonia treated	—	100	—	7	0.30
<b>Grapeseed oil</b>					
Crude oil	58	—	—	65	4.00
Ultrafiltered	48	—	—	5	3.60
Ultrafiltered ammonia treated	12	—	—	5	0.50
<b>Rice bran oil</b>					
Crude oil	—	—	13R + 70Y	300	16.00
Ultrafiltered	—	—	9R + 60Y	<10	31.00
Ultrafiltered ammonia treated	—	—	6R + 60Y	<10	1.30

<sup>a</sup>Diaflo PM membrane at 4 bar, 20°C using stirred cell system.

<sup>b</sup>2 cell.

**TABLE 7. Composition of Crude, Degummed, Superdegummed, and Ultrafiltered Soybean Oil.**

Components	Crude Soybean Oil	Degummed Soybean Oil	Superdegummed Soybean Oil	Ultrafiltered Soybean Oil
Phospholipids, %	2–3	0.3–0.8	0.05–0.08	0.03
Non P	0.15–0.3	0.02–0.1	Reduced Amount	<50
Glycolipids, %	0.10–0.15	0.02–0.03	Reduced Amount	<1
Free sugar, %	0.01–0.02	—	N/A	N/A
Lipoproteins, %	ca. 0.005	0.001	N/A	N/A
Amino acids, %				
Potassium, ppm	300–400	70–100	N/A	<5.0
Calcium, ppm	100–150	50–120	<5	<5.0
Magnesium, ppm	80–130	50–100	<5	<0.1
IronI, ppm	1–10	0.5–5.0	<0.50	<0.1
Carotenes, ppm	25–30	20–25	—	<20
Green pigment, ppm	1–2	1–2	—	<0.5
Free fatty acids, %	0.5–1.6	0.5–1.6	0.5–1.6	0.5–1.6

superior to those obtained from the conventional degumming process. Data for the rapeseed miscella, treated with UF and KOH, indicate that the residual phospholipids and metals in the oil are low enough to be removed by either silica gel or bleaching clay treatments (Table 8). PC content of the lecithins obtained from conventional, Alcon (deactivated the enzymes before solvent extraction), and ultrafiltration

**TABLE 8. Analysis of Rapeseed Oil Before and After Miscella UF Process (With and Without the Addition of KOH).**

	P ppm	FFA %	K ppm	Fe ppm	Cu ppm	S ppm	Lovibond 23'' R G B
Crude Oil	294	1.30	39.0	3.20	0.30	19	8.2 + 80 + 5.1
UF treated oil without KOH refining	7	1.30	2.0	0.13	0.04	9	6.0 + 70 + 1.2
UF treated oil with KOH Refining	3	0.03	0.7	0.01	0.01	4	4.2 + 50 + 0.0

Final oil was bleached with 1.5% bleaching clay and deodorized; taste of refined product was stable for 3 months, and the frying properties were excellent. Membrane: Polysulfone PM 10 (Amicon).

**TABLE 9. Composition (%) of Lecithin from Normal Soy Oil, Alcon Process, and Membrane-Separated Oils.**

Phospholipid	Lecithins from Normal Soy Oil	Lecithins from Alcon-process	Lecithins from Membrane Process
PC	32	46	51.2
PE	21	23	14.2
LPC	2	2	2.0
PI	14	8	0.3
PA	17	19	3.6
Other P-Lipids	4	2	2.6

K-, Ca-, and Mg-amounts in oils or lecithin are not available.

processes were 32%, 46%, and 51.2% (Table 9). The high PC content is one of the most desirable components of the phospholipid mixtures because of its excellent functional property.

Japanese workers (23–26,30) and Koseoglu et al. (27–29) have also shown that complete removal of phospholipids from the crude oils is possible with a polyimide ultrafiltration membrane. Polyimide membranes are described in U.S. Patent

**TABLE 10. Comparison of Products Obtained Both from Membrane and Conventional Processing of Sunflower Oil.**

Dewaxed Products from	Wax	FFA %	Color	Phospho- Lipids %	POV %	AOM (19.2 hrs) %
Membrane processed, bleached, and deodorized <sup>a</sup>	0.006	0.04	0.3R + 3.5Y	<0.001	0.0	11.0 POV
Conventional processing including refining, bleaching, and deodorization	0.006	0.04	0.3R + 3.1Y	<0.001	0.0	12.0 POV

<sup>a</sup>Bleached with acid-activated bleaching clay and deodorized at 260°C for 1.5 hr at 3 mm Hg.

4,414,157 of Iwama et al. (25), and U.S. Patent 4,545,940 issued to Mutoh et al. (31) describes a membrane used for the dewaxing of triacylglycerol oils that consists of a copolymer of ethylene and tetrafluoroethylene. In addition to waxes, phospholipids and free fatty acids were also retained by this membrane, but the disclosed flux is too low to be economical.

Mutoh et al. (31) dewaxed vegetable oil by cooling to allow the wax to crystallize followed by temperature-adjusted ultrafiltration. The authors claimed that, in addition to wax removal, this process also reduces phospholipids, FFAs, and water content in vegetable oils. The wax, FFA color, peroxide value (PV), and phospholipids contents of the oil were exactly the same, and the AOM values were found to be comparable. Iwama (26) completed a large-scale evaluation with 25 tons of soybean oil and reported that the combined membrane degumming and physical refining is an economically viable alternative to conventional processing of soybean oil. The solvent-resistant commercial polyimide ultrafiltration membrane (20,000 MWCO) NTU-4220 (Nitto Electric, Tokyo, Japan) was effective at 40–50°C to separate the phospholipids selectively. Increase in pressure from 2 kg/cm to 4 kg/cm increased the flux from 60 l/m<sup>2</sup>.h to 90 l/m<sup>2</sup>.hr. The feed oil concentration is reported to be less than 50%, and the best results were obtained in the range of 20–30% oil in the feed. The concentration did not influence the selectivity of phosphorous rejection rates. The purified oil recovery rates were 97–98% during the batch experiments. The results from long-term evaluations (1400 hr) show that miscella flux was steady with no major fouling. The long-term storage experiments and stability test showed no apparent difference in the quality of the oils obtained from chemical or membrane-based technologies. The five-stage membrane-based miscella degumming process was tested, and data were collected for 250 tons/day of soybean oil production facility. A five-stage process may require 1400 square meters of membrane area, and the total investment cost could be recovered within three years. The processing conditions for soybean oil purification using a combination of capillary (CM) 50,000 MWCO polyimide ultrafiltration membranes and tubular (TM) membrane systems are given in Table (11). A combined CM and TM

**TABLE 11. Processing Conditions and Data from Retentate for Soybean Oil Purifications with Polyimide and Membranes.**

Membrane system	Flow rate 1/min	Membrane processing conditions			Retentate	
		Pressure kg/cm <sup>2</sup>	Temperature °C	Treatment time, min	Phospho-Soybean lipids, %	oil, %
Polyimide membrane 50,000 MWCO						
CM	150.8	0.8	55	—	67.3	32.7
TM	14.0	3.0	55	1578	96.5	3.5
CM & TM		Same as CM and TM		454	96.4	3.6

Abbreviation: CM, capillary membrane system setup; TM, tubular membrane system setup; CM & TM, capillary and tubular membrane systems.



TABLE 12. AOCS Color Measurements of Membrane-Processed Cottonseed Oils.

Membrane	Color Readings 1" Cell			β-Carotene Readings	
	Crude Oil Retentate	Permeate	Retentate	Crude Oil	Permeate
Polyamide	2.5R/16Y 12.7	0.4R/3Y	7.4R/50Y	12.5	16.5
Polyvinylfloride	3.6R/24Y 74.3	0.4R/3Y	4.3R/28Y	67.2	4.5

system recovers 96.6% of the oil in the permeate fraction. The total phospholipid rejection rates were around 99.9%.

Semi-pilot plant scale trials with crude oils containing 220–580 ppm phosphorous (28) show that nonaqueous UF separations might be used to degum and partially decolorize crude domestic oils before physical refining. No permeates from two commercially available membranes contained phosphorous, thus indicating complete removal of hydratable and nonhydratable phospholipids (28).

Soybean oil does not have as much of a color problem as do cottonseed and peanut oils. Reductions of yellow color were insignificant, but 50% reductions in red color values were achieved. Beta-carotene content was not influenced by the membrane treatment, although chlorophyll contents of the permeates were reduced. The best results were obtained with cottonseed oil, where color readings were reduced from 2.5 R–16 Y in the crude oil to 0.4 R–3 Y. Beta-carotene content was significantly reduced by polyvinylfloride membrane (Table 12).

Theoretically, triacylglycerols and phospholipids have similar molecular weights, which make them difficult to separate by membrane technology. However, the structural differences are exemplified in nonpolar solvents such as hexane. Phospholipids are surfactant in nature, and in hexane miscella, they form micelles with a molecular weight of 20,000 Da or more.

The maximum pore size used to separate phospholipid micelles, in which color pigments and other impurities are physically bound, can be in the range of 10,000–50,000 Da depending on the polymer type. Considerable swelling occurs with polysulfone membranes, which, in turn, affects the membrane chemistry drastically and reduces flow rates and in some cases totally closes the pores. Similar results have also been observed with polyamide and fluorinated polymers.

The MW descriptions of the membranes in hexane may not be applicable because of swelling and the changes in the surface chemistry of the polymers used. A sizable reduction in flow rates may be observed with hexane-oil miscellas. The stability of micelles apparently is a function of solvent type, charges and location of the phosphorous group, and specific impurities of the crude oil.

The micellar-enhanced ultrafiltration MEUF technique, based on addition of surfactants and chelating agents to complex and enhance removal of undesirable compounds, show considerable promise in membrane degumming applications. The natural substances such as phospholipids act as surfactants to form large micelles that will be rejected by the membrane.

**TABLE 13. Effect of Membrane Type on Phosphorus Rejection Rate and Permeate Flux.**

Membrane Type	Miscella I		Miscella II	
	Flux (1/m <sup>2</sup> /h)	Phosphorus Rejection (%)	Flux (1/m <sup>2</sup> /h)	Phosphorus Rejection (%)
Polyimide	2.87	99.3	0.86	83.3
Polyamide	6.46	98.1	4.31	78.7

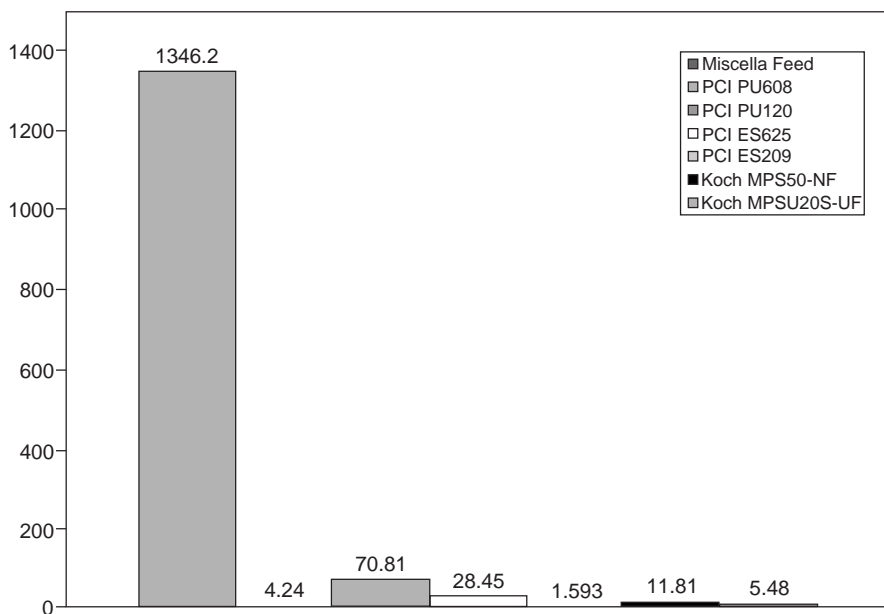
In recent tests (29), polyimide membranes showed higher rejection rates with hexane and isopropyl alcohol (IPA) than that of the polyamide membrane. The permeate flux of polyamide membrane with the crude cottonseed oil-hexane and IPA miscellas (20% by weight) were 2.25–5 times of the polyimide membrane (Table 13). The highest fluxes obtained with hexane and IPA miscellas were 6.6 l/m.hr and 1.4 l/m.h, respectively.

The influence of temperature on permeate is significant when the process is carried on under 150 psi and with a feed concentration of 20 weight%. Flux is mainly a function of viscosity and can be increased by 400% when the temperature of the mixture is increased from 20°C to 50°C.

Recent tests by Sun (32) have shown that polyimide membranes have higher rejection rates than those of polyamide membranes, but polyamide membranes have higher flux. The highest flux obtained with hexane miscella and polyamide membranes was 6.6 LMH. The phosphorus rejection rates of 98.1–99.3% were obtained with hexane miscella. The addition of surfactants increased the phosphorus rejection rate from 83.3–78.7% to 96.4% with IPA miscella. The added surfactants facilitated the formation of large phospholipid clusters. Koseoglu et al. (33) reported that membranes made of polyamide were least affected by hexane, but that a membrane made from a fluorinated polymer was deteriorated by hexane.

The recent U.S. patent by Koseoglu et al. (34) illustrates a process for refining and degumming of oils by ultrafiltration that significantly improves the quality of the processed oils and lecithin products and is more economical and environmentally suitable than other known methods. The process includes the steps of (1) contacting the oil with an ultrafiltration membrane wherein the ultrafiltration membrane comprises a polymer or copolymer of a vinylidene difluoride monomer; (2) separating the oil into a permeate fraction having a reduced phosphatide, color, and free fatty acids content and a retentate fraction having an increased phosphatide content; and (3) treating the retentate fraction to produce a lecithin product. This process will allow the use of ultrafiltration membranes that can be profitably in the degumming of solutions of oils in organic solvents (including phosphatides) by an ultrafiltration process.

The ultrafiltration membranes according to this process (i.e., comprised a polymer or a copolymer of a vinylidene difluoride monomer) are (1) hexane-resistant; i.e., they retain their strength and do not swell in hexane so that they retain their



**Figure 6.** Phosphorous content of permeates obtained from various membranes.

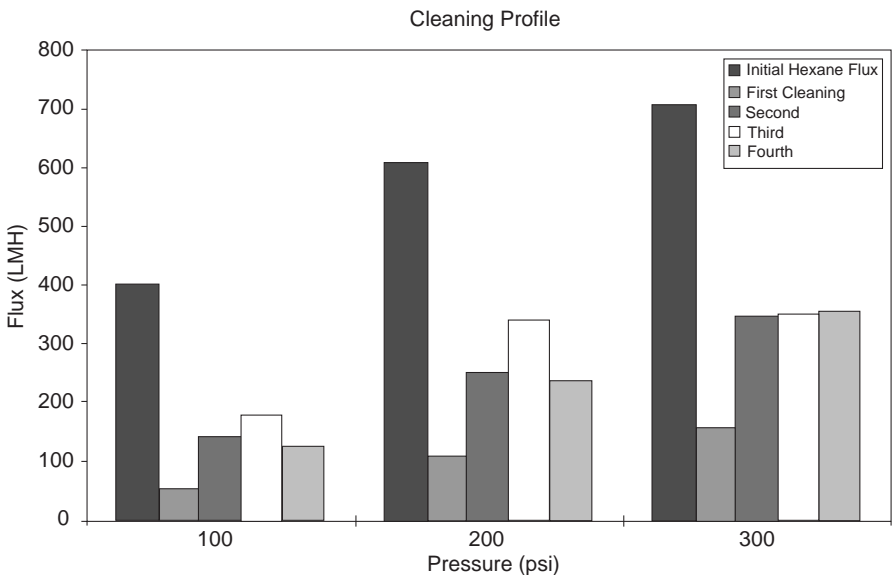
permeability and selectivity; (2) withstand the pressure drop over the membrane during ultrafiltration; (3) have a good permeability for triacylglycerol oil and its solvents, which results in a relatively high flux through the membrane during the ultrafiltration process; (4) retain free fatty acids, coloring pigments, and phosphatides from pressed oil and/or miscella and thus show a high selectivity; and (5) do not unduly suffer from fouling; i.e., rinsing the membrane with solvent, permeate, or miscella at regular (but not too frequent) intervals restores the membrane flux to a fully acceptable and constant level. Figure 6 shows phosphorous content of permeates obtained from various membrane modules. The phosphorous content of the crude oil was reduced drastically from 1346 ppm to 1.5 levels in the permeate.

Good results in degumming miscella have been obtained by using a polyvinylidene difluoride (PVdF)-membrane according to the present invention with a molecular weight cutoff (MWCO) of 30,000 to 200,000 Da. Lower MWs, as, for example, 9000 Da, can also be used, but they are only slightly more selective and their flux is lower. The preferred MW is about 5000 to about 500,000 Da. The most preferred MW is about 10,000 to about 250,000 Da. Using a membrane with a higher MW leads to a higher flux and thus to savings in the investments required for a given degumming capacity but can lead to a decrease in selectivity. However, it has been found that this decrease can be effectively counteracted by forming a small amount of soap in a pressed oil or miscella being processed by partially neutralizing the free fatty acids present therein. Consequently, the membranes according to the process can also profitably be used for degumming crude pressed oils.

When a miscella comprising solvent, triacylglycerol oil, and phosphatides is fed to such an ultrafiltration module, its membrane will retain the phosphatides but allow the solvent and the triacylglycerol oil to pass through the membrane. Accordingly, the retentate will have a higher phosphatide content than the feed and the permeate will have a significantly lower phosphatide content than the feed, which means that the refined oil resulting from evaporating the solvent from this permeate and from bleaching the still residue can be physically refined to yield a high-quality, fully refined edible oil.

The ultrafiltration mode in the process according to the method is a cross-flow filtration, which means that the miscella is pumped along the ultrafiltration membrane and that only a fraction of the solvent and oil present in the miscella permeates through the membrane per pass through the membrane tube. Accordingly, the retentate can be recycled to the feed of the module and/or it can be fed to a second and/or subsequent modules to attain a higher phosphatide concentration in the retentate (and thus a higher yield of degummed oil). Membrane cleaning with hexane is effective (Figure 7), and there is no need for a water/detergent-based cleaning system. Figure 7 illustrates the flux levels after four consecutive tests.

Diluting the retentate with fresh solvent and its subsequent concentration by ultrafiltration will thus allow a substantially oil-free solution of phosphatides to be obtained. A substantially oil-free lecithin powder product can be produced from this solution by evaporating the solvent, for instance, in a spray tower. Conventional oil-free lecithin powder is normally produced by de-oiling conventional standard lecithin, using either acetone or a liquefied hydrocarbon, such as propane. In conventional processes, this de-oiling constitutes a separate process step, and thus



**Figure 7.** Cleaning profiles with hexane for the solvent membranes after four tests.

**TABLE 14. Analytical Data That Indicate Results Obtained by Subjecting Crude Soybean Oil to the Process.**

Property/sample	Permeate	After Treatment with Rice Hull Ash	After Treatment with Bleaching Earth	After Physical Refining
Free fatty acids (wt%)		0.17	0.16	0.02
Lovibond color Y/R		30/10	40.00/5.40	1.00/0.10
Peroxide value		1.80	0.60	0.00
Anisidine value		1.20	1.40	1.00
Chlorophyll, ppm		595.00	25.00	20.00
Phosphorus, ppm	0.63	0.06	0.00	0.10
Calcium, ppm	0.02	0.02	0.02	0.02
Magnesium, ppm	0.02	0.02	0.02	0.02
Iron, ppm	0.02	0.00	0.00	0.02
Copper, ppm	0.00	0.00	0.01	0.00
Nickel, ppm	0.00	0.00	0.00	0.02
A.O.M., h				18.00

makes de-oiled lecithin an expensive product. Producing a substantially oil-free lecithin powder product according to the present method is thus more economical than the conventional process. Crude soybean oil containing 1799-ppm phosphorus and a free fatty acid content of 0.61 wt% was treated in a membrane system and a permeate resulted that had a phosphorus content of 6.3 ppm in the oil. The solvent was evaporated, and the resulting still residue was treated with 0.20 wt% citric acid treated rice hull ash under vacuum at 99°C for a period of 15 min, after which the adsorbent was removed by filtration. Subsequently, the oil was bleached at 105°C and under vacuum while using 0.95 wt% bleaching earth (Filtrol 105 WF) for a period of 15 min; again, the adsorbent was removed by filtration. The filtrate was subjected to a physical refining process at 257°C for a period of 74 min. Samples were taken and analyzed. The analytical data given in Table 14 clearly show that excellent results are obtained by subjecting crude soybean oil to the process according to the present method and then to standard bleaching and physical refining treatments. The taste of the fully refined oil was judged to be 9 on a scale of 1–10, and its storage stability was fully acceptable.

*Economics of Membrane Degumming:* Membrane technology can be applied to edible oil refining to (1) simplify the process, (2) reduce energy consumption, and (3) reduce wastewater production. According to the U.S. Department of Energy, the potential energy savings from implementation of this technology in the United States alone is estimated to be 7.2–35.3 trillion BTU per year. In 2 years, with 25% market penetration, 14.5 million gallons/year of water and 27,670 tons/year of solid waste could be eliminated (18). In addition, loss of neutral oil with gums could be reduced by 75% (18). If the energy savings are combined with other savings, such as the reduced capital investment, waste reduction, and recovery of neutral oil and phospholipids, the total impact of this new technology to the whole edible oil industry could be doubled (18). Figure 5 shows diagrams for the existing chemical refining process, the physical refining process, and the proposed membrane refining process.

*Energy Savings:* Energy usage in refineries and expected savings resulting from the introduction of new membrane process can vary with the age (actually the extent of modern practices), and it may range from 2000 to 4000 BTU/lb of oil processes. In fact, most oilseed mills and refineries in the United States are old, and their energy usage is about 4000 BTU/lb. As these plants are less efficient, more energy savings can be expected.

The following energy savings estimates include considerable practical information we have collected from our industry. Data on membrane life, effects of fouling, and changes in feed stock are needed to answer the remaining questions.

Approximately 16.3 billion lb of crude soybean, cottonseed, corn, and peanut oil were refined in the United States during 1993 (34), which includes the following oilseeds: 12.675 b soybean, 1.2 b corn, 1.045 b cottonseed, 186 m peanut, 375 m sunflower, and 849 m canola. Older plants (Plant A: 4000 BTU/lb oil) could save 40% of their total energy consumption. However, for newer plants (Plant B: 2000 BTU/lb oil), the savings is expected to be around 10%. Therefore, the range energy saving from degumming, refining, and bleaching by the new process is estimated to be around  $3266 \times 10^9 - 26,128 \times 10^9$  BTU/year.

According to industry experts, energy usage in waste treatment is about 7–10% of the total consumption depending on the age of the plant. The energy savings expected from elimination of wastewater treatment in edible oil refineries could be in the range of  $2286 \times 10^9 - 6532 \times 10^9$  BTU/year. These estimates do not include reductions in capital investments. For example, elimination of wastewater treatment results in at least \$1.5 million savings in capital investment for each plant built based on this technology.

Based on our calculations, the total energy savings expected from successful implication of membrane processing into edible oil processing can range from 7.2 to 35.3 trillion BTU per year in the United States alone. These calculations are based on the data available from the energy audits of various oil mills and refineries and U.S. Department of Commerce publications. The refining losses caused by entrapment of neutral oil in soapstock and hydrolysis of neutral oil during the caustic refining is around \$157.7 millions/year. In addition, the volume of neutral oil lost with the bleaching clay is about \$26.3 millions/year.

The other savings that can be obtained from utilization of the technology is \$22.06 per ton of oil processed, which include reduced solid waste, neutral oil losses, bleaching clay usage, and higher value of the byproducts. The additional costs for physical refining will be balanced by the savings from elimination of the oil drying costs.

According to a recent DOE report (15), steam energy savings from partial or full implication of membrane technology in membrane refining, degumming, and bleaching is expected to be at least 15–21 trillion BTU's/yr. In addition, if reduced oil losses, steam savings, and other savings from such as reduced capital investment, wastewater treatment, and cooling water are combined with savings from neutral oil and steam usage, the total impact of this new technology to the U.S. edible oil industry will be doubled.

*Waste Reduction:* In many cases, wastewaters from vegetable oil processing mills are discharged to municipal treatment systems or are treated on-site and

discharged directly into rivers or streams. Direct on-site discharge treatment typically includes pH adjustment to acidic range, gravity separation, pH readjustment to near neutrality, chemical coagulation with dissolved air flotation, biological treatment, and, in some cases, filtration through granular media. Many plants have found that discharge to municipal systems (despite high costs) is more economical than on-site treatment. However, because of the increased disposal charges and governmental disposal limits, more edible oil processors are in search of new inexpensive, efficient technologies to eliminate these high BOD acidic waters.

The average wastewater production for per 100 lb of crude oil processed is 14.1 lb. The total amount of wastewater produced by the U.S. edible oil industry on a yearly basis (based on 1993 production data) is 23.02 million gallons. In addition, currently 106,890 tons of used bleaching clay is disposed of. The disposal sites for this kind of solid waste is limited. The used bleaching clay is regularly trucked to the disposal site.

## 6. CONCLUSIONS

The new membrane method (Figure 5) will simplify the whole process to almost a single-step operation. The process not only removes all phospholipids, but it also removes most coloring pigments and some free fatty acids. Bleaching requirements are reduced because of entrapment of some coloring pigments during the membrane separation process. A great deal of profit should be realized because of improved productivities and by eliminating the chemical process. Moreover, it is emphasized that the membrane process can be carried out without water and chemicals, which results in no wastewater streams. This result would reduce costs by eliminating installations for treating effluents. It is meaningful that hexane removal by distillation after degumming results in the improvements of distillation efficiency. Additionally, it would be profitable because the membrane process makes it possible to omit the waxing process and to recover phospholipids from the concentrated side. Energy savings and wastewater elimination approach 7.26–35.33 trillion BTU/yr and 23.02 million gallons/year, respectively. Decreased oil losses and decreased bleaching earth requirements, 53,445 tons/year, are the other potential advantages of membrane processing. The advantages of this unique process are as follows:

- Elimination of degumming process.
- Neutral oil loss is reduced by 80%.
- Does not require phosphoric acid for nonhydratable phosphatide.
- Produces a value-added product such as lecithin.
- Elimination of caustic refining.
- No need for acidulation process, and FFA can be sold as is.
- 100% elimination of waste waters from degumming, acidulation, and water washing steps.
- Complete elimination of oil drying process.
- Reduction in bleaching requirements during bleaching process.

- In a conservative estimate, 50% reduction in bleaching clay use (55,445 tons) and disposal is obtained.
- Neutral oil loss is reduced because of miscella bleaching.
- Ability to use physical refining for oils like soybean and cottonseed.

Thus, soybean, cottonseed, peanut, or canola crude oils from this process can easily be physically refined, which would provide a considerable advantage that imported palm oil enjoys. Specifically, palm oil, a major price competitor of domestic oils, like cottonseed, soybean, and peanut in world markets, is processed by “physical refining”—a method in which the oil is subjected to a high-temperature-high-vacuum process that distills off only the free fatty acids and undesirable odor and flavor compounds. However, to date, physical refining has not been applicable to domestic oils because of their relatively high (2–5%) contents of phospholipids (“lecithins,” “gums”). These compounds become “hydrated” and mucilaginous during degumming and/or caustic refining, and they carry along the occluded neutral oil during their removal by centrifugation.

This process alone, in addition to the advantages summarized above, shows the following benefits of the physical refining that will also be obtained. The various costs such as installation (22%), capital investment (20%), steam usage (28%), cooling water (7%), process makeup water (85%), wastewater treatment (63%) electrical power (62%), and refining loss (60%) are lower compared with that of the chemical refining process.

## REFERENCES

1. S. S. Koseoglu and D.E. Engelgau, *J. Amer. Oil Chem. Soc.*, **67**, 239–249 (1990).
2. G. Hoffman, *The Chemistry and Technology of Edible Oils and Fats and Their High Fat Products*, Academic Press, San Diego, California, 1989.
3. F. D. Gunstone, J. L. Harwood, and F. B. Padley, *The Lipid Handbook*, 2nd ed., Chapman & Hall, New York, 1994, p. 64.
4. L. A. Jones, and C. C. King, *Cottonseed Oil*, National Cottonseed Products Association, Inc. and The Cotton Foundation, Memphis, Tennessee, 1990.
5. G. R. List, in D. R. Erikson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soy Oil Processing and Utilization*, American Oil Chemists’ Society, Champaign, Illinois, 1980, p. 362.
6. R. A. Carr, *J. Amer. Oil Chem. Soc.*, **55**, 765 (1978).
7. L. Lin, K. C. Rhee, and S. S. Koseoglu, in S. S. Koseoglu, K. C. Rhee, and R. Wilson, eds., *Proceedings of 1996 AOCS World Conference and Exhibition on Oilseed Processing and Edible Oil Refining*, AOCS Press Champaign, Illinois, 1998.
8. A. K. S. Gupta, U.S. Patent 4,062,882, 1977.
9. S. S. Koseoglu, *Oils & Fats Intl.*, **5**, 16 (1991).
10. T. L. Mounts, Refining, in D. R. Erikson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soy Oil Processing and Utilization*, American Oil Chemists’ Society, Champaign, Illinois, 1980, p. 139.



11. G. R. List, J. W. King, J. H. Johnson, K. Warner, and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **70**, 473 (1993).
12. G. Haraldsson, *J. Amer. Oil Chem. Soc.*, **60**, 251 (1983).
13. R. Ohlson and C. Svensson, *J. Amer. Oil Chem. Soc.*, **53**, 8 (1976).
14. K. J. Moulton and T. L. Mounts, *J. Amer. Oil Chem. Soc.*, **67**, 33 (1990).
15. C. M. D. Mohr, D. E. Engalgau, S. A. Leeper, and B. L. Charboneau, *Membrane Application and Research in Food Processing*, Noyes Data Corp., Park Ridge, New Jersey, 1989.
16. S. S. Kulkarni, W. F. Edward, and N. N. Li, Ultrafiltration, in W. S. Winston and K. K. Sirkar, eds., *Membrane Handbook*, Van Nostrand, Reinhold, New York, 1992, p. 393.
17. I. Bemberis and K. Neely, *AIChE Symp. Ser.*, **250**, 65 (1986).
18. S. S. Koseoglu and D. E. Engalgau, *J. Amer. Chem. Soc.*, **67**, 239 (1990).
19. A. K. S. Gupta, U. S. Patent 4,062,882, 1977.
20. A. K. S. Gupta, U. S. Patent 4,093,540, 1978.
21. A. K. S. Gupta, U. S. Patent, 4,533,501, 1985.
22. A. K. S. Gupta, *Fette Seifen Anstrichm.*, **3**, 79 (1989).
23. K. Hiromitsu, U. Nobuo, and O. Hiroo, *Jpn. Kokai Tokkyo Koho JP*, **82**, 63,398 (1982).
24. K. Hiromitsu, U. Nobuo, and O. Hiroo, *Jpn. Kokai Tokkyo Koho JP*, **57**, 149,399 (1982).
25. A. Iwama, and Y. Kazuse, U. S. Patent 4,414,157, 1983.
26. A. Iwama, in *Proceeding of the World Conference on Biotechnology for the Fats and Oils Industry*, J. AOCS Press, Champaign, Illinois, 1989, p. 244.
27. S. S. Koseoglu, K. C. Rhee, and E. W. Lusas, *AOCS Meeting*, New Orleans, Louisiana, 1987.
28. S. S. Koseoglu, K. C. Rhee, and E. W. Lusas, in *Proceeding of World Conference on Edible Oils*, 1990, p. 182.
29. L. Lin, K. C. Rhee, and S. S. Koseoglu, *J. Membrane Sci.*, **134**, 101 (1997).
30. S. Tanashashi, K. Nagano, M. Kasai, F. Tsubone, A. Iwama, Y. Kazuse, K. Tasaka, and Y. Isooka, U. S. Patent 4,787,981, 1988.
31. Y. Mutoh, K. Matsuda, M. Oshima, and H. Ohuchi, U. S. Patent 4,545,940, 1985.
32. S. Sun, Thesis, Texas A&M University, College Station, Texas, 1994.
33. S. S. Köseoglu, J. T. Lawhon, and E. W. Lusas, *J. Amer. Oil Chem. Soc.*, **67**, 315 (1990).
34. S. S. Köseoglu, W. E. Farr, W. De Greyt, and M. Kellens, U.S. Patent 6,797,172, 2004.
35. L. P. Raman, N. Rajagopalan, and M. Cheryan, *Oils & Fats Intl.*, **10**, 28–38 (1994).
36. T. F. Keurentjes, T. M. Sluijs, R. J. H. Franssed, and K. Riet, *Ind. Eng. Chem. Res.*, **31**, 581–587 (1992).
37. L. P. Raman, M. Cheryan, and N. Rajagopalan, *J. Amer. Oil Chem. Soc.*, **73**, 219–224 (1996).
38. N. Kumar, S. Krishna, and D. N. Bhowmick, *J. Amer. Oil Chem. Soc.*, **73**, 399–401 (1996).
39. F. P. Cuperus and J. T. P. Derksen, *Lipid Technol.*, **9**, 101–107 (1995).
40. S. S. Koseoglu, K. C. Rhee, and E. W. Lusas, in D. R. Erickson, ed., *Proceedings of the World Conference on Edible Fats and Oils Processing: Basic Principles and Modern Practices*, AOCS Press, Champaign, Illinois, 1990, p. 182.
41. B. Jirjis, H. Muralidhara, and D. D. Otten, U.S. Patent 6,207,209, 2001.

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## *Margarine Processing Plants and Equipment*

**Klaus A. Alexandersen**

When designing margarine processing plants and choosing the equipment to be installed, a wide range of considerations have to be made with regard to issues like actual processing, hygiene, sanitation, and efficiency.

In margarine production, oils and fats usually are considered to be the most important raw materials used, as oils and fats are significant in relation to the characteristics of the finished margarine. The type of oils or fats used has considerable influence on the crystallization characteristics during margarine processing, which has to be considered when choosing the equipment involved in the margarine processing line. The criteria involved in choosing this equipment are to a certain extent based on knowledge about product characteristics, polymorphism, and crystal structure of margarine and related products.

In this chapter, crystallization of oil and fat products, margarine processing equipment and packaging methods, processing methods, and specific process flows are discussed. Various oil types exhibiting interesting crystallization habits are reviewed along with certain specialized margarine or fat products. Storage of finished products as well as production quality control and hygiene will also be covered.

## 1. CRYSTALLIZATION OF OIL AND FAT PRODUCTS

### 1.1. Product Characteristics

The rheological characteristics of finished margarines are expressed in terms such as *consistency, texture, plasticity, hardness, structure, and spreadability* (1).

These characteristics are related to a number of variable factors. These are temperature, concentration of the disperse phase or solid fat content, crystal size, crystal size distribution, crystal shape, interparticle forces of van der Waals' type and mechanical treatment (2).

The two dominating factors are the amount of solid triglycerides (or solid fat index) and the processing conditions during production (3). Formulation or choice of oil blend allows control of the solid content, which, for identical processing conditions, is directly related to the consistency and type of crystalline structure formed (3–5). Processing conditions (rate and degree of cooling, mechanical working, final product temperature, etc.) regulate the type of crystals formed and the morphology and extent of intertwining of the solid structure that holds the liquid oil (6).

The term *morphology* is used to denote the general relation of the physical behavior and performance of fats and oils to their crystal structure and the molecular configuration of their triglyceride components (7).

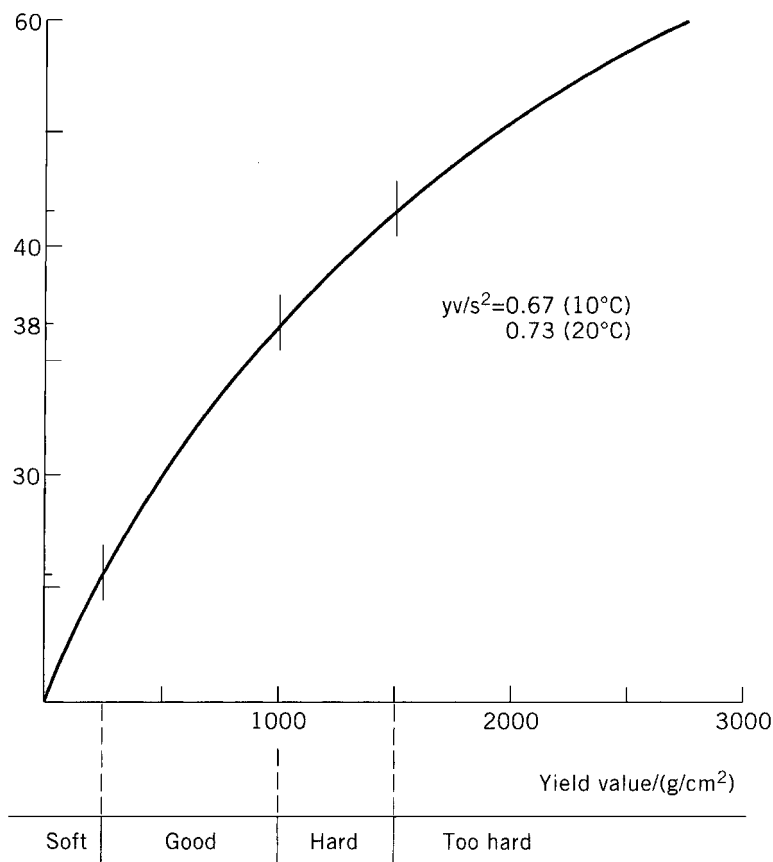
The curve describing the relationship between the solid fat content of a fat and its hardness is not a straight line. Hardness decreases sharply when solid fat content goes below a certain value at which the material loses some of the characteristic plastic properties (2). Haighton (3, 8) has reported the hardness of margarine in terms of yield value to have a strong correlation to the solid content under constant processing conditions, as shown in Figure 1.

### 1.2. Polymorphism and Crystal Structure

It has been reported extensively that fats solidify in more than one crystalline type (2–23). Triglycerides exhibit three main crystal types— $\alpha$ ,  $\beta'$ , and  $\beta$ —with increasing degrees of stability and melting point. The molecular conformations and packings in the crystal of each polymorph have been reported. In the  $\alpha$  form, the fatty acid chain axes of the triglyceride are randomly oriented and the  $\alpha$  form reveals a freedom of molecular motion with the most loosely packed hexagonal subcell structure.

The  $\beta'$  form and the  $\beta$  form are of an extended chain conformation with orthorhombic and triclinic subcell structures, respectively. In the  $\beta'$  form alternating fatty acid chain axes are oppositely oriented, whereas in the  $\beta$  form all fatty acid chain axes are oriented in one way (9, 10).

Crystals of the  $\alpha$  form are fragile, transparent platelets approximately 5  $\mu\text{m}$  in size. They are extremely transitory and require quite low temperatures to exist.  $\beta'$  crystals are tiny needles seldom more than 1  $\mu\text{m}$  in length.  $\beta$  crystals are large



**Figure 1.** Hardness of margarine vs. percentage solid in fat (3). Courtesy of J. Amer. Oil Chem. Soc.

and coarse, approximately 25–50  $\mu\text{m}$  in length and can grow to over 100  $\mu\text{m}$  during extended periods of product storage. The  $\beta$  form is responsible for product quality failure in “sandy” and “grainy” margarines (7). In severe cases this can lead to separation of the oil usually described by the term *oiling out*. Storage temperature that is too high, inadequate oil blend formulation, or process conditions promote this product failure.

In the manufacture of margarine, the emulsion is processed in a scraped-surface heat exchanger that must supercool the melted fat quickly in order to form as many crystal nuclei as possible (11).

The fat is believed to first crystallize in the  $\alpha$  form, which is transformed more or less rapidly to the  $\beta'$  form depending on the crystal habit of the fat, rate of cooling, and the amount of mechanical work applied (5, 7, 12, 13).

$\beta'$  is the crystal form desired in margarines as it promotes plasticity (4, 5, 13). The  $\beta'$  crystal form tends to structure as a fine three-dimensional network capable

of immobilizing a large amount of liquid oil (6). Large  $\beta$  crystals do not tend to give a three-dimensional structure.

Both Wiedermann (4) and Thomas (5) have grouped various oils and fats according to their crystal habits. As an example, soybean, sunflower seed, corn, coconut, and peanut oils show a  $\beta$  tendency. Cottonseed oil, palm oil, tallow, and butter oil have a  $\beta'$  tendency. Oil blend formulation has a significant influence on the crystal form attained by a margarine or shortening. The suitability of a fat or oil for margarine formulation is very much dependent on the crystal size present, amount, and habit of these crystals (13). Incorporation of a higher melting  $\beta'$  tending oil to a basestock can induce the crystallization of the entire fat into a stable  $\beta'$  form (5). The effects of such formulation practice and processing conditions have been studied extensively by Rivarola et al. (6) for blends of hydrogenated sunflower seed oil and cottonseed oil. For strong  $\beta$  tending hydrogenated sunflower seed oil, it was found that with increasing cooling rate, the tendency to crystallize in the  $\beta'$  form increased. For blends of hydrogenated sunflower seed oil and strong  $\beta'$  tending hydrogenated cottonseed oil it was concluded that even at quick cooling rates, small quantities of the  $\beta$  form are formed.

In certain margarines formulated mainly on hydrogenated oils, such as sunflower seed oil and canola oil, with very strong  $\beta$  tendency, the problem of sandiness can be pronounced. Addition of crystal-modifying agents or crystal inhibitors to such margarines can retard the development of sandiness by delaying the transformation from the unstable  $\alpha$  form to the stable  $\beta$  form. The addition of sorbitan esters stabilizes the intermediate  $\beta'$  form and helps prevent the formation of the  $\beta$  form (15, 16). Sorbitan tristearate is effective as a crystal inhibitor in margarines. It is assumed that sorbitan tristearate can be accommodated by the  $\beta'$  crystal network of the triglycerides and by stearic hindrance prevent the formation of the more densely packed  $\beta$  crystal form (17, 18).

In margarine with a good consistency, the fat crystals have formed a three-dimensional network consisting of primary and secondary bonds. The crystals may vary in shape and appearance in the form of small needles or platelets with lengths ranging from less than 0.1 to 20  $\mu\text{m}$  or more (3, 6). They do not behave as individual particles and can grow together, forming a strong network (primary bonds). They may also show a tendency to agglomerate, forming tiny porous crystal clusters with considerable fewer contact points (secondary bonds) (3). As a result of this and depending on the resulting crystal form obtained, branched and intertwining long chains are formed (6). These chains are responsible for forming the three-dimensional network. The primary bonds are strong and are not readily reestablished when broken by mechanical work. Secondary bonds are weak and readily reestablished when broken by application of mechanical work. As mentioned earlier, processing conditions involving fast cooling rates and application of a certain amount of mechanical work tend to produce margarines with a better stability and consistency. It is generally accepted that a larger amount of primary bonds are established if margarine is allowed to crystallize without sufficient degree of mechanical work. This results in a product exhibiting excessive posthardening and a hard and brittle texture (19). Due to this, it is advantageous to crystallize

the product as much as possible in the scraped-surface heat exchanger to achieve the desired spreadability or consistency.

### 1.3. Palm Oil

Crystallization and processing of palm oil with satisfactory results in a scraped-surface heat exchanger line for margarine and shortening requires some attention due to the slow crystallization phenomena observed in palm oil.

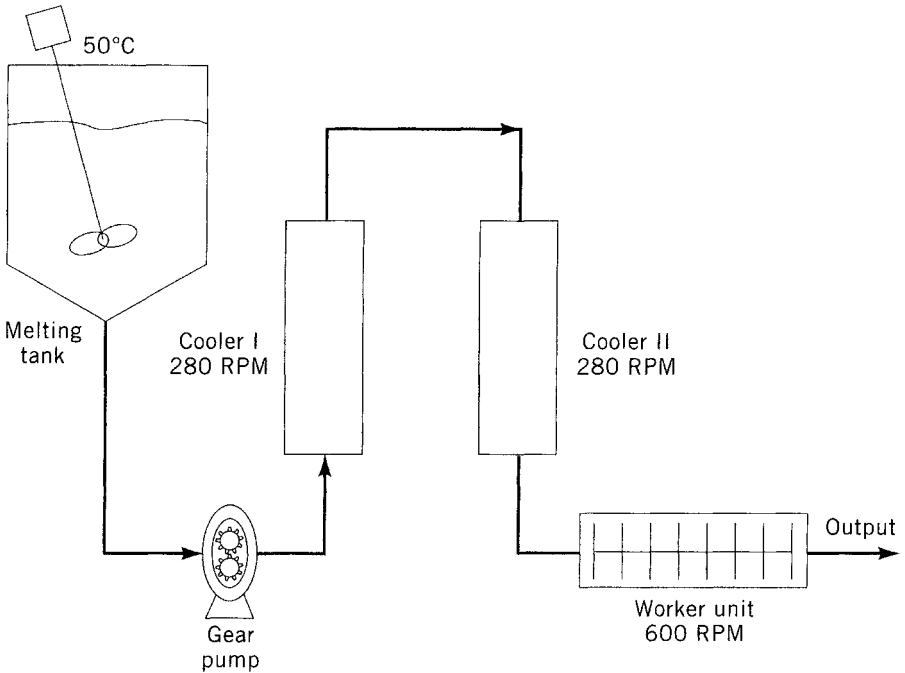
The polymorphism, crystallization, formulation, and processing of palm oil has been commented upon and studied extensively (20–25, 26–34). The slow crystallization of palm oil and the subsequent posthardening phenomenon and product graininess is a drawback in products formulated with high palm oil contents and could be a limiting factor to its use (24, 25). It has been shown that the rate-determining step in the crystal growth mechanism of triglycerides is the orientation of molecules at the crystal faces (20). In palm oil the  $\alpha$ -polymorph transformation to the  $\beta'$  (i.e., the  $\alpha$  lifetime) is unusually long, which is apparently due to the high level of diglycerides present (approximately 6%) (20, 21). The problem of posthardening in product formulated with high palm oil contents can be influenced by choice of proper processing conditions and storage time (21–23).

Lefebvre (35) hypothesized that crystals, in general, are formed before or early in the worker unit (B unit) (see Section 2.3), when a low flow rate is used in a scraped-surface heat exchanger. The important slow processing of the product leads to a fine crystallization and the destruction of the intercrystal bonds of the primary type. With a higher flow rate, crystals appear late in the worker unit and partially during packaging. Crystallization is then coarser and intercrystal bonds are only slightly damaged, all of which is less favorable.

This hypothesis relates very well with the observations made by Oh et al. (22) during pilot-plant-scale crystallization and processing of palm oil in a scraped-surface heat exchanger line for margarine and shortening, as shown in Figure 2.

Palm oil from the same batch was processed with flow rates *A* and *B* of, respectively, 28 kg/h and 55 kg/h. Different flow rates result in different retention times for products *A* and *B* in the coolers and the worker unit. Product outlet temperatures from cooler II of, respectively, 12°C and 14°C (54°F and 57°F) were observed. The outlet temperature from the worker unit were, respectively, 19–20°C (66–68°F) and 20–21°C (68–70°F).

Refrigerant temperatures remained constant for both flow rates. Product *A* was found to have sufficient time to be more uniformly stabilized before leaving the process line. Product *B* was found to have attained insufficient time to be uniformly stabilized and resulted in a finished product in the quasi-equilibrium state. Crystal growth in product *A* was not substantial during 10 days storage at 20°C (68°F), whereas the crystal growth for product *B* was significant under the same storage conditions. It was concluded that better processing conditions may overcome the problem of slow crystallization of palm oil and also avoid the effect of posthardening during storage. Different compositions of palm oil and palm oil fractions give rise to different crystallization behaviors. Hydrogenated palm oil



**Figure 2.** Schematic diagram of pilot plant (22). Courtesy of The Palm Oil Research Institute of Malaysia.

has the highest stability in the  $\beta'$  crystal form followed by palm oil and then palm stearin.

The preceding observations relate well with observations in industrial-scale scraped-surface heat exchanger processing lines.

In industrial-scale processing lines, it has been found to be advantageous to process palm-oil-based industrial margarines with an additional worker unit installed between the cooling cylinders as shown in Figure 3 (26). This increases the product's retention time in the processing line and allows a slight increase in the flow rate without compromising the product quality.

Generally, the recommended flow rate for palm-oil-based industrial margarines is approximately 60% of the nominal capacity of a scraped-surface heat exchanger process line for industrial margarine (27); for example, a scraped-surface heat exchanger with a nominal capacity of 3000 kg/h for oil blends based on oils such as soybean oil or cottonseed oil will, for oil blends based on palm oil, have a capacity of approximately 1800 kg/h.

In connection with crystallization of palm-oil-based products it should be noted that the tempering practice for industrial margarines and shortening at 26.7°C (80°F) was designed especially for hydrogenated oils. This tempering procedure tends to generate lower solid fat content at temperatures below 26.7°C (80°F) and raise it above 26.7°C (80°F). It is generally unsuitable for palm oil, palm-kernel oil, and coconut oil (21).

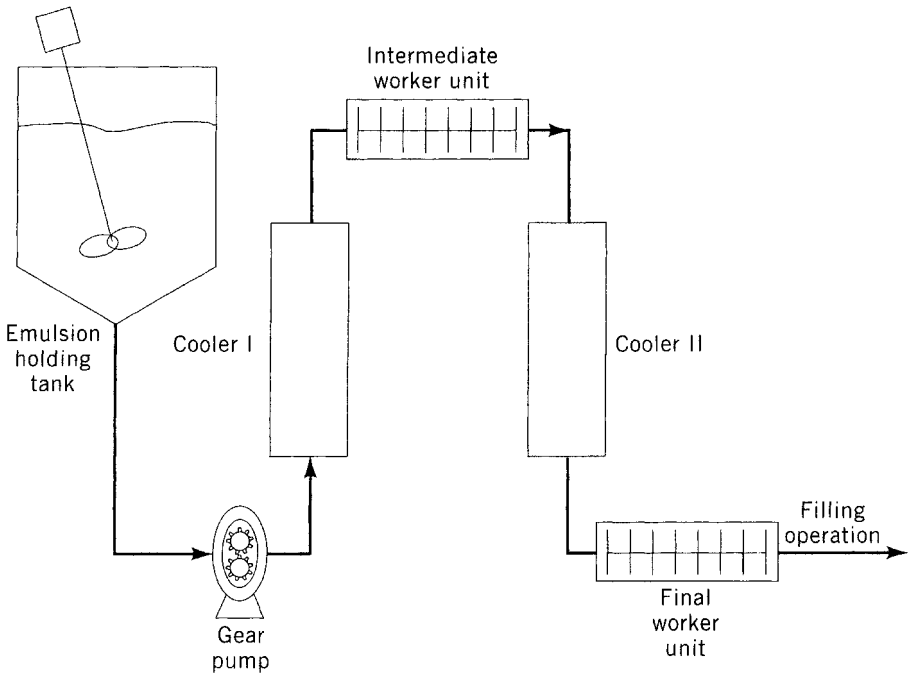


Figure 3. Schematic diagram of industrial source plant.

#### 1.4. Canola Oil and Sunflower Seed Oil

We have noted earlier that the crystallization of strongly  $\beta$  tending sunflower seed oil blends can be influenced favorably toward the  $\beta'$  polymorph form by addition of a high melting hardstock of the hydrogenated cottonseed oil (6) as well as by addition of sorbitan tristearate (17, 18). It should be noted for the formulation and crystallization of margarines based on sunflower seed oil blend that interesterification of oil blends is a possible route to minimize posthardening. It is possible to produce table margarine with good consistency and a linoleic acid content of 36% as well as a trans-isomeric fatty acid content of less than 2% based on an oil blend prepared by interesterification. Interesterification of a blend consisting of 60% sunflower seed oil, 15% coconut oil, and 25% hydrogenated sunflower seed oil [melting point 70.7°C (159°F)] and an iodine value (IV) of 8.5 can achieve this.

Interesterification has been reported to change the crystallization tendencies of oil blends in such a way that the crystal size in certain interesterified oil blends is smaller than in the similar noninteresterified oil blends (36–39). List et al. (40) found that interesterification of oil blends made from fully hydrogenated soybean oil and soybean oil affects the polymorphic transition from the undesirable  $\beta$  form to the desirable  $\beta'$  form thus avoiding graininess in finished margarine products. Interesterification of blends of palm oil fractions is also a possibility in margarine formulation producing margarines with very low or “zero trans” fatty acid contents



(41, 42). With today's health conscious discussions in the media and the use of transisomeric fatty acid content in margarines as a marketing parameter, the inter-esterification of oil blends may possibly gain some momentum in the future.

It is well documented that hydrogenated canola oil has a tendency to crystallize in the  $\beta$  polymorphic form due to its triglyceride homogeneity (it has about 95% of 18-carbon fatty acids) (43). Crystallization of  $\beta$  tending canola oil blends (low-erucic-acid rapeseed oil) can be influenced by addition of an oil with  $\beta'$  tending crystallization of different origin. When processing canola-oil-blend-based margarines for tub or stick packaging, the industry follows a different formulation principle than for sunflower seed oil blends, which are usually used for soft margarines with high linoleic acid contents. Canola oil constitutes approximately 42% of all vegetable oils consumed in the margarine production in Canada (44), whereas soybean oil constitutes the majority of all oils supplied for the production of margarine in the United States (45).

Canola oil contains 5% palmitic acid compared to 11% for soybean oil. Palm oil contains high levels of palmitic acid, approximately 44%, and it has been found that the addition of palm oil to canola-oil-based oil blends for margarine production has a beneficial effect on their polymorphic stability (30). When palm oil is mixed with canola oil, the homogeneity of the fatty acid chain length is reduced, which promotes  $\beta'$  crystalline stability (43). Based on the solid fat content found in stick margarine in North America, it is advantageous to manufacture margarine from canola oil by incorporating palm oil at a level of at least 15%, after hydrogenation of canola oil, or at a level of 10%, before hydrogenation of canola oil. This greatly delays the polymorphic transition from the  $\beta'$  to the  $\beta$  form (43, 46, 47). The amount and point of addition can affect the transition to the  $\beta$  polymorph as hydrogenation changes the physical properties of an oil blend (46).

The high content of diglycerides (about 6%) in palm oil and the  $\beta'$  stabilizing effect of diglycerides probably do not have any significant influence on the polymorphic behavior of canola oil blends with palm oil levels as above. The diglyceride content in canola oil blends is only raised slightly by addition of palm oil in the above levels (46).

It has been found that the  $\beta'$  stabilizing effect increased with the level of added palm oil and that this stabilizing effect is most likely due to the decrease in fatty acid homogeneity and, thus, increased triglyceride diversity (43, 46). This is attributed to the increased range of fatty acid chain lengths, which in turn increases the irregularity in the crystal network. Increased irregularity in the crystal network increases the polymorphic stability (46).

A new type of canola oil containing high levels of palmitic acid possesses better  $\beta'$  stability in the hydrogenated form (30, 46). The stabilizing effect of palmitic acid, mentioned by Wiedermann (4), is related to its level in the solid fat fraction, which is increased by addition of palm oil or when the palm oil is partially hydrogenated (46). In general, the more diverse the triglyceride structure of the highest melting portion of the fat, the lower the  $\beta$  forming tendency (48).

To illustrate this, the triglyceride composition of some fully hydrogenated oils are indicated in Table 1.

**TABLE 1. Percent (%) Triglyceride Composition and Mono- and Diglyceride Content of Fully Hydrogenated Oils (%).**

Carbon Number	Hard Fats					
	Soybean	Beef Fat <sup>a</sup>	Rapeseed	Rapeseed Blend	Cottonseed	Palm
44	—	0.2	—	—	—	—
46	—	1.4	—	0.1	—	0.5
48	0.2	7.5	—	3.4	0.9	6.4
50	3.3	21.0	1.6	8.8	13.6	40.0
52	27.6	44.9	11.6	15.2	43.5	41.9
54	66.7	24.5	28.3	25.9	40.5	10.7
56	1.7	0.4	6.7	6.2	1.3	0.4
58	0.5	—	6.8	7.2	—	—
60	—	—	12.3	9.0	—	—
62	—	—	31.9	23.6	—	—
64	—	—	0.8	0.8	—	—
Monto	0.4	0.1	0.5	0.5	0.3	0.9
Di	3.6	2.0	3.7	4.4	5.8	8.2

<sup>a</sup>Glycerides contain odd-numbered and branched fatty acids.

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In Table 1, the most  $\beta'$  stable fat is palm oil hard fat. This may be explained by its unique composition, and its balanced C48–C54 triglyceride content with an equally balanced C50–C52 content (49).

In accordance with the above, it has been found that slightly hydrogenated palm oil delayed polymorphic transition from  $\beta'$  to  $\beta$  considerably, compared to no palm oil addition (43).

Stick margarine of good quality and melting point, 35°C (95°F), based on a canola oil blend with palm oil addition, can be produced in a scraped-surface heat exchanger line for margarine, according to the flow outline in Figure 4. A reduced flow rate of approximately 85%, compared to the nominal capacity of the scraped-surface heat exchanger (A unit) (see Section 2.2), is recommended. It should also be noted that the intermediate worker unit (B unit) (see Section 2.3), with variable-speed drive inserted between the cooling cylinders, should have a relative volume of approximately one third of the volume of the intermediate worker unit indicated in Figure 3, based on a given flow rate and heat exchange area (50).

### 1.5. Specific Heat and Heat of Fusion

In the solid state, the specific heat of oils and fats shows little change as molecular weight varies. An increase in specific heat can be observed with increased unsaturation. In the liquid state, specific heat increases slightly with molecular weight but decreases slightly with less unsaturation. In general, there is little variation among natural oils and fats (21).

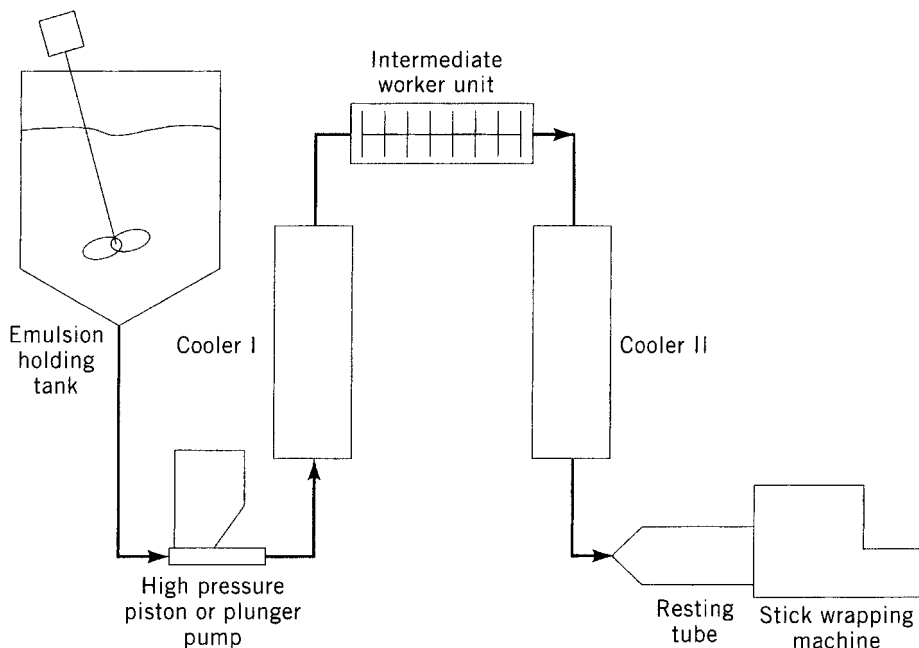


Figure 4. Schematic diagram stick (table) margarine plant.

The specific heats of liquid oils and fats, including palm oil, palm kernel oil, and coconut oil, may be taken as (21).

$$C_p(\text{kcal/kg}) = 0.47 + 0.00073 \times T,$$

where  $T$  is temperature in  $^{\circ}\text{C}$  ( $1 \text{ Btu/lb} = 0.252 \text{ kcal/kg}$ ).

A specific heat of  $0.514 \text{ kcal/kg}^{\circ}\text{C}$  for the fat phase of a retail margarine with 82% fat content and  $0.607 \text{ kcal/kg}^{\circ}\text{C}$  for the same margarine has been reported (19).

It is difficult to determine the latent crystallization heat in oil blends for margarine production due to their complexity.

The heat of fusion normally increases with bigger chain lengths and decreasing unsaturation in the triglycerides. Blends of triglycerides have less latent heat of crystallization than the similar nonblended triglycerides (19).

Timms (21) has heat of fusion to  $17.7\text{--}22.3 \text{ kcal/kg}$  for milkfat,  $24\text{--}31 \text{ kcal/kg}$  for fully hardened milkfat,  $26\text{--}29 \text{ kcal/kg}$  for cocoa butter in the  $\beta'$  polymorph,  $22.6 \text{ kcal/kg}$  for refined, bleached, and deodorized (RBD) palm oil,  $29.7 \text{ kcal/kg}$  for RBD palm kernel oil,  $26.0 \text{ kcal/kg}$  for RBD coconut oil,  $31.6 \text{ kcal/kg}$  for fully hardened palm kernel oil, and  $31.2 \text{ kcal/kg}$  for fully hardened coconut oil. The heat of fusion is an empirical physical property dependent on the thermal history or tempering of the oil.

Calvelo (19) has reported the total heat of crystallization ( $J_c$ ) for a specific retail margarine with 82% fat content to be  $33.4 \text{ kcal/kg}$ .

## 2. PROCESSING EQUIPMENT FOR MARGARINE AND RELATED FAT PRODUCTS

Choice of equipment for the processing line is very important for the production of margarines. For each piece of equipment in the production line, special design features have to be considered for various margarine types to ensure that the complete processing line has all the necessary capabilities.

Besides the necessary emulsion preparation equipment (see Sections 4.1–4.3) such as process tanks, plate heat exchangers, and centrifugal pumps, the essential equipment for production of margarines is discussed in the following sections.

### 2.1. High-Pressure Feed Pumps

The margarine emulsion is usually fed from a holding tank to the scraped-surface heat exchanger (A unit) by a high-pressure positive-displacement pump of the plunger or piston type with product contact parts in 316 stainless steel. Pumps with ceramic pistons are available for special applications. Normally, pumps with two or three plungers or pistons are standard in order to minimize discharge pressure pulsations in the process line. A high-pressure piston pump for margarine production is illustrated in Figure 5.

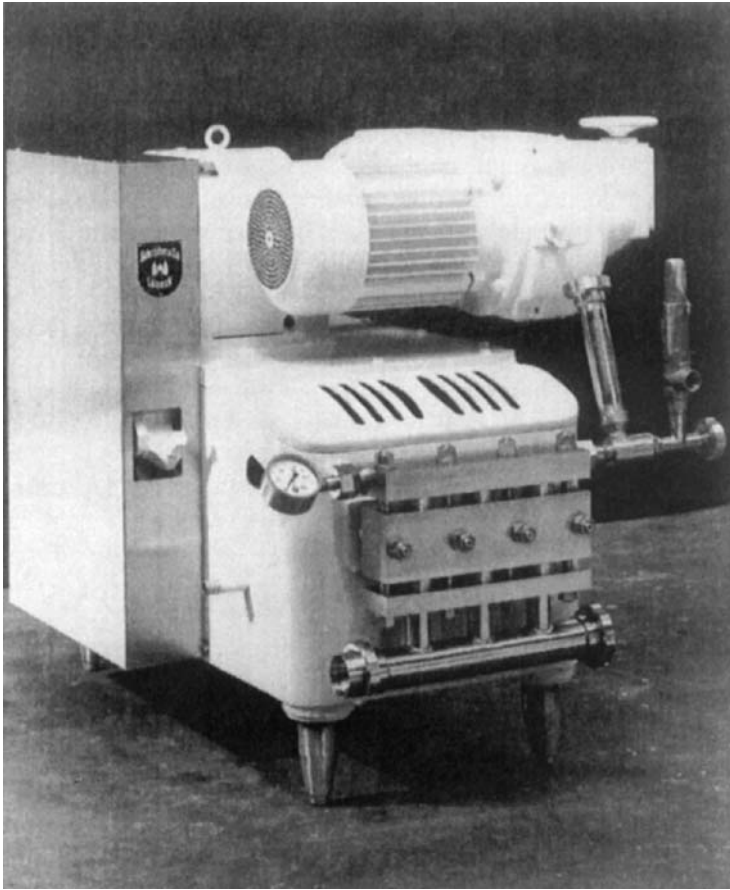
To further minimize possible pressure pulsation, the pumps can be installed together with a pulsation dampener mounted at the discharge. Pulsation dampeners are air pressurized or spring loaded to ensure a smoother product flow in the process line. Slow rotational speed of the pump's crankshaft also helps to minimize pressure pulsation.

The high-pressure pumps are normally supplied with a pressure relief valve and associated product piping to protect the scraped-surface heat exchanger equipment downstream and the pump itself, should a blockage of the production line occur.

A filter is normally installed in the suction line to the high-pressure pump to protect the pump and the hard chromium-plated scraped-surface heat exchanger cylinder from any foreign matter in the margarine emulsion.

Depending on the designed maximum product pressure of the downstream scraped-surface heat exchanger and the various types of margarine produced, high-pressure positive-displacement pumps with maximum discharge pressures of 40 bars (about 600 psi), 70 bars (about 1030 psi), or 120 bars (about 1800 psi) are normally installed in the process line.

Production of industrial margarine for semiliquid filling does not normally generate product line pressures as high as, for example, puff pastry margarine. Gear pumps are normally installed as an alternative to high-pressure positive-displacement pumps in the production of industrial margarine or shortening for semiliquid filling (26, 51). Gear pumps for this application normally can deliver a maximum discharge pressure of 26–33 bars (about 390–500 psi). The drawback for the application of gear pumps in margarine processing is that this type of pump tends to slip at higher discharge pressures (52).



**Figure 5.** High-pressure piston pump for margarine production. Courtesy of Schroeder & Co., Luebeck, Germany.

## 2.2. High-Pressure Scraped-Surface Heat Exchanger

Scraped-surface heat exchanger equipment, specifically designed for margarine production, is available from Cherry-Burrell Votator Division of Louisville, Kentucky, United States, Crown Chemtech Ltd. of Reading, U.K., Gerstenberg & Agger A/S of Copenhagen, Denmark, and Schroeder & Co. (Tetra-Laval owned) of Luebeck, Germany, under the respective trademarks Votator, Chemetator, Perfector, and Kombinator.

The scraped-surface heat exchanger (A unit) is the centerpiece of equipment of the margarine processing line, where initial cooling, supercooling, and subsequent induced nucleation and crystallization take place (3, 53). The A unit has to have a high degree of flexibility with regard to variation of process conditions for different product types and formulations (51, 53).

The scraped-surface heat exchanger normally consists of one or more horizontal heat transfer cylinder assemblies. The cooling cylinder of a cylinder assembly is usually made from commercially pure nickel or steel, ensuring high heat transfer coefficients. The cooling cylinder is surrounded by an insulated outer jacket containing refrigerant (normally ammonia or Freon 22). The inside hard chromium-plated surface of the cooling cylinder is continuously scraped clean during operation by a rotating shaft mounted with free-floating blades. The blades are thrust against the cylinder wall mainly by the centrifugal force resulting from the high rotational speed of the shaft. The annular gap between the cylinder wall and the shaft has been reported to be in ranges from 3 to 22 mm (52), but a more typical range is 5–17 mm (3, 51–54).

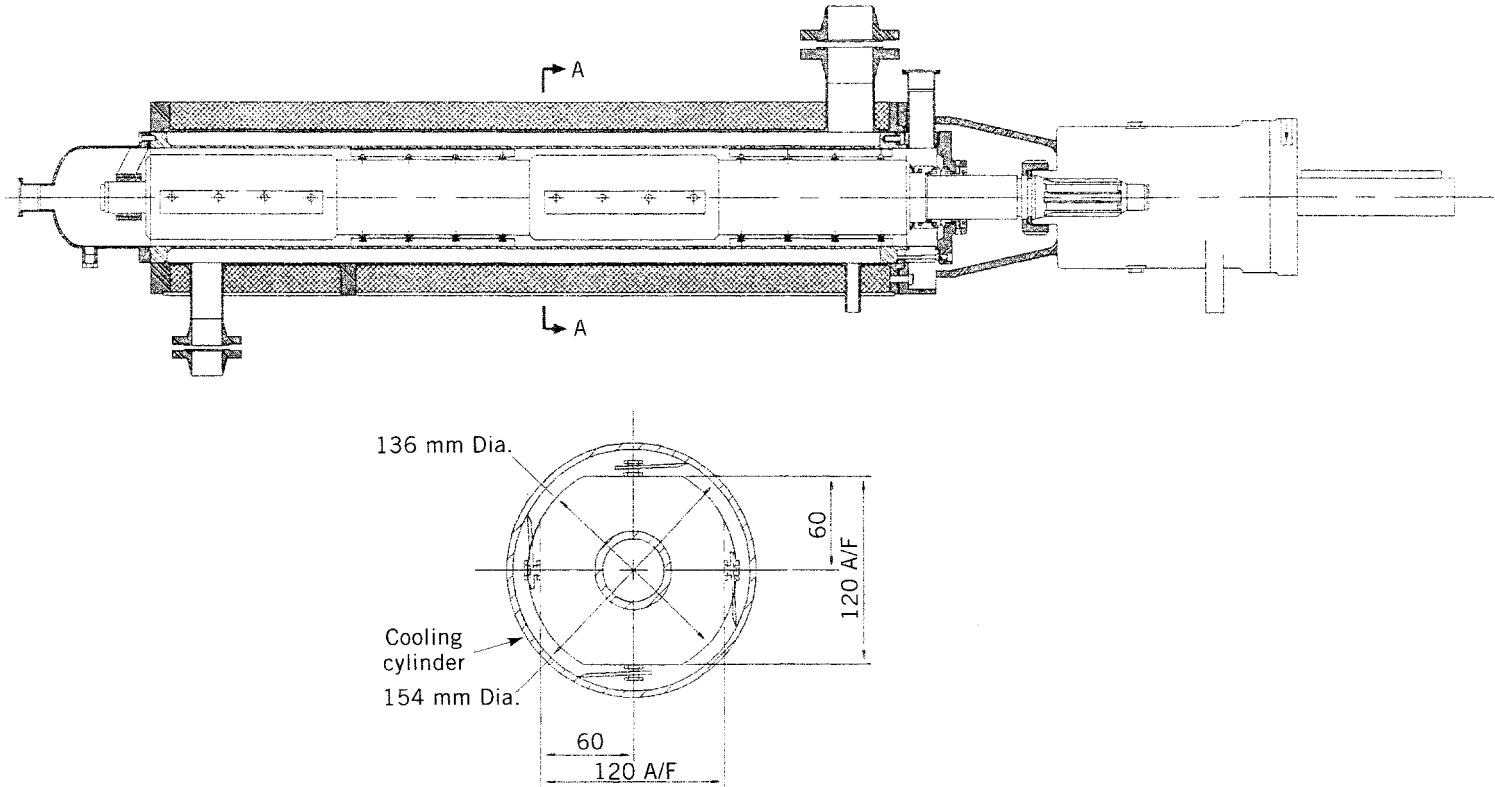
When margarine emulsion passes through the space between the shaft and cylinder wall, a thin crystallized product film is constantly and very rapidly scraped off the cylinder wall and remixed with warmer product because of the scraping action of the blades and the shaft's high rotation speed. This causes rapid crystal nucleation, further emulsification of the product, very high overall heat transfer coefficients, and a homogeneous cooling of the margarine emulsion under precise temperature control of the product being crystallized (53–55).

The rotational speeds of shafts normally range from 300 to 700 rpm (51–53) and shafts are normally mounted with two, four, or six rows of blades (53). The blades are fixed to the shafts by specially designed pins and are movable at their fixing points. Figure 6 illustrates the design and operation of a scraped-surface heat exchanger based on a longitudinal view of the A unit and a cross-sectional view of the cooling assembly. The shaft is mounted with four rows of blades in a staggered configuration. The annular gap in this situation varies from 9 to 17 mm.

In the crystallizing product, there is a rapid increase in the solid content during the passage through the cooling cylinder. Also, the viscosity of the product increases accordingly with the temperature drop. At a certain point during this process, a critical shaft speed is reached. Beyond this speed, no additional mixing is obtained, and the power input required to rotate the shaft at a higher speed will more than offset any heat transfer benefits resulting from more frequent scraping of the cylinder wall (54, 55).

In order to prevent buildup of crystallizing product on the shaft, warm water is normally circulated through the shaft to ensure a clean shaft surface at all times (51–54). The warm water is normally pumped into the shaft at a point near the thrust/axial bearing assembly and exits close to the water inlet point based on the inside construction of the shaft (52). The water circulation facility is also beneficial after a temporary production stoppage, as the warm water helps to melt solidified product and, thus, facilitates the restart of the A unit.

*Energy Balance.* The above-mentioned temperature drop, crystal nucleation, and partial crystallization of the product during the passage through the A unit involve an overall energy balance including specific and latent heat of the product as well as other energy source inside the equipment. The power input through the blade shaft is transferred to the product and the cylinder wall as heat ( $Q_m$ ). A small amount of



**Figure 6.** Scraped-surface heat exchanger. Courtesy of Crown Chemtech U.S.A., a division of Crown Iron Works Co., Minneapolis, Minnesota.

heat is also added to the process through the warm water circulation inside the shaft ( $Q_w$ ).

According to this, the energy in form of heat in the A unit can be expressed as follows (19):

$$Q_t = FC_p(T_1 - T_2) + FJ_cW_2Y + Q_m + Q_w, \tag{1}$$

where  $F$  is the flow rate,  $C_p$  the product's specific heat,  $T_1$  the emulsion's inlet temperature,  $T_2$  the product exit temperature,  $J_c$  the latent heat of crystallization in the fat,  $W_2$  the solid fat content at the exit from the A unit, and  $Y$  the fat content of the margarine emulsion.

In a stationary condition, the heat  $Q_t$  will presumably be transferred through the cylinder wall at an ammonia evaporation temperature of  $T_f$ , which makes it possible to define the heat transfer coefficient  $U$  as

$$Q_t = UAT \ln, \tag{2}$$

where  $A$  is the heat transfer area and  $T \ln$  is a logarithmic value defined as

$$T \ln = T_1 - \ln(T_1/T_2)T_2, \tag{3}$$

where  $T_1 = (T_1 - T_f)$  and  $T_2 = (T_2 - T_f)$ .

If the product at the exit from the A unit has a solid fat content of  $W_2$  at temperature  $T_2$  and is left to crystallize under stationary conditions, the degree of supercooling will be reduced with time, as the crystallization continues until a certain temperature  $T_a$  has been reached. Based on this we have

$$C_p(T_a - T_2) = J_cY(W_a - W_2), \tag{4}$$

where  $W_a$  is the solid fat content at temperature  $T_a$ . Based on sufficient time to achieve a stable situation,  $W_a$  can be determined from the solid fat curve in the product at temperature  $T_a$ .

From formula (4) the solid fat content at the exit of the A unit,  $W_2$ , can be calculated as follows:

$$W_2 = W_a - \frac{C_pT}{J_cY}, \tag{5}$$

where  $T = (T_a - T_2)$ .

Formulas (1), (2), and (5) make it possible to relate process variables such as the flow rate  $F$ , the emulsion temperature  $T_1$ , and the ammonia evaporation temperature  $T_f$  with parameters contributing to the consistency of the margarine such as the solid fat content at the exit of the A unit  $W_2$ .

As the crystallization of a fat product demands both a rapid temperature drop and time for crystal nucleation and crystal growth, sufficient retention



time for the product in the A unit is required. The retention time can be calculated from

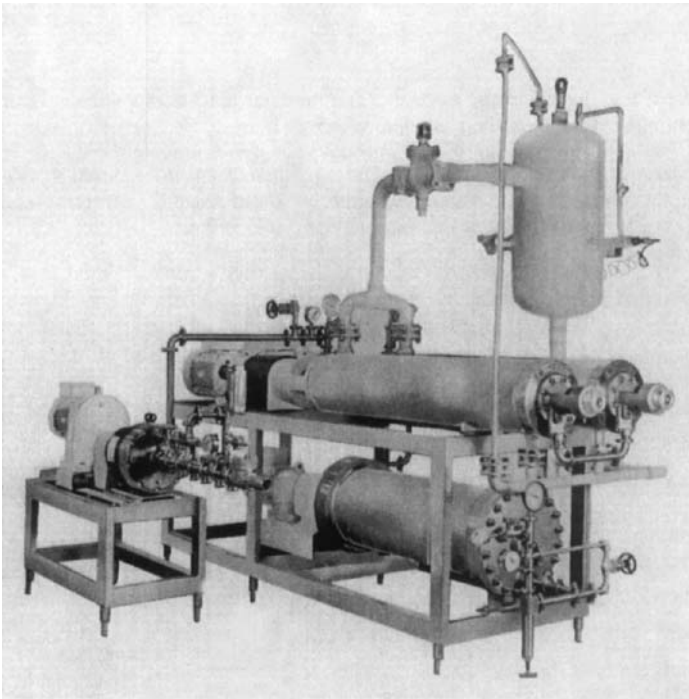
$$T_r = V/F, \quad (6)$$

where  $F$  is the product flow rate and  $V$  is the product volume in the A unit.

*Shaft Design.* The high viscosity margarine products exhibit during processing in the A unit increases the significance of factors such as flow rate, shaft rotation speed, turbulent flow conditions in relation to shaft design, blade configuration, and annular gap between the shaft and cylinder wall (51). This is due to the viscosity's influence on flow properties, created turbulence, increased effect of mechanical work, and obtained mixing and heat transfer.

Several shaft or cylinder designs are available today in A units for margarine processing. The A units can be grouped according to whether they are mounted with eccentric shafts, oval shafts, sectioned shafts, or oval tubes.

Eccentric shafts have been in wide use in the past and were developed by the Votator Division of Louisville, Kentucky, and are claimed to provide more intensive cooling for high-melting bakery margarine as well as a certain amount of working and compression action similar to that given by the Complector of the older, open-chill drum system (52) (see Section 5.2).



**Figure 7.** Votator scraped-surface heat exchanger unit. Courtesy of Cherry-Burrell Votator Division, Louisville, Ky.

Figure 7 shows a new, all stainless steel Votator A unit complete with high-pressure feed pump and worker unit for production of industrial margarines and shortenings.

In the literature, one can find theoretical and practical studies relating to heat transfer conditions in scraped-surface heat exchangers (19, 54), which cover factors such as specific weight, specific heat, latent heat of crystallization, dry matter content, retention time, and overall heat transfer conditions.

A review of some of the fluid mechanics and heat transfer aspects of scraped-surface heat exchangers currently available for margarine processing has been given by N. Hall Taylor (56). A summary by N. Hall Taylor outlining the important physical phenomena occurring under different circumstances in the A units is given in the following discussion.

*Heat Transfer and Viscous Dissipation for Newtonian Fluids.* Because the gap width is small relative to the shaft radius, the annular space can be represented on the basis of a two-dimensional flow model. This is illustrated in Figure 8.

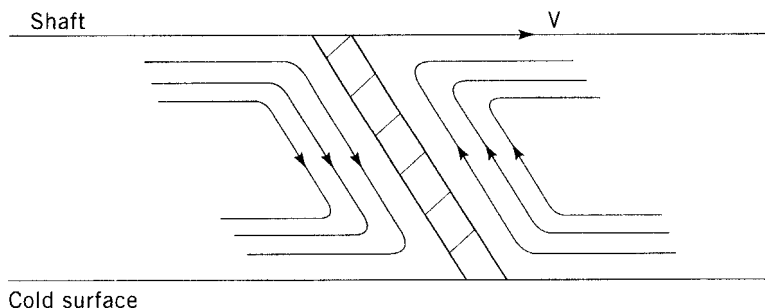
Figure 8 indicates the important action of the scraper blade. Removal of material at the front allows fresh warm material to flow down the back of the blade to be brought into contact with the cold surface. Two things then happen. First, the material adjacent to the surface is slowed down to develop a velocity profile. Second, the material starts to transfer heat into the wall.

This is a transient fluid flow and heat transfer problem. Figure 9 illustrates the growth of these layers adjacent to the wall as seen from a coordinate system traveling with the blade.

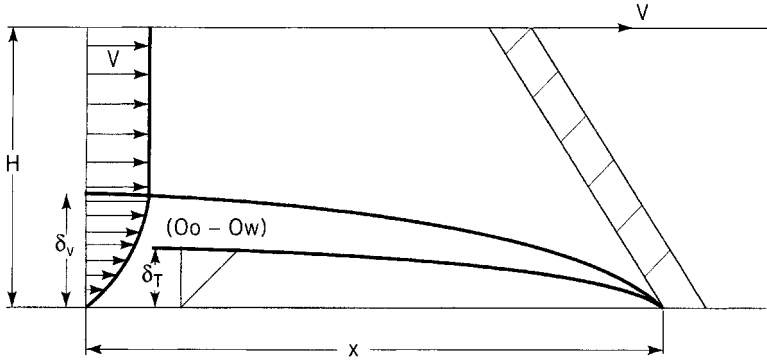
In Figure 9, the upper line represents the viscous layer, which shows the progressive development of the linear velocity profile. The thickness at any position relative to the blade is given approximately as

$$\delta_v = 2 \left( \frac{\nu x}{V} \right)^{1/2},$$

where  $\nu$  is the kinematic viscosity. The material outside this viscous layer continues to travel at the rotational velocity  $V$ .



**Figure 8.** Fluid movement in the proximity of the blade (56). Courtesy of N. Hall Taylor, Crown Chemtech Ltd., Reading, United Kingdom.



**Figure 9.** Velocity profile behind the blade (56). Courtesy of N. Hall Taylor, Crown Chemtech Ltd., Reading, United Kingdom.

The inner line in Figure 9 represents the corresponding thermal layer and shows the development of the thermal gradient. Again the material outside this layer remains at a constant temperature above the wall temperatures (i.e.,  $\theta_0 - \theta_w$ ). The thickness of the thermal layers ( $\delta_T$ ) is given by

$$\delta_T = 2 \left( \frac{\alpha x}{V} \right)^{1/2},$$

where  $\alpha$  is the thermal diffusivity.

Thus the ratio of the thickness of the two layers at any position is solely related to the Prandtl number of the material:

$$\frac{\delta_v}{\delta_T} = \left( \frac{V}{\alpha} \right)^{1/2} = Pr^{1/2}.$$

For foodstuffs under low shear conditions, the Prandtl number is large, i.e., the viscous layer is much thicker than the thermal layer.

Within the viscous layer, all the viscous dissipation is taking place. The scale of viscous heat generation/unit volume ( $p$ ) at any point is given by

$$p = \mu \left( \frac{du}{dy} \right)^2,$$

where  $du/dy$  is the velocity gradient.

It can be shown that within the viscous layer:

$$p = \frac{\rho V^2}{4t},$$

or in coordinates relative to the blade:

$$p = \frac{\rho V^3}{4x}.$$

This result is only true provided the thickness of the viscous layer is less than the gap width ( $H$ ); i.e.,  $\delta_v < H$ .

By integrating the viscous dissipation terms over the volume of the viscous layer, one can derive an equivalent heat flow that has to be removed in addition to any cooling that takes place. This is given by

$$q_v = \rho V^3 \left( \frac{v}{LV} \right)^{1/2},$$

where  $L$  is the distance behind the blade at which the viscous boundary layer is destroyed. This can either be due to the next blade (so that  $L$  is the distance between the blades) or because of some turbulent motion in the liquid. The cause of such turbulent action will be discussed in detail later.

The heat transferred from the thermal layer to the wall can also be estimated and averaged over the mixing length. This gives

$$q_T = k\Delta\theta \left( \frac{V}{\alpha L} \right)^{1/2}.$$

Thus, increasing the rotational velocity will improve the rate of heat transfer (proportional to  $V^{1/2}$ ). However, at the same time the heat that has to be removed from viscous dissipation is increasing (proportional to  $V^{5/2}$ ).

The net cooling flux is

$$q_c = q_T - q_v.$$

This indicates that for a given material there will be an optimum velocity at which the greatest cooling is achieved. It also suggests that under certain conditions  $q_T = q_v$  and no net cooling is achieved.

This will occur if

$$V^2 > C_p \Delta\theta \text{Pr}^{-1/2}$$

or

$$V^2 > C_p \Delta\theta \left( \frac{k}{C_p \mu} \right)^{1/2}.$$

The velocity at which this takes place will decrease as the viscosity increases. In a margarine process the most critical section is in the final scraped-surface heat exchanger (SSHE) and then toward the exit end. Here there is the greatest viscosity (highest Pr) and also the smallest temperature difference between the wall and the material ( $\Delta\theta$ ).

In most cases this critical velocity is well above the maximum operating velocity of the SSHE.

*Non-Newtonian Fluids.* Most foodstuffs and margarines are non-Newtonian fluids. This means that the effective viscosity varies with the shear applied to the material.

A useful representation of this behavior is as follows:

$$v = v_0 \left( \frac{\gamma_0}{\gamma} \right)^k,$$

where  $\gamma$  is the shear rate, essentially equal to the velocity gradient;  $v_0$  is a reference viscosity at the reference shear rate  $\gamma_0 = s^{-1}$ ; and  $k$  is a constant typically in the range 0.6–0.7. It should also be remembered that viscosity is generally strongly dependent on temperature. This can typically be represented by

$$v = v_R \left( \frac{\theta}{\theta_R} \right)^n,$$

where  $v_R$  is a reference viscosity at temperature  $\theta_R$  and  $n$  is a constant in the range 2–3.

The influence of shear on viscosity has a very significant influence upon the rate of development of the viscous boundary layer.

The shear in the viscous layer is given by

$$\gamma = \frac{V}{\delta_v}.$$

Thus, just behind the blade, the shear will be very high (since  $\delta_v$  is very small), and this means that the effective viscosity will be low. Consequently, the layer will move away from the wall more gradually than in the Newtonian fluid case.

A simplified equation for the thickness of the viscous layer for non-Newtonian fluid is

$$\delta_v = \delta_{v_0} \left[ \frac{\delta_{v_0} \gamma_0}{V} \right]^{k/2-k},$$

where

$$\delta_{v_0} = 2 \left( \frac{v_0 x}{V} \right)^{1/2}.$$

The influence of the shear factor can be seen from the following table, which shows the derived thickness ( $\delta_v$ ) as a function of  $\delta_{v_0}$  for a velocity  $V$  of 2 m/s, equivalent to 300 rpm:

$\delta_{v_0}$	$\delta_v$
1	0.02
2	0.06
4	0.18
8	0.50
16	1.41

This illustrates that the viscous layer is an order of magnitude smaller for the non-Newtonian material when compared to a corresponding Newtonian material.

The thermal diffusion process, however, is not affected by the shear and so the same equations as before apply. Thus, the thickness of the thermal layer becomes closer to that of the viscous layer.

This analysis indicates that, for high-melting-point margarines, there is likely to be a very thin layer close to the cooling surface in which a linear profile is developed. This is a region of high shear, which effectively lowers the viscosity within this region. Outside this layer the material is moving uniformly with the rotating shaft. This condition is often referred to as *mass rotation*.

There is, however, a number of instabilities that induce vortices, and these can delay the onset of the mass rotation condition. The next section will discuss the cause of these instabilities.

*Flow Instabilities.* For clarity, these instabilities will be discussed in terms of Newtonian fluids, although similar, more complicated behavior will occur with non-Newtonian fluids.

- 1. Instability behind the blade.** The flow situation is equivalent to the analysis of the transition from laminar to turbulent flow along a plate parallel to the direction of flow and is shown in Figure 10. Instability is predicted to start at Reynolds numbers greater than 580, although observable disturbances need a higher value, say 1000.

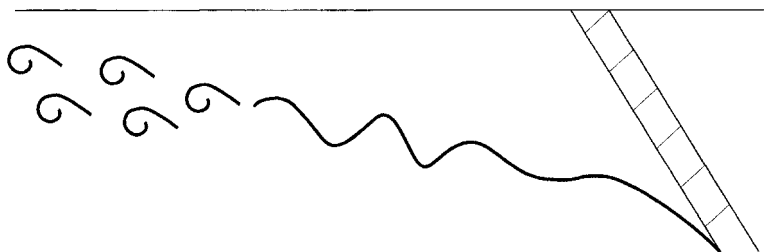
Thus this type of disturbance will occur when

$$\text{Re} = \frac{Vx}{\nu} > 1000.$$

On the basis of the earlier discussion, this implies a mixing length ( $L$ ) of

$$L = \frac{1000\nu}{V}.$$

For water  $\nu = 10^{-5}$  and so if  $V = 2$  m/s,  $L = 5$  mm. For an oil of 1000 cP,  $L$  will be about 5 m, in which case this instability will not be observed since the distance to the next blade is only 0.2 m.



**Figure 10.** Instability behind the blade (56). Courtesy of N. Hall Taylor, Crown Chemtech Ltd., Reading, United Kingdom.

**2. Instability within the annular space.** The rotation of the fluid in the annular space means that a centrifugal pressure gradient exists across the gap, the higher pressure being at the wall. This pressure gradient is given by

$$\frac{dp}{dr} = \frac{\rho V^2}{r}.$$

Ignoring the velocity gradients, this implies a pressure difference across the gap of

$$\Delta p = \rho V^2 \frac{H}{R},$$

where  $R$  is the shaft radius.

For our standard SSHE,  $H = 16$  mm and  $R = 61$  mm so that at 300 rpm  $\Delta p$  is 0.01 bar, with the pressure at the cylinder wall being slightly higher than at the shaft surface.

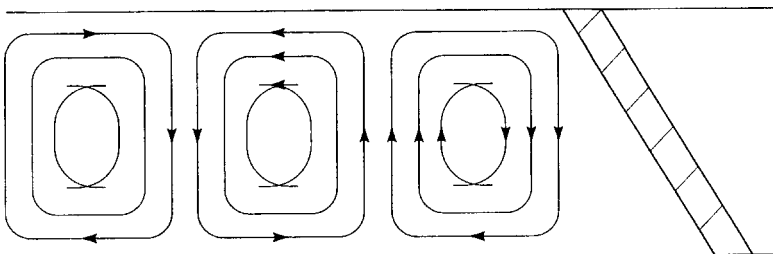
Although the pressure difference seems small compared to the local operating pressure of say 50–70 bars, it is still capable of inducing a circulation pattern. Thus, by Bernoulli's equation, this pressure difference can accelerate the liquid (ignoring viscous effects) to a velocity  $u$  given by

$$\frac{1}{2} \rho u^2 = \Delta p = \frac{\rho V^2 H}{R},$$

$$\frac{u}{V} = \left( \frac{2H}{R} \right)^{1/2}.$$

Hence for the standard SSHE,  $u = 1.37$  m/s.

The significance of this centrifugal effect is that it can cause a series of fairly stable vortices to be set up between and travel with the blades. Figure 11 shows this effect. This implies that the outer dimension of the vortex is equal to the gap width  $H$  and that the mixing length  $L$  lies somewhere between  $H$  and  $2H$ . Because this is about a tenth of the distance between the blades, the heat transfer should be increased by a factor of 2–3.



**Figure 11.** Vortices behind the blade (56). Courtesy of N. Hall Taylor, Crown Chemtech Ltd., Reading, United Kingdom.

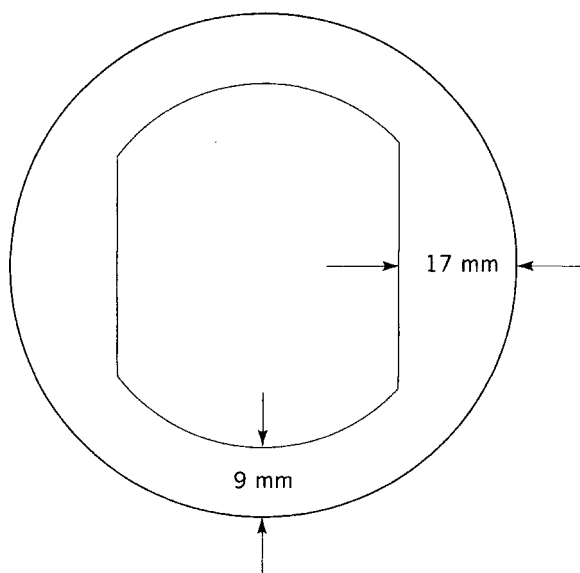
The vortices need some time to establish, and they will be most persistent if the ratio of the distance between the blade ( $\pi R$ ) to the gap width ( $H$ ) is close to an integer. For the standard SSHE the ratio is about 13.

- 3. Enhancement of annular gap instabilities.** The instability of the previous section will be suppressed by higher viscosities, again reverting to mass rotation. There are different methods used to overcome this with varying degrees of success:

Oval tubes  
Oval shafts  
Eccentric shafts  
Sectioned shafts

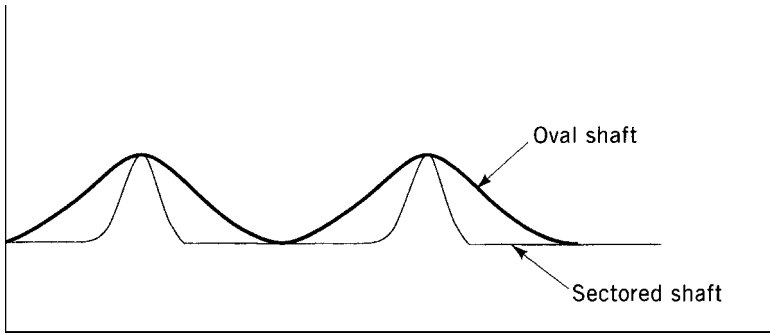
The first three are clear from their description. The sectioned shaft equipped with staggered blades has large flats to accommodate the blades on opposite sides, so that the gap widths vary between 9 and 17 mm. Figure 12 shows a diagram of such a shaft. The effect of the staggered blades is that the position of the flat is rotated through  $90^\circ$  with each successive blade set. This arrangement has other advantages and will be explained later.

The last three design concepts listed have the common feature that the gap width at a point on the cooling cylinder will vary as the shaft rotates. In the case of the oval tube the gap width varies when seen from a point rotating with the shaft.



**Figure 12.** Sectioned shaft (56). Courtesy of N. Hall Taylor, Crown Chemtech Ltd., Reading, United Kingdom.





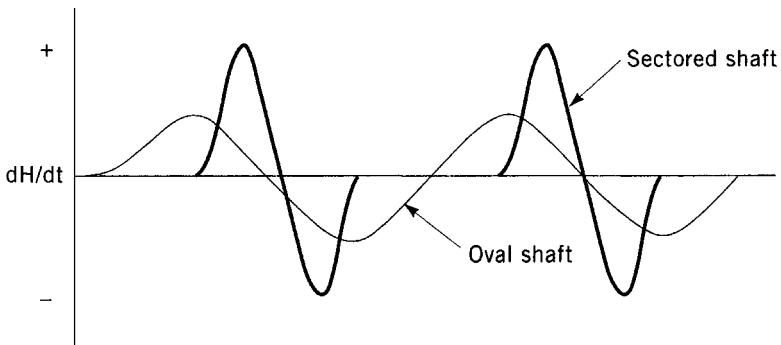
**Figure 13.** Influence of shaft type on gap width (56). Courtesy of N. Hall Taylor, Crown Chemtech Ltd., Reading, United Kingdom.

Figure 13 illustrates this variation in gap width for the case of the oval shaft and the sectored shaft. An eccentric shaft would also have a sine-type function but with only one maximum per revolution.

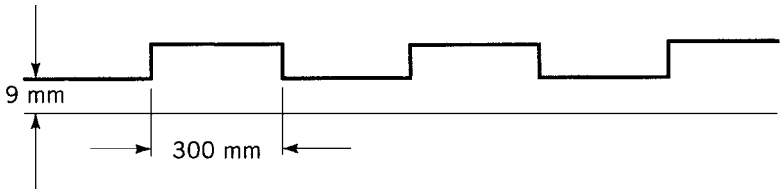
The key feature of these designs is that the change in gap width creates a radial velocity equal to  $dH/dt$  at the shaft surface. This also has the ability to generate vortices within the gap.

As Figure 14 illustrates, the advantage of the sectored shaft is that it has pulses of much greater velocity than the oval shaft followed by periods in which the turbulence is allowed to develop.

- 4. Axial flow.** The axial velocity of the material through the annular gap is at a much lower velocity than the rotational velocity. It can, however, still contribute to the creation of instabilities when the staggered blade configuration is used. This is because, as the material progresses through the cylinder, it encounters variations in gap width as illustrated in Figure 15. At each of the changes in cross-sectional areas there is the possibility to induce turbulence.



**Figure 14.** Influence of shaft type on rate of change gap width (56). Courtesy of N. Hall Taylor, Crown Chemtech Ltd., Reading, United Kingdom.

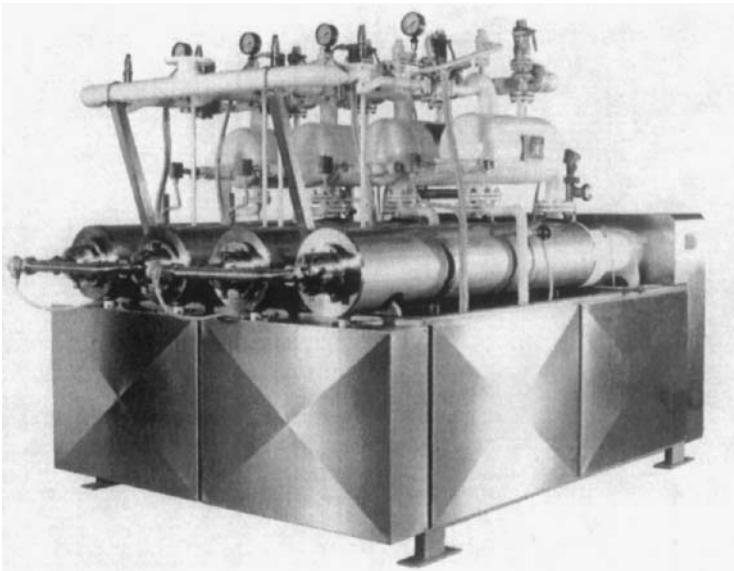


**Figure 15.** Change of gap with axial flow (56). Courtesy of N. Hall Taylor, Crown Chemtech Ltd., Reading, United Kingdom.

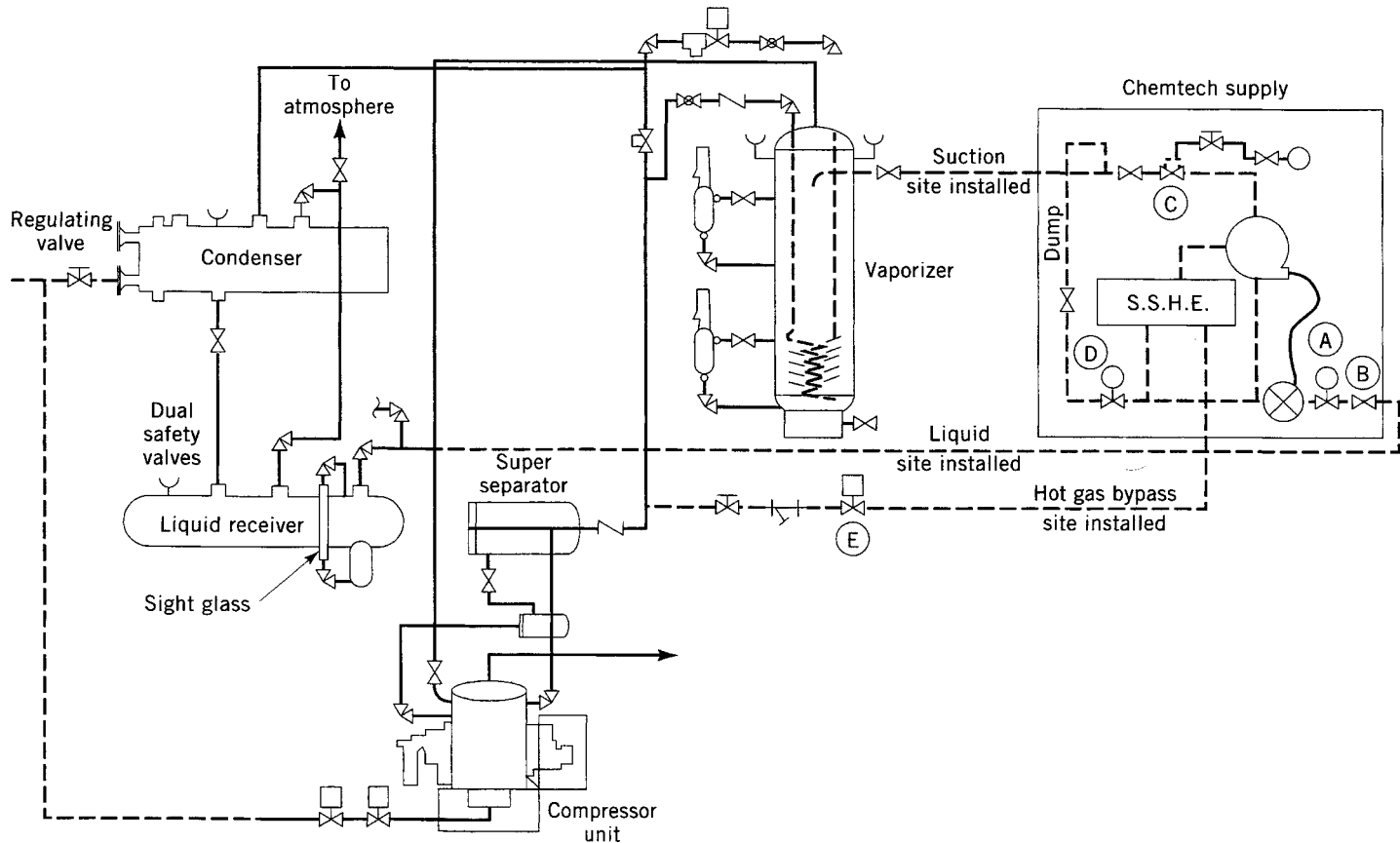
*Refrigeration System and Scraped-Surface Units.* Scraped-surface heat exchangers for margarine production are, as mentioned, designed for direct expansion refrigerants such as ammonia and Freon 22. Advantage is taken of the high rate of heat transfer due to surface boiling of the refrigerant (54).

A-units with individual refrigeration systems per cooling cylinder assembly are available from most suppliers. From Figure 16, an A-unit with four cooling cylinders with individual refrigeration systems can be seen. Each cooling cylinder is mounted with a surge drum above the cylinder. The surge drum is part of the refrigeration system of each cylinder. Figure 17 shows how the refrigeration system of an A-unit cooling cylinder assembly operates.

During normal operation, all stop valves around the A-unit are open. The liquid refrigerant inlet solenoid valve (A) is open, allowing liquid to pass through the level control valve (B) and into the bottom of the refrigerant jacket surrounding the



**Figure 16.** Chemetator SSHE for margarine processing. Courtesy of Crown Chemtech Ltd., Reading, United Kingdom.



**Figure 17.** Schematic diagram of refrigeration system. Courtesy of Crown Chemtech U.S.A., a division of Crown Iron Works Co., Minneapolis, Minnesota.

cylinder. Rapid heat transfer through the cooling cylinder wall from the warm product inside the cylinder causes a considerable proportion of the liquid refrigerant to vaporize upon contact with the outside wall of the cooling cylinder. Gas and entrained liquid are discharged from the top of the jacket into the surge drum. To ensure flooded conditions at all times in the jacket, a liquid level is maintained in the surge drum by a sensor linked via a capillary tube to the control valve (B).

Gas leaves the surge drum via a pressure regulating valve (C) and the suction to the suction trap of the fridge plant. The system is controlled by the pilot valve mounted on the control valve (C). On manual systems, this is adjusted by hand to give the desired temperature indication on the pressure gauge. On automatic systems, this is linked via controller to the liquid temperature measuring device (G) (57).

A current measuring device on the drive motor to the A-unit detects a rise greater than a predetermined level above the normal running current, typically 10%, for the specific product being processed. This automatically closes the liquid inlet valve (A) and the pressure regulating valve (C) while keeping the A-unit's shaft rotating (54, 57). Normally, a warning signal is given to the operator of a potential freeze-up, which may be prevented if the problem can be identified and corrected. The system is then reset manually. If the problem is identified, such as failure of the high-pressure feed pump, it is possible to prevent a certain freeze-up by operating a hot-gas system either manually or automatically. This system is lined electrically, so that it will only operate if valves A and C are closed. Selecting the hot-gas option opens valves E and D. This immediately allows hot gas from the high-pressure discharge side of the compressor to be introduced directly into the refrigerant jacket of the A unit. The pressure in the jacket and surge drum rises and forces all the liquid out via valve D and the suction line into the suction trap of the refrigeration plant. Once the liquid is ejected, and assuming that the A-unit shaft is still rotating, the hot-gas system can be switched off manually or automatically through an electrical time delay relay. The system will then be ready for restart when the original problem has been corrected.

In certain parts of the world, power cuts can occur frequently and cause problems in the operation of A units for margarine production. Due to this, A units are usually mounted with various features in the refrigerant system to minimize the downtime related to power cuts. The hot-gas option is one feature. At the moment of the power cut, valves D and E will automatically open and valves A and C will close. Although the fridge compressor will also stop running, the residual hot gas in the condenser and pipework will cause an immediate rise in the pressure in the refrigerant jacket of the A unit. Although the A-unit shaft has stopped rotating, this should allow it to rotate freely when power is restored. It is, however, important that this should nevertheless be checked manually after all necessary safety precautions have been taken by isolating the drive motor locally or at the electrical control panel (57).

Following a power cut, product feed failure, or any other abnormal conditions, it is possible that the A unit will be frozen solid. In this situation, the hot-gas system can be operated, as described, together with the warm water circulation through the

A-unit shaft to ensure rapid melting of the solidified margarine inside the cooling cylinder.

Other systems used in A units to help prevent freeze-up situations, as described above, operate by a drop tank principle, where the refrigerant is removed from the refrigerant jacket with the aid of increased refrigerant pressure in the system without installation and activation of a hot-gas system.

### 2.3. Worker Units

Fats require time to crystallize. This time is provided in crystallizers normally called worker units, or B units. These are cylinders with larger diameters mounted with pins on the inside of the cylinder walls (stationary pins) and on the rotors (rotating pins) (3, 54, 55). The pins fixed to the concentric rotor are mounted in a helical arrangement that intermesh with the stationary pins of the cylinder wall (55). Worker units can be installed either between cooling cylinders of a multicylinder. A unit or after the A unit (3, 4, 51, 54, 55, 58). Worker units have the benefit of giving the margarine emulsion time to crystallize under agitation by the pins of the rotating rotor (see Section 1).

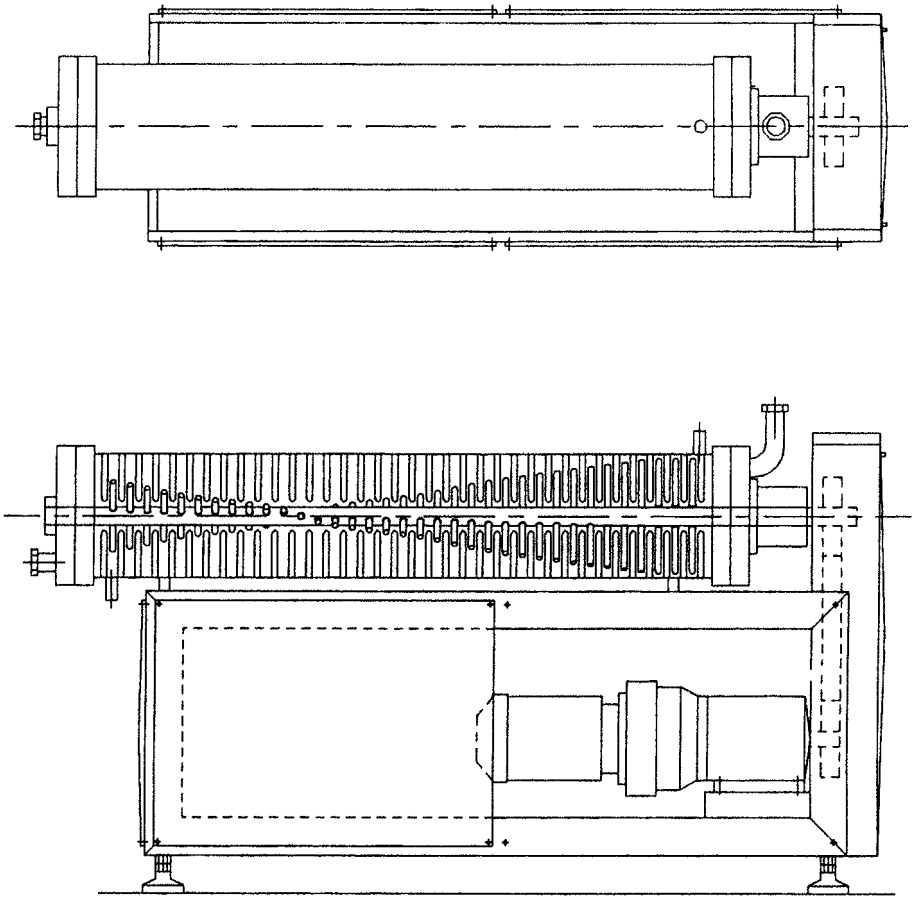
The worker unit is normally mounted with a heating jacket for tempered water on the cylinder and often also equipped with its own built-in water heater and circulation pump for the tempered water. This is advantageous in preventing product buildup on the cylinder wall and allows better product temperature control during the passage through the worker unit. Product temperature increases of 2°C or more due to release of latent heat of crystallization and mechanical work can be observed in the worker unit (3).

Worker unit cylinders usually have product volumes ranging from 35 L up to approximately 105 L per cylinder. B units with up to three worker cylinders mounted on the same support frame are available on the market. Each worker cylinder usually has its own individual drive with fixed or variable speed for maximum flexibility during processing of margarine. The design of a worker unit is illustrated in Figure 18.

### 2.4. Resting Tubes

When producing margarine for stick or block wrapping, a resting tube is normally connected directly to a packaging machine to allow the product sufficient time to attain a hardness that is suitable for wrapping (3, 4, 54, 55). During production of table margarine for stick wrapping, the product will commonly pass through the cooling cylinders of the A unit and a possible intermediate worker unit (B unit) inserted between the cooling cylinders. From the A unit, the product enters the resting tube connected directly to the packaging machine (3).

The intermediate worker unit normally has a lesser product volume than final worker units used in production of soft table margarine for tub filling. The purpose of limiting the amount of work given to the product is first to produce a product that is not too soft to be handled in the automatic stick wrapping machine. Second, it is



**Figure 18.** Worker unit (B unit) with one cylinder. Courtesy of Crown Chemtech U.S.A., a division of Crown Iron Works Co., Minneapolis, Minnesota.

to prevent the aqueous phase of the margarine from being dispersed in an extremely fine state of subdivision (54), which could have a negative effect on the flavor release. Finally, too intensive working of a table margarine, with its higher solid fat content compared to a soft margarine, could cause the product to attain an unpleasant, greasy consistency (59). Too greasy a consistency could also cause the wrapping material to stick to the product, which would result in a poor presentation of the product to the consumer. Figure 4 illustrates the process flow for production of table margarine for stick wrapping.

The margarine is forced through the resting tube by the pressure of the high-pressure feed pump. Resting tubes are normally fitted with screens or perforated plates (55) to allow a minimal degree of work to be given to the product to ensure optimal crystallization and plasticity.

Resting tubes for table margarine and similar products are made up of flanged sections with lengths varying from approximately 450 mm (17.5 in.) to approximately 900 mm (35 in.) (54, 60). This allows the product volume of the resting tube to be varied in accordance with the physical characteristics of the solidifying margarine (54). Resting tubes for table margarine production commonly have diameters ranging from approximately 150 to 180 mm (6 to 7 in.) (54, 60). Resting tubes for production of puff pastry margarine usually have diameters ranging from approximately 300 to 400 mm (12 to 16 in.). The flanged section in these resting tubes has a length of up to approximately 1000 mm (39 in.). The volume of resting tubes for puff pastry margarine is normally considerably larger than for other products to allow sufficient time for development of the special consistency required in puff pastry margarine (see Section 5.2).

Some equipment suppliers recommend using one single resting tube for feeding table margarine to the packaging machine, whereas others recommend the use of two connecting, parallel resting tubes. When one of the two resting tubes has been filled with product, a motor-actuated rotary valve automatically switches the flow of product to the second resting tube. The product in the first resting tube remains static until the second resting tube has been filled.

The construction of a resting tube usually involves the required inlet adaptor, flanged sections, screens or perforated plates, and an outlet connection flange for direct linkup to the packaging machine. Alternatively, the resting tube could also be mounted with an outlet extrusion nozzle, in case the product is fed to the packaging machine through the older, open hopper system. Resting tubes are normally jacketed for warm water circulation to minimize the friction between the margarine and the stainless steel wall of each section. This helps prevent channeling of the product and reduces the overall discharge pressure required at the high-pressure feed pump.

Figure 19 shows resting tubes of varied sizes for puff pastry margarine.

## 2.5. Packaging Equipment

Margarine products are packed in several ways depending on margarine type, product consistency, and consumer preferences. In the U.S. market, consumer retail margarines and related products, including butter blends, cover a variety of products packaged in different ways (61). These can be grouped as follows:

- Margarine in quarter-pound sticks
- Margarine in one-pound solids
- Margarine patties
- Soft margarine in tubs
- Spreads in quarter-pound sticks or one-pound solids
- Soft spreads in tubs
- Diet products in sticks or tubs
- Liquid margarine in squeeze bottles.

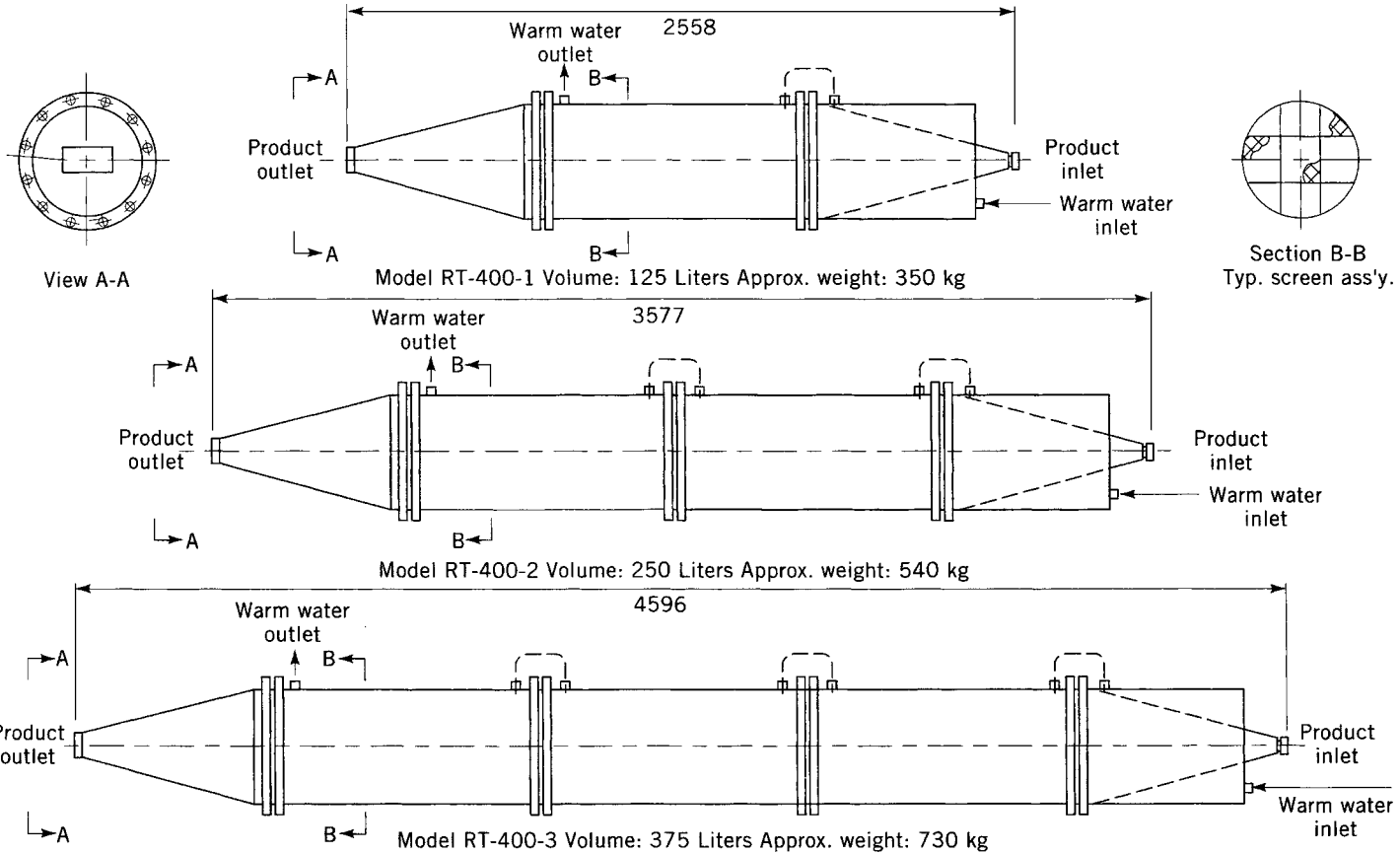


Figure 19. Resting tubes for puff pastry margarine. Courtesy of Crown Chemtech U.S.A., a division of Crown Iron Works Co., Minneapolis, Minnesota.

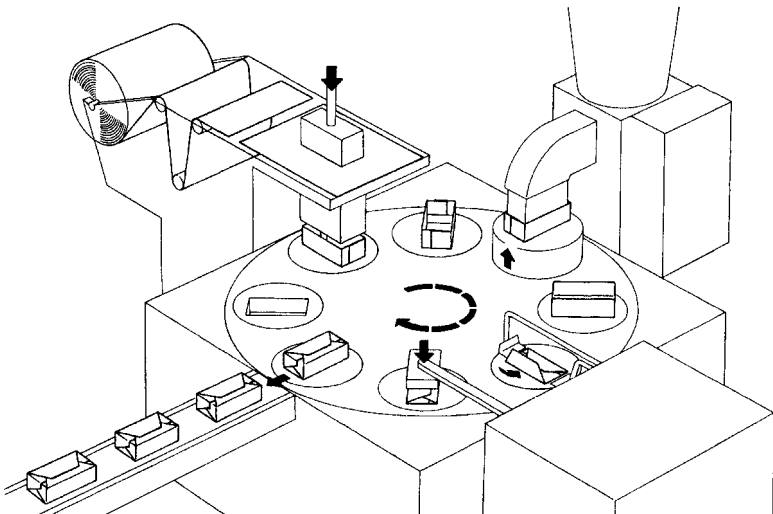


Margarines for food service or industrial use are normally filled into 50-lb plastic bag lined cartons, wrapped in blocks of 5 lb or bigger, or supplied in bulk.

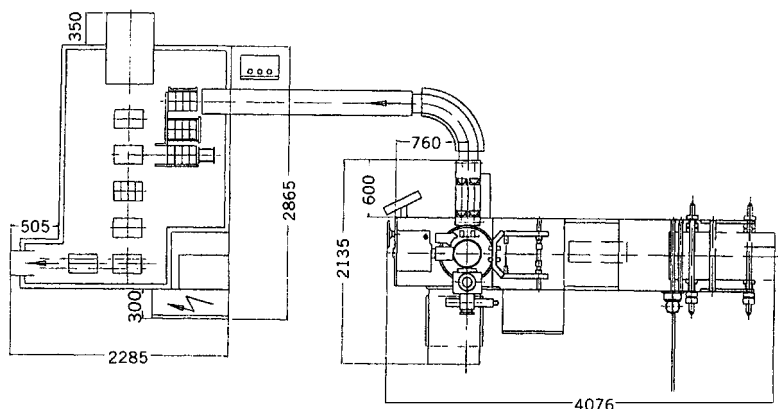
*Stick/Solid Retail Margarine.* Two basic types of stick or solid packaging machinery are used in the United States (62). The first of these forms is a molded print that forces product into a measuring and molding chamber. The molded print is removed from the chamber and then wrapped and cartoned. On the second type of machine, the product from the resting tube is filled directly into a cell that is prelined with a preformed wrapper bag. The wrapping is then folded and ejected from the cell.

The second type of machinery is the more widely used type in the U.S. margarine industry. The principle operation of the second type of machine is shown in Figure 20. This type of packaging machine was originally developed by Benz & Hilgers GmbH of Neuss, Germany, and today a wide range of machines for stick or solid wrapping based on this concept exist (63). The concept shown in Figure 20 involves a machine with drive elements running in an oil bath. The product can be fed to the machine either by a trough with feed worms or by direct linkup to a resting tube. Machines of this type can also be fed by a vertical funnel with a special scraper/agitator mounted. This type of feeding arrangement is normally used when wrapping miniportions at low hourly capacity.

In Figure 20, the wrapping material is fed continuously from a changeable reel and is cut crosswise by a knife system before arriving at the bag forming station. A plunger guides the wrapper through folding channels to form the bags, then positions the bags exactly into the cells located in the intermittently running rotary table in the center of the machine (64). The positioned bags are transported by the rotary table to the dosing station. At the dosing station, the cells are lifted



**Figure 20.** Forming of the wrapper bag, filling, and folding. Courtesy of Benz & Hilgers GmbH, Neuss, Germany.



**Figure 21.** Example of a packaging line. Courtesy of Benz & Hilgers GmbH, Neuss, Germany.

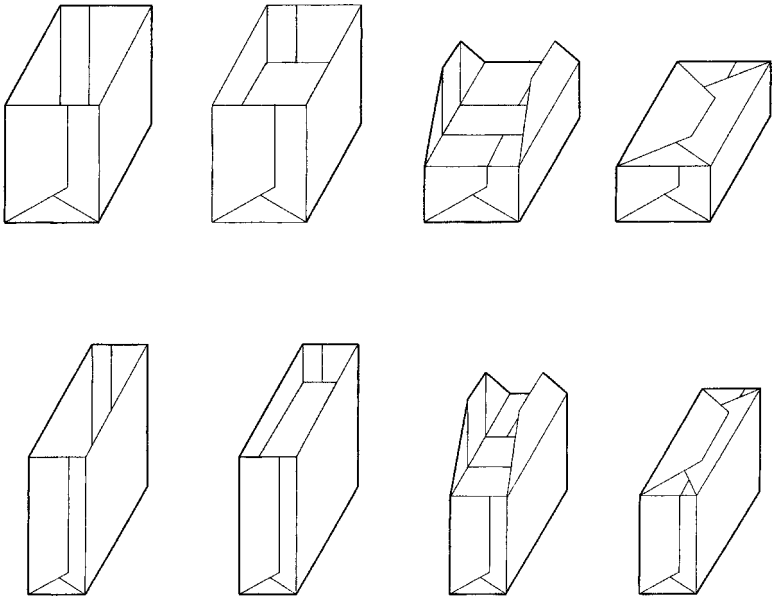
with the wrapper inside to ensure air-free filling of the product. Product is filled into the preformed wrapper bags by the dosing station utilizing a dosing cylinder with a piston. After subsequent folding and calibrating station, sharp-edged sticks or solids are transported out of the packaging machine to the cartoning machine. Figure 21 shows an example of a packaging line including the stick wrapping machine and an attached cartoning machine.

This packaging operation is more suitable for softer products than the system where the product is molded before wrapping (62). Furthermore, the described system normally operates with a bottom fold principle, which facilitates the folding and closing operation during wrapping of softer product (64). A more economical length-side fold principle can also be used in the packaging operation, saving wrapping material. The two folding principles are shown in Figure 22.

The wrapping materials used in the wrapping operation shown in Figure 20 may be parchment, laminated aluminum foil, plastic-coated material, or plastic foil (63). For packaging of margarines, the first two wrapping materials are commonly used.

Generally, packaging lines as shown in Figure 21 used in the margarine industry are becoming quite sophisticated, involving electric and electronic monitoring systems to control the functional sequences of the machinery. Monitoring systems cover registration of production data, identification of end of wrapping material roll, product pressure control, photoelectric wrapper registration, and automatic control of dosing volume by integrated check weigher (63). Computer-aided machine diagnostic systems can also be installed in packaging machinery. This involves a programmable logic controller (PLC) monitoring system, which helps to avoid faults in the packaging operation, to identify reasons for failure, and to control production data.

High-speed, fully automatic packaging lines for stick wrapping of margarine with speeds up to 240 sticks per minute are widely used in the U.S. margarine industry. Such lines include fully automatic cartoning machines for inserting four



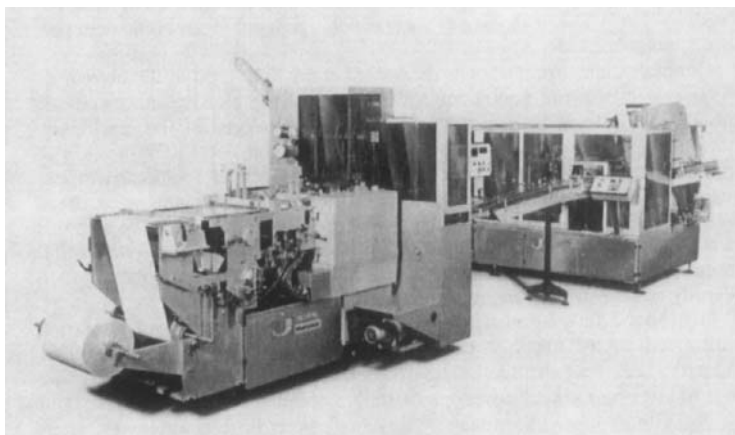
**Figure 22.** Packets with bottom and length-side fold. Courtesy of Benz & Hilgers GmbH, Neuss, Germany.

quarter-pound sticks into one carton, for example. The cartons can then be packed into cases in semiautomatic case packers or fully automatic wrap-around case packers. Finally, the packaging lines can also include automatic palletizing machines. Figure 23 shows a fully automatic, high-speed stick wrapping machine complete with cartoning machine.

*Soft Tub Margarine.* In the North American market, soft margarine and spreads are usually filled into tubs made from either polypropylene (PP) or polyethylene (PE). Polypropylene allows for a thinner wall of the tubs and is more rigid than PE. Due to the more rigid structure of PP, tubs made from PP can crack. Tub made from PE have a smaller tendency to crack, as PE is more flexible. Due to this, lids are normally made from PE. Polyethylene gives a better weight control during the manufacture of tubs, whereas PP in larger quantities is cheaper than PE. Polypropylene and PE have equal properties in permeability of ultraviolet light and air (oxidation) (65).

Tub filling machines for margarines and spreads are available from several U.S. equipment manufacturers such as Rutherford of Rockford, Illinois, Phoenix Engineering of Wisconsin, and Osgood of Clearwater, Florida.

In tub filling operations, it is normally required for hygienic and easy cleaning procedures that the filling machine have a clear separation of the mechanical drive and the product conveyor. Furthermore, it is advantageous to have filling machines that prevent product or cleaning agents from entering the mechanical drive (66). Cleaning of tub filling machines is normally limited to those parts in the conveyor



**Figure 23.** Stick wrapping machine with cartoner. Courtesy of Benz & Hilgers GmbH, Neuss, Germany.

area that are in contact with the product. The dosing module and the entire area in contact with the product can be automatically CIP (clean-in-place) cleaned in more sophisticated machines.

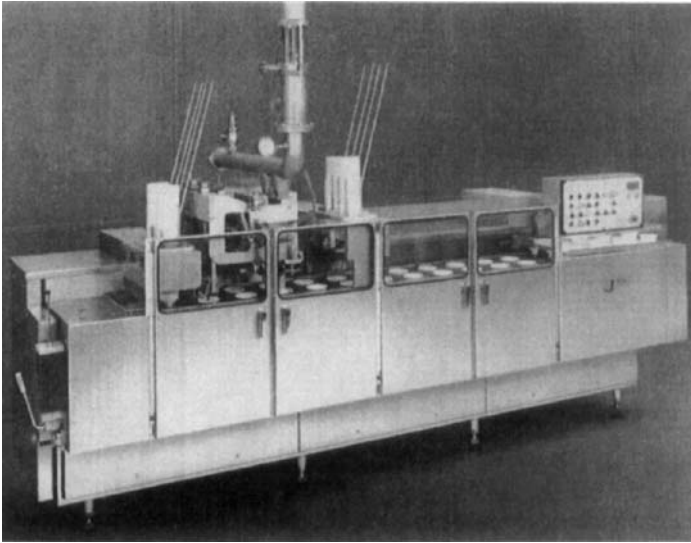
Most tub filling machines are in-line machines with up to four tracks depending on the requirements of filling volume and capacity.

Tub filling machines can be fitted with a variety of functions depending on whether the margarine is packaged in tubs with a heat-sealed membrane or coverleaf under the lid, for example. The main functions of a tub filling machine for margarine normally are (67):

- Tub feeding station with magazine
- Direct product feed with pneumatically operated compensating piston
- Dosing device with filling nozzles
- Feeding of snap-on lids
- Press-on station for lids
- Date coding device
- Off-conveyor
- Control panel

Optional functions usually include:

- Tub cleaning or sterilization device
- Automatic CIP cleaning system
- Coverleaf station with magazine
- Sealing membrane station with magazine
- Other functions



**Figure 24.** Fully automatic tub filling and closing machine. Courtesy of Benz & Hilgers GmbH, Neuss, Germany.

In margarine production, the packaging line for tubs can be completed with wraparound case packers and palletizers (67). A fully automatic tub filling machine is shown in Figure 24. A device for the simultaneous quantitative regulated filling of liquid or soft plasticized substances, such as butter, margarine, pastes, or the like, by means of nozzles into adjacently arranged containers with the assistance of at least one control element interchangeably switchable from filling to discharging and at least one dosing piston has been described (68).

*Industrial Margarines.* These products are usually filled into plastic-bag-lined cartons of various sizes. Special bakery margarines, such as puff pastry margarines, are normally wrapped in blocks of approximately 1–25 kg. Alternatively, puff pastry margarine can be packed in plates or sheets of 1–5 kg (68, 69). Edmunds and Budlong (69) have given a detailed description of a continuous sheeting and packaging machine for puff pastry margarine and related products.

Block and plate wrapping machines for margarines are available today from C. Bock & Sohn Maschinenfabrik of Norderstedt, Germany, and Gerstenberg & Agger A/S of Copenhagen, Denmark.

Block packing machines are today quite sophisticated, and it is possible to wrap different block sizes in one machine. Block packing machines can be delivered with special slicing equipment for slicing the block during extrusion but before the final wrapping as illustrated in Figure 25. Block packing machines can, if required, be installed for automatic CIP cleaning, which is important especially in connection with butter production (70).

Figure 25 shows a fully automatic block production line where the product to be wrapped is fed from the SSHE plant into a dosing station. With the help of product

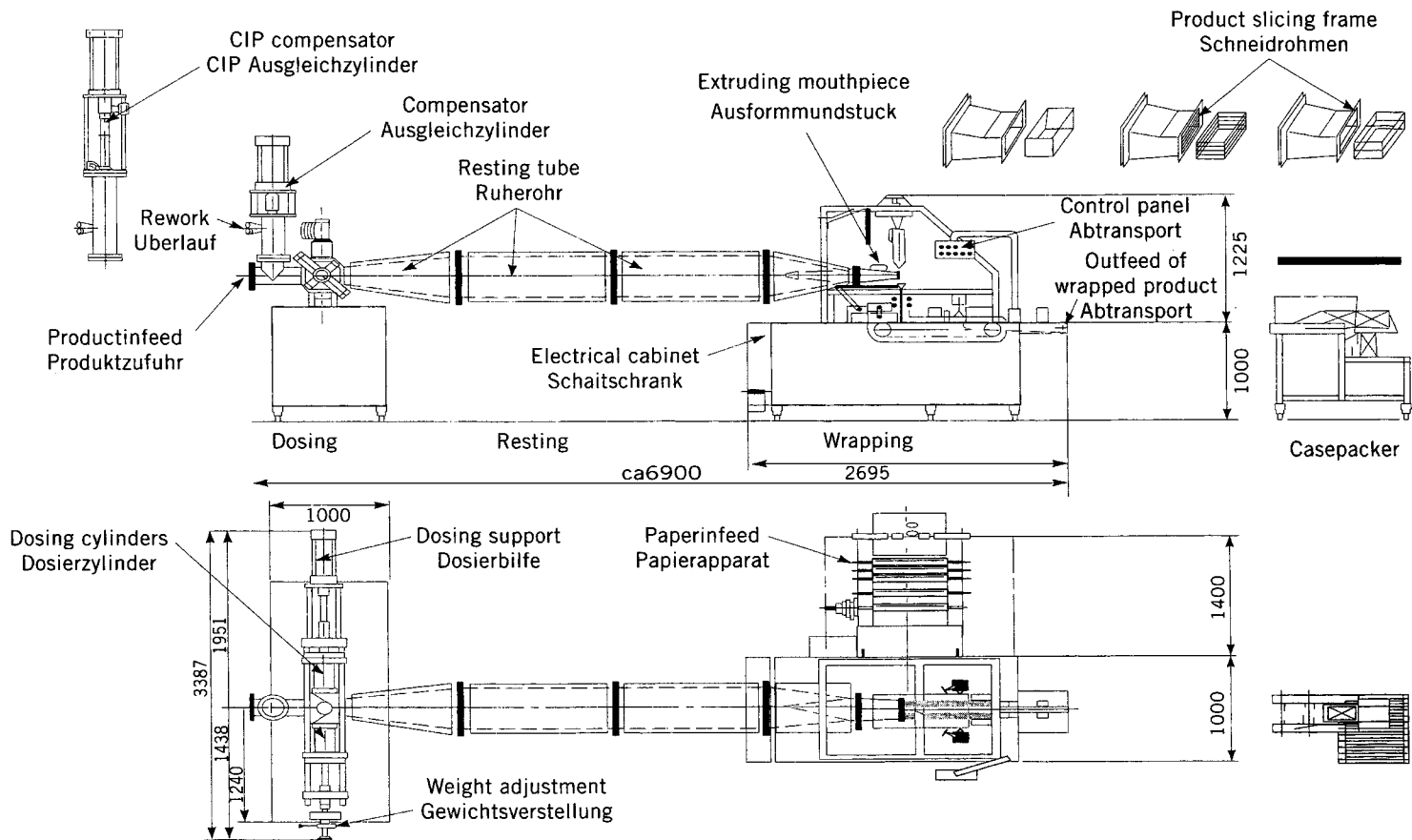


Figure 25. Fully automatic block wrapping machine. Courtesy of C. Bock & Sohn Maschinenfabrik, Norderstedt, Germany.

and compensator pressure, it is passed on through two laterally placed cylinders via the resting tube toward the mouthpiece of the block packing machine. Exact weight control is achieved by the piston stroke of the coupled dosing pistons mounted in the two cylinders. The extrusion nozzle of the block packing machine is equipped with a special cutoff device that cuts the product vertically from top to bottom after finished dosing. The wrapper is fed from the reel, cut, and positioned automatically under the extrusion nozzle or mouthpiece. The product block arrives onto the wrapper, which is supported by a transport plate. Each wrapper will be controlled in its final position before dosing takes place. A no-wrapper/no-dosing device is mounted in the machine. Vacuum will hold the wrapper correctly on the transport plate while the block moves toward the folding level. Here the prefolded block will be transported by a chain conveyor to the various folding stations. The wrapped and folded block leaves the machine on a transport belt (69).

Modern sheet wrapping production lines function after the same principles except that the product is extruded as a sheet or plate from the mouthpiece vertically into a plate turner. Before the extrusion, the wrapper is positioned and follows the product into the plate turner. The plate turner is driven by a four-step gear drive rotating the plate turner 90° while the cross-folding takes place between each dosing/extrusion cycle. In a horizontal position the plate is pushed out on a conveyor belt and transported through a permanent folding device for end folding below the wrapped plate (70).

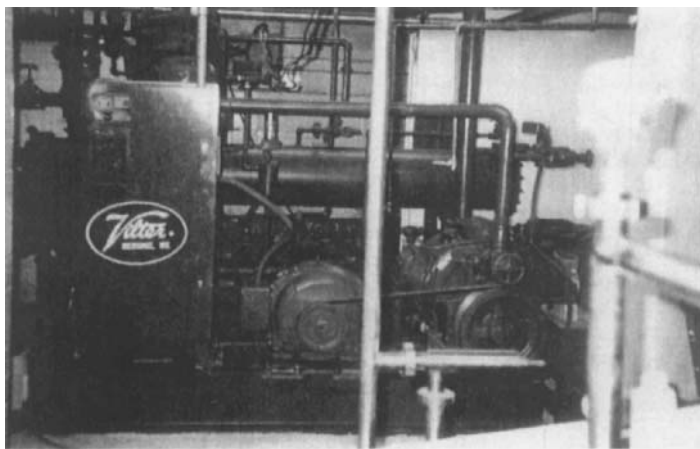
## 2.6. Refrigeration Plants

Refrigeration is a key operation in the margarine production plant. In the margarine industry, Freon 22 and ammonia were widely used as refrigerants. New regulations phasing out the use of chlorofluorocarbons (CFCs) are in place in many countries for environmental reasons (see Section 3). Plans for phasing out a hydrochlorofluorocarbon (HCFC) such as Freon 22 (R-22) are currently being made or in some countries are already in place (52, 71). The layout of an ammonia compressor plant servicing an SSHE for margarine production can be seen in Figure 17 (see Section 2.2).

Ammonia systems consist of a compressor designed to compress the low-pressure ammonia gas from the SSHE. The gas is then discharged from the compressor into the condenser. When ammonia is under a pressure of 150 psi (10 bar), it will liquify at a temperature of 25.6°C (78°F) (71). Condensers can be of the air-cooled or water-cooled type covering also evaporative condensers (72). From the condenser, the liquid ammonia flows to the receiver. The receiver in which the high-pressure ammonia liquid is stored maintains a constant supply of refrigerant to the SSHE.

Figure 26 shows a packaged ammonia compressor system designed for servicing an SSHE in margarine production. The system is skid-mounted from the factory for easy installation. Only the condenser of the system is supplied loose.

Ammonia compressor systems used in margarine plants are usually equipped with highly efficient superseparators for removal of lubrication oil from the



**Figure 26.** Packaged ammonia compressor system. Courtesy of Cremeria Americana SA, Mexico.

ammonia (71). Lubrication oil carried over into the ammonia will eventually reduce the heat transfer efficiency of the SSHE, as the oil will be deposited as a thin film on the outside wall of the cooling cylinder. This can reduce the heat transfer considerably. Compressors of the reciprocating piston type or screw compressors are normally installed depending on compressor cost at various capacities or individual preferences (71). The screw compressors, with their highly efficient coalescing separators, reduce the amount of oil in the system considerably (70).

The use of ammonia as a refrigerant in margarine plants offers certain advantages as well as disadvantages. The advantages are cost, efficiency, detection, and environment (70). The quantity of refrigerant needed to charge an ammonia system is substantially less than for other systems, which provides additional savings. Ammonia is the most efficient of the commonly used refrigerants. Easy detectability of ammonia leaks is an advantage compared to R-22, taking into consideration the latest enforcement laws by the U.S. Environmental Protection Agency (EPA). Finally, ammonia is biodegradable and has no impact on the ozone layer (71).

The disadvantages are toxicity and flammability. Ammonia has a corrosive effect on tissues and can cause laryngeal, bronchial spasm and edema, which lead to obstructed breathing. Ammonia's flammability range in air is 16–25% by volume. It is usually characterized as hard to ignite (71). A suitable ammonia detection system with alarm should be installed and well maintained. Detectors should sound an alarm at the lowest practical level, not to exceed 1000 ppm.

Due to the disadvantages of ammonia, a number of regulations and standards provide safe practice procedures for the use of ammonia as a refrigerant. Details on mechanical requirements of refrigeration systems can be found in ANSI/ASHRAE Standard 15, Safety Code for Mechanical Refrigeration. Piping requirements should comply with ANSI B31.5, Refrigeration Piping (70). Many local and national codes must also be complied with in many states.



### 3. REFRIGERANTS FOR THE FUTURE

A number of new refrigerants have been proposed during the last several years as candidates to replace R-22 and R-502 in industrial refrigeration systems (73). International accords such as the Montreal Protocol on CFC production and other accords concerning pollution and gas emissions to the atmosphere in particular prompt a review of the refrigerants used in the margarine industry (52, 74).

Studies of the CFC refrigerant's ozone depletion and its effect on the ozone layer and global warming have reached such serious conclusions that both national and international accords are in place to protect the environment (52, 71, 74).

R-22 is an HCFC refrigerant considered to have an ozone depleting effect only 5% of that of a CFC refrigerant such as R-12 (52). Replacements for R-502 are being announced earlier than replacements for R-22 by refrigerant manufacturers. This is due to the early deadlines for ending production of ozone-depleting CFC refrigerants such as R-115, which is a component of R-502 (73). New refrigerants to replace R-502 and R-22 are discussed in detail in the literature (73, 74). Well-known biodegradable but toxic ammonia currently is emerging as the leading replacement refrigerant (71).

The industry should already consider the effects of the new environmental policies on its possible need for new refrigeration equipment or for modification of existing equipment (71, 74).

New alternative refrigerants may exhibit different heat transfer characteristics and may quite importantly require different discharge pressures than R-22 under similar temperature conditions (73). This should be considered very carefully, and all safety procedure and regulations as well as pressure vessel codes should be followed closely when modifying existing refrigeration plants (72). Considerable information on R-22 and R-502 replacement refrigerants has been developed by the Alternative Refrigerants Evaluation Program (AREP). AREP's purpose is to identify the most promising non-ozone-depleting refrigerants (73).

Fluorocarbon products that do not contain chlorine and/or bromine (i.e., fully fluorinated and hydrofluorinated [HFC] products) are not stratospheric ozone-depleters, and production of these products is not being eliminated by the Montreal Protocol. They are, however, restricted by the U.S. Clean Air Act and must be recovered rather than released to the atmosphere.

As a result of the Montreal Protocol and Kyoto Protocol and subsequent amendments and ratification by individual countries, there are current and proposed regulations limiting the production, consumption, and trade of CFCs, HCFCs, and HFCs. Over the past two decades, the global fluorocarbons market has undergone a number of major transitions toward a greater use of non-ozone-depleting HFCs and non-global-warming, nonfluorocarbon alternatives in emissive or potentially emissive applications.

Compared with the United States, the European Union has been significantly more aggressive in its production reduction to date and scheduled reduction of HCFC production, and it is considering restrictions in the use of HFCs in compliance with Kyoto Protocol goals to limit the emissions of global warming gases.

HFCs, FCs, and other fluoro-based compounds are some of the alternatives to HCFCs and CFCs (75).

#### 4. PLANT LAYOUT AND PROCESS FLOWSHEET

In margarine production, raw materials account for about 50% of the margarine cost, actual production costs account for 20%, and other costs are 30% of the total (35). Well-managed formulation and efficient, accurate metering/weighing systems for the various raw materials in the emulsion preparation plant are essential factors for cost-efficient margarine production (35, 76–78).

Table 2 can be used to illustrate the significance of the cost of the various ingredients in a specific recipe for production of 1 ton of margarine.

Microcomputers, allow the optimizing of formulation cost or least-cost formulation. One method is to select from the formula file according to fluctuations in raw materials prices. The high number of formulas required can make this task quite difficult unless computers are used to sort out the least-cost formula. Production schedules and previous purchases of raw materials will also have to be considered (35).

Another method is to create new formulations by minimization. Here formula cost is optimized against constraints. These constraints are based on finished product characteristics in relation to raw material characteristics. Production constraints relate to raw material properties, existing and new processes as well as productivity in the plant. It is essential to compare formulas and processes in order to optimize productivity by minimizing metering or weighing errors during emulsion

**TABLE 2. Ingredient Cost (79).**

Ingredient	% in Recipe	U.S. \$/Ton Margarine
Soybean oil, hydr. 44/46°C (111.2/114.8°F)	32.00	190.30
Soybean oil, hydr. 34/36°C (93.2/96.8°F)	4.00	23.79
Soybean oil	44.00	213.22
Emulsifier	0.20	5.98
Lecithin	0.20	1.61
Color (carotene)	0.005	12.65
Aroma	0.02	8.05
Water	16.935	0.14
Salt	2.00	3.91
Milkpowder	0.50	23.00
Potassium sorbate	0.10	8.40
Citric acid	0.04	2.53
	100.0	493.58

From Crown Wurster & Sanger, Minneapolis, Minnesota, with permission.

preparation and the use of unsuitable formulas. This will help to minimize the amount of product that has to be recycled. In a high-productivity setting, reworked or recycled product should constitute no more than 0.1–0.2% of the total plant production. Product specifications, fulfillment of these specifications, and product consistency as well as expected technical performances of the product are quality constraints. Depending on the quality control efficiency, recycling losses may reach 0.2–0.4% of the total production. Raw material quality is usually the cause (35).

Emulsion preparation systems play a very important role for achieving the above productivity and thus the desired profitability in margarine production. Three general systems are normally used for metering and mixing the various ingredients into a water-in-oil emulsion. These are (80):

- A continuous metering pump system
- A batchwise scale tank system
- A batchwise flowmeter system

#### 4.1. Continuous Metering Pump System

The margarine industry, like other food processing industries, is continuously involved in optimizing productivity through rationalization to minimize production costs. To achieve this a proper production method and production installation must be chosen allowing optimal capacity at minimal labor cost, maintenance cost, space, and energy requirements. At the same time the high product quality and productivity must be assured (81).

Continuous emulsion preparation using a metering pump system has been successfully used during the last decades in the margarine industry to meet the above requirements (76, 78) and is considered to be a very flexible installation (53). Well-known suppliers of metering pump systems are Bran + Luebbe Inc. of Buffalo Grove, Illinois, and American Lewa Inc. of Holliston, Massachusetts.

In connection with the use of continuous metering pump systems, metering or dosing can be defined as the addition of a defined ingredient flow or amount (ingredient flow is equal to the ingredient amount added over a specified time period) to a process tank, a mixer, or a process (81).

To allow metering, the ingredient flow (i.e., ingredient amount) must be transported, metered, and added. The metering pump covers the three operations of transport, metering, and addition in one step. Thus, the metering pump differs from regular pumping applications by two characteristics (81):

1. The flow is easily adjustable in a defined way.
2. Pressure and viscosity variations have no or only minimal influence on the flow.

A metering pump consists of drive with gear reducer and a pumphead, where the gear reduces the rotary motion of the drive motor and converts it into a reciprocating

plunger motion. Suction and discharge valves work alternately according to the plunger stroke. The capacity is determined by plunger diameter, stroke length, and stroking speed and can be adjusted manually, electrically, or pneumatically (81–83).

A combined adjustment of stroke length and stroking speed will allow the proportional metering of two or more ingredient flows based on the use of multiple pumpheads (79). Due to the flexibility of the metering pump, margarine emulsion preparation can be fully or partly automated by the use of a metering pump system with multiple pumpheads. For example, only two pumpheads are used for metering of the oil phase and the water phase.

Plunger diameter, stroke length, and type of stroking speed adjustment can be chosen individually for each pumphead in the multiple pumphead metering pump system (81–83).

In margarine production it is possible to install a multipumphead system with individual pumpheads for each ingredient or ingredient group used in the emulsion preparation (76, 81). All pumpheads can be driven by one single motor with gear reducer, which is an advantage from an energy consumption point of view. The proportional metering of each ingredient is adjustable through the stroke length in each pumphead. The total capacity of the metering pump system is adjustable according to the product demand of the crystallization line and the packaging operation (76, 78, 81).

Figure 27 shows a multipumphead metering system that uses an individual pumphead for each ingredient. A system capable of accurately metering up to 16 ingredients, with dosing accuracies of  $\pm 0.1\%$ , has been reported (76, 78). Dosing pump suppliers guarantee accuracies better than  $\pm 0.5\%$  (82, 83).

Maintenance of a stable emulsion in the continuous metering system's total product flow is critical for an efficient margarine production and is achieved through the use of specially designed static in-line mixers. These are installed in the main pipelines downstream of the metering system as can be seen from Figure 27.

Low-pressure or high-pressure metering pump systems can be installed according to user's preference and required plant design. Figure 27 shows a high-pressure system, where the emulsion flow from the system passes directly to the SSHE of the crystallization line. In low-pressure systems, the emulsion flow from the system passes the static mixers and a possible in-line plate pasteurizer before entering a balance tank. The balance tank is usually equipped with an agitator and high and low level switches for control of the drive of the metering pump system. From the balance tank, the emulsion is then pumped by a separate high-pressure piston pump to the SSHE.

Due to the flexibility of the multipumphead metering system virtually any margarine formula can be processed within the range of fat content and water content for which the system is originally laid out during the design of the overall processing capabilities (76, 78). Failsafe devices assure that the system is stopped automatically if an ingredient fails to flow. The use of a balance tank offers some advantages in this connection.

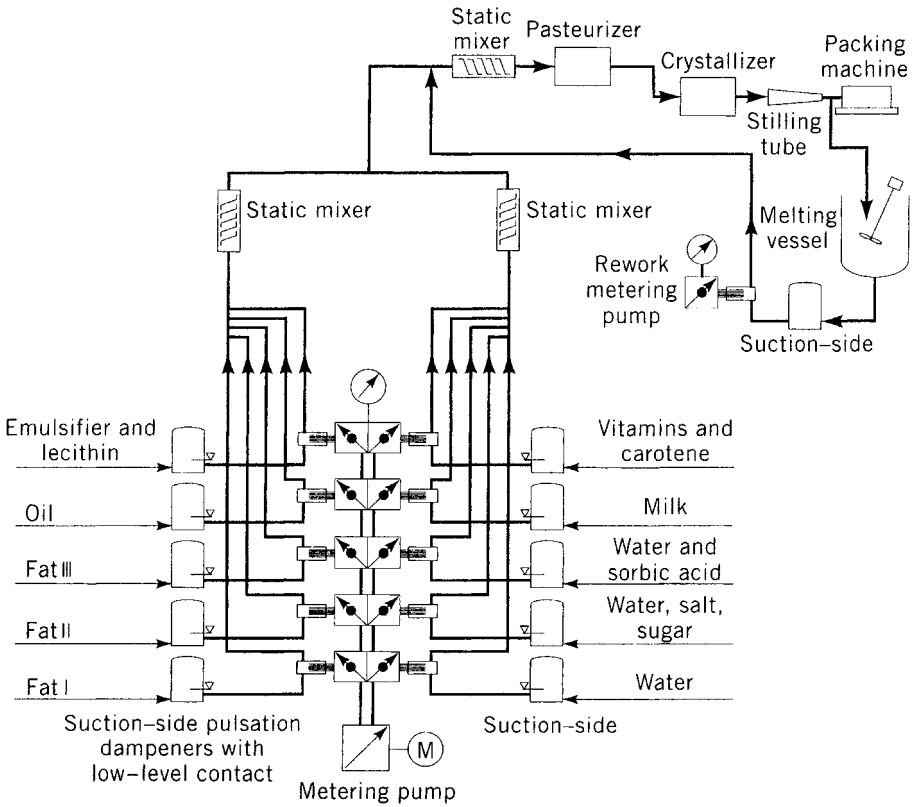


Figure 27. Multipumphead metering system (74). Courtesy of Food Engineering.

Accumulating excess margarine from the packaging operation can be returned to the main fresh emulsion flow by a separate rework metering pump as shown in Figure 27. This helps guarantee a uniform quality in the final product (78), as well as minimizes waste.

Adjustment of the multipumphead metering system according to the recipes to be produced and other required functions can be done automatically and integrated into a control system based on the use of a PLC. The control system can be connected to a possible main computer system in the margarine plant, allowing for registration of process parameters and other statistical information used in production control (81–83).

The described principles of a multipumphead metering system have been reported to offer several advantages in the margarine emulsion preparation (78). These are

*Dosing accuracy of ±0.1%.* Accurate dosing of raw materials can save margarine producers significantly in the cost of ingredients.

*Improved hygiene.* The totally enclosed system keeps the product safe from contamination and permits easy cleaning and disinfection.

*Single system convenience.* All elements for pumping, metering, mixing, and controlling mounted within one unit result in minimal floor space requirement and if layout is appropriate, allows easy maintenance.

*Consistent quality and composition of the emulsion.*

## 4.2. Scale Tank System

Scale tank systems or automatic batching systems are used in the margarine industry in order to meet today's requirements with regard to automation, accuracy, labor cost reduction, productivity, and inventory control (75, 82).

Automation in today's margarine industry means that all actions needed to operate the process with optimal efficiency are ordered by a control system on the basis of instructions that have been fed into the control system in the form of a control program.

In an automated process the computer-based control system continuously communicates with every controlled component and transmitter. The control system monitors and controls the process through signals received and sent covering areas such as (84):

- Actuation of components in the process through output (command) signals
- Input (feedback) signals from valves and motors informing the control system that the component in question has been actuated
- Input (analog) signals from transmitters covering temperature, pressure, and other parameters that provide information on the actual status of process variables
- Input signals from monitoring transmitters in the system that report when a given condition has been attained. Such conditions could be maximum or minimum level in a process tank, preset maximum temperatures, etc.

The logic unit of the control system processes the signals for optimal process control, which means that product losses and consumption of service media and energy are kept at an absolute minimum.

The automated control system has the following control tasks (84):

- On/off or digital control
- Analog control
- Monitoring
- Reporting

These control tasks cover areas such as controlling start/stop of motors, opening and closing of valves, agitation start/stop, pasteurization control, selection of product routes and filling valves, control of pumping capacities and weighing systems for formulation and blending, registration of fault conditions, interlocking of functions and various process sections, self-diagnostic fault finding, data logging,

materials consumption and inventory reporting, maintenance in relation to equipment operational hours, optimization of process in relation records of energy consumption, quality assurance, and total plant supervision (84).

The possibilities for automation are quite extensive. For each margarine production plant different levels of automation may be required or possible. The automation level for a plant is decided and planned according to factors such as (84):

- Selected or installed process equipment and its affect on automation level
- Requirements with regard to level of operator interactions and labor
- Required degree of reporting within the plant in relation to quality control, inventory control, and accounting

Examples of automation in margarine production have been reported (76–78). Automation based on the use of scale tanks for automatic batching has been reported in detail for a U.S.-based plant for production of margarine and blends containing butter (77).

Oils required for the margarine production in the described plant may arrive by railroad tank car or road tank truck and are unloaded by connecting the vessel's discharge system to the receiving pump of the plant. A sanitary flowmeter registers the amount of product received and transmits this information to the processing computer for inventory control. Storage tanks for the received oils are normally of the stainless steel silo type. The tanks are equipped with both heating and cooling controls for maintaining a constant oil temperature and are flooded with nitrogen to prevent oxidation of the oils. Oils are pumped from the storage tanks to the batching system in hot-water heated jacketed pipelines to keep the oils from solidifying (77). Oil storage tanks could be mounted with level controls capable of reporting the oil level in each storage tank to the processing computer. In this way the computer can monitor whether the oil level in a storage tank is large enough to meet the batch requirements.

Milk required for the production is received in a similar manner and pasteurized before storage in a refrigerated tank until required for batching. A portion of the milk may be used for combining with salt for brine milk.

Minor ingredients such as sodium benzoate, potassium sorbate, citric acid, cream, emulsifier, and butter are stored in individual, stainless steel tanks. Each of these ingredients are weighed, during the batch formulation, in a smaller stainless steel tank suspended from an electronic loadcell (77). Microingredients such as vitamin A, vitamin D, carotene, color, and flavor are also stored in stainless steel tanks and enter the system through piston-type metering pumps. The batching system consists of two larger stainless steel tanks suspended from an electronic loadcell and are used for weighing the oils and the milk ingredients.

Through a keyboard, the computer operator can enter the formulas and number of batches required for the production each day. The computer can hold numerous formulas. A sequential weighing of each ingredient designated by the formula used is started by computer command. The ingredients weighed are discharged into one

of two blending tanks after which the microingredients are metered into the blend tank. At this stage the computer control system automatically commences a new weighing cycle. The prepared batch in the blending tank is transferred to a surge tank before transfer to the balance tank feeding the SSHE lines (77).

The computer control system is capable of displaying the formula of the batch, desired weights for each ingredient being batched, and total weight of the entire batch. Blend tank status, ingredient tank status, overweight or underweight conditions, and batch tank status are monitored by the control system, which will automatically alert the operator should a fault condition occur.

The described automated batching system offers important advantages with regard to data processing and hard-copy printing of the results of the production day. These are (77):

- Summary report of the amount of each ingredient weighed
- Summary of the amount of formulas run
- Inventory of ingredients remaining in storage in various tanks
- Data transfer to main computer for accounting purposes

Automated batching systems using scale tanks in margarine production offer a good solution toward higher productivity, better inventory control, accuracy in formulation, reduced labor requirement, and a consistent product.

### 4.3. Flowmeter System

A flowmeter-based system is an alternative to the metering pumps system and the scale tank system in the emulsion preparation. Flowmeter-based systems can also be automated through computer control covering automatic start/stop of feed pumps, opening/closing of valves, registration of raw materials consumption, etc.

Flowmeter-based systems are used quite commonly in the margarine emulsion preparation (80). These systems are a good alternative in margarine plants where only a minimal degree of automation is desired due to the lower labor costs and local requirements. Figure 28 illustrates a margarine plant using flowmeters for metering the ingredients for the margarine emulsion preparation.

Batch controllers for each flowmeter are mounted in the main control panel. The emulsion preparation cycle begins when the operator enters the desired quantities of each oil type into the batch controller for the oil flowmeter. The operator selects the proper outlet valve of one of the oil storage tanks, selects the proper feed pump, and activates the batch controller. The selected outlet valve will then open automatically and the selected feed pump will start automatically. The preselected oil quantity is metered into the emulsion preparation tank. When the desired quantity has been metered, the batch controller automatically activates the closing valve downstream of the flowmeter, stops the pump, and closes the outlet valve. The operator now selects the outlet valve and feed pump for the second oil type through a switch system, reactivates the batch controller, and the described sequence is repeated.



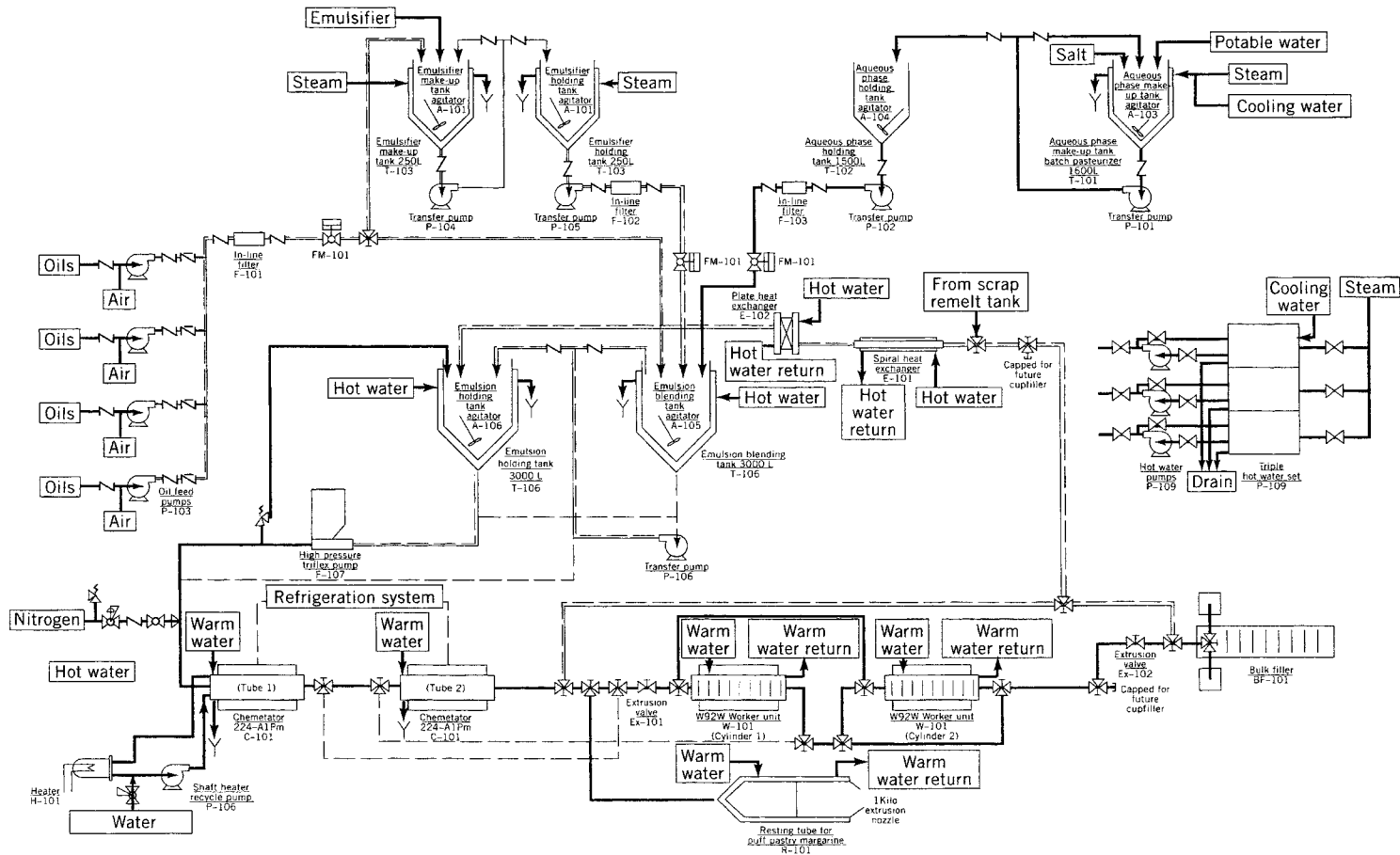
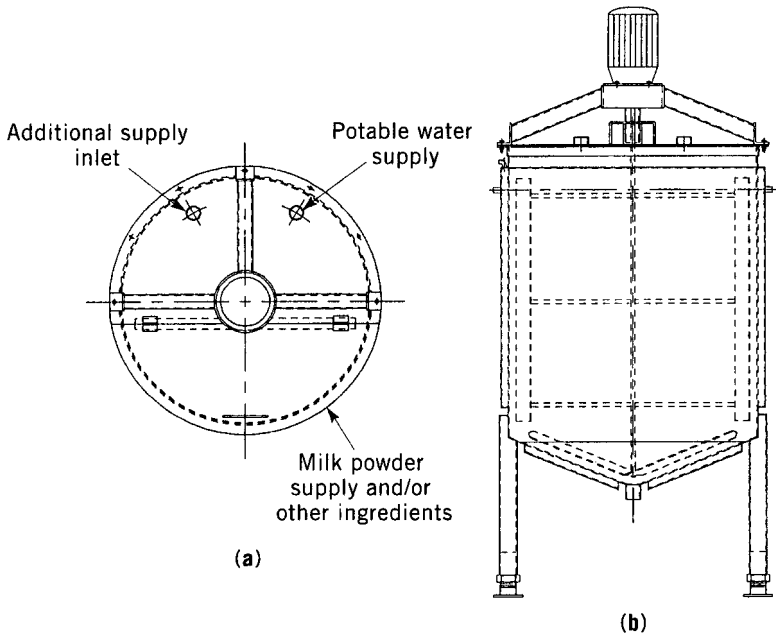


Figure 28. Flowmeter-based emulsion preparation. Courtesy of Crown Chemtech U.S.A., a division of Crown Iron Works Co., Minneapolis, Minnesota.

Individual oil feed lines and flowmeters for each oil type can be installed for optimal accuracy. When metering of all oil types for the oil blend is completed, the operator enters the desired quantity of emulsifier solution into a second batch controller. The sequence is repeated, but this time for metering the emulsifier solution, which has been pre-prepared in designated tanks. The same sequence is finally repeated for the prepared water–milk phase through a third batch controller and flowmeter after a proper period of time, allowing sufficient mixing of the oil blend and emulsifier solution in the blending tank.

The water–milk phase preparation system in Figure 28 is based on the use of a batch mixing and pasteurization tank. A defined quantity of water is added to the batching tank. Milk powder is added to the tank and mixed with the water during heating. The tank is equipped with a special agitator designed to prevent burning of protein on the tank wall. Heating and cooling of the prepared batch takes place in the tank by steam heating of the jacket of the tank. When the desired temperature of 75–78°C (167–172°F) has been reached, heating is stopped and cooling is commenced by circulating chilled water through the heating/cooling jacket of the tank. Figure 29 illustrates the described batch mixing and pasteurization tank.

The pasteurized batch is transferred to a holding tank for use in the emulsion preparation. The process of mixing and pasteurization of a batch takes less than 2 h (84). The water–milk phase can alternatively be prepared in a mixing tank and pasteurized using a modern type of multisection plate pasteurizer. The prepared



**Figure 29.** Batch mixing and pasteurization tank. Courtesy of Crown Chemtech U.S.A., a division of Crown Iron Works Co., Minneapolis, Minnesota.

water–milk phase is pumped from the mixing tank to the plate pasteurizer, where the product undergoes successive stages of treatment such as preheating, heating to 75–78°C (167–172°F), holding at that temperature for 15–20 s, cooling, and chilling in a continuous flow. The preheating and cooling stages are combined in a regenerative section where the outgoing pasteurized product gives up its heat to the incoming product. This greatly reduces the thermal energy demand (84).

Figure 30 illustrates a possible layout of the equipment of the margarine processing line shown in the flow diagram in Figure 28.

Pasteurization of the water–milk phase is a very important process. The pasteurization kills microorganisms that cause disease. If infections occur, the reason is either that heat treatment has not been properly performed or that the water–milk phase has been reinfected after pasteurization (84). Due to this it is important to monitor the pasteurization process carefully in order to make sure that the water–milk phase is treated in the prescribed manner. Proper storage conditions for the pasteurized batch before use in the emulsion preparation are also important. Pasteurization of the complete margarine emulsion as shown in Figure 27 is often done to minimize the risk of reinfection and to ensure the best possible storage properties of the finished margarine product.

Thorough cleaning and disinfection of the equipment are essential parts of margarine operations to ensure optimal hygienic conditions. Combined with proper processing such as pasteurization, proper cleaning procedures help to ensure optimal product shelf life.

Extensive development has and is taking place in the area of cleaning and disinfection techniques. A wide range of detergents and disinfectants is available today, complicating the choice of suitable cleaning agents for particular food processing operation. Economic pressures have speeded up the mechanization and automation of the cleaning operations.

The degree of cleanness can be defined by the following terms (84):

**Physical cleanness:** removal of all visible dirt from the cleaned surfaces.

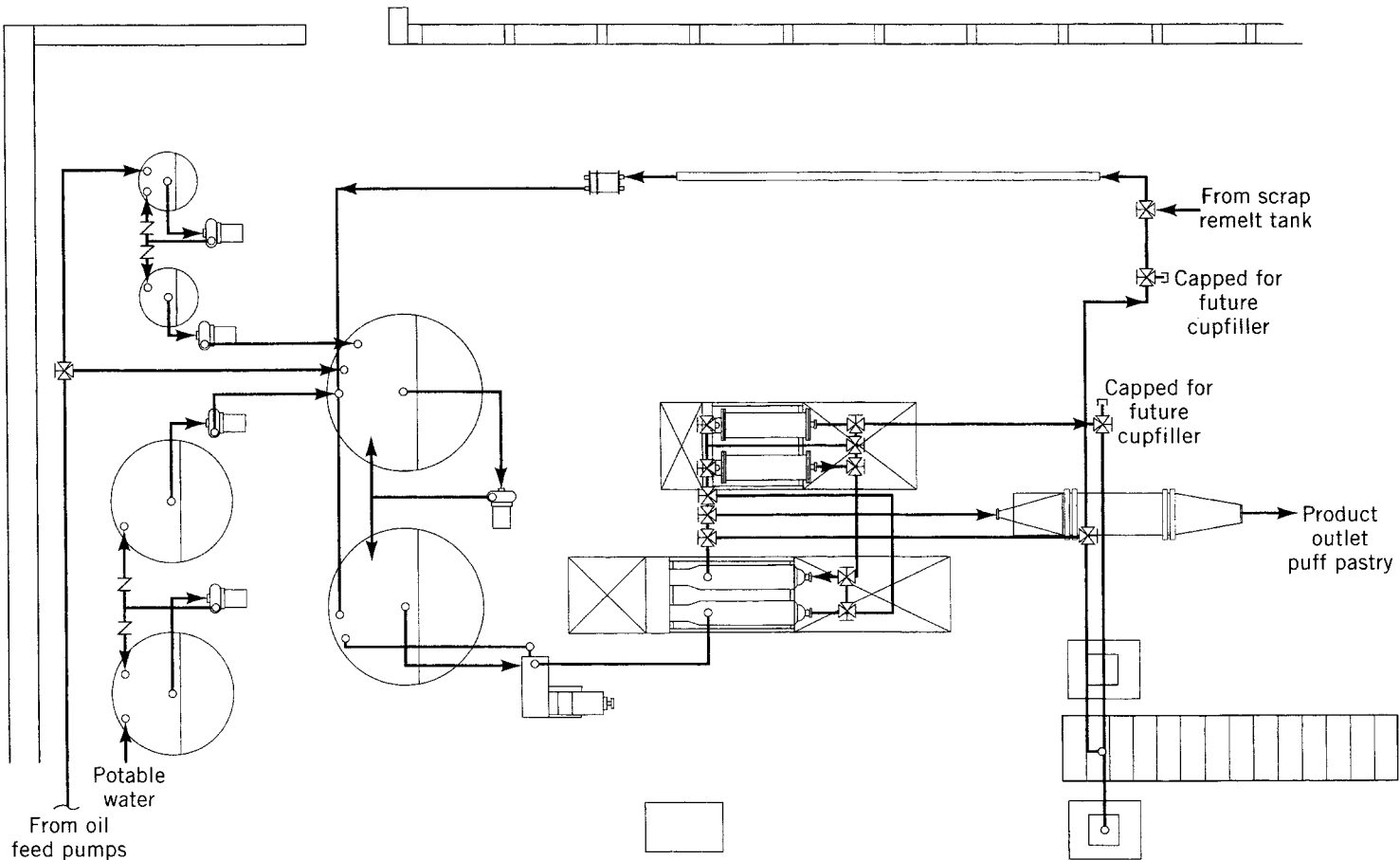
**Chemical cleanness:** removal of all visible dirt as well as microscopic residues, which can be detected by taste or smell but are not visible to the naked eye.

**Bacteriological cleanness:** obtained by disinfection that kills all pathogenic bacteria and most, but not all, other bacteria.

**Sterility:** destruction of all microorganisms.

Even today, some items of equipment in the margarine production can be found not to be designed for easy cleaning and draining. Tanks with flat bottoms and inadequate drainage points can be found. Pipes are found with unnecessary bends, blank ends, and unsatisfactory valves. Such installations are very difficult to clean and could lead to the buildup of stagnant products.

During the design and erection phase of new plants, full consideration should be given to problems of cleaning. Cleaning operations must be performed strictly according to a carefully planned procedure in order to achieve the required degree



**Figure 30.** Layout of a margarine processing line. Courtesy of Crown Chemtech U.S.A., a division of Crown Iron Works Co., Minneapolis, Minnesota.

of cleanness. The cleaning cycle in a margarine operation usually comprises the following steps (84, 85):

Removal of residual fat and milk solids in the plant by means of drainage and forcing product out with water or compressed air.

Preliminary wash with warm water about 49°C (120°F) for loosening fat and milk solids adhering to the sides of the equipment.

Cleaning with alkaline detergent solution at 60–70°C (140–158°F) for approximately 30 min to remove all traces of fat, milk solids, and other residues from the interior of the production line. All blank ends and valves not suitable for CIP should be removed and washed by hand.

Postrinsing with clean, warm water to remove the last traces of detergent.

Disinfection by means of heating with steam or hot water, alternatively disinfecting with chemical agents such as chlorine and other halogen compounds, benzoic acid washing, or quaternary ammonium salts. In the latter case, the cycle is concluded with a final rinse.

Cleaning in place (CIP) can be defined as circulation of cleaning liquids through machines and other equipment in a cleaning circuit (84). This method of cleaning has replaced the older practice of stripping down valves and other difficult to clean equipment in many margarine factories. The CIP method is essentially the same as the method described above (85).

The passage of the high-velocity flow of liquids over the equipment surfaces generates a mechanical scouring effect that dislodges dirt deposits. This only applies to the flow in pipes, heat exchangers, pumps and valves, etc. The usual technique for cleaning of tanks is to spray the detergent on the upper surfaces and allow it to run down the walls. The mechanical scouring effect is often insufficient but can to some extent be improved by the use of specially designed spray nozzles or cleaning turbines. Tank cleaning requires large volumes of detergent that must be circulated rapidly (84).

#### **4.4. Storage of Finished Product**

Storage conditions play quite an important role for the overall quality of margarine products. Insufficient or improper storage conditions can lead to several product failures such as sandiness or graininess, oiling out, lack of plasticity, brittleness, or microbiological spoilage for sensitive product types (86).

Margarines are usually stored in palletized cartons or boxes in refrigerated storage rooms built with insulated walls and insulated ceiling for optimal energy utilization. The margarine pallets are usually placed individually in a rack system to allow for proper air circulation around each pallet. During the initial period of storage, the temperature change in the product is not uniform across the pallet load. The cartons or boxes on the outer layers reach storage temperatures well before those in the middle of the pallet (52). This could lead to differences in product structure depending on whether the product is located in the outer layer or in the middle of the pallet.

Recently, this problem has been addressed by a very simple solution. Specially designed spacers are inserted between each layer of cartons on the pallet. The airflow is in this way facilitated throughout the pallet, and heat exchange between the product and the environment is achieved more efficiently. The spacers are designed in such a way that they enable the air to circulate as it flows, thereby ensuring that temperature stabilization is carried out quickly. Systems for inserting and retrieving the spacers have been developed. Spacers can be inserted or removed in less than 1 min and do not increase the height of pallets significantly as they are only approximately 20 mm in thickness each (52).

Retail margarines are usually stored at 5–10°C (40–50°F) at the point of manufacture for 1–2 days before shipment, so that the crystal structure can become fully developed and stabilized. With lower melting point fats now used in most margarines, especially in polyunsaturated table margarines and low-fat spreads, and also because of the water present, most margarines today require that the refrigeration is maintained throughout the distribution chain and in the consumer's home (45, 86–88). Specialty margarines such as puff pastry margarine should be stored 2–4 days at 12–16°C (54–61°F) to allow time to stabilize the special texture and plasticity desired prior to dispatch or cold storage (89) (see Section 5.2).

Studies of the effect of storage conditions on quality of retail margarines, such as polyunsaturated margarines, have tended to focus on the changes in physical and chemical properties that occur during storage. The effect of storage on the sensory properties of the product also has great importance to the manufacturer, distributor, and the consumer (88).

Storage conditions affect sensory properties such as color, flavor, texture, and general acceptability (88). Sensory values for these properties decline with storage time. For polyunsaturated retail margarine it has been found that storage at 5°C (41°F), alternatively 10°C (50°F), did not result in significant differences in the product with regard to color and texture. Product stored at 5°C (41°F) exhibited significantly better flavor results than product stored at 10°C (50°F). High-quality shelf life of polyunsaturated retail margarine is seen to be approximately 8 months when stored at a constant 5°C (41°F), 6 months at 10°C (50°F) (88).

Low-fat spreads with 40% fat content and containing protein usually have a shelf life of 8–10 weeks and water-based low-fat spreads of about 4 months based on storage at temperatures below 10°C (50°F) (90) (see Section 5.1). Very low fat spreads with fat contents below 20% and with a water continuous emulsion character require low pH, ultra high temperature processing, and possibly aseptic filling procedures to allow closed shelf lives comparable to conventional low-fat spreads (91).

## **5. PROCESSING OF LOW-FAT SPREADS, PUFF PASTRY MARGARINE, AND PUFF PASTRY BUTTER**

Low-fat spreads, puff pastry margarine, and puff pastry butter are all very interesting products from an equipment and processing point of view as they require processing techniques that are quite different from those used in the processing of conventional retail margarine.

## 5.1. Low-Fat Spreads

*Introduction.* Under the influence of official dietary recommendations, product pricing structure, and evolving consumer lifestyles, low-fat spreads have progressed during the last decade from being food alternatives to butter and margarine to the present standing of a product in its own right. This market trend toward reduced fat consumption has led to a significant reduction in the consumption of butter both on the U.S. and the European Community (EC) markets (91–95).

Margarine consumption has remained fairly steady with a slight upward trend in the EC market lately (approximately +1% per annum) (93), whereas the U.S. market from 1991 to 1992 showed an overall reduction of 2.2% even though the consumption of low-fat margarine and spreads showed an increase of 49.8% (96).

Low-fat spreads were first introduced in the market in Great Britain in 1968 and have a significant market share today of approximately 26% in Great Britain (93). The production of low-fat spreads is traditionally complex and there are many variations on the same theme as the technology becomes more advanced. Low-fat spreads are inherently unstable, since the bulk of the product comprises water-soluble ingredients, while an acceptable texture is normally only achieved with a water-in-oil emulsion. Therefore, the tendency of the emulsion will be to become oil in water, and once this occurs the reaction is invariably irreversible, resulting in high wastage. Additionally, if the emulsion is unstable, although the product may not be fully “reversed,” the texture will be open and coarse and unacceptable (90).

In the yellow spreads market, oil-in-water spreads have recently been introduced and are relatively new. One drawback for these products is their stringent requirements for ultra high temperature processing and aseptic filling to achieve acceptable shelf lives. Low-fat spreads (40% fat) containing protein usually have a shelf life of 8–10 weeks and water-based low-fat spreads of about 4 months based on storage at temperatures below 10°C (50°F) (90).

Table 3 illustrates low-fat spreads available with fat contents ranging from 60% to as little as 5%. Below about 20% fat content products of a water continuous emulsion character are prevalent (91).

**TABLE 3. Some Low-Fat Spreads.**

Low-Fat Spreads	Approximate Composition (% Fat)
Vegetable fat spreads	60
	40
Vegetable/butterfat blended spreads	40
Butterfat spreads	40
Very low fat spreads	20–30
Water continuous spreads	15
	9
	5

Adapted from Ref. (91), with permission.

*Formulation.* Several patents have been issued covering low-fat spreads formulation and processing indicating that critical process control and/or significant levels of water binding agents are required (91, 92, 97, 98).

From a formulation point of view, low-fat spreads can be grouped as follows:

Without protein and without stabilizer added

Without protein but with stabilizer added

With low protein level and with stabilizer added

With high protein level and with stabilizer added

With low protein level and with stabilizer and thickener (fat replacer) added

To further illustrate and summarize the complexity of low-fat spreads formulation and possible ingredients to be used, a typical formulation of a 40% fat content low-fat spread is shown in Table 4 of functional properties of possible ingredients in

**TABLE 4. Low-Fat Spread at 40% Fat—Typical Formulation.**

Component	Ingredients	%
Oil blend	Hydrogenated vegetable oil Vegetable oil	37–40
Emulsifier	Mono and diglycerides Lecithin Polyglycerol ester	0.25–1.0
Color	Beta carotene including vitamins A and D Annatto	0.001–0.005
Flavor	Butter extract Organic acids Ketones Esters	100–200 ppm
Stabilizer	Maltodextrin Gelatin Modified starch Sodium alginate	1–3
Preservative	Potassium sorbate Sorbic acid	0.1–0.3
Water with protein source	Buttermilk Skim milk Whey Caseinate Soy	50–60
Salt	Salt	1–2
Starter culture	<i>S. Cremoris</i> <i>S. Diacetylactis</i> <i>S. Leuconostoc</i>	Trace
Sodium-hydroxide	—	0.1
Sodium-hydrogen	Acid regulator	0.1–0.4
Trisodium-citrate	Acid regulator Buffer	0.1–0.4



low fat spreads formulation. Table 5 indicates a summary of recipes for various types of low-fat spreads.

*Processing.* Low-fat water-in-oil emulsions with fat contents of 40% or lower have been found to be quite sensitive to line pressures and cooling rate in the SSHE line. Fill temperatures are higher than with corresponding 50% fat products because the emulsion is more viscous. If fill temperature is too low, the product will build up in the tub with excessive lid contact causing crumbly product and water leakage. If too much crystallization occurs in the process, the shearing forces of processing and filling may break the emulsion. Therefore, low-fat products are more easily prepared by use of high liquid oil content and low solid fat index (SFI) blends. The higher liquid oil content improves the emulsion stability by more adequately separating the increased number of aqueous-phase droplets. Careful blend selection and processing ensures that quite butterlike textures can still be produced. In the case of low-fat butter, the production is more difficult due to higher SFI values for butter oil at lower temperatures.

Low-fat butter or dairy spreads can also be produced from an oil-in-water dairy cream or premixed cream with a fat content adjusted to the desired percentage in the low-fat dairy spread using phase inversion. For product stability reasons, emulsifier (approximately 1% distilled monoglyceride) and stabilizer (hydrocolloids such as gelatin or sodium alginate) are added in smaller quantities to the prepared cream. This is necessary to prevent free water in the finished stored product. Minor ingredients, such as flavor and color, can also be added. The cream is prepared during controlled agitation and temperature and passed through the SSHE line at a rate of 40–50% of normal capacity. High SSHE (A unit) shaft speeds as well as increased shaft speeds in the required worker unit (B unit) are preferred to achieve phase inversion. Constant flow rate and exact temperature control are necessary for proper phase reversion, crystallization, and working of the product (90–92, 99).

In general, vegetable-oil-based and butter-oil-based low-fat spreads as well as blended low-fat spreads containing both vegetable oil and butter oil can be produced continuously. This is achieved by crystallizing a batchwise or continuously prepared water-in-oil emulsion in an SSHE process line.

The process line for this purpose is normally especially designed to ensure crystallization and texturization of the product to take place under controlled conditions and within the processing equipment. The manufacture under high degree of agitation with minimal shear precedes the processing of the emulsion, providing an emulsion of the correct phase (water–oil) and water droplet size.

The flow diagram in Figure 31 shows such a process line using SSHEs for pasteurization of the prepared water-in-oil emulsion, crystallization of the emulsion, and reworking of the crystallized emulsion.

Typically, the bulk liquid oils are transferred from the storage facility to the emulsion mixing vessel at 55–60°C (131–140°F). Oil-soluble ingredients, such as emulsifier, color, and flavor, are added in a separate vessel to minimize their storage time at elevated temperatures. Emulsifiers are used to lower the surface tension between the water and oil phases, thereby stabilizing the liquid emulsion before

**TABLE 5. Basic Formulations for Reduced-Fat Spreads.**

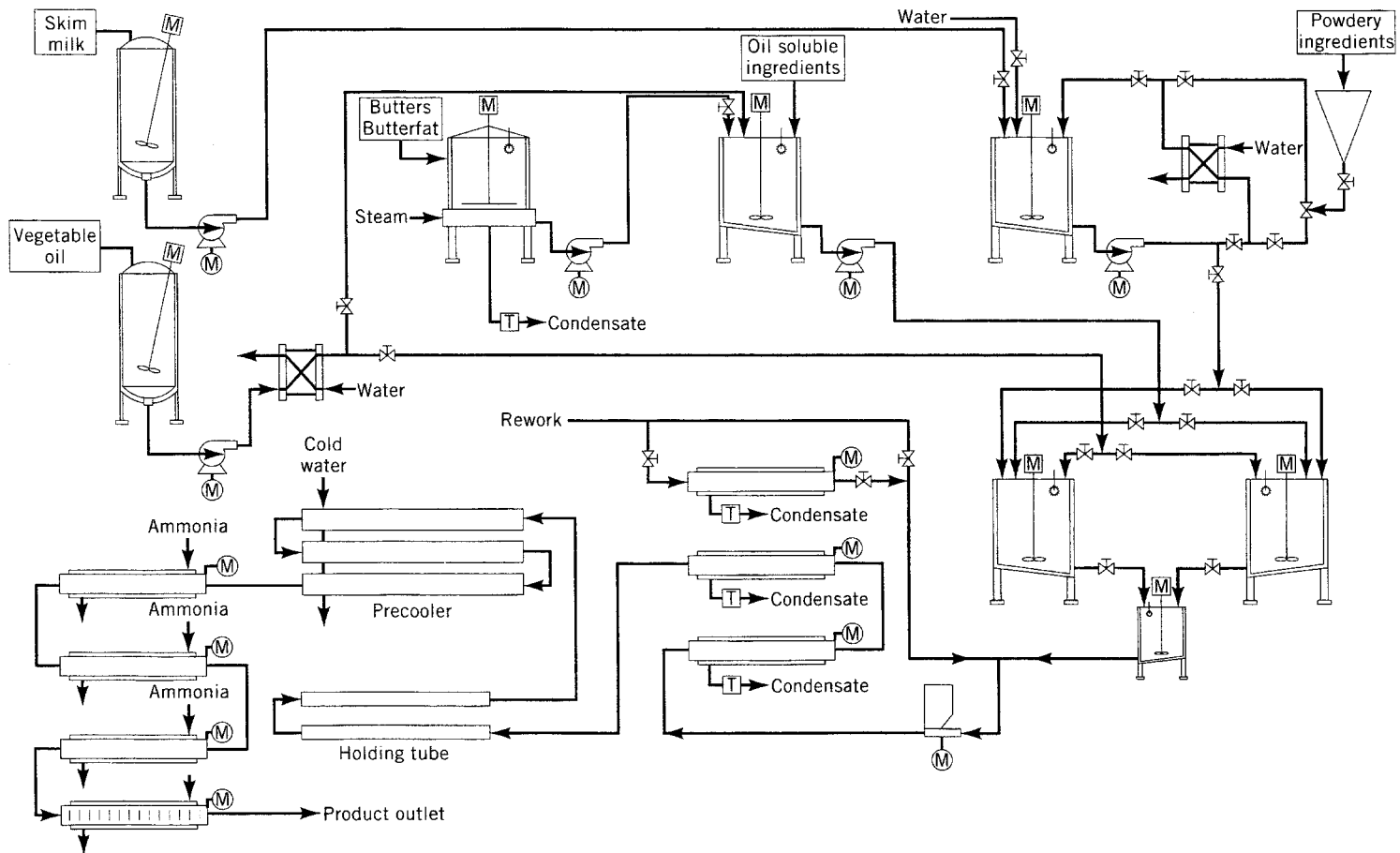
Ingredient	Product Type (Fat Content)						
	60%	40% Water Only	40% Water Plus Stabilizer	40% Low Protein Level	40% Higher Protein Level	20% Based on EPO42031 5A2	10% Oil in Water Based on EPO29856 1A2
Composition, % Fat <sup>a</sup>	59.5	39.5	39.5	39.5	39.5 <sup>b</sup>	19.6	10.0
Emulsifier (distilled monoglycerides)	0.4 (IV 55)	0.6 (IV 80)	0.6 (IV 80)	0.5 (IV 55)	0.6 (IV 55)	0.4 (IV 55)	—
Lecithin	0.1	0.1	0.1	—	—	0.1	—
Beta carotene, ppm	4	3	3	3	4	5	5
Flavor/vitamins, %	0.02	0.01	0.01	0.01	0.01	0.01	0.01
Water (salt) (adjust to pH 4.8–6.2 with lactic acid if required)	39.0	59.8	59.3	57.4	51.7	69.7	86.3
Gelatin	—	—	—	1.5	2.0	5.0	3.0
Thickener	—	—	0.5	—	—	3.5 <sup>c</sup>	9.0 <sup>c</sup>
Skim milk powder	1.0	—	—	1.0	—	—	—
Sodium caseinate	—	—	—	—	6.0	1.5	0.5
Potassium sorbate	—	—	—	0.1	0.1	0.1	0.1
Flavor	—	0.01	0.01	0.02	0.1	0.1	0.1

<sup>a</sup> Typically liquid 76 hard fraction 24 (slip point 42–44°C), i.e., palm stearin.

<sup>b</sup>Can also contain butteroil.

<sup>c</sup>Starch based.

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**Figure 31.** Schematic diagram of SSHE process line for production of low-fat spreads. Courtesy of Crown Chemtech U.S.A., a division of Crown Iron Works Co., Minneapolis, Minnesota.

crystallization takes place. This is necessary to ensure a homogeneous product and a fine dispersion of the aqueous phase. The use of emulsifiers gives greater numbers of smaller water droplets in the product, resulting in a light texture and good flavor release.

Milk proteins and soy lecithin can also affect the water droplet size. Proteins and lecithin tend to increase the drop size (91, 100).

The aqueous phase is prepared in a separate vessel and would typically comprise skimmed milk, whey, or water. Salt and various acidity regulators are added to the water phase along with an adjustment of the acidity. Finally, a bulking agent is added to yield the optimum viscosity for a particular formulation.

The influence of the viscosity and functionality of the aqueous phase on emulsion stability, spreading, and eating characteristics of the product are significant. In high-protein low-fat spreads, the protein's function is to create a three-dimensional network responsible for immobilizing the water (94). The functional properties for a given protein are greatly influenced by the environment (i.e., other ingredients such as stabilizers) in which the protein is present during the emulsification process (101).

The heated aqueous phase is added to the oil phase under controlled conditions creating a good-quality water-in-oil emulsion. Critical parameters at this stage include the temperature of the two phases, water phase viscosity and functionality, addition rate, and type and speed of mixing.

The prepared emulsion is fed via a balance tank to a high-pressure pump, usually of a piston variety to a series of in-line SSHEs. Once in the pasteurizer heating cylinders, the product is pasteurized and held prior to being subjected to precooling and prepared for crystallization. Cooling, stabilizing, and texturizing of the emulsion are continuously undertaken within a series of A and B units.

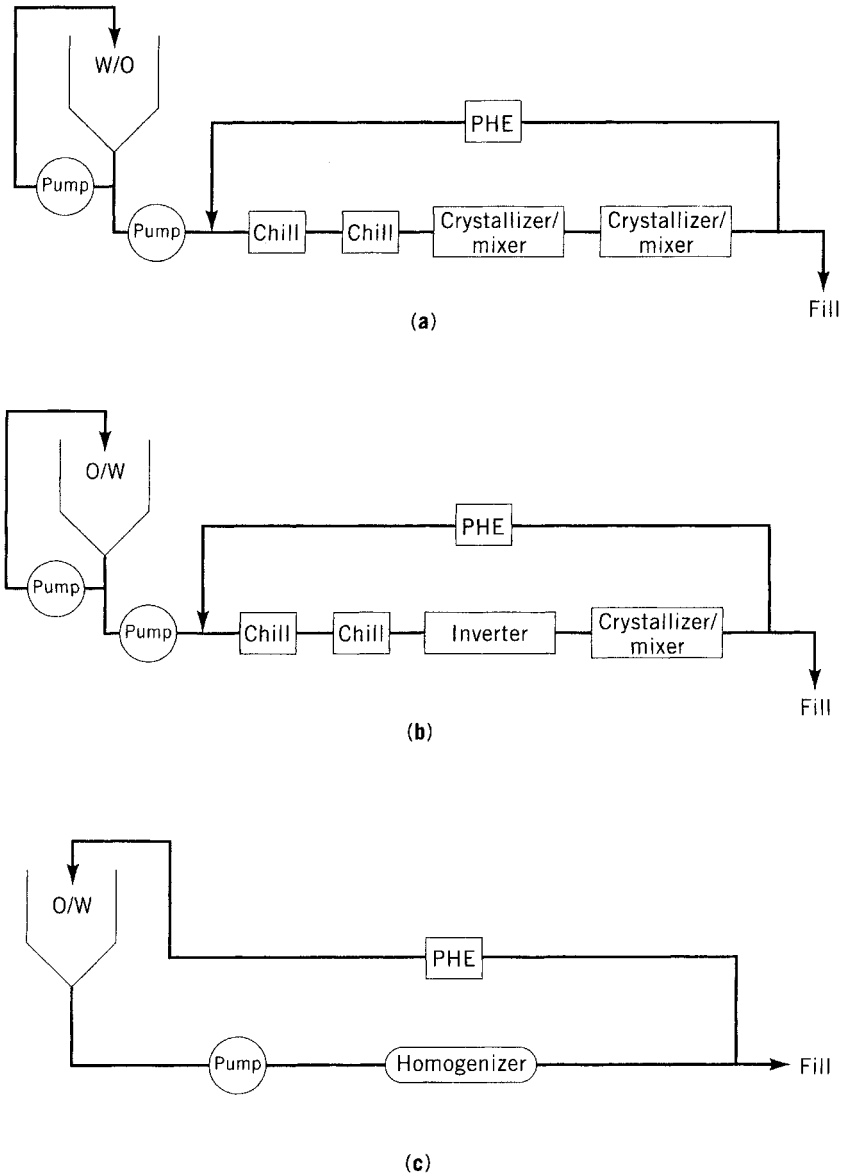
The emulsion is rapidly supercooled with vigorous agitation by the scraping and blending action of the knife blades of the A unit. During the passage through the A unit, a thin film of crystallized emulsion is continuously scraped off the walls of the cooling cylinders and mixed with warmer emulsion. The water droplet size is reduced further during this step and the reduction is dependent on emulsion viscosity, shaft speed, and retention time. The process continues until the emulsion leaves the last cylinder and enters a worker unit for final texturization. Due to the presence of higher amounts of solidified fat in the product during its passage through the worker unit, water droplets can recombine during this process step. Typical process conditions (25–40% fat) would be as follows (90): aqueous phase temperature 45°C (113°F), oil phase temperature 60°C (140°F), emulsion temperature 52°C (125.6°F), pasteurization temperature 85°C (185°F) for 15 s, precool temperature 40°C (104°F), final cooling temperature 12°C (57.6°F), temperature at filler 16°C (60.8°F).

Ammonia/Freon evaporation temperatures would vary depending on throughput.

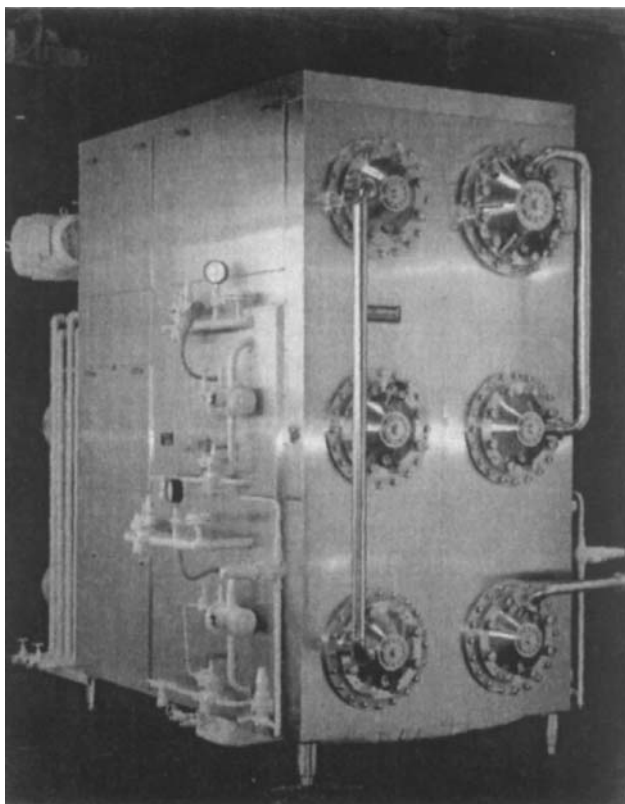
For stick wrapping, the produced product passes to a resting tube connected directly to the stick wrapping machine. When the product is filled into tubs, it is conveyed directly from the after-treatment worker cylinder to the filling machine.

Excess product from the packaging operation is continuously remelted in a rework SSHE in a controlled manner and returned to the system via the balance tank or a positive pump facility for adding reclaimed material.

Figure 32 illustrates and summarizes the basic process lines used for the production of different types of low-fat spreads.



**Figure 32.** Basic process line for low-fat spreads (91). (a) Conventional processing; (b) inversion processing; (c) method of oil in water spreads.



**Figure 33.** Scraped-surface heat exchanger. Courtesy of Schroeder & Co., Luebeck, Germany.

Figure 33 shows an SSHE with four cooling cylinders, one pin worker, and one inversion crystallizer mounted for production of low-fat spreads using the inversion technique.

## 5.2. Puff Pastry Margarine

*Introduction.* Puff pastry is quite different from other margarine types in its properties and especially its plasticity (102). The plasticity of puff pastry margarine is essential for preparation of puff pastry of high quality. Puff pastry is made from very thin layers of dough and margarine, which bake to a light and flaky structure of good volume and uniform appearance. This laminated structure is achieved by a special dough handling procedure, where the dough is folded and rolled together with the puff pastry margarine resulting in a finished puff pastry dough with alternating layers of dough and margarine (87, 102, 103).

The function of the puff pastry margarine is to act as a barrier between the dough layers both during rolling to prevent them from fusing together and to prevent the formation of a three-dimensional structure between the gluten protein in each thin dough layer during baking (104).

The demands on the properties of puff pastry margarine can be summarized as follows (80):

A high degree of plasticity over a wide temperature range.

Sufficient plasticity for stretching rolling in the dough preparation to ensure unbroken homogeneous thin layers of margarine in the dough. This is necessary for the laminated structure and volume of Danish pastry.

The absence of softness or greasiness when worked.

Choice of type of SSHE, shaft and blade design, flow rate and required product retention time, oil blend formulation, as well as process temperature profile along with several other factors have a significant influence on the final quality of any puff pastry margarine (51, 105).

*Formulation.* To ensure the above properties of a puff pastry margarine, the oil blend formulation plays quite an important role. When formulating a suitable oil blend for puff pastry margarine, several factors such as local climatic conditions, temperature and methods used during dough preparation, consumer (baker) preferences, and desired quality of the finished baked Danish pastry must be considered (51). Puff pastry margarine normally has a fat content of 80% and oil blends giving a flat SFI curve are sought. Tallow, lard, shea fat, palm oil and, to a certain degree, hydrogenated fish oil are the most suited fats for production of plastic puff pastry margarine (87, 106).

Palm-oil-based puff pastry margarine, where palm stearin, hydrogenated palm oil, and palm kernel oil are the components of the oilblend, reportedly performs better than tallow-based puff pastry margarine (103). This can be attributed to the fact that it is easier to produce a vegetable-oil-based puff pastry margarine with a good plasticity in an SSHE process line (105). Tallow-based puff pastry margarine produced on the open chill drum system has excellent plasticity.

One hundred percent soybean-oil-based puff pastry margarine cannot be characterized as a typical puff pastry margarine oil blend (105). Soybean-oil-based puff pastry margarine has relatively poor plasticity. Hydrogenated soybean oil in combination with hydrogenated palm oil can give very good baking results as well as a margarine with an excellent texture and plasticity (51). An example of such an oil blend is as follows: hydrogenated soybean oil, 44°C (111.2°F), 40%; hydrogenated soybean oil, 38°C (100.4°F), 20%; hydrogenated palm oil, 42°C (107.6°F), 35%; liquid soybean oil, 5%.

Emulsifiers of the monoglyceride type with or without polyglycerol ester added are usually added to the oil blend of the puff pastry margarine at a level of 1% of the overall recipe of the margarine (106, 107). Emulsifiers influence the crystallization of the margarine both during processing and storage resulting in improved plasticity (106). They also ensure that the emulsion is heat stable during baking. Soy lecithin is usually added at a level of 0.8% to facilitate the emulsifier effect.

Low pH of the water phase of the puff pastry margarine will have a pronounced effect on the lift in the finished pastry. Low-calorie puff pastry margarine with 60% fat content has been reported (94).

*Processing.* Puff pastry margarine with optimal properties has for many years been produced on the open chill drum/vacuum complector system available in the market from Gerstenberg & Agger A/S, Copenhagen, Denmark.

Here a thin layer (less than 1 mm) is applied directly on the surface of the open chill drum. Crystallization takes place during complete rest and during very rapid cooling. Afterward, the margarine flakes are rested in large hoppers for crystallization to progress before separate kneading under vacuum in a complector occurs (80).

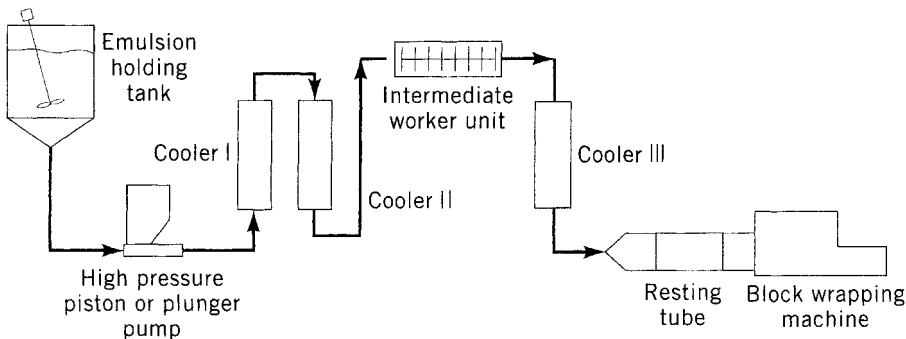
Due to this system's disadvantages in space requirements, labor demand, and hygiene, production of puff pastry margarine in SSHE process lines has developed significantly over the past two decades. Today, the majority of all puff pastry margarine is produced in SSHE lines.

Choice of the required SSHE equipment is very important for the production of high-quality puff pastry margarine. For each piece of equipment in the production line, special design features have to be considered to ensure that the complete production line has all the necessary capabilities for puff pastry margarine. This relates to the previously mentioned required properties of puff pastry margarine. A detailed discussion of required design features in the process line can be found elsewhere in this Chapter (see Section 2.2).

It should be noted that process lines for vegetable-oil-based puff pastry margarine differs somewhat from the process lines for animal-oil-based puff pastry margarine with regard to the equipment sizing and layout (51).

A general flow diagram for a puff pastry margarine processing line can be seen in Figure 34.

Normally, it is recommended to use a process line where the SSHE is equipped with multiple cooling cylinders (80). This is advantageous in the production of puff pastry margarine as successive steps of cooling, working, and cooling of the product promote the development of the consistency and the plasticity desired. The desired properties of puff pastry margarine depend not only on oil blend formulation but also very much on the three-dimensional crystal structure formed during crystallization and storage (105).



**Figure 34.** Schematic diagram for SSHE process line for production of puff pastry margarine.



During the product passage through the multiple cylinders of the SSHE, the product is supercooled and, to a certain degree, crystallized. When mechanical work is applied to the product during the cooling process by the blades of the A unit or by the pins of the intermediate worker unit's (B unit) shaft, two types of crystal structures will appear: a primary and a secondary.

The bindings between the crystals of the secondary structure are weak and even though destroyed by the application of mechanical work, they reestablish themselves very quickly when the mechanical work is eliminated. The bindings in the primary structure are, on the contrary, very strong and when destroyed by mechanical work, they will not reestablish easily. It is widely accepted in the industry that the primary structure has a tendency to be formed if insufficient mechanical work is applied. This leads to products with a brittle and hard texture. A more detailed discussion of crystallization and crystal structures can be found in Section 1.1.

Normally, the retention time in the A-unit alone is not sufficient for crystallization of puff pastry margarine due to the special texture required. For this reason, it is advantageous to insert an intermediate worker unit (B unit) between the cooling cylinders to allow time for the crystallization to progress further during agitation under the absence of cooling (51, 104). Please refer to Figure 34.

To reduce the postcrystallization to a minimum to ensure the development of the proper crystal structure and desired plasticity, it is necessary to prolong the cooling and working of the puff pastry margarine in the SSHE line (105). This is achieved by reducing the capacity of the process line to a level of only 50–60% of the capacity obtainable for regular margarine on the same A-unit.

The crystallization process is normally controlled through a variation of flow rate, refrigerant evaporation temperature, or by changing the layout of the intermediate worker unit and cooling cylinders according to the oil blends used.

Besides the influence of temperature, blending, and time (capacity) on the texture and quality of the puff pastry margarine, the volume and design of the final resting tube (Figure 34) have a significant influence in relation to the oil blends used (51, 80, 105). During the passage through the resting tube, a minor product temperature increase indicating minimal postcrystallization can normally be observed before the product enters the packaging operation.

### 5.3. Puff Pastry Butter

*Introduction.* The flavor of butter and butter fractions is very attractive to the human palate due to their content of very short chain fatty acids. Furthermore, the word *butter* is appreciated by the consumer. Due to this, there has been a growing interest in recent years, especially in Europe, to use butter stearin and other butter fractions in pastries such as Danish pastries and croissants (108, 109). The properties desired in puff pastry butter are similar to those outlined for puff pastry margarine in Section 5.2.

*Formulation.* Oil blend formulation for puff pastry butter requires the same consideration as for puff pastry margarine with regard to usage temperature, solid

**TABLE 6. Possible Oil Blends for Recombined Butters for Various Bakery Applications.**

Oil	Bakery (%)	Croissant (%)	Puff Pastry (%)
Butter oil, drop point 32°C (89.6°F)	20	15	5
Butter stearin, drop point 40°C (104°F)	75	80	95
Soft butter stearin, drop point 24°C (75.2°F)	0	5	0
Butter olein, drop point 18°C (64.4°F)	5	0	0
Total	100	100	100

fat contents, plasticity, emulsifier dosage, and pH of the water phase. Some possible oil blends (110) are shown in Table 6.

*Processing.* Design and layout of the SSHE line as well as the processing conditions basically follows the same pattern as outlined for puff pastry margarine in Section 5.2.

## 6. PRODUCTION CONTROL, QUALITY CONTROL, AND SANITATION

The success of a margarine manufacturer depends on many factors including marketing, productivity, and changes in ingredient costs. The foundation for continued success is the quality of the product itself. To ensure a constant high quality of the product, production and quality control as well as sanitation in the margarine plant are quite important disciplines.

Production control through registration of process parameters, formulation, flow rates, ingredient consumption, and other production variables has been described in Sections 4.1, 4.2, and 4.3. Cleaning and disinfection procedures have been described in Section 4.3.

Quality of raw materials and finished products can be determined through instrumental techniques and ultimately by sensory evaluation. Results from these quality control methods may be compared to minimum standards available either by law or set by the marketplace (111).

### 6.1. Raw Materials

Management of quality requires that the specifications and regularity of properties of mixture are fulfilled. The desired specifications are obtained when formulas are made for raw materials of standard quality. This quality has to be maintained (35). Fats and oils are obviously the raw materials of major importance in a margarine production. When a margarine plant is not integrated with a refinery, increased control of raw materials and stabilization of manufacturing parameters through the creation of specifications, acceptable by many fats and oils suppliers, is important (35).

When a margarine plant is integrated with a refinery, the raw materials characteristics are generally obtained in the refinery. The quality control in the margarine plant will essentially concentrate on the production parameters and on finished product examination (35).

Quality control systems usually used for judging the quality of oils and fats or oil blends used in margarine production could evaluate color, color stability, flavor, flavor stability, free fatty acid, peroxide value, active oxygen method (AOM) stability, iodine value, slip melting point, fatty acid composition, refractive index, crystallization rate, and solid fat/temperature relationship (solid fat index) (5, 91, 112, 113).

Refractive index, iodine value, AOM stability, and peroxide value provide standardized methodology for those factors affecting oxidative stability (5, 113).

Solid fat index, melting points, penetration, and viscosity are normally used to measure factors affecting consistency and texture (5, 35, 112, 113). Color is most frequently measured by the Lovibond procedure (5).

Determination of crystallization rate and solid fat index can be done conveniently using pulsed nuclear magnetic resonance (NMR) techniques (91).

## 6.2. Finished Products

Testing techniques for the evaluation of physical properties and other properties of finished margarine products as well as low-fat spreads have been stated to include (4, 91): appearance, oral melting characteristics, oil exudation, slump (collapse), penetrations, spreadability, emulsion viscosity at 35°C (95°F), emulsion drop size, and electrical conductivity.

Oil exudation results from a reduction in the volume of the fat crystal network over time and is due to the formation of strong primary or secondary bonds. External pressure also is an influencing factor that is particularly relevant with wrapped products stacked at a low point in a pile in storage. Empirical tests include measuring the oil exuded under controlled pressure on absorption into pre-weighed paper (91).

Spreadability can be evaluated by spreading the product in a consistent manner on a suitable surface such as greaseproof paper or cardboard. The results may vary from smooth and homogeneous to very coarse and showing visible water drops (91). In this way hardness, softness, homogeneity, and water stability may be evaluated along with the spreadability (92).

The stability of the water-in-oil emulsion is quite important in low-fat spreads, and electrical conductivity gives a measure of this. Electrical conductivity can be followed during production through suitably designed measuring cells mounted in the process line or be measured directly on product samples in tubs (91).

Light microscopy can give a good impression of the drop size distribution, which is an important characteristic especially for indicating potential microbiological hazards in water-in-oil products (91). A very simple test for judgment of the droplet size distribution in margarine is the use of dyed type of absorbent paper (indicator paper) specially prepared for such purpose (91, 92, 114).

Appearance, color, oral melting characteristics, and flavor quality are factors that can be judged through sensory evaluation by trained panels (4, 35, 91).

### 6.3. Microbiology and Plant Sanitation

Microorganisms are classified into three main groups, depending on their method of reproduction (84, 85).

*Molds.* The category of molds comprises a fairly heterogeneous group of multicelled, threadlike fungi (84). Most molds reproduce by spores of various types. The spores usually have thick walls and are relatively resistant to desiccation and heat (84). When the colonies are fully developed, they become visible to the naked eye and can be described by expressions such as “hairy.” Mold colonies can occur in various colors depending on type of food product (83).

*Yeasts.* They are single-cell organisms of spherical, elliptical, or cylindrical shape (84). These usually reproduce by budding. The yeast cell begins to grow a small bud on the cell wall, which then increases in size until it is the same as the parent cell (84, 85). It then breaks free and the process starts again.

*Bacteria.* This group consists of single-cell organisms that mostly reproduce by binary fission (84, 85). That is, a mature organism starts dividing in the center, resulting in the formation of two identical organisms. Under ideal conditions, this fission can take place every 20 min, so one bacterium held under the optimum conditions would result in many millions of bacteria in 24 h (84).

*Development of Microorganisms.* Microorganisms require certain basic conditions for growth. Temperature is the greatest single factor affecting growth, reproduction, and food deterioration (84). Bacteria can only develop within certain temperature limits that vary from one species to another. The thermal death point for bacteria falls into two classes (84, 85). The first is the simple type of bacteria, which is killed by heating to 70°C (158°F) for 30 min. The second is the bacteria type that forms a special heat-resistant state (spores), which enables the bacteria to withstand adverse conditions. These are killed by steam treatment at 120°C (248°F) for 30 min (84, 85).

The thermal death point for molds and yeasts is heating to 60°C (140°F) for about 30 min (84). Bacteria cannot grow in the absence of moisture (82, 83). Thus, they will not grow in dry oils and fats or in any other form of dry material. Free water, even to the extent of one fourth of 1%, however, is sufficient to permit the growth.

Microorganisms usually require other conditions for growth such as protein, sugar, trace elements, and vitamins (85). Some are very sensitive to, and may be, inhibited by acidic or alkaline conditions; others are not. Salt will destroy some types, while others will grow only in strong salt solutions.

*Microorganisms in Relation to Margarine.* Margarine consists of oils and fats and water that is finely dispersed in the oil blend as well as other ingredients as indicated earlier. Normally, microorganisms cannot grow in fat and oil, which means the microbiological rancidity only appears in the water droplets and on the surface of the margarine (115). The composition of the water

phase, therefore, plays a very important part in the storage quality of the margarine (85).

The growth of bacteria, but not yeast and molds, can be controlled by the combined effects of the salt concentration and the pH (or acidity) of the water phase. A reduced salt concentration requires the margarine to be more acid (lower pH) to give the same measure of protection against the growth of bacteria (85, 115). In practice, margarines can be divided into three groups according to their salt content. These are low salt margarines (0–1% salt), medium-salt margarines (1–2.5% salt), and high-salt margarines (over 2.5% salt).

Nearly all forms of bacteria could survive and possibly grow in low-salt margarines. Due to this, it is important to produce a low-salt margarine with a very low initial total bacteria count. To achieve this, very good cleaning procedures and an overall high plant and equipment hygiene needs to be maintained (85). Furthermore, it is quite important that the water dispersion in the low-salt margarine is as fine as possible as smaller water droplets furnish less nourishment for microorganisms in contaminated water droplets (85, 115). Finally, low-salt margarines should have a pH range of 4.0–5.0 (85).

For medium-salt margarines, the initial total bacteria count should be kept low but a water dispersion that is a little coarser can be allowed. Also, the water phase can have a slightly higher pH of about 5.5 (85).

High salt levels in margarine (over 2.5%) should be combined with a high pH (pH 6) as a low pH in high-salt margarines induces a greater rate of chemical rancidity (oxidation) in the margarine (85, 115).

As the growth of molds and yeast in margarine is not prevented through the combination of salt concentration and pH (acidity), the only protection against the development is the size of the water droplets (85). It is, therefore, important that the correct amount of emulsifying agents is used and that the processing conditions are such that a tight and stable emulsion can be prepared in a controlled manner. Based on the above, it follows that some microorganisms can and will grow either in the emulsion preparation system or the margarine production units. The regular and efficient cleaning of the plant is, therefore, of the highest importance.

The previously mentioned thermal death point of most microorganisms is about 60–70°C (140–158°F). The thermal death point is the temperature at which the organisms, when heated in a water solution will die (85). The presence of fat and milk solids will protect them from the effect of heat, and they can, therefore, withstand far higher temperatures. Pasteurization of the water phase or the liquid emulsion improves the microbiological keeping properties. After pasteurization, care should naturally be taken to ensure that the emulsion of the margarine is not exposed to contamination (115).

It is vitally important that people working in a margarine plant observe cleanliness and the elementary rules of hygiene such as thoroughly washing their hands before starting to work and after visiting the restrooms, paying immediate attention to cuts and other wounds, never working with a dirty or loose bandage or with an open wound, and never touching any foodstuffs more than is absolutely necessary.

People known to suffer from gastroenteritis should be removed from sensitive areas of the production line (85).

#### **6.4. Margarine Production Building Facilities**

During the design of plants and buildings, consideration should be given to Good Manufacturing Practices (GMPs as defined by Title 21, Code Federal Regulation, Part 110) for microbiological control and ease of cleaning. This would allow the ideal condition for margarine production but, in practice, margarine production in some cases still takes place in buildings with exposed beams, for example, which act as dust and dirt traps, which constitute a microbiological risk. The following outlines some good manufacturing practices for facilities in order to establish hygienic margarine production.

A margarine plant should be located in such surroundings and in relation to other plants that there are no environmental hazards affecting the hygienic aspects (116). On the outside of all entrances to the rooms of the plant, suitable areas should be covered with asphalt, concrete, or other materials that prevent the accumulation of water and allow proper cleaning of the area.

The plant should have the necessary rooms for production and storage including cold storage as well as a separate room or another suitable facility for the storage of packaging materials, additives, cleaning materials, and detergents (116). Separate canteen and locker rooms as required for the personnel should be available. Further, the plant should be laid out in such a way that finished products can be transported in a hygienic manner from the storage room to distribution trucks.

For the use of the personnel, sufficient number of restrooms with handwashing facilities should be available and located in such a manner that there is no direct access between restroom areas and production rooms, storage rooms, or other rooms where margarine, food additives, or other foodstuffs are located, unless the restroom area contains a special front room that separates the restroom from the production facilities.

Fixed installations for heating, power generation, or compressors for refrigeration, which could pollute the air, should be installed in a separate, effectively ventilated room (116). The production rooms, with exception of the cold storage room, should be adequately ventilated (85, 116). In the production room and product handling areas or in their close vicinity, there should be handwashing facilities installed with cold and warm water (116). Soap and disposable towels should be available at the handwashing facility (116). The floors should be smooth and easily cleaned and made of materials that are adapted to the use of each room. The walls should be smooth, free of ledges, and easily cleaned up to a suitable height (116).

The production room itself should have a minimum ceiling height of 2.5 m and the floors should be watertight and made from concrete with acid brick tiles or other suitable material. The floors should slope appropriately toward drainage points. The walls should be covered with glazed tiles or other suitable material at a minimum height of 1.7 m and the transition between the floor and wall should

be watertight and made for easy cleaning (116). The floor should be kept fat free and washed regularly with a mild alkaline detergent (85).

Unless the building and the production room, as well as other rooms, are kept clean, there is always a serious danger of the plant being reinfected after washing, which negates all the precautions taken with regard to cleaning and hygienic procedures in the plant.

## REFERENCES

1. L. H. Wiedermann, *J. Amer. Oil Chem. Soc.*, **49**(8), 478 (1972).
2. J. M. deMan, *J. Dairy Sci.*, **47**, 1194 (1964).
3. A. J. Haighton, *J. Amer. Oil Chem. Soc.*, **53**(6), 397 (1976).
4. L. H. Wiedermann, *J. Amer. Oil Chem. Soc.*, **55**(11), 823 (1978).
5. A. E. Thomas III, *J. Amer. Oil Chem. Soc.*, **55**(11), 830 (1978).
6. G. Rivarola et al., *J. Amer. Oil Chem. Soc.*, **64**(11), 1537 (1987).
7. C. W. Hoerr, *J. Amer. Oil Chem. Soc.*, **37**(10), 539 (1960).
8. A. J. Haighton, *J. Amer. Oil Chem. Soc.*, **36**(8), 345 (1959).
9. E. S. Lutton, *J. Amer. Oil Chem. Soc.*, **49**(1), 1 (1972).
10. K. Sato and T. Kuroda, *J. Amer. Oil Chem. Soc.*, **64**(1), 124 (1987).
11. L. deMan et al., *J. Amer. Oil Chem. Soc.*, **66**(1), 128 (1989).
12. U. Riiner, *Lebensm.-Wiss. u. Technol.*, **3**(6), 101 (1970).
13. U. Riiner, *Lebensm.-Wiss. u. Technol.*, **4**(6), 175 (1971).
14. I. Wilton and G. Wode, *J. Amer. Oil Chem. Soc.*, **40**(12), 707 (1963).
15. N. Garti et al., *J. Amer. Oil Chem. Soc.*, **58**(12), 1058 (1981).
16. N. Garti et al., *J. Amer. Oil Chem. Soc.*, **59**(4), 181 (1982).
17. J. Schlichter Aronhime et al., *J. Amer. Oil Chem. Soc.*, **64**(4), 529 (1987).
18. N. Krog, *J. Amer. Oil Chem. Soc.*, **54**(3), 124 (1977).
19. A. Calvelo, RITA 86, *Heat Transfer during the Production of Margarines and Low Fat Spreads*, Molinos Rio de la Plata S. A., Buenos Aires, Argentina, 1986.
20. U. Riiner, *Lebensm.-Wiss. u. Technol.*, **4**(3), 76 (1971).
21. R. E. Timms, *J. Amer. Oil Chem. Soc.*, **62**(2), 241 (1985).
22. F. C. H. Oh et al., *PORIM Bulletin No. 15*, Palm Oil Research Institute of Malaysia, November, 1987, pp. 11-17.
23. K. G. Berger and W. B. Wright, *PORIM Occasional Paper No. 17*, Palm Oil Research Institute of Malaysia, January, 1986.
24. M. L. Duns, *J. Amer. Oil Chem. Soc.*, **62**(2), 408 (1985).
25. M. Maclellan, *J. Amer. Oil Chem. Soc.*, **60**(2), 368 (1983).
26. R. Day, *Production of Shortening and Cake Margarine for Packaging in Cartons*, Crown Chemtech Technology Memorandum No. 10, Reading, United Kingdom, 1993.
27. Internal Memorandum, Crown Wurster & Sanger, Minneapolis, Minnesota, unpublished, 1993.
28. K. Kawamura, *J. Amer. Oil Chem. Soc.*, **56**(8), 753 (1979).

29. K. Kawamura, *J. Amer. Oil Chem. Soc.*, **57**(1), 48 (1980).
30. P. H. Yap et al., *J. Amer. Oil Chem. Soc.*, **66**(5), 693 (1989).
31. K. P. A. M. van Putte and B. H. Bakker, *J. Amer. Oil Chem. Soc.*, **64**(8), 1138 (1987).
32. K. G. Berger, *PORIM Technology No. 5*, Palm Oil Research Institute of Malaysia, August, 1981.
33. N. Sudin et al., *Palm Oil Developments No. 18*, Palm Oil Research Institute of Malaysia, April, 1993, pp. 1–3.
34. F. C. H. Oh and K. G. Berger, *PORIM Bulletin No. 2*, Palm Oil Research Institute of Malaysia, May, 1981, pp. 13–24.
35. J. Lefevre, *J. Amer. Oil Chem. Soc.*, **60**(2), 295 (1983).
36. R. Duterte, *Rev. Francaise Corps Gras*, **19**(10), 587 (1972).
37. R. Duterte, *Rev. Francaise Corps Gras*, **23**(10), 547 (1976).
38. E. Becker, *Fette, Seifen, Anstrichmittel*, **61**, 1040 (1959).
39. A. Jakubowski, *Rev. Francaise Corps Gras*, **18**(7), 429 (1971).
40. G. R. List et al., *J. Amer. Oil Chem. Soc.*, **54**(10), 408 (1977).
41. H. Traitler and A. Dieffenbacher, *J. Amer. Oil Chem. Soc.*, **62**(2), 417 (1985).
42. T. Y. Kun and A. Ibrahim, *Palm Oil Developments No. 15*, Palm Oil Research Institute of Malaysia, September, 1991, pp. 9–14.
43. F. J. Massiello, *J. Amer. Oil Chem. Soc.*, **55**(2), 262 (1978).
44. C. F. Shen et al., *ELAEIS*, **2**(1), 143 (1990).
45. *INFORM*, **1**(3), 174 (1990).
46. O. L. Brekke, in D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, eds., *Handbook of Soy Oil Processing and Utilization*, American Soybean Association, St. Louis, Missouri, and the American Oil Chemists' Society, Champaign, Illinois, 1980.
47. P. H. Yap et al., *J. Amer. Oil Chem. Soc.*, **66**(12), 1784 (1989).
48. P. H. Yap et al., *J. Amer. Oil Chem. Soc.*, **66**(12), 1792 (1989).
49. M. M. Chrysam, in T. H. Applewhite, ed., *Bailey's Industrial Oil and Fat Products*, 5th ed., Wiley-Interscience, New York, 1985, p. 62.
50. L. deMan et al., *J. Amer. Oil Chem. Soc.*, **66**(12), 1777 (1989).
51. K. Alexandersen, *Guidelines for Selection of Required Chemetators, Worker Units and Resting Tubes in Relation to Capacity and Product Type, Technology Memorandum No. 5*, Crown Wurster & Sanger/Crown Chemtech Ltd., Minneapolis, Minnesota, 1993.
52. K. Alexandersen, *Vegetable Puff Pastry Margarine for Extrusion, Technology Memorandum No. 1*, Crown Chemtech Ltd., Minneapolis, Minnesota, 1993.
53. K. K. Rajah, in V. K. S. Shukla and F. D. Gunstone, eds., *Oils and Fats in the Nineties*, International Food Science Centre A/S, Denmark, 1992, pp. 254–282.
54. Ref. 48, p. 78.
55. N. T. Joyner, *J. Amer. Oil Chem. Soc.*, **30**, 526 (1953).
56. C. E. McMichael, *J. Amer. Oil Chem. Soc.*, **33**(10), 512 (1956).
57. N. Hall Taylor, *The Operation of Scraped Surface Heat Exchangers with Very Viscous Materials during Cooling, Technology Memorandum No. 2*, Crown Chemtech Ltd., Reading, United Kingdom, 1993.



58. D. Finch, *Refrigerant Hot Gas By-pass System, Technology Memorandum No. 8*, Crown Chemtech Ltd., Reading, United Kingdom, 1993.
59. G. W. Reigel and C. E. McMichael, *J. Amer. Oil Chem. Soc.*, **43**(12), 687 (1966).
60. *Grindsted Technical Memorandum No. TM 101-2e*, Grindsted Products, Brabrand, Denmark.
61. Internal Memorandum, Crown Chemtech Ltd., Reading, United Kingdom, 1994.
62. *Margarine Statistics Report*, National Association of Margarine Manufacturers, Washington D.C., January 15, 1993.
63. Ref. 48, p. 80.
64. Benz & Hilgers GmbH, Filling and Wrapping Machines, Neuss, Germany, 1994.
65. Benz & Hilgers GmbH, Multipack 8362, Neuss, Germany, 1994.
66. Internal Memorandum, Wurster & Sanger, Div. of Crown Iron Works Co., Minneapolis, Minnesota, February, 1993.
67. Benz & Hilgers GmbH, Benhil Tub Filling and Closing Machine 8270, Neuss, Germany, 1994.
68. Benz & Hilgers GmbH, Copparapid 8270, TI 5/90, Neuss, Germany, 1994.
69. L. Langenhahn, A. Mooshammer, G. Fischer (to Benz & Hilgers, GmbH), U.S. Pat 4,830,068 (May 16, 1989).
70. R. S. Edmunds and T. L. Budlong, *J. Amer. Oil Chem. Soc.*, **53**(1), 30 (1976).
71. W. Altrock, personal message, Chr. Bock & Sohn Maschinenfabrik, Norderstedt, Germany, 1994.
72. M. Arnold, *Food Proc.*, December (1993).
73. Vilter Manufacturing Company, Milwaukee, Wisconsin.
74. K. E. Hickman, *ASHRAE J.*, **1**, 42 (1994).
75. N. E. Miller, *Consulting-Specifying Engineer*, **1**, 26 (1994).
76. "Fluorocarbons," *Chemical Economics Handbook*, SRI Consulting, Menlo, Park, Calif., 2004.
77. Field Report, *Food Eng.*, September (1981).
78. R. A. Elliott, *Am. Dairy Rev.*, **40**(6), 28 (1978).
79. Anonymous, *Food Eng. Int'l.*, **4**, 38 (1981).
80. Internal Memorandum, Crown Wurster & Sanger, Minneapolis, Minnesota, July, 1993.
81. K. Alexandersen, *Margarine Production*, Presented at the World Conference on Edible Oils and Fats Processing, Cannes, France, 1985.
82. C. V. Karstedt, *Fette Seifen & Anstrichm.*, **81**, 65 (1979).
83. Bran + Luebbe Inc., Metering Pumps and Systems, Buffalo Grove, Illinois, 1994.
84. American Lewa Inc., Metering Pumps in the Processing of Oils and Fats, Holliston, Massachusetts, 1994.
85. *Dairy Handbook*, ALFA-LAVAL, Food Engineering AB, Lund, Sweden.
86. B. V. Smith, *Plant Hygiene and Microbiology in the Production of Margarine*, Internal Memorandum, Crown Wurster & Sanger, Minneapolis, Minnesota, 1990.
87. K. Alexandersen, *Storage Conditions for various Margarine and Shortening Products, Technology Memorandum No. 4*, Crown Chemtech Ltd., Minnesota, 1993.

88. A. Jart, *Some Fat Containing Foods*, vol. 3, 2nd ed., *Fat Technology*, Royal Veterinary & Agricultural University of Copenhagen, Denmark, 1977.
89. R. L. McBride and K. C. Richardson, *Lebensm.-Wiss. u. Technol.*, **16**(4), 198 (1983).
90. *Puff Pastry Margarine*, *Technical Memorandum No. TM 103-1e*, Grinsted, Brabrand, Denmark.
91. A. Richards, *Continuous Production of Typical Low Fat Spread at 25–40% Fat*, *Technology Memorandum No. 9*, Crown Chemtech Ltd., Reading, United Kingdom, 1993.
92. D. J. P. Moran, *PORIM Technology No. 15*, Palm Research Institute of Malaysia, 1993.
93. J. Madsen, *Low-Calorie Spreads and Melange Production in Europe*, *Technical Paper No. TP 107-1e*, Grinsted, Brabrand, Denmark.
94. T. P. Pantzaris, *Palm Oil Developments No. 18*, Palm Oil Research Institute of Malaysia, 1993, pp. 3–7.
95. H. Kifli, *Palm Oil Developments No. 18*, Palm Oil Research Institute of Malaysia, 1993, pp. 8–9.
96. R. Leysen et al., *J. Amer. Oil Chem. Soc.*, **63**(6), 727 (1986).
97. Margarine Statistics Report, National Association of Margarine Manufactures, Washington D.C., January 15, 1993.
98. J. Lefebvre, *J. Amer. Oil Chem. Soc.*, **60**(2), 295 (1983).
99. Ref. 48, p. 74.
100. A. Pedersen, *Low Fat Butter and Spreads*, Special Issue of *Danish Dairy & Food Industry Worldwide* (1990).
101. W. V. Nieuwenhuyzen, *J. Amer. Oil Chem. Soc.*, **58**(10), 886 (1981).
102. C. J. Cante et al., *J. Amer. Oil Chem. Soc.*, **56**(1), 71A (1979).
103. J. Madsen, *Puff Pastry Margarine*, *Technical Paper No. TP 101-1e*, Grinsted, Brabrand, Denmark.
104. T. Y. Kun and A. S. H. Ong, *Palm Oil Developments No. 4*, Palm Oil Institute of Malaysia, 1986, pp. 17–18.
105. A. Pedersen, *Puff Pastry Butter*, Special Issue of *Danish Dairy & Food Industry Worldwide* (1988).
106. J. Madsen, *Postcrystallization in Puff Pastry Margarine*, *Technical Paper No. TP 102-1e*, Grinsted, Brabrand, Denmark.
107. J. Madsen, *Puff Pastry Margarine*, *Technical Memorandum No. TM 103-2e*, Grinsted, Brabrand, Denmark.
108. J. Madsen, *Emulsifiers Used in Margarine, Low-calorie Spread, Shortening, Bakery Compound and Filling*, *Technical Paper No. TP 105-1e*, Grinsted, Brabrand, Denmark.
109. E. Deffense, Multi-step Butteroil Fractionation and Spreadable Butter, *Sonderdruck aus Fett Wiss. Technol.-Fat Sci. Technol.*, **13**, 1987.
110. R. E. Timms, in R. C. Cambie, ed., *Fats for the Future*, Ellis Horwood Limited, Chichester, England, 1989, pp. 251–261.
111. K. Alexandersen, *Recombined Butter Products for Bakery Applications*, *Technology Memorandum No. 11*, Crown Chemtech Ltd., Minneapolis, Minnesota, 1994.
112. T. L. Mounts and K. Warner, in D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb., eds., *Handbook of Soy Oil Processing and Utilization*, American

Soybean Association, St. Louis, Missouri, and the American Oil Chemists' Society, Champaign, Illinois, 1980.

113. E. G. Latondress, *J. Amer. Oil Chem. Soc.*, **58**(3), 185 (1981).
114. A. Jart, *Fedtkemi*, vol. I, 2nd ed., *Fat Technology*, Royal Veterinary & Agricultural University of Copenhagen, Denmark, 1977.
115. Indicator Paper for the Ascertainment of Loose Water, *Technical Memorandum No. TM 16-1e.*, Grinsted, Brabrand, Denmark.
116. Basic Principles for Producing a Margarine with Good Keeping Properties, *Technical Memorandum No. TM 107-1e.*, Grinsted, Brabrand, Denmark.
117. Bekendtgørelse om margarinefabrikker m.m., *Directive No. 198* Danish Ministry of Environment, Copenhagen, Denmark, May 20, 1985.

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## *Extrusion Processing of Oilseed Meals for Food and Feed Production*

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### **1. INTRODUCTION**

There are several different definitions of extrusion depending on the manufacturer as well as the purpose of the extrusion. For the purpose of this chapter, the best definition of extrusion can be explained as “continuous cooking under pressure, moisture and elevated temperature” (1). During the process of extrusion, several operations take place, such as grinding, hydration, mixing, shearing, thermal treatment, gelatinization, protein denaturation, destruction of micro-organisms and some toxic compounds, shaping, expanding, and partial dehydration (2). The use of extruders has been expanding rapidly in the food, feed, and oilseed industries over the past few years, although basic extruder technology has been around for a long time. It has been used in one form or another in many industries. New designs of extrusion equipment have increased the range of their application in food, feed, and oilseed processing.

## 2. TYPES OF EXTRUDERS

There are several types of extruders available in the market: some have single and some have twin-screw shafts. The extruder can be considered either wet or dry (3). Today, in the oilseed industry, no one uses twin-screw extruders because of their cost and complexity. Most of the oilseed industry uses either wet or dry (single-screw) extruders. All the extruders are basically screw pumps through which material is forced, while in the meantime, subjected to heat, cooking, and shear forces. Several parameters affect the quality of the end product when using extrusion technology. Serrano and Villalbi (4) list the following:

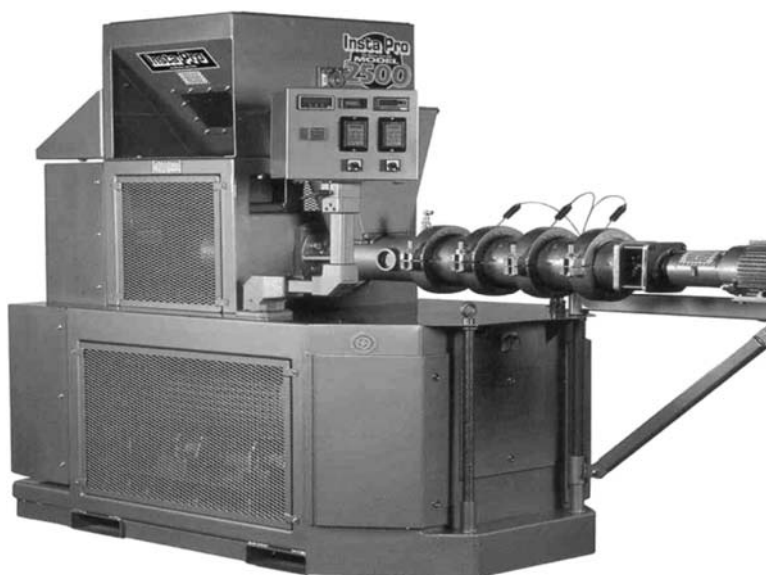
- Particle size of the oilseeds
- Extruder shaft speed
- Preconditioning moisture and temperature levels
- Residence time, percentage of moisture added, and temperature reached within the extruder barrel
- Barrel configuration of the screw and shear locks
- Die design and restriction in the die

The majority of these parameters are closely interrelated, and the key to obtaining a quality product is to find the best combination of three different types of extruders, namely dry extruders, segmented single-screw extruders, and interrupted flight extruders/expanders, which are discussed in this chapter.

### 2.1. Dry Extruders

The term “dry extruder” is applied to a particular type of single-screw machine. These machines were originally designed solely for processing whole soybeans. For this operation, the addition of moisture was not necessary because the high oil content of the beans acted as a lubricant during extrusion. Thus, the machines could reasonably be referred to as “dry extruders.”

The dry extruder is a continuous bioreactor. It is a relatively energy efficient, continuous cooker of both traditional and nontraditional ingredients. It handles relatively dry and viscous material and improves texture and handling characteristics. This type of extruder uses friction as the sole source of heat accompanied by pressure and attrition, compared with methods that use steam, water, or other forms of preconditioning. Heat and pressure are developed by passing the product being extruded through a barrel by means of a screw with increasing restriction. Then, the sudden decrease in pressure when the product is discharged into the atmosphere results in the expansion of the products (5). A typical dry extruder is shown in Figure 1. The term dry extrusion, as opposed to wet extrusion, refers to the fact that this type of extruder does not require an external source of heat or steam and it capitalizes its source of heat by internal friction for cooking (6). As noted earlier, the dry extruder was developed for processing whole soybeans on the farm. The dry

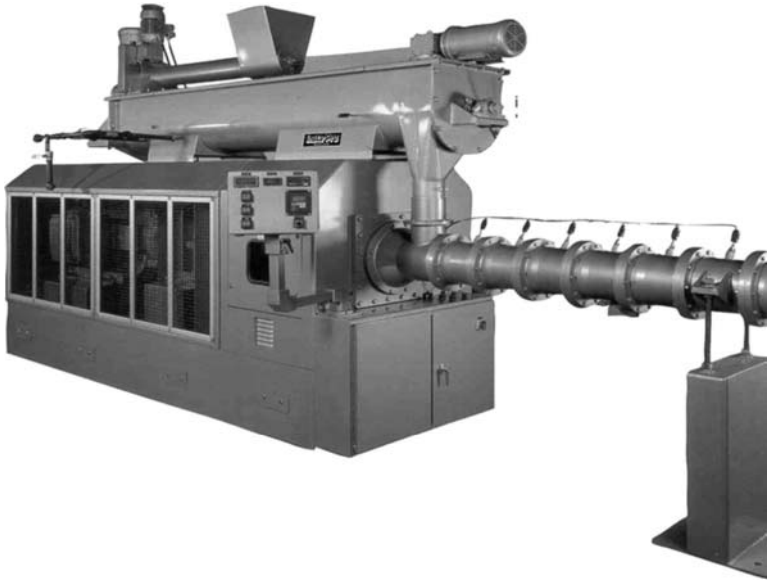


**Figure 1.** A typical dry extruder. Courtesy of Insta-Pro International, Des Moines, Iowa. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp.>)

extruder can process the ingredients that have a wide moisture content range, i.e., 10–30%, depending on the formulation. If the material has lower initial moisture contents, then drying of the material after extrusion cooking is not necessary. Usually, in dry extrusion, moisture is lost in the form of steam at the exit, and this moisture loss depends on the initial start up moisture in the material. Dry extruders have the option of water injection during extrusion. Usually, starchy material requires some moisture in order to gelatinize (7).

Recent experience has shown that these machines tend to process whole soybean more efficiently if the material is preconditioned with steam prior to extrusion. This finding, together with other recent work, has shown that simple extruders can be made more versatile and efficient in the context of producing compounded feed products (such as aqua feeds and pet foods) if raw materials are preconditioned with steam before extrusion. This has led to the retrofitting of many of these “dry extruders” with steam conditioners and has hence blurred the distinction between “dry” and “wet/steam” extruders (8). It has thus become a little confusing to continue to call these simple machines “dry extruders.” A typical dry extruder with a preconditioner is shown in Figure 2.

Many oilseeds and pulses contain inhibitory compounds that prevent their effective use as feedstuffs in the raw state. Dry extrusion is an excellent means of inactivating these inhibitory compounds, making the oilseeds and pulses usable as feed ingredients (8). Basically, the effect is a result of the susceptibility of the inhibitory compounds to heat denaturation. Oilseeds and pulses that can be effectively processed by dry extrusion include soybeans, linseed, groundnuts (peanuts), rape, field beans, peas, and lentils, among others. Dry extrusion can be used for materials such



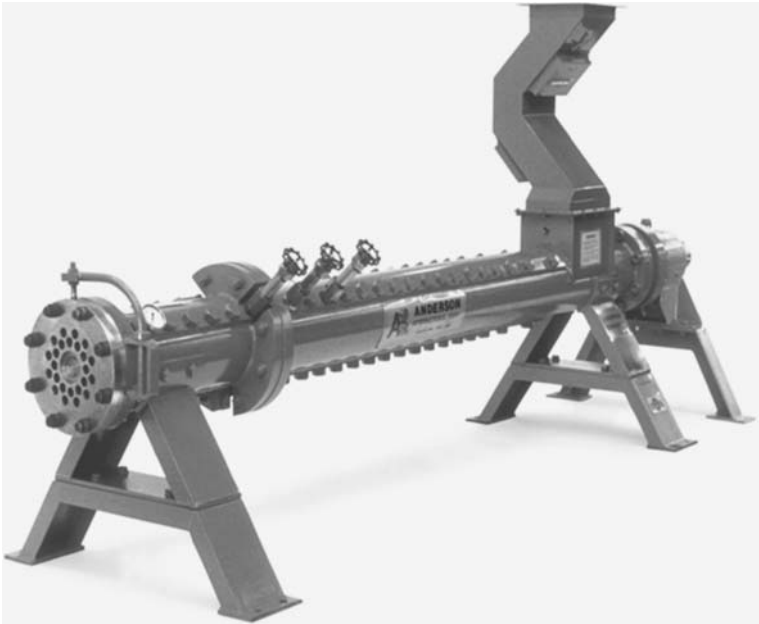
**Figure 2.** A typical dry extruder with preconditioner. Courtesy of Insta-Pro International, Des Moines, Iowa. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

as rice bran, and can be extrusion processed to inhibit the development of rancidity by denaturing intrinsic lipase enzymes (8). This allows such materials to be stored prior to conventional oil extraction with less danger of oil quality deterioration. Also, by extruding materials such as soybeans immediately prior to processing through an expeller, oil recovery capacity can be dramatically improved. This technique is suitable for smaller scale operators (8).

## 2.2. Interrupted Flight Extruders

The basic design for most of today's interrupted flight extruders, also called expanders, was developed and introduced in the United States by the Anderson International Company (Cleveland, Ohio) as the "Anderson International Grain Expander" for processing cereals (9). Domestically made expanders were sold for processing rice bran in Brazil in 1965, Ecuador in 1969, and Mexico in 1970. This design was applied to preparing soybeans and cottonseed for solvent extraction in Brazil in the early 1970s. Brazilian-made expanders were brought back to the United States for processing cottonseed in the late 1970s. An estimated 70% of the domestic tonnage of soybeans and cottonseed processed in the United States is now prepared for solvent extraction by interrupted flight extruders. Currently, machines of similar design are made in the United States, Brazil, India, Switzerland, and Germany (10). A typical expander is shown in Figure 3.

An interrupted flight extruder is mechanically different from other extruders because it was developed from a screw-press. A screw-press and an interrupted

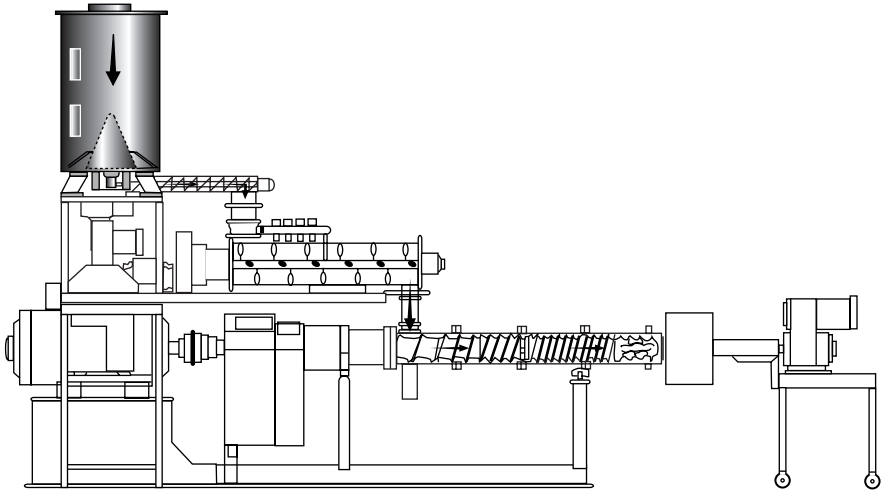


**Figure 3.** A typical expander/extruder. Courtesy of Anderson International, Cleveland, Ohio. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

flight extruder are similar in that a revolving worm shaft pushes the material through a cylindrical barrel and out through an opening at the barrel's end. However, a screw-press is a more massive and costly machine. It generates more pressure, and it has a slotted-wall barrel that permits oil to flow out from the solids (11). However, an interrupted flight extruder is essentially an extruder with an interrupted flight screw with bolts extending from the barrel into the space between the cut flights to increase shear in the conveyed product. Although extruders often are equipped with steam-heated/water-cooled jackets, commercial interrupted flight extruders usually are not jacketed and rely on direct steam injection for heat control.

The expander-extruder's internal mechanism consists of a rapidly rotating worm shaft, having individual worms with an interrupted flight positioned inside a smooth-walled barrel equipped with removable stationary pins protruding from the barrel and intermeshing with the interruptions of flight. The purpose of intermingling of the rotating worms with stationary pins is to provide a high shear, and a turbulent mixing action, which kneads the solid formulation with the injected water and steam to provide a rapid and uniform absorption of the injected moisture into all of the solid matter. As the steam is absorbed, it releases its heat of vaporization, which helps elevate the temperature of the mixture. Frictional heat also is generated by the rapid motion of the flights, further elevating the temperature as they compact and work the mixture, subjecting it to higher and higher pressure as it is forced through the length of the barrel. By the time the mixture reaches





**Figure 4.** *T* typical segmented single-screw extruder. A Courtesy of Wenger Manufacturing, Sabetha, Kansas. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

the end of the length of the barrel, it is thoroughly cooked and placed under pressure high enough so that all of the water is contained in the liquid state even though temperatures may reach 120–150°C (12).

### 2.3. Segmented Single-Screw Extruder

Segmented single-screw extruders are most widely applied to cooking extrusion design in the food and feed industry. Some oilseed processors also use this type of extruder to make full-fat soy meal for food and feed application. Wet means that steam and water can be injected into a barrel during processing. Typically, the barrel of these machines is also equipped with heating and cooling jackets. They process a greater tonnage of extruded product than any other extruder design (9). A typical segmented single-screw extruder is shown in Figure 4.

## 3. WHY PROCESS OILSEED WITH EXTRUSION?

There are several different reasons for which industry uses extrusion technology to process oilseeds. Some of the main reasons to process oilseed with extrusion are as follows:

1. To deactivate the antinutritional factors in oilseeds for use in animal feed and human food
2. To physically remove the oil from seed rather than using solvent extraction

3. To facilitate solvent extraction of the oil by producing collets
4. To produce food ingredients by partially removing the oil from different oilseeds

### 3.1. Processing Oilseed with Extrusion for Animal or Human Uses

There are eight major oilseed meals in the world (soybean meal, cottonseed meal, rapeseed meal, sunflower seed meal, peanut or groundnut meal, linseed meal, copra meal, and palm kernel meal). Soybean meal represents more than 50% of the total oilseed meal production. Raw soybeans cannot be used as such for animal feed or human food. There are several different antinutritional factors that have been identified in raw soybeans, and these factors affect the digestion of soybeans in the stomach. These factors are (1) trypsin and chymotrypsin inhibitors, (2) phytohaemagglutinins (Lectins), (3) urease, (4) allergenic factors, and (5) lipases and lipoxygenases. All of these antinutritional factors can be deactivated, modified, or reduced through proper heat treatment to minimize or eliminate their adverse effect. As all these inhibitors are proteins, caution should be taken to assure that no destruction of the oilseed protein occurs (13).

### 3.2. Main Objectives for Processing Soybeans with Extrusion

Extrusion processing of soybeans is done for several purposes as summarized below:

1. *To destroy growth inhibitors that are inherent in soy proteins.* Growth inhibitors may be defined as any substance in foods that exerts a deleterious physiological effect on man or animals as reviewed by growth or metabolism studies.
2. *To debitter proteins.* The most effective process of extrusion cooking involves the application of moisture, and research confirms that an effective method of reducing the intensity of the raw flavor is to treat with steam.
3. *To denature protein.* Denaturation of protein is the thermal processing of protein that lowers protein solubility and destroys the biological activity of enzymes and toxic proteins. For most mature species, lowering protein solubility renders them more digestible. Many species, however, immediately after birth will assimilate more protein if it is available in a highly soluble form. On occasion, studies have indicated that the adult stage (such as in shrimp) can also effectively use soluble proteins. Extrusion cooking can be managed to process protein to the degree of solubility desired.
4. *To rupture spherosomes or lipid bodies,* thus freeing the oil for greater calorie availability and use.
5. *Inactivation of fat-splitting enzymes,* particularly lipoxidases.

Microscopic evaluation of the structure of soybeans reveals individual cells that are 30–50 microns in diameter. These individual cells contain protein bodies that

are 6–10 microns in diameter and spherosomes (lipid bodies) that are 0.2–0.5 microns in diameter. During extrusion processing, the spherosomes coalesce into large oil droplets and the protein bodies burst with subsequent curdling. Extrusion cooking also tends to rupture the cell walls, releasing the contents and resulting in a confluent mass (14). Knowledge of this structure can be used to achieve the desired final product through careful processing. The full-fat soy that is used in feeds for monogastric animals (broilers, layers, turkeys, ducks, pigs, fish, crustaceans, etc.) requires quite different functional characteristics than full-fat soy used in milk replacers for calves. It also appears that full-fat soy used in various fish and shrimp diets requires processing standards that differ from feeds produced for broilers and layers (14).

Full-fat soybean meal is an excellent source of energy and protein with special value in diets for poultry when high nutrient concentration is required. This is the case for all turkey diets, most broiler diets, and feeds for laying hens early in the production cycle, as raw bean contain antinutrients.

There are several different methods to process full-fat soybeans for reducing the antinutritional factors, i.e., cooking/autoclaving, microwave treatments (micronization), roasting/toasting (salt bed roasting, heated ceramic roasting, rotary drum drier, grain drier), and extrusion. All these methods have their advantages and disadvantages. Extrusion cooking has some additional advantages that other methods do not offer. For example, the high-temperature short-time (HTST) cooking process will minimize degradation of food nutrients while improving the digestibility of protein by denaturation. Also, during extrusion cooking, most of the oil cells rupture, making oil available for the animal. The critical factor during extrusion cooking is the prevention of over- or underprocessing in order to reduce the nutritional value.

### **3.3. General Principles of Soybean Processing by Extrusion**

Since the 1960's, when whole soybeans first began to be used in intensive animal production, there has been a massive increase in the quantities of soy produced around the world. Although the bulk of this production is processed into a host of useful products ranging from margarine to ink, a significant proportion is now used as full-fat soy for animal feeding. Part of this increase in full-fat soybean meal production and use has been a result of recognition of the value of this material as a concentrated source of nutrients (8).

The high energy and protein content of full-fat soybeans make them particularly useful for formulating high nutrient density rations for the intensive production of modern livestock. It should be noted that the exact nutritional composition of full-fat soybeans will vary depending on the variety of soybean used together with the conditions prevailing during the cultivation and harvesting of the beans and the processing technique employed (8). Raw soybeans cannot be used in significant quantities in nonruminant diets because of the presence of antinutritive factors that can adversely affect animal productivity and health. The two most important of these factors are protease inhibitors (trypsin and chymotrypsin) and haemagglutinins.

Soybeans also contain lectins, urease, saponins, goitrogenic factors, rachitogenic factors, allergic factors, and metal chelating factors, all of which can have a negative impact on performance (15). Fortunately, many of these antinutritive factors are heat labile, and adequate heat treatment renders full-fat soybean meal safe for use in nonruminant diets. Hence, given a cost effective means of heat processing, the nutritional potential of this valuable raw material can be unlocked.

### 3.4. Why Process Soybean with Extrusion?

Whole soybeans contain not only high-quality protein (38–42%), but also a rich source of energy as a result of the oil they contain (18–22%). They have the potential of supplying major amounts of both energy and protein to all types of livestock and poultry feed. Collective terms that are being used to describe processed soybeans range from “full-fat soybeans,” “heat-processed soybean seeds,” “heat-treated soybeans,” to simply “whole soybeans.” More specific references are seldom used to correctly identify the processing method.

Although many reviews and publications are available regarding the nutritional value of processed whole soybeans, few have realized the effect of the processing method on the nutritional value, particularly, the energy value. The following is a summary of selective relevant publications of interest.

1. *Poultry*. Wiseman (16) reported considerable differences among different processing methods on the apparent metabolizable energy (AME) and nitrogen retention (NR) values for 2.5-week-old chickens. His data indicates that extruded soybeans have the highest metabolizable energy (ME) value (17.88 MJ/kg and 17.69 MJ/kg for wet and dry extrusion, respectively) followed by soybean meal and added oil (17.46 MJ/kg), micronized (15.8 MJ/kg), toasted (15.56 MJ/kg), jet sploded (14.25 MJ/kg), and raw soybeans (13.53 MJ/kg). The highest NR value was achieved with a dry extruder (84%) followed by toasted (67%), micronized (66%), jet sploded (63%), soybean meal plus oil (60%), wet extrusion (56%), and raw soybeans (30%).

The National Research Council (NRC) for poultry (17) used the collective term “heat processed soybean seed.” It reported an ME value of only 3300 kcal per kilogram (13.8 MJ/kg), which is not much different from that of raw soybeans listed above. No mention to the method of processing was made.

Zhang et al. (18) studied the effect of extrusion and expelling on the nutritional quality of conventional and Kunitz trypsin inhibitor-free soybeans in chicken. Their results indicated that increasing the extrusion temperature of conventional soybeans (CSB) to 138°C and 154°C significantly increased the TME<sub>n</sub> compared with CSB extruded at 104°C or 121°C (3815 kcal/kg DM, 3936 kcal/kg DM vs. 3665 kcal/kg DM, 3678 kcal/kg DM, respectively). Extruding Kunitz trypsin inhibitor-free soybeans at 104°C, 121°C, and 138°C resulted to similar TME<sub>n</sub> as that of CSB extruded at 138°C and 154°C.

The expelled CSB meal had a  $TME_n$  value of 3265 kcal/kg DM. They also reported that amino acid digestibility of CSB extruded at 138°C or 154°C did not differ from that of extruded KFSB. Expelling of CSB had no significant effect on amino acid digestibility values when compared with extruding alone.

Stillborn et al. (19) compared the effect of three processing techniques on broiler performance. They reported a significantly higher body weight and better feed efficiency ( $P < .05$ ) with whole soybeans processed by the dry extruder as compared with the wet extruder or the roaster.

Sell (20) found no significant differences ( $P > .05$ ) in either growth rate or feed efficiency in broilers when fed diets from 0 days to 53 days of age containing up to 47% extruded whole soybeans in the starter diet and 32% in the finishing diet as compared with the control soybean meal plus animal/vegetable fat diet. He also reported no significant differences ( $P > .05$ ) in turkey tom performance in a 140-day feeding trial when fed 22.2% extruded whole soybeans as compared with the control soybean meal plus animal/vegetable fat diet.

Waldroup and Hazen (21) compared feeding soybean meal, roasted soybeans, dry extruded soybeans, and raw soybeans to laying hens. Their results showed that hens fed the extruded soybeans had the highest egg production and the best feed conversion as compared to the control soybean meal diet. Hens that were fed the raw soybeans showed inferior results compared with the other groups.

It is widely accepted that the nutritional value of dietary proteins depends on their amino acid composition and how effectively these amino acids are used by the animal receiving the diet. In an experiment designed to assess the effectiveness of different heat treatments on the amino acid digestibility of full-fat soybeans, McNab (22) reported that although the trypsin inhibitor (TIU) was variable among the different processes, it was considered to have been reduced to safe levels in all cases (2 TIU/mg). His results showed that autoclaving and extrusion by the dry extruder provided the highest digestible amino acids (93.1% and 92.5% for autoclaved and dry extruded, respectively) as compared with toasted (94.1%), micronized (90.4%), micronized after water addition (87.9%), continuous toasting (87.6%), or wet extrusion (85.6%).

The results presented above illustrate the need to evaluate products and processes effectively to establish the correct nutritional properties.

2. *Swine*. Marty and Chavez (23) studied the effect of heat processing on digestible energy and other nutrient digestibilities of full-fat soybeans fed to weaner, grower, and finishing pigs. Their results showed that extruded soybeans had superior crude protein digestibility (86.4%) when compared with other heat treatments (79.8%, 79.8%, 80%, and 78.2% for jet sploded, micronized, roasted, and solvent-extracted soybean meal, respectively). Their results indicate that extrusion was most beneficial for weaner pigs as compared with growing and finishing stages.

Kim et al. (24) conducted metabolism studies on nursery pigs to determine the effect of extruding or roasting on the nutritional value of "Williams 82 Soybean" with (+K) and without (-K) gene expression for the Kunitz trypsin inhibitor. They reported that apparent values for nitrogen digestibility, biological value, percent nitrogen retention, gross energy digestibility, and metabolizable energy were greater for pigs fed extruded soybean as compared with pigs fed roasted soybean. All the above criteria were greater for pigs fed (-K) as compared with (+K) soybeans. The control soybean meal fed pigs showed intermediate results as compared with extruded and roasted groups. These results indicate that the NRC ME value of 1664 Kcal/lb for full-fat soy products should be revised to indicate the type of processing used.

In a study to determine the effect of roasting and extrusion on the ileal digestibility of nutrients in growing and finishing pigs, Kim et al. (25) concluded that ileal digestibilities of dry matter, gross energy, nitrogen, and various amino acids tend to be greater for extruded soybeans, intermediate in soybean meal, and lowest for roasted soybeans. These trends were true for both growing and finishing pigs.

Hancock et al. (26) reported that the improved feed efficiency for pigs fed extruded sorghum and soybeans indicate that the ME value of sorghum grain is dependent on the processing method, and the NRC value for ME of heat-processed soybeans is probably too low, at least for dry-extruded whole soybeans. Their data showed 6% increase in dry matter digestibility and 14% increase in nitrogen digestibility as a result of extrusion of soybeans and sorghum as compared with soybean meal-sorghum-soy oil nonextruded control diet.

3. *Ruminants.* Aldrich and Merchen (27) studied the effect of heat treatment of whole soybean on protein digestion by ruminants. They reported that increasing the extrusion temperature from 220°F to 320°F at 20°F increments resulted in a linear decrease of in situ degradation of soybean protein. As expected, raw soybean protein degraded very fast. The extent of degradation was 84.1%, 45.7%, 40%, 40.9%, 36.7%, and 30.4% for the raw, 220°F, 240°F, 260°F, 280°F, 300°F, and 320°F treatments, respectively. In other words, extruded soybean at 320°F had a bypass protein value of 69.6% as compared with 15.9% for raw soybeans.

Precision-fed cecectomized roosters assay was used to determine the total amino acid digestibility both before and after ruminal incubation. Unincubated raw soybean had a value of 68.5% compared with 87.7% for extruded soybean at 320°F. Digestibility of residues of extruded soybeans (average of 90% for 240°F, 280°F, and 320°F treatments) following in situ incubation were higher than those of raw soybeans (82%) after incubation. This reflects the differences in trypsin inhibitor activities and underscores the fact that the postruminal digestibility of the ruminal escape protein from raw soybeans is likely to be lower than that of the escape protein of extruded soybeans.

Socha and Satter (28) conducted a study to determine the production response of early lactation cows fed either solvent-extracted soybean meal, raw soybeans, extruded whole soybeans, or roasted soybeans with alfalfa silage as the sole forage source. They reported that dry matter intakes were lower for cows on the raw and roasted soybean treatments. Cows on the extruded soybean diet produced more milk, milk protein, and more 3.5% fat-corrected milk than cows fed the other diets. Body weight changes and body condition scores did not differ among the various treatments.

### 3.5. Effect of Extrusion on Soybeans

**3.5.1. Effect on Proteins** Proteins are denatured by the cooking process, an effect related to the temperature created. Proteins are made up of amino acids, which are known as the building blocks of protein. Amino acids are held together by primary bonds, whereas the molecules are held together by secondary bonds. The cooking action of the extruder breaks down the secondary bonds, but does not create sufficient heat to destroy the amino acids or the primary bonds.

This effect is similar to that caused by cooking an egg. The secondary bonds hold the protein in an egg together in a certain way. When the egg is heated, the protein is denatured, or the secondary bonds are broken. When these bonds are broken, the product loses its distinctive physical appearance. If the egg was cooked to extreme temperatures, the primary bonds holding the amino acids together would be destroyed, thus affecting their nutritional value. The extrusion process does not adversely affect the primary bond, but does create sufficient heat to denature or break down the secondary bonds.

It is this effect of denaturization that is useful when extruding oilseeds. Soybeans have an enzyme, urease, which can be denatured by heat created in the extruder. Enzymes are proteins, and when they are denatured, they lose their effectiveness. Urease breaks down urea to ammonia.

Another protein in soybeans that is destroyed by extrusion is the trypsin inhibitor, which is produced in the pancreas. Without the action of trypsin, the animal cannot use protein, as it is trypsin that splits or hydrolyzes the protein molecule. Other less important enzyme inhibitors that are denatured by the extruder relate to fats and the carbohydrate fraction of a diet. As the heat needed to deactivate enzymes is less than that needed to prepare oilseeds for oil extraction, the effect on the amino acids is much less severe, thus making them more available to the animal or higher in digestibility.

**3.5.2. Effect on Energy** The energy fraction of the diet can be broken into two parts: carbohydrate and fat. First, the carbohydrate fraction, which is primarily starch, is greatly affected by extrusion or heat and expansion.

Starch is basically a granular mass of molecules. Each starch molecule is like a chain with many side branches and is interconnected into a tightly held mass. Raw starch has little water absorbing capacity, and if dried after being soaked, goes back to its original state. One of the ways to test for starch is to pass light waves through

the granule and measure its polarization. As starch passes through the extruder, it is gelatinized, and when it leaves the extruder it expands. This effect is caused by breaking down the granular structure and opening up the molecular chain. This is similar to the effect that occurs by cooking starch in the presence of heat and water. The difference is that with the dry extruder, the effect takes place at much lower moisture levels and much quicker. Once the starch granule is gelatinized, it cannot revert to the original granular state, and it now is water soluble and the light waves are not polarized as they pass through the material. In a dry extruder, the only moisture needed to gelatinize the starch is the amount needed to lubricate the extruder and allow the starch to pass out of the orifice of the extruder. As the material leaves the dry extruder, the inherent moisture vaporizes, and the starch gel quickly stiffens after the liquid has vaporized. The amount of expansion that takes place is dependent on the amount of starch in the formula. With formulas containing 40–50% starch, the normal expansion will equal 2–3 times. For this reason, most extruded formulas are reground, returning the bulk density more closely to the original product.

**3.5.3. Effect on Fat** Extrusion, or heat and expansion, effects fat in two ways. First, fat or oil is encapsulated within the cells of oilseeds. When a raw oilseed is ground, the resulting product will be dry. During extrusion, the oil is released from the cells making the product oily. The extent to which cells are ruptured in a dry extruder has not been measured.

If starch is present when an oilseed is extruded, the fat and starch will form a complex that is not soluble in ether, the normal method used for fat analysis. Studies have shown that fat will enter a complex with starch on the basis of a 1 to 10 ratio. An acid hydrolysis is necessary to measure the correct and actual fat level in such a case.

Heat generated by the dry extrusion method does not adversely effect fat quality. Temperatures of the magnitude of 350–400°F are needed to produce off-odor development, whereas temperature for extrusion is generally in the 250–300°F range.

**3.5.4. Effect on Fiber** The effect of extrusion on fiber is related primarily to its bulk density. As a result of the mechanical action, grinding, and attrition, the hollow structure of fibrous materials is crushed, thus changing the bulk density. High fiber ingredients are normally low in moisture and take on moisture slowly, which requires some type of preconditioning prior to extrusion.

**3.5.5. Effect on Nutritional Value** The beneficial nutritional effects of extrusion vary with species, age, and type of ration being fed, but, in general, these benefits can be expected of the protein, energy, and fiber content of the ration by the following factors.

- Denaturing the enzymes, urease, lipase, amylase, and the trypsin inhibitors
- Improving starch digestion as measured by rate and efficiency of gain
- Increasing the bulk density of highly fibrous materials



The mechanisms by which extrusion improves the digestibility and use of energy are not fully understood. Certain studies would indicate that the combination of heat, pressure, and moisture is involved in increasing the susceptibility of starch to enzymatic digestion. Other studies indicate that the protein-binding effect of the starch granule may be disrupted, allowing easier access of microbial and animal enzymes to the starch granule.

In general, studies have shown a marked improvement in performance when extruded grains have been used in rations for young animals. This effect is a result of the somewhat undeveloped digestive tract of young animals as compared with the older animal. Improvements in gain and efficiency have also been noted for animals on high concentrated rations. The denaturation of enzyme inhibitors allows the normal enzymatic breakdown and use of proteins, fats, and carbohydrates.

The majority of the poultry, swine, and other feed industries use full-fat soybean meal in their formulation. Ninety five percent of full-fat soybean meal is produced through the extrusion process.

#### **4. SOYBEANS CAN BE CONVERTED INTO FULL-FAT SOY BY USING DRY OR WET EXTRUDERS**

There are a number of different products on the international market all being sold as full-fat soybean meal. These products are produced by a variety of processes, all of which have a different impact on the nutritive value of the product and its quality in terms of antinutritional factor levels. The following processes can be applied to the production of full-fat soybean meal: boiling/autoclaving, roasting/toasting, micronizing, jet-sploding, and extruding (wet or dry).

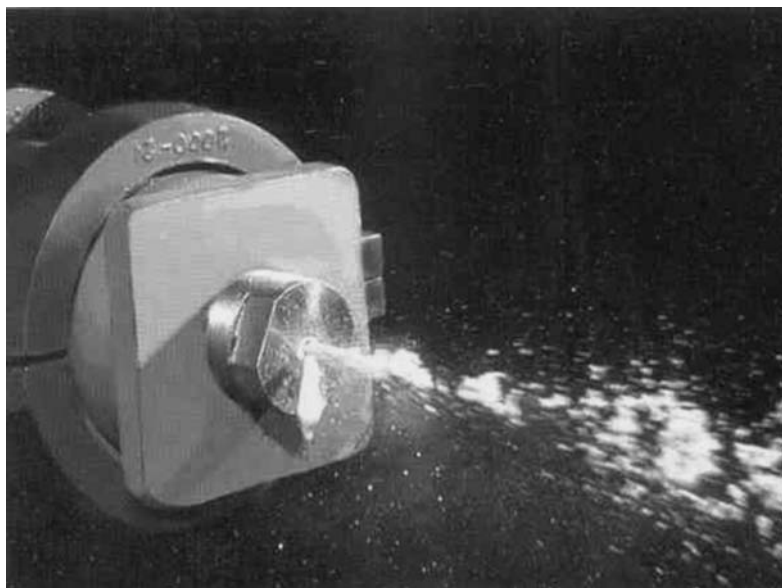
Extrusion is a continuous process and has obvious advantages over simple batch-cooking processes like boiling or autoclaving. It is also quicker than boiling as the beans have to be kept at boiling point for about 30 min to achieve reasonable levels of inhibitor destruction. The main variables associated with product quality and process efficiency as far as soybean processing is concerned are time, temperature, moisture content, and the degree of physical damage needed to render the oil content more digestible. Extrusion is a readily controllable process, whereas temperature, time (to some extent), moisture content, and the degree of physical damage can all be manipulated. The net result of this is that a very high-quality product can be produced when the extrusion process is properly handled (8).

##### **4.1. Processing of Soybeans with Dry Extruders**

The term dry extrusion, as opposed to wet extrusion, refers to the fact that this type of extruder does not require an external source of heat or steam (29). Dry extruders generate heat and pressure mechanically as a result of the frictional and shear forces produced within the extruder barrel. Provided that adequate operating temperatures are achieved (138°–150°C), this combination of heat and pressure is sufficient to substantially denature the important antinutritive factors in soybeans and render

the material usable in feeds. Dry extruders are single-screw extruders with a segmented screw put together around the shaft. In between the screw, a restriction (steam lock, choke plate) of different diameters can be placed to increase the cook and shear. When material moves in the barrel and comes across these restrictions, it is unable to pass through, and consequently, pressure builds up and a back flow is created. Usually, these restrictions are arranged in such a way that they increase in diameter toward the die end of the screw creating more pressure and shear as they reach the die. This buildup of pressure and temperature, together with shear stresses developed, tends to plasticize the raw materials into viscous paste or a puffed shape, depending on the raw material. In dry extrusion, pressure and temperature should be at a maximum just before leaving the die. The die design and opening also plays a very important role in pressure buildup. Different dies are used for different material and shapes. The cooking range in a dry extruder can be 180–320°F with very high pressure. As soon as the material leaves the extruder dies, pressure is instantaneously released from the products, which cause internal moisture to vaporize into steam, making the product expand (30). Figures 5 and 6 show the processing of full-fat soybeans by dry extrusion.

Most standard dry extruders are manufactured in such a way that the speed of rotation of the main shaft is around 550 revolutions per minute (rpm). This shaft speed is ideal for general purpose use where everything from cereal starch to full-fat soybeans needs to be processed on the same machine. However, if the machine is to be dedicated exclusively to full-fat soybean meal production, a slightly higher shaft speed (around 620 rpm) is desirable in combination with an



**Figure 5.** A dry extruder extruding full-fat soybean. Courtesy of Insta-Pro International, Des Moines, Iowa. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)



**Figure 6.** A dry extruder making full-fat soybean meal. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

extended three chamber barrel, as this will give a higher throughput per hour without comprising product quality.

In dry extrusion, whole oilseeds can be used, and this type of extruder has the ability to grind the oilseeds during extrusion processing. It is also argued that the grinding action of the extruder screw may also increase nutrient availability by rupturing cell walls, which release the oil and make it more biologically available. In addition, lecithin and the natural tocopherols in the beans may also be liberated by this grinding action. Dry extruders capitalize their source of heat through internal friction for cooking, and therefore, no external steam or heat is injected into an extruder barrel during cooking. The dry extruder can process the ingredients that have a wide range of moisture contents, i.e., 10–30%, depending on the formulation. If material has a lower initial moisture content, then the drying of the material after extrusion cooking is not necessary. Usually, in dry extrusion, we lose moisture in the form of steam at the exit, and this moisture loss depends on the initial startup moisture in the material. The heat and pressure generated in the extruder barrel typically raises the temperature to 135–150°C. This temperature and pressure is sufficient to denature the antinutritional factors in the soybeans and rupture the oil cells. This can be accomplished only through optimum processing and good quality control measures. If too much heat and pressure is applied, then significant damage may be done to the protein component of the soybeans, thereby reducing digestibility and availability in nonruminants. Thus, the process must be carefully controlled to ensure sufficient heat is applied to denature antinutritive factors without excessive cooking, which would damage the protein component.

Recently, several developments have occurred in dry extruder design and its components (8). Recent research has shown that if the starting material can be preconditioned with steam, the efficiency of the extruder increase almost doubled (8). Researchers also found that, in some applications, a longer barrel will work much better than the normal barrel used for soybeans (8). Several dry extrusion operations for full-fat soy have retrofit their dry extruder with steam preconditioning. Woodroffe (8) reported that retrofitting a dry extruder with a steam preconditioner can improve the processing capabilities up to 70%. Such technology can also reduce the wear of the extruder barrel components by 20%, reduce the electrical power cost by around 40%, improve the destruction of antinutritional factors, improve the protein efficiency ratio of full-fat soybean meal, allow the production of a finely textured end product, and avoid the shrinkage of extruded full-fat soybean meal as a result of lower moisture contents. With care, full-fat soybean meal can be produced with the use of a steam conditioner, but without the necessity of adding a drier to the processing line, as moisture is lost both at the extruder die and during cooling. However, the availability of a drier increases the flexibility of processing options and may yield a material with better keeping qualities.

Dry extruders can successfully process soybeans without the additional use of steam conditioning. This may be advantageous in terms of oil availability. However, the use of a steam conditioner in conjunction with dry extruders will increase throughput, improve inhibitor destruction, and enhance the biological value of the protein in the end product.

As nothing is added or taken away in the full-fat soy process, the gross composition of full-fat soy will depend on a constant moisture basis. The most serious variation can come with the level of moisture of the beans and the percentage of impurities, such as dust, straw, or weed seed among others. Full-fat soy will obviously contain the same level of oil as found in whole soybeans on a similar moisture basis. The average oil content of full-fat soy will be 17–18% if determined by the Soxhlet method using petroleum ether. This figure will be higher if oil is determined by the acid hydrolysis method. The oil in full-fat soy, which is properly processed by extrusion, is very stable and provides a remarkably long shelf life for such a high-fat product. The long shelf life can be explained by the fact that full-fat soy contains a high level of tocopherol and lecithin (4%) that inhibit oxidation of the full-fat product. At the same time, during extrusion, heat will destroy the enzymes lipase and lipoxigenase that cause rancidity. If full-fat soy is not processed at the proper temperature, it will show the signs of rancidity, i.e., increased peroxide value and free fatty acid level. This is no doubt a result of the incomplete destruction of lipoxidases. A moisture level above 12% will favor hydrolytic rancidity that triggers the oxidative rancidity and mold growth.

The degree of destruction of trypsin inhibitors is influenced by moisture content as well as processing time and temperature. With dry extruders, the highest reduction in trypsin inhibitor activity (TIA) appears to occur at the temperature range of 138°C to 150°C and a process moisture content of 20%. It is almost impossible to achieve these parameters without using steam preconditioning, as water injection directly into the extruder barrel can only be done successfully in very limited

quantities during whole soybean processing. This has led many users of dry extruders to fit steam conditioners to their machines to aid in full-fat soybean production. Extruder barrel component wear is reduced by approximately 20%. In addition, the protein efficiency ratio (PER), a measure of the efficiency with which animals use protein from feedstuffs of full-fat soybean meal, seems generally to be greater for steam preconditioned material, and it is easier to make a finely textured end product if a steam preconditioner is employed.

## 4.2. Processing of Soybean with Wet Extruder/Expanders

There are mainly two types of wet extruders that are being used to process soybeans: (1) segmented single-screw extruders and (2) interrupted flight extruders/expanders.

**4.2.1. Segmented Single-Screw Extruder** A typical single-screw extruder consists of a live bin, a feeding screw, a preconditioning cylinder, an extruder barrel, a die, and a knife. A live bin provides a buffer of raw material at the inlet so the extruder can operate continuously and without interruption. This bin should be equipped with a means of preventing the bin discharge from becoming bridged or blocked off. This bin is typically operated between high- and low-level limits.

A variable speed feeding screw must be used to continuously and uniformly discharge material from the bin and feed it to the extruder, as uniform feeding of the raw material is essential for the consistent and uniform operation of an extruder. The extrusion rate of the food extruder is typically controlled by a feeder screw or other metering devices. Therefore, the production of the single screw is dependent on the screw speed. This feeding screw must meet a variable speed to achieve the desired feed rate for extrusion. This feeder screw can feed directly into the extruder inlet throat or into a preconditioning cylinder (31).

As single-screw extruders have a relatively poor mixing ability, they are generally used with material that has been either premixed or preconditioned. Preconditioner is used to add the steam and water in the raw material before it enters into an extruder barrel. In general, preconditioning prior to extruding will enhance any extrusion process that would benefit from higher moisture and longer retention time. Preconditioning of the raw material can improve the life of wear components in the extruder barrel by several folds. The total capacity of the single-screw extruder will increase by using the preconditioner. By preconditioning the raw material, product quality can be improved greatly. Preconditioning prior to extrusion greatly increases extrusion throughput and enhances deactivation of growth inhibitors (32).

The single-screw extruder barrel assembly is composed of a jacketed head, a rotating extruder shaft, screws, shearlocks, a stationary barrel housing, a die, and a knife. Screws are the key elements of the single-screw extruder, as its geometry influences the unit operation of the extruder. The extruder bore may be of uniform diameters from inlet to discharge. It can be tapered, decreasing in a bore diameter from inlet to discharge, or it can be of a uniform diameter with the final segment of the barrel being tapered or decreasing in diameter. The screw design, including a

variable pitch constant depth, increasing root diameter, increasing number of flights, shear-locks, and decreasing diameter, is most frequently used in the industry (33).

A single-screw barrel can be divided into three processing zones: the feeding zone, the kneading zone, and the final cooking zone (34). The feeding zone generally has deep channels that receives the feed. The preconditioned or dry material enter in this zone and are conveyed to the kneading zone. Water may be injected at this point to help develop a uniform dough and to improve the heat transfer in the extruder barrel. As the material is conveyed into the kneading zone, its density increases because of the addition of water and steam. In this zone, screw pitch decreases and the flight angle decreases to facilitate the mixing and a higher degree of fill. This zone applies compression, mild shear and thermal energy to the feed, and extrudate begins to lose some of its granular definition. By the end of this zone, the feed material is a viscoamorphic mass at or above 100°C (35). The reduced slip at the barrel wall prevents the material from turning with the screw, and this is referred to as “drag flow” (36). A continuous screw channel serves as a path for “pressure-induced flow,” as the pressure behind the die is usually much higher than that at the inlet. “Leakage flow” also occurs in the clearance between the screw tip and the barrel wall. The flight of the screw may be interrupted in this area to further increase mixing via leakage flow (37). The mechanism of shear begins to play a dominate role because of the barrel fill condition in this zone. Steam and water can be injected in the early part of this zone. Steam injection will increase the thermal energy as well as moisture into the extrudate. As the extrudate moves through the kneading zone, it begins to form a more integral flowing dough mass, and will typically reach its maximum compaction. The material will exhibit a rubbery texture similar to a very warm dough. At this stage, the material enters into its final cooking zone. The screw flights in this zone are typically shallow and have a short pitch. The function of this zone is to compress and pump the material in the form of a plasticized mass to the die. Temperature and pressure typically increase most rapidly in this region because of the extruder screw configuration. Shear is highest in this zone, and temperature of the products reaches the maximum and is held for less than five seconds before the product is forced through the die (38). As the product discharges through the die into a region of lower pressure, it expands as a result of moisture vaporization.

**4.2.2. Interrupted Flight Extruder/Expander** Interrupted flight expanders are used for two different applications on full-fat soybean. One is to transform flaked, full-fat soybean into porous collets for subsequent solvent extraction. The other is to make full-fat soy for animal feeds. Using expanders to make full-fat soy is an attractive way for a feed mill to produce its own urease-free, trypsin inhibitor-free soy meal directly from raw beans. The soybean should be ground. It doesn't have to be dehulled, preheated, or premoistened. Die openings of one-quarter inch diameter with one-eighth inch land are sufficient. Trypsin inhibitor level in the extruded full-fat soy is usually 2–8 international units per milligram on a fat-free basis. Urease activity can be reduced to undetectable levels, but it is best to

see some activity, 0.1, for example, to ensure that the soybean has not been overcooked (39).

Full-fat soy exits the expander as a meal that flashes some of the moisture that had been injected as steam. After flashing, the meal still contains 15%–20% moisture. Moisture is then reduced in an apron type dryer/cooler. The air in the dryer is sometimes not preheated. The hot soy meal contains sufficient heat to drive off the moisture, provided it contacts enough low humidity air to carry the moisture away.

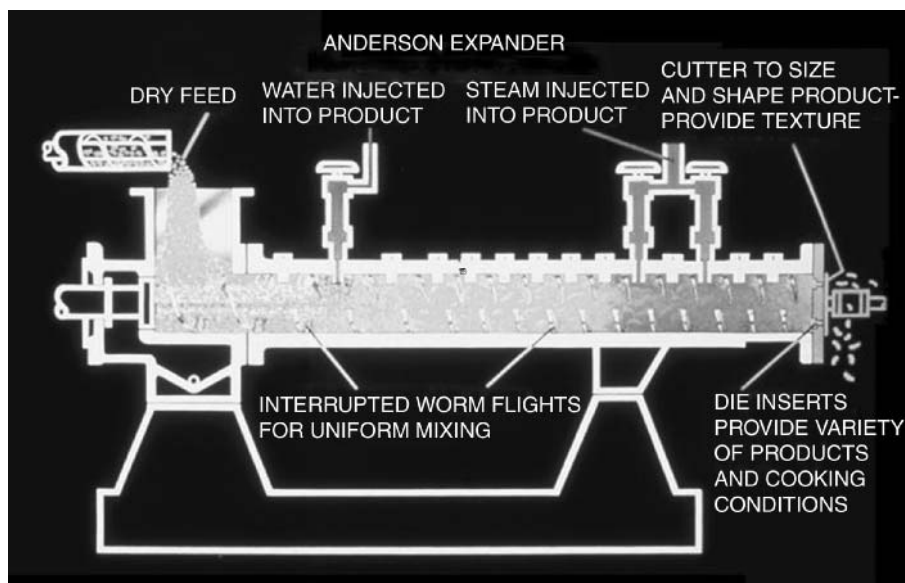
As expanders cook within 20 seconds, they counteract the activity of troublesome enzymes, such as lipase in rice bran (40) and urease in soybean (41). The short time between enzyme activation and inactivation destroys the enzyme before it has time to cause damage. An expander is even more effective than the horizontal, atmospheric pressure cookers described earlier.

Canola, for example, contains enzymes that release phosphorus compounds into the oil. If canola is processed through a stack cooker, the enzymes, which were triggered into action when the canola was flaked, exposing them to air, have several minutes to release phosphatides into the oil before the slow-acting stack cooker brings the canola to a high enough temperature to inactivate the enzymes. An expander brings the canola to full temperature in 20 seconds, and the enzymes do not have enough time to release phosphatides. This results in a significant reduction of phosphorus compounds in the finished oil; 48 ppm compared with 350 ppm when using stack cookers. Reduced chlorophyll, free fatty acid, peroxide value, and greenish color are added benefits. Zhang et al. (42) saw a similar situation with phosphorus in soybean oil. They saw that conventionally prepared soybean yielded degummed oil with 184 ppm phosphorus, whereas extrusion-prepared soybean yielded degummed oil with 67 ppm phosphorus (42).

In this application, the expander replaces the cooking tray in a stack cooker and cooks the oilseed at 190–221°F. If the oilseed is going to a solvent extractor, it would continue to a prepress or collet expander (same as flakes from a cooker). If the oilseed is going to a fullpress, then drying to optimum crushing moisture (2–3%) is still required.

In wet extrusion, soybeans are usually ground or flaked because this type of extruder will not grind soybeans during the extrusion process. The cooking of soybeans depends mainly on the addition of steams and some friction from the extruder. The resulting full-fat soy will contain approximately 13–18% moisture content depending on the original moisture of soybeans. These full-fat soybeans must be dried first and then cooled for proper storage (43). A typical expander (how it works) is shown in Figure 7.

There are two major sources of nutritional variation in full-fat soy. The first variation depends on the variety of seed used. Varieties grown in the United States show major differences in their fat and protein content when compared with varieties grown in Brazil, Argentina, and other countries. The second major source of variation is the equipment characteristics and the processing techniques. Also, the energy level in full-fat soy is influenced by the processing tech-



**Figure 7.** A cut view of expander (how it works). Courtesy of Anderson International, Cleveland, Ohio. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

nique; including temperature, duration of the process, and oil cell rupture. Feed manufacturers need to ensure that the products they receive are safe, of known quality, and as constant in composition as possible. In order to achieve these objectives, it is required to control the quality of the incoming and of the final products. There are several different tests that can be performed on full-fat soy, including both fat and protein content. Tests should be performed to check the antinutritional factors in soybean meal. Undercooking of soybean will not completely eliminate these factors, whereas overcooking will compromise the protein quality and makes it less nutritive for the animal. Therefore, extra caution should be taken during extrusion to control the proper cooking temperature and residence time in the extruder barrel.

Properly processed and good quality full-fat soy can be extremely efficient and profitable in animal as well as human nutrition. The heat applied to the beans releases additional aromas and flavors, which encourage both piglets and other domesticated mammals to eat more (44). Part of this improvement could be a result of the inactivation of the lipoyxygenases in the bean, promoting the quality and storage life of the end product (45). The use of full-fat soy started in 1960 has already revolutionized the entire feed industry, the nutrition of animals, and aquatic species, as well as the food industries around the world. Presently, full-fat soy is being used in poultry (broiler and layers), swine, ruminants (beef and dairy cows), and aquaculture. A properly processed full-fat soy can be used in bakery products, soy breads, cakes and doughnuts, sweet goods, chocolate and confections, spreads, and wafer fillings.



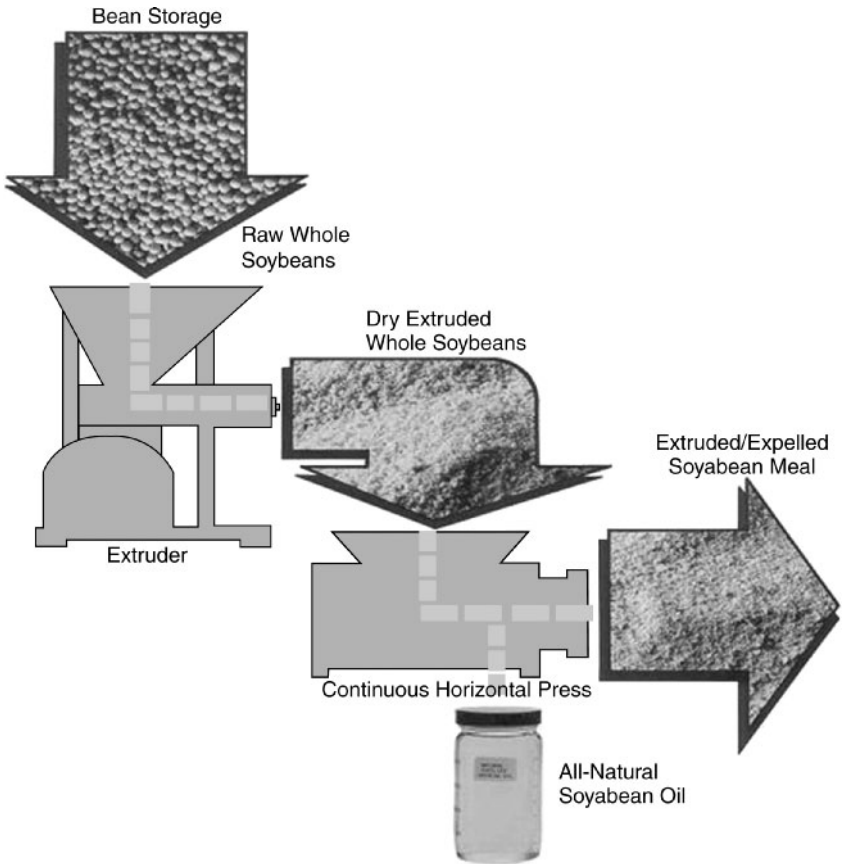
## 5. EXTRUSION-EXPELLING OF OILSEEDS

The traditional method of oilseed processing is based on solvent extraction to separate the oil from the meal in soybeans and other oilseed crops. Solvent-extraction technology thrives on economies of scale. It requires heavy capital investments, high technology, and well-developed infrastructure for collection, storage, and distribution of raw material and finished goods. The main advantage of solvent extraction is the high efficiency of oil extraction and lower cost of production. The major concerns for this process are the emission of hexane vapors into the atmosphere, which create considerable environmental concerns. Indeed, the solvent-extraction industry is under pressure to limit the level of emissions to the environment (46). In this situation, any process that does not use the solvents would find favorable consideration under these circumstances.

## 6. EXTRUSION-EXPELLING OF SOYBEANS

In this process, soybean oil is removed using an expeller by first heating the soybeans for a long time and then passing them through the expeller. The disadvantage of this process is that it gives low extraction efficiency and fluctuation in the quality of protein meal and oil, and a high cost of production. Prolonged heat treatment of soybeans prior to pressing, nonuniform heating as a result of variation of seed size within seed lots, and the need for multipass pressing have adverse effects on the quality of meal and oil. Also, there is not enough heat to destroy all the anti-nutritional factors causing off-flavor to the meal making it unsuitable for human consumption. Nelson et al. (47) modified this traditional method by adding dry extruders to extract the oil from soybeans as a pretreatment. Dry extruders are single-screw autogeneous machines that operate at low moisture and require minimum auxiliary equipment. It has been found that coarsely ground whole soybeans at 10–14% moisture can be extrusion cooked in less than 30 seconds at a temperature of about 130–135°C. It was discovered that the semifluid extrudate can be immediately pressed in a continuous screw-press to obtain high-quality oil and press cake. The main advantage of adding a dry extruder in this process is that only a single pass is required from the press in order to get oil down to 6% in the meal. Protein content of this meal is in the range of 46–50% and can be used for food or feed application. A typical process and their products are shown in Figures 8 and 9.

Extrusion prior to expelling greatly increases the throughput of the expeller over the rated capacity. An oil recovery of up to 70% can be achieved in a single pass. The high-temperature short-time extrusion cooking process eliminates the prolonged heating and holding of raw material in conventional expelling. The oil and the meal produced by this method are remarkably stable as a result of the fact that extrusion results in releasing the natural antioxidants (tocopherols) from the beans. Table 1 shows analysis of dry extruded-expelled soybean meal. Hill (48) stated that, when compared with solvent-extracted crude soybean oil,



**Figure 8.** A flow diagram of extruding expelling of oilseeds. Courtesy of Insta-Pro International, Des Moines, Iowa. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

the extruded-expelled soy oil contained lower amounts of peroxides, free fatty acids, trace minerals, sterols, phosphatides, unsaponifiable matter, moisture, and volatile matter. Table 2 shows characteristics of extruded-expelled solvent-extracted crude and refined, bleached and deodorized (RBD) soybean oils. The expelled soy oil contained the same amount of pigments, fatty acids, and triacylglycerols as crude solvent-extracted oil. He also reported that the high stability of extruded soy oil is a result of the high levels of natural antioxidants (tocopherols), while also having low levels of phospholipids and peroxides. Table 3 shows the effects of processing steps on the content of minor compounds in expelled soybean oil.

Extrusion-expelling concept is being used in the United States, Russia, and other countries to process soybeans and other oil seeds. Recently, extrusion-expelling technology has been adopted commercially by some soybean producers, grain elevators, entrepreneurs, and cooperatives in the United States and elsewhere



**Figure 9.** Different products (oil, full-fat meal, and partially defatted meal) from the extrusion and expelling process. Courtesy of Insta-Pro International, des Monies, Iowa. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

(49). Initially, extrusion-expelling technology was developed for soybean, but units have been installed to process other oilseeds. Table 4 shows analysis of extruding-expelling of soybean, cotton, canola, and sunflower seed. Said (49) reported extruding and expelling of cottonseed to produce a meal with a low content of gossypol and a residual oil content of 5–7%. The meal is sold as dairy, beef, and sheep supplements that provide high rumen bypass protein and is easily

**TABLE 1. Proximate Analysis of Dry Extruded-Expelled Soybean Meal.**

Component	%
Dry matter	94.0
Crude protein	46.0
Ether extract	7.0
(acid hydrolysis)	7.7
Crude fiber	5.5
Ash	5.3
Undegradable protein	50.0–60.0
Free fatty acid	0.5
Linolenic acid	0.4
Linoleic acid	0.9
Lecithin	0.2

Source: Insta-Pro International, Des Monies, Iowa.

**TABLE 2. Characteristics of Extruded-Expelled, Solvent-Extracted Crude, and RBD<sup>a</sup> Soybean Oils.**

Component	Crude Extruded-Expelled	Crude Solvent-Extracted	Commercial RBD
Unsaponifiable matter (%)	0.98	1.60	0.30
Peroxide value (meg/kg)	0–1.00	2.40	0.00
Moisture and volatile matter (%)	0.03	0.30	0.06
Iodine value	1.33	1.32	1.33
Free fatty acids	0.14	0.30–0.70	0.05
Phosphatide (%) <sup>b</sup>	0.20	1.5–2.5	0.01–0.05

Source: Insta-Pro International, Des Moines, Iowa.

<sup>a</sup> Refined, bleached, and deodorized.

<sup>b</sup> % phosphorus x 31.7.

**TABLE 3. Effects of Processing Steps on Content of Minor Compounds in Expelled Soybean Oil.**

Oil Sample	Chlorophylls (ppm)	Free Fatty acid (%)	Peroxide Value (meg/kg)	Tocopherol (ppm)
Crude extracted-expelled	0.42	0.16	0.10	1516
Degummed	0.40	0.15	8.24	1477
Refined	0.32	0.08	7.02	1430
Bleached	0.00	0.08	1.58	1236
Deodorized	0.00	0.03	0.00	790

Source: Insta-Pro International, Des Moines, Iowa.

**TABLE 4. Analysis of Extruding Expelling of Soybean, Cotton and Sunflower Seeds.**

Oilseed*	Raw Oil	Raw	Oil	Extruding Temperature °F	Expelled	Expelled
	Content %	Protein Content %	Removal Efficiency %		Meal Oil Content %	Meal Protein Content %
Soybean	18–22	36–40	65–67	300–320	6–9	42–46
Cotton	20–22	20–22	65–70	230–250	6–8	26–30
Canola	35–40	22–26	72–75	250–260	8–11	32–37
Sunflower	35–45	16–22	72–75	240–260	10–13	22–27

\*All values used for oil protein content are expressed in average ranges. Actual content varies because of varietal differences and growing conditions. Operator management also influences the quality of the end product.

**TABLE 5. Analysis of Extruded/Expelled Cottonseed Oil.**

Component	%
Moisture	Trace
Insoluble Matter	0.23
Unsaponifiable Matter	0.92
Total MIU	1.15
Total Fatty Acids	92.83
Free Fatty Acids	1.10
Oxidized Fatty Acids	1.59
Saponification Number	185.80
Acetone Insolubles	1.00
Metabolizable Energy Value	3939.00 Metabolizable Energy Calories Per Pound 8666.00 Metabolizable Energy Calories Per Kilogram
Iodine Value	96.70
Free Gossypol	5300.00 ppm

Source: Insta-Pro International, Des Moines, Iowa.

digestible as a good source of amino acids and energy. The crude oil is being sold for refining. Table 5 shows analysis of extruded-expelled cottonseed oil.

Extrusion-expelling technology is also being used in Russia, Latvia, Lithuania, and other newly independent states for extruding and expelling sunflower seed. The oil is being used for human consumption and meal for animal feed. Similar installations have been built to process rapeseed and peanuts (49). Table 6 shows the quality characteristics of soybean meals recovered in commercial practices by different oil-extraction processes.

**TABLE 6. Quality Characteristics of Soybean Meals Recovered in Commercial Practice by Different Oil Extraction Processes.**

Property	Processing Method		
	Extruding-Expelling	Solvent Extraction	Screw-Pressing
Moisture (% as is)	6.90	11.70	11.00
Oil (%) <sup>a</sup>	7.20	1.20	5.60
Protein (%) <sup>a</sup>	42.50	48.80	43.2
Urease ( $\Delta$ pH)	0.07	0.04	0.03
KOH solubility (%)	88.10	89.10	61.60
PDI (%)	18.10	44.50	10.60
Rumen bypass (%)	37.60	36.00	48.10
Color (Hunter L)	65.80	69.10	51.50
Trypsin inhibitor (mg/g)	5.52	5.46	0.30
Trypsin inhibitor (TIU/g)	12,250.00	5275.00	2000.00

<sup>a</sup>At 12% moisture basis.

Source: Insta-Pro International, Des Moines, Iowa.

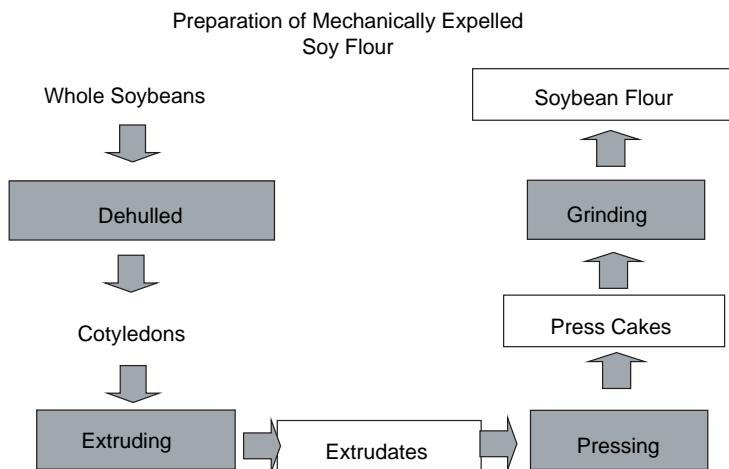
## 7. NUTRITIONAL ADVANTAGES OF EXTRUSION-EXPELLING OF OILSEEDS

Several researchers have studied the extruded-expelled meal for feeding trials for poultry, swine, and beef cattle. When compared with solvent-extracted meal, extruded-expelled meal is much better in nutrition and animals performance.

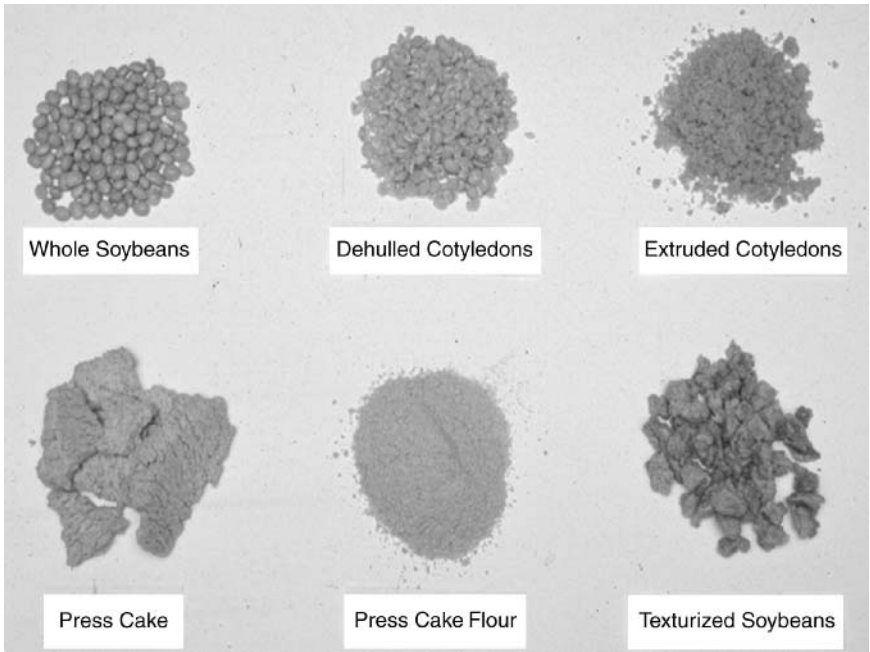
According to Said (50), an extrusion-expelling facility for 7000 tons to 8000 tons of oilseed per year is based on operating six days a week and 24 hours a day, and can be built for as little as \$175,000 to \$250,000. Operational costs average \$25 per ton for such a facility, covering both fixed and variable cost, including electrical, labor, maintenance, and depreciation. Soybean and cottonseed processors in the United States are showing a net profit of \$20–30 per ton of oilseed processed by the extrusion-expelling process. This profit can increase if the extrusion-expelling processor starts refining its own oil.

The existing technologies for making extruded full-fat and partially defatted soybean meals have been modified, and an extruder texturizing step has been added to produce mild-flavored texturized soy products, which can be made in dispersed locations without solvent-extraction plants (51, 52). This process has been fine tuned for food-grade dehulled soybean to produce soy flour with high protein dispersibility index (PDI) to make texturized vegetable protein. According to Lusas and Riaz (53), good quality soybean flour was produced using an Insta-Pro dry extruder, which has PDI of 45.

A flow sheet for making partially defatted soy flour is shown in Figure 10. Food-grade soybeans are cleaned, cracked and dehulled, and extruded by a dry extruder to free the oil. The oil is then removed by a screw-press. The press-cake is ground and adjusted to 21% moisture content and is formed



**Figure 10.** A flow diagram of making partially defatted soybean flour.

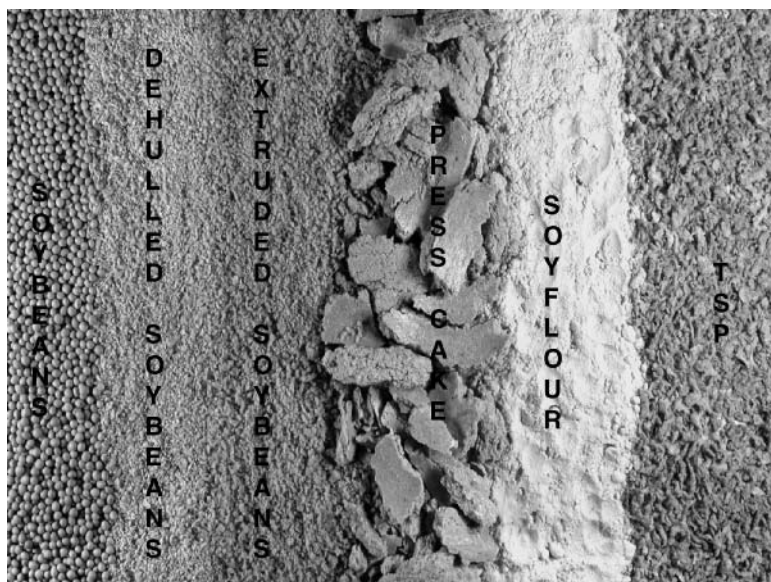


**Figure 11.** Different stages of soybean processing to make low-fat soybean flour by extrusion and expelling process. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

into shreds or chunks using the same extruder or another extruder. If desired, caramel color or other ingredients can be added to the mix before texturization (54, 55). The extruded products hydrate readily, resemble ground or chunk meat, and retain a chewy texture when cooked. Figures 11 and 12 show different stages of soybeans during processing into textured products and after hydrating in the water respectively.

The advantages of using the extrusion-expelling method to make texturized vegetable protein are as follows (56):

- Soybeans do not have to be first converted into flour or concentrates in centralized oil mills before texturization.
- Preparation of texturized soybeans can be done in simple dispersed facilities with small-size extrusion and screw-pressing equipments—tractor power take-off-driven if necessary.
- The texturized product is extremely bland because of early deactivation of lipoxygenases and other enzymes.
- The pressed oil is more bland than that obtained by typical solvent extraction or screw-press processing: it has a nutty-like flavor and might be acceptable with minimum processing in developing countries.

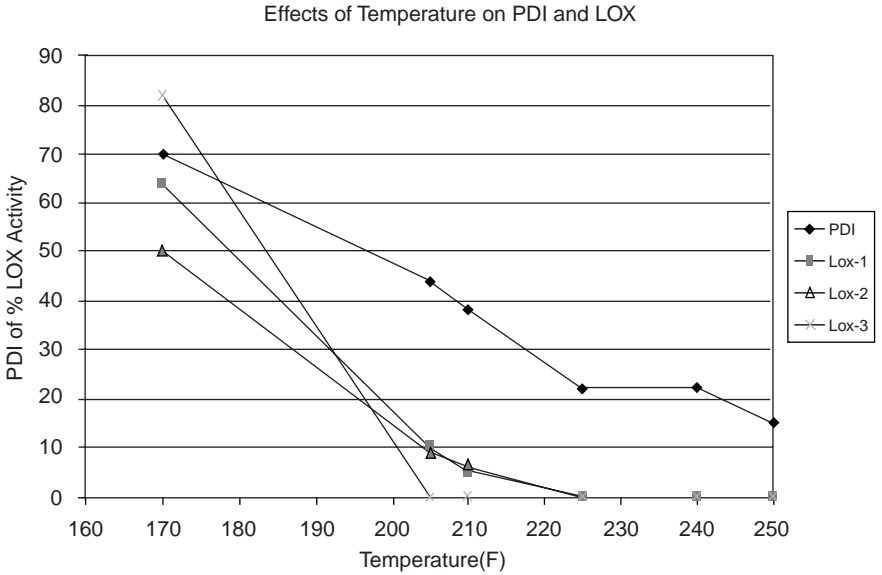


**Figure 12.** (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

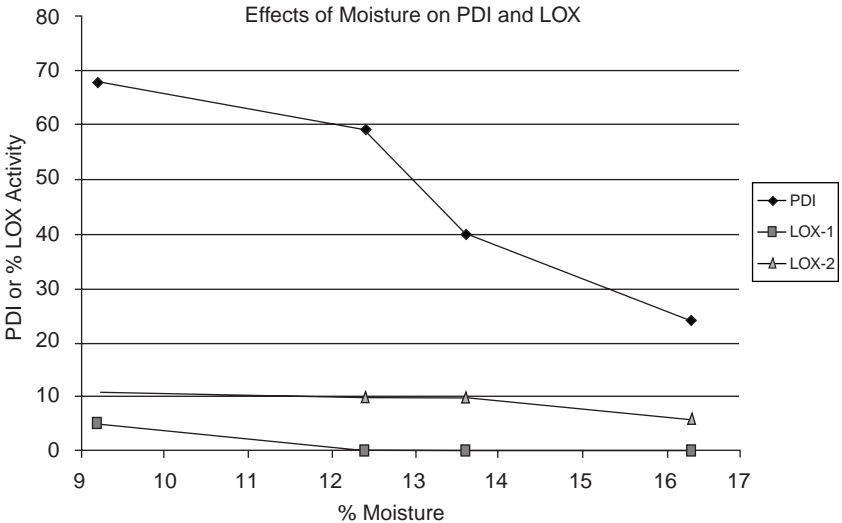
- Both the textured soy protein and oil products retain natural antioxidants and have good shelf life.
- Identity preservation is another factor that is becoming important in soybean processing. This may be a result of the need for specific varieties of specific applications or to separate organically grown varieties or to maintain identity of GMO/nonGMO varieties. The large scale and fully automated structure of the solvent-extraction industry does not lend itself to segregation of varieties through the processing cycle. Extrusion-exPELLING on a small scale can ensure identity preservation and could take advantage of the developing market for identity preserved soybean products.

Although the process is simple in concept, several principles must be considered. “Beany” and other off-flavors often occur in soy products because of the activity of lipoxigenases and other enzymes. As shown in Figure 13, the lipoxigenases that also probably approximate the activity of other indigenous enzymes are inactivated at about 105–107°C (57). Yet, at this temperature, the protein has a PDI of approximately 25—still enough for later texturization if a high shear extruder is used. As shown in Figure 14, increased moisture also accelerates reduction of PDI, and extrusion cooking at higher than 13% moisture can be detrimental to future texturization (57). Arresting enzymatic activity by essentially instantaneous heating when extruding the soybeans in the extruder is critical for obtaining mild-flavored products.





**Figure 13.** Effect of extrusion temperature on protein dispersibility index (PDI) and lipoxygenase enzymes activity (LOX).



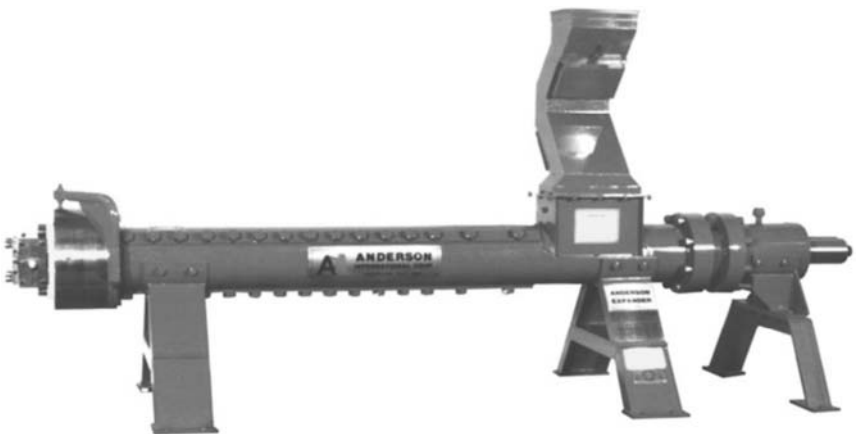
**Figure 14.** Effect of moisture on protein dispersibility index (PDI) and lipoxygenase enzymes activity (LOX) during extrusion at 99°C.

## 8. MECHANICAL CRUSHING WITH EXPANDERS

Interrupted flight expanders are also used to prepare oilseeds for mechanical crushing. Soybean can be passed through a high shear interrupted flight extruder equipped with a rotating cone point at the end of the shaft to shear and force the bean to flow between adjustable jaws. This converts the unbroken beans into a frothy meal of almost fluid-like consistency (58). Fluidization is caused by moisture boiling through the freshly liberated oil. The soybean, at 10% moisture and ambient temperature, for higher capacity, can be cracked before entry into the expander. High shear developed by the expander pulverizes the soybean and ruptures the oil cells. Heat generated by shaft friction raises the temperature to 275°F. This causes the moisture to flash from 10–14% down to 6–7% as the soybean exits into atmospheric conditions.

These high-pressure oilseed expanders are fitted with adjustable jaw chokes in place of die plates (Figure 15) and are operated under low-moisture, high shear conditions. Under these conditions, they can transform cracked or uncracked oilseeds, with or without preheating, into frothy, semifluid extrudates that flash to 5–7% moisture en route to the screw-press. A rotating cone point inserted between two laterally positioned, stationary jaws, generates the shear (59). The shear can be increased or decreased by adjusting the proximity of the cone point to the jaws and the opening between the jaws.

An eight inch diameter expander, with 150 HP drive, can process preheated, cracked soybean at 6000 lbs/hr, producing full-fat soy with low levels of urease and trypsin inhibitor. Also, the hot product can pass into a screw-press (after its moisture is allowed to flash to 5–7%). As most of the oil cells have been ruptured by the expander, a screw-press can process the soybean at three times the capacity it would have had with unexpanded soybean.



**Figure 15.** A dry expander. Courtesy of Anderson International, Cleveland, Ohio. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp.>)

## 9. EXTRUSION OF OILSEEDS BEFORE EXTRACTION

Preparation of oilseeds prior to extraction has always been important for good extraction results. Expanders accomplish so much in the preparation stage that better extraction is achieved, while reducing the requirements for cracking and flaking.

The first oil-bearing material that was expanded before solvent extraction was rice bran (60). Rice bran is a fine powder, making it very difficult to extract. Rice bran also contains the enzyme, lipase, which splits trigacylglycerols into free fatty acids. Lipase can raise the free fatty acid in the bran's oil approximately 3–7 percentage points every day (61). Cooking in an expander inactivates lipase and converts the bran into porous, sponge-like collets that allow rapid percolation of the solvent. Inactivation of enzymes, coupled with conversion into sponge-like collets, was a significant improvement in rice bran processing (60).

Another application of the expander is to form porous collets from oilseeds that contained less than 30–33% oil (such as soybean and cottonseed) as well as from pre-pressed oilseeds of higher oil content. The main objective of the expander/extruder is to reshape flaked soybean or cottonseed into porous collets. Flaked oilseeds extract well if the flakes are of good quality, but if the flakes are thick or if they are crumbly, or if the feed already contained fines, then extraction suffers. Interrupted-flight expanders convert poor quality flakes and fines into easily extracted collets. Figure 16 shows an expander making collets for extraction. Even compared with good flakes, expanded collets offer significant advantages because collets are larger, heavier, stronger, and more porous than flakes. This permits faster solvent flow (because of larger particles), greater extractor capacity (because the heavier, more porous collets occupy less space), and better drainage (because the collets are strong enough not to crumble as easily as flakes do) (62).

The oilseed reaches 235°F at 10–13% moisture at the die plate under a pressure of 30–40 atmospheres. All the water (natural moisture, injected steam, and liquid water) is compressed into the liquid phase. When the product leaves the high-pressure interior of the expander, some of the moisture flashes to reach equilibrium at atmospheric pressure. The flashing inflates the collets with internal pores and surface cracks, giving the collets a sponge-like structure. Figure 17 shows typical soybean collets made with expander for solvent extraction.

Some oilseeds are mechanically screw-pressed to remove approximately half the oil and then solvent extracted to remove the rest. Expanders can transform the press-cake into porous collets. Steam injected into the expander raises cake moisture by 2–4%. This must be done before the cake has cooled and hardened. Once cool and hard, the denatured protein in the cake can no longer be transformed into a gelatinous, inflatable condition. If oilseeds of high oil content are pre-pressed to 15–30% oil, they extrude similarly to flaked soybean (at 18% oil) and flaked cottonseed meals (at 30–33% oil).

According to Watkins et al. (63), the use of an expander offers several advantages in processing soybeans for extraction.

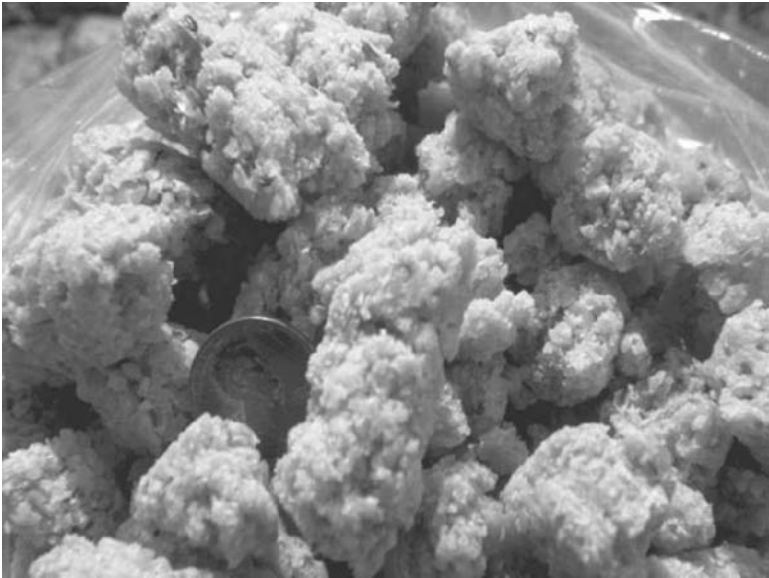
- The seed is finely homogenized in the expander, freeing the oil for rapid extraction.
- Dense collets are formed (which weigh more per cubic foot).



**Figure 16.** An expander making soybean collets for solvent extraction. Courtesy of Anderson International, Cleveland, Ohio. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

- The collets are porous and do not restrict percolation of the solvent through the extractor bed, as it may occur with fragile flakes.
- More oil is recovered by solvent from collets as compared with flakes.
- The solvent drains more completely from the extracted collets, resulting in more complete removal of oil.
- Less energy is required for desolventizing the collets compared with flakes.

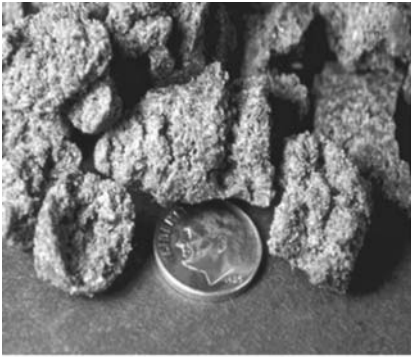
Figures 18 and 19 show collet made from pre-press canola, rice bran, sunflowers, and cottonseeds respectively.



**Figure 17.** A typical soybean collets made by expander. Courtesy of Anderson International, Cleveland, Ohio. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)



**Figure 18.** Collets made from pre-press canola and rice bran by expander for solvent extraction. Courtesy of Anderson International, Cleveland, Ohio. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

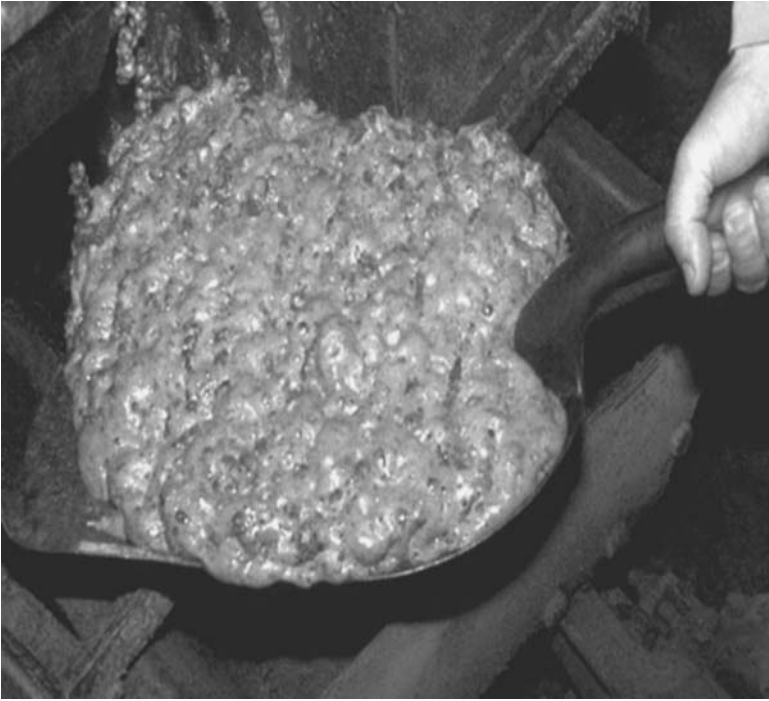


**Figure 19.** Collets made from sunflower and cottonseeds by expander for solvent extraction. Courtesy of Anderson International, Cleveland, Ohio. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

## 10. EXTRUSION OF HIGH OILSEEDS

Oilseeds containing more than 30–33% oil, such as safflower, sunflower, peanut, canola, and rapeseed, would cause problems in an expander because more oil would be liberated than the collet could reabsorb. Figure 20 shows a high content of oil that can not be processed in a closed wall expander. Pockets of oil would accumulate within the expander and disturb or stop steady-state operation. In an effort to reduce oil level, extracted meal is sometimes mixed with the fresh oilseed. This does reduce the oil level, but the reprocessing of extracted solids increases the load on the expander, the solvent extractor, and the desolventizer. This may force the plant to run at a reduced capacity. Williams (64) developed a slotted-wall expander that can make collets from high oil materials by allowing the liberated oil to escape through the slotted-wall drainage cage. A typical expander with a slotted wall is shown in Figure 21.

Field trials with canola (65) and other oilseeds (66) show that the slotted-wall expander could extrude full-fat oilseeds at high oil levels and produce collets at 20–30% oil. Such expanders are used on full-fat safflowers, sunflower, peanut, canola, and rapeseed. The oilseeds are prepared by drying, or by cooking and drying and flaking, similar to the way they are for screw-pressing. The slotted-wall

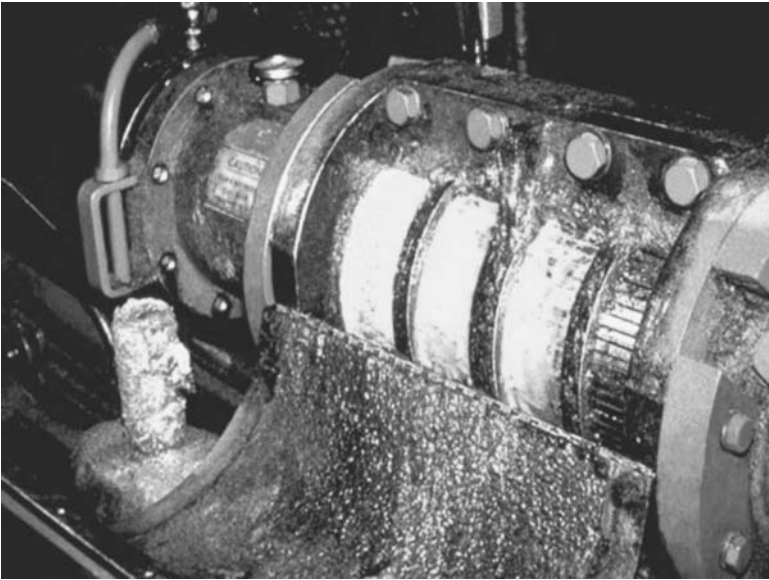


**Figure 20.** A view of high oilseed crops coming from the expander. Courtesy of Anderson International, Cleveland, Ohio. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

expander accepts then full-fat (42% to 45% for some), and allows the easily liberated fat to escape through the barrel wall. The partially deoiled solids (22–35% fat depending on the oilseed) continue along the length of the barrel, being converted into a tacky semiplastic mass (67). The solids finally discharge (through die) as continuous strands that inflate with internal tiny pores and surface cracks. A typical



**Figure 21.** A typical slotted-wall expander. Courtesy of Anderson International, Cleveland, Ohio. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)



**Figure 22.** A typical slotted-wall expander in operation. Courtesy of Anderson International, Cleveland, Ohio. (This figure is available in full color at <http://www.mrw.interscience.wiley.com/biofp>.)

slotted-wall expander in operation is shown in Figure 22. The cracks weaken the strands and cause the strands to break apart into collets of approximately 0.5–2'' in length (68). These collets usually are of the right porosity and length to present no difficulty in solvent extractors. However, the length of the collets, the diameter, and the internal porosity are easily influenced by manipulating extrusion conditions. Preparation for most oilseeds is to crack or flake the seeds and heat them to approximately 60–70°C while reducing the moisture to approximately 8% and being careful not to denature the protein.

## REFERENCES

1. M. N. Riaz, Practical Short Course, Feeds and Petfood Extrusion, Texas A&M University, College Station, Texas, January 25–30, 2004.
2. N. Said, Practical Short Course, Feeds and Petfood Extrusion, Texas A&M University, College Station, Texas, January 25–30, 2004.
3. M. N. Riaz, in M. N. Riaz, ed., *Extruders in Food Application*, Technomic Publishing Co., Connecticut, 2000.
4. X. Serrano and E. Villalbi, *Nutrition Implication*, Bellaterra, Barcelona, Spain, pp. 188–197.
5. W. B. Wijeratne, Practical Short Course, Texturized Vegetable Protein and Other Soyfoods, Texas A&M University, College Station, Texas, September 14–19, 2003.



6. N. Said, in M. N. Riaz, ed., *Extruders in Food Application*, Technomic Publishing Co., Connecticut, 2000.
7. N. Said, *Practical Short Course, Feeds and Petfood Extrusion*, Texas A&M University, College Station, Texas, January 26–31, 2003.
8. J. M. Woodroffe, *American Soybean Association Technical Bulletin FT33* (1995).
9. M. N. Riaz, in R. Guy, ed., *Extrusion Cooking*, CRC Press, Boca Raton, Florida, 2001.
10. E. W. Lusas and L. R. Watkins, *Extrusion Workshop, Soy/Nutritive Foods*, ALADEES (Association Latinoamericana de Extrusion de Alimentos Enriquecidos con Soya), Chihuahua, Mexico, August 23–25, 1990.
11. M. Williams, in M. N. Riaz, ed., *Extruders in Food Application*, Technomic Publishing Co., Connecticut, 2000.
12. M. N. Riaz and M. Williams, in J. L. Kvamme and T. D. Phillips, eds., *Petfood Technology*, Watt Publishing, Mt. Morris, Illinois, 2003.
13. M. N. Riaz, *Proceedings of the Third International Soybean Processing and Utilization Conference*, The Japan Society for Food and Science Technology, and ISPUC-III, Tsukuba, Ibaraki, Japan, October 15–20, 2000, pp. 375–376.
14. G. Rokey and B. Plattner, *Process Description "Full Fat Soy"*, Wenger Manufacturing, Sabetha, Kansas, 2001.
15. N. Said, *Practical Short Course, Feeds and Petfood Extrusion*, Texas A&M University, College Station, Texas, January 26–31, 2002.
16. J. Wisman, *Feed International*, Watt Publishing, Mt. Morris, Illinois, 1984.
17. NRC, *Nutrient Requirement of Poultry*, 9th rev. ed., National Academy Science, Washington, D.C., 1994.
18. Y. E. Zhang, C. M. Parsons, K. E. Weingartner, and W. B. Wijerante, *Poultry Sci.*, **72**: 2299–2308 (1993).
19. H. Stillborn, L. I. Ndife, B. L. Bowyer, H. M. Hellwig, and P. W. Waldroup, *Poultry Misset International*, 1987, pp. 20–23.
20. J. L. Sell, *Iowa State University Poultry Newsletter*, 1984, pp. 3–7.
21. P. W. Waldroup and K. R. Hazen, *Nutrition Report Int.*, **18**, 99–103.
22. J. M. McNab, 26th Annual Georgia Nutrition Conference for the Feed Industry, November 9, 1989.
23. B. J. Marty and E. R. Chavez, *Can. J. Anim. Sci.*, **73**, 411–419 (1993).
24. J. H. Kim, J. D. Hancock, R. H. Hines, and M. S. Kang, *Kansas State University Swine Day*, 1994, pp. 176–181.
25. J. H. Kim, J. D. Hancock, R. H. Hines, and T. L. Gugle, *Kansas State University Swine Day*, 1994, pp. 58–62.
26. J. D. Hancock, R. H. Hines, and T. L. Gugle, *Kansas State University Swine Day*, 1994, pp. 92–94.
27. C. G. Aldrich and N. R. Merchen, in Triple "F" Nutrition Conference, Des Moines, Iowa, 1995.
28. M. I. Socha and L. D. Satter, M.Sc. Thesis, University of Wisconsin, Madison, Wisconsin, 1991.
29. N. Said, *Practical Short Course, Feeds and Petfood Extrusion*, Texas A&M University, College Station, Texas, January 21–26, 2001.

30. M. N. Riaz, Practical Short Course, Snack Food Processing, Texas A&M University, College Station, Texas, March 14–19, 2004.
31. G. Rokey, Practical Short Course, Feeds and Pet Food Extrusion, Texas A&M University, College Station, Texas, January 25–30, 2004.
32. B. Strahm, in M. N. Riaz, ed., *Extruders in Food Application*, Technomic Publishing Co., Connecticut, 2000.
33. G. Hertzal, Practical Short Course, Texturized Vegetable Protein and Other Soyfood, Texas A&M University, College Station, Texas, September, 14–19, 2004.
34. C. Mercier, P. Linko, and J. M. Harper, *Extrusion Cooking*, American Association of Cereal Chemists, St. Paul, Minnesota, 1989, pp. 3.
35. J. M. Faubion, R. C. Hoseney, and P. A. Seib, *Cereal Food World*, **27**, 212–216 (1985).
36. R. C. Miller, in R. B. Fast and E. F. Caldwell, eds, *Breakfast Cereals and How They are Made*, American Association of Cereal Chemist, St. Paul, Minnesota, 1990, pp. 135–193.
37. G. Rokey, Practical Short Course, Aquaculture Feed Preparation, Nutrition and Feed Management, Texas A&M University, College Station, Texas, August 24–29, 2003.
38. J. M. Harper, *Food Tech.*, **32**(7), 67–72 (1978).
39. M. A. Williams, Practical Short Course, Aquaculture Feed Preparation, Nutrition and Feed Management, Texas A&M University, College Station, Texas, August 24–29, 2003.
40. M. A. William, in T. H. Applewhite, ed., *The Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, AOCS Press, Champaign, Illinois, 1989, pp. 100–102.
41. M. A. William, *Feed Management*, **42**, 20 (1991).
42. F. Zang, S. S. Koseoglu, and K. C. Rhee, *J. Amer. Oil Chem. Soc.*, **71**, 1145 (1994).
43. M. Williams, Practical Short Course, Feeds and Petfood Extrusion, Texas A&M University, College Station, Texas, January 25–30, 2004.
44. J. H. Murray, 2nd International Conference on Designing & Engineering New Food Plant, Chicago, Illinois, 1987, p. 8.
45. M. Kouzeh-Kanani, D. J. van Zuilichem, J. P. Roozen, and W. Pilnik, *Lebensmittel Wissenschaft Und Technologie*, **15**, 139–142 (1982).
46. W. B. Wijeratne, Proceedings of the Third International Soybean Processing and Utilization Conference, The Japan Society for Food and Science Technology, and ISPUC-III, Tsukuba, Ibaraki, Japan, October 15–20, 2000, pp. 371–374.
47. A. I. Nelson, W. B. Wijeratne, S. W. Yeh, and L. S. Wei, *J. Amer. Oil Chem. Soc.*, **64**, 1341–1347 (1987).
48. S. E. Hill. M.S. Thesis, University of Illinois, Urbana, Illinois, 1990.
49. N. Said, *INFORM*, **9**(2), 139–144.
50. N. Said, Practical Short Course, Aquaculture Feed Preparation, Nutrition and Feed Management, Texas A&M University, College Station, Texas, August 24–29, 2003.
51. E. W. Lusas and M. N. Riaz, *Extrusion Communique*, **9**: 15–18 (1996).
52. M. N. Riaz, Practical Short Course, Vegetable Oil Extraction, Texas A&M University, College Station, Texas, May 17–22, 1998.
53. E. W. Lusas and M. N. Riaz, *Siposio Brasileiro de Eextrusao de Alimentos: Massas Alimenticias e Productos Extrudados*, Centro de Convencoes da Unicamp, Campinas, Sao Paolo, Brazil, March 27–29, 1995.

54. M. N. Riaz and E. W. Lusas, Proceedings of the 3rd International Conference on the Impact of Food Research on New Product Development, Karachi, Pakistan, HEJ Research Institute of Chemistry, 1997, pp. 69–76.
55. M. N. Riaz, *Freisinger Tage 99. Prospects for Plant Protein in Foods*, Fraunhofer-Institute Verfahrenstechnik und Verpackung IVV, Freisinger, Germany, June 10–11, 1999.
56. E. W. Lusas and M. N. Riaz, Institute of Food Technologists Annual Meeting, New Orleans, Louisiana, June 22–26, 1996.
57. S. Zhu, M. N. Riaz, and E. W. Lusas, *J. Agri. Food Chem.*, **44**, 3315–3318 (1996).
58. M. A. Williams, *Extrusion Communiqué*, **6**: 12 (1993).
59. M. A. Williams, *Feed Management*, **44**, 25 (1993).
60. M. A. Williams and S. Baer, *J. Amer. Oil Chem. Soc.*, **42**, 151 (1965).
61. M. A. Williams, *Oil Mill Gazette*, **95**, 10 (1989).
62. E. W. Lusas and L. R. Watkins, *J. Amer. Oil Chem. Soc.*, **65**, 1109 (1988).
63. L. R. Watkins, W. H. Johnson, and S. C. Doty, *Oil Mill Gazette*, **94**, 30 (1989).
64. M. A. Williams, (to Anderson International Corp), U.S. Patent 4,901,635, 1990.
65. M. A. Williams, *Oil Mill Gazette*, **95**, 10 (1989).
66. M. A. Williams, *INFORM*, **1**, 959 (1990).
67. M. A. Williams, *Oil Mill Gazette*, **10**, 20 (1995).
68. M. A. Williams, *INFORM*, **6**, 289 (1995).

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## *Fatty Acids and Derivatives from Coconut Oil*

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### 1. THE WORLD'S FATS AND OILS OUTPUT

The oleochemical industry is fairly well developed and its future secure because of a reliable supply of raw materials. The world's fats and oils output has been growing rapidly over the past few decades, far beyond the need for human nutrition. The world's production and consumption of natural oils and fats has grown from 79.2 million t in 1990 to 117 million t in 2001. Malaysia, Indonesia, and Argentina are notable excess-supply producers; India, the European Union countries, and China are notable high-demand areas that supplement regional production through imports (1).

The principal raw materials from which the natural fatty acids are derived are tallow, crude tall oil, coconut, palm kernel, and soybean oils.

Many new fatty acid plants have been built in Southeast Asia, which is a major source of coconut and palm oils used as raw materials for the production of C8–C14 fatty acids. Altogether, those countries (excluding China and India) have producers of fatty acids from oil splitting with a capacity of 1.5 million t. Significant amounts of the increasing production are being exported to other areas, including the United States, Western Europe, and Japan (2).

The Philippines, a major producer of coconut oil, established its first oleochemical plant of limited capacity in 1967. The plant produced only 3,000 t/year of coco

fatty alcohols using KAO's technology via the methyl ester route. This was followed by a moderate-size plant, a joint venture between KAO Japan and a local partner commissioned in 1980, producing 25,000 t of coco fatty alcohol and a host of oleochemical derivatives.

COCOCHEM's cocochemical plant, established in 1984, was the biggest in the region during that period, with a splitting capacity of 70,000 t crude coconut oil and producing 36,000 t of fatty alcohols, 9,000 t of glycerine, and various cuts of fatty acids and fatty alcohols. The plant uses the Lurgi technology of the hydrogenation of fatty acids to produce fatty alcohols. Recent data for the Phillipines listed a capacity of 25,000 t of alcohol products from coconut oil by the fatty acid hydrogenation process for Coccochem and 4,000 t for Colgate. Phillipinas Kao produced 30,000 t of alcohol products from coconut oil using the methyl ester hydrogenation process (3). Consumption of fatty acids in the United States, Western Europe, and Japan totaled 2.5 million t in 2001. There was a sharp decline in production and consumption of fatty acid in North America after the events of September 2001. However, growth is expected at the rate of 2.3% for the consumption of split acids for the period 2000–2006. Growth in Western Europe is expected at the rate of 1.2%/yr for the period 2001–2006. Japan's consumption is expected to decline (2).

## 2. THE ROLE OF COCONUT OIL IN THE OLEOCHEMICAL INDUSTRY WORLDWIDE

Coconut oil and palm kernel oil, a coproduct of palm oil, comprise less than 5% of the total natural fats and oils, but they are important feedstocks of the oleochemical industry.

Coconut oil is commercially a major source of lauric acid. Together with palm kernel oil and, to a small extent, babassu oil, it belongs to the so-called lauric oils, which are characterized by their high lauric oil content of approximately 50%.

The lauric oils are highly desirable materials in the oleochemical industry worldwide because of the importance of the lauric fraction especially in the manufacture of soaps and detergents (4).

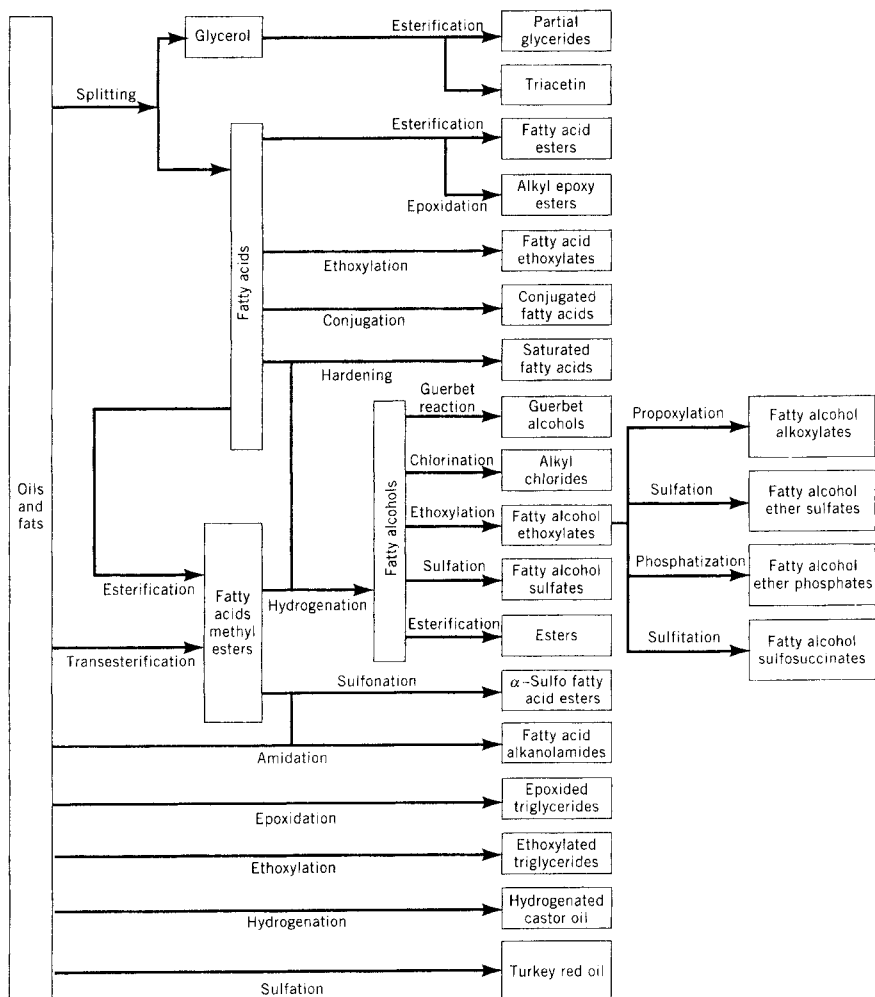
Coconut oil is well positioned because it has the unique advantage of having its fatty acid composition falling within the carbon chain spectrum highly desired by the oleochemical industry where the C12 and C14 fatty acid fractions are sought after. Table 1 shows the fatty acid composition of coconut oil and palm kernel oil.

The caproic to capric (C6–C10) fatty acid fractions comprising approximately 15% are good materials for plasticizer range alcohol and for polyol esters. The latter are used in high-performance oil for jet engines and for the new generation of lubricants. These acid fractions are also the basic material for the manufacture of medium-chain triglycerides, a highly valued dietary fat.

The C12–C18 fatty acid fractions, approximately 85% of the coconut oil fatty acid composition, are the primary raw materials for detergent-grade fatty alcohols. Coconut oil is a primary source of basic oleochemicals and a host of other oleochemical derivatives. Figure 1 indicates some of the major processes by which

**TABLE 1. Fatty Acid Composition of Coconut and Palm Kernel Oils (5).**

Fatty Acid	Formula	Coconut Oil (%)	Palm Kernel Oil (%)
Caproic	$C_6H_{12}O_2$	0.2–0.8	0–1
Caprylic	$C_8H_{16}O_2$	6–9	3–5
Capric	$C_{10}H_{20}O_2$	6–10	3–5
Lauric	$C_{12}H_{24}O_2$	46–50	44–51
Myristic	$C_{14}H_{28}O_2$	17–19	15–17
Palmitic	$C_{16}H_{32}O_2$	8–10	7–10
Stearic	$C_{18}H_{36}O_2$	2–3	2–3
Oleic	$C_{18}H_{34}O_2$	5–7	12–19
Linoleic	$C_{18}H_{32}O_2$	1–2.5	1–2

**Figure 1. Oleochemical raw materials and their derivatives (6).**

various oleochemicals and their derivatives can be obtained from fats and oils. These processes are applicable to coconut oil with the exception of epoxidation and direct sulfation because coconut oil lacks the necessary unsaturation to initiate the two reactions. As can be seen from Figure 1, a great number of oleochemicals can be derived from natural fats and oils. These oleochemicals find increasing use in various applications.

### 3. TYPES OF FATTY ACIDS AND DERIVATIVES FROM COCONUT OIL AND THEIR GENERAL APPLICATIONS

Coconut oil is one of the most important raw materials for the oleochemical industry. The whole range of its fatty acid composition is used as the starting material for a wide variety of oleochemical products. Fatty acids are the building blocks that, with proper selection and application of oleochemistry, are converted to higher valued products.

Coconut oil is considered a saturated oil. From Table 1, it can be seen that coconut oil has approximately 92% saturated fatty acid, from caproic to stearic, and only around 8% unsaturated fatty acid, composed of oleic acid and linoleic acid.

#### 3.1. Types of Fatty Acids from Coconut Oil

Fatty acids obtained by the high-pressure splitting of coconut oil, as discussed later in this Chapter, are distilled and can be fractionated into various fractions or individual cuts. Types commercially available are the following (7).

1. *Whole distilled coconut fatty acid.* A refined product whose fatty acid composition is identical to that of the original oil.
2. *Caprylic, capric acid.* The low-molecular-weight fraction comprising around 55% C8 and 40% C10 fatty acid fractions with small amounts of C6 and C12 fractions.
3. *Topped coconut fatty acid.* The C12–C18 fraction after topping off the C8–C10 fraction.
4. *Lauric, myristic acid.* The medium-chain fatty acid fraction comprising approximately 72% C12 and 26% C14 fatty acid fractions with traces of C10 and C16 fatty acid fractions.
5. *Lauric acid.* A pure-cut C12 fatty acid with a purity of 99% minimum with traces of C10 and C14 fatty acid fractions.
6. *Myristic acid.* A pure-cut C14 fatty acid with a purity of 98% minimum and traces of C12 and C16 fatty acid fractions.

There is a market for individual cuts of fatty acids of high purity. These are highly desired by certain industries, such as the cosmetic industry, and command higher prices.

Table 2 shows the typical composition and properties of these types of fatty acids as produced by United Coconut Chemicals Philippines, Inc.



**TABLE 2. Fatty Acid Product Specifications (7).**

Fatty Acid Products	Product Code	Iodine Value	Acid Value	Saponification Value	Titer (°C)	Unsaponifiable Matter (% maximum)	Color Lovibond 5.25 inches		Approximate Carbon Chain Distribution								
							Y	R	C6	C8	C10	C12	C14	C16	C18	C18 : 1	C18 : 2
							(maximum)	(maximum)									
Whole distilled coconut fatty acid (C8–C18)	Philacid 0818	6–10	268–274	269–275	21–25	0.5	15	1.5	0.5	7.5	6.5	48	18	9	2	7	2
Caprylic–capric acid (C8–C10)	Philacid 0810	0.8 maximum	355–365	356–366	1–5	0.5	—	—	4	55	39	3	—	—	—	—	—
Topped coconut fatty acid (C12–C18)	Philacid 1218	8–12	254–260	254–260	25–29	0.5	15	1.5	—	—	1.0	55	22	10	2.5	8	2
Lauric acid (C12)	Philacid 1200	0.3 maximum	279–281	279–281	42–43	0.5	8	0.8	—	—	0–1	99 min.	0–1	—	—	—	—
Myristic acid (C14)	Philacid 1400	0.3 maximum	245–247	245–247	53–54	0.5	8	0.8	—	—	—	0–2	98 min.	0–2	—	—	—
Lauric–myristic acid (C12–C14)	Philacid 1214	0.5 maximum	268–273	268–273	33–35	0.5	10	1.0	—	—	1	72	26	1	—	—	—

### 3.2. Oleochemical Derivatives from Coconut Oil and Their General Applications

Coconut fatty acids and their various fractions, aside from being used directly, are converted further to other derivatives. Their range of application covers a broad spectrum in the oleochemical industry. As shown in Figure 1, fatty acids can undergo different processes in the manufacture of various oleochemical derivatives. Among the more common products and applications are the following:

*Fatty Acids.* A large volume of coconut fatty acids are used as major components in toilet soap manufacture. Its high lauric content provides the quick lathering properties of toilet soap.

*Fatty Acid Esters.* Different fatty acid fractions can be esterified with a monoalcohol or a polyol to yield various esters. Polyol esters of trimethylol propane or pentaerythritol and C8–C10 fatty acids are the bases for high-performance lubricants. Re-esterification of C8–C10 fatty acid with glycerol yields a medium-chain triglyceride, a low-viscosity, highly stable oil. Medium-chain triglyceride is used as a solvent for flavors, in the surface treatment of dried fruits, and as a high-energy, readily digestible dietary fat.

Esterification with monoalcohol, such as isopropanol and myristic acid, yields isopropyl myristate, an important cosmetic ingredient. Glyceryl monoesters and wax esters find application as food emulsifiers, mold release agents, and lubricants for the plastic industry.

*Fatty Alcohols.* Fatty alcohol is considered a basic oleochemical manufactured by high-pressure hydrogenation of fatty acids or fatty acid methyl esters. The majority of the fatty alcohol produced is further subjected to various processes, such as sulfation, ethoxylation, amination, phosphatization, sulfitation, and others.

Fatty alcohol can be fractionated to separate the C8–C10 fraction, known as plasticizer range alcohol, and the C12–C18, known as the detergent range alcohol. The plasticizer range alcohol is a liquid with good dissolving power. It can be used in a limited way as a solvent for printing inks and lacquers. Esterification with a polycarboxylic acid, such as phthalic anhydride, yields an excellent plasticizer especially for PVC.

The C12–C14 alcohol finds special application as lubricant additives and in the formulation of bearing and hydraulic oils. The C16–C18 fatty alcohol finds application as a defoamer, as a solubility retarder for syndet bars, and as a consistency giving factor in creams, lipstick, pastes, and polishes.

By far, the greatest application of fatty alcohol is in the manufacture of fatty alcohol sulfate and fatty alcohol ether sulfate. These materials possess good foaming properties and ready biodegradability and are extensively used as base surfactants for laundry detergent products, shampoos, dishwashing liquids, and cleaners.

*Polyglycol Ethers.* Polyglycol ethers, produced by the reaction of fatty alcohol with ethylene oxide, constitute the most important class of nonionic surfactants. They possess good wetting properties, produce relatively low foam, and are highly effective at low temperature and low concentration. They are used as textile auxiliaries, in dishwashing liquids, degreasing products, and liquid cleaner formulations.

*Other Specialty Surfactants.* Monoalkyl phosphate, fatty alcohol ether phosphate, and fatty alcohol sulfosuccinate are some of the specialty surfactants derived from fatty alcohol with specific applications in the cosmetics and other chemical industries.

*Fatty Amides.* Cocomonethanolamide and cocodiethanolamide formed by the reaction of fatty acids or esters with monoethanolamine or diethanolamine are popularly used as foam boosters for shampoos and detergent products.

*Fatty Amines.* Fatty amines are the most important nitrogen derivatives of fatty acids. They are produced by the reaction of fatty acids with ammonia and hydrogen. They are the bases for the manufacture of quaternary ammonium compounds used as fabric softeners and biocides. Fatty amine oxides are mild to the skin with good cleaning and foaming properties and find application as a shampoo ingredient. The above mentioned products are but some of the oleochemical derivatives from coconut fatty acids (5).

### 3.3. Principles and Methods in the Manufacture of Oleochemicals

Oleochemicals, by their very name, may be defined as chemicals from oil. These could be natural fats and oils, or oils of petrochemical origin. To have a clear distinction, oleochemicals derived from natural oils are termed natural oleochemicals, whereas those derived from petrochemicals are termed synthetic oleochemicals (8).

The natural oleochemicals are obtained from natural oils with the least change in the structure of the carbon chain fraction. In contrast, synthetic oleochemicals are built up from ethylene to the desired carbon chain fraction or from oxidation of petroleum waxes.

Fats and oils are renewable products of nature. One can aptly call them “oil from the sun” where the sun’s energy is biochemically converted to valuable oleochemicals via oleochemistry. Natural oleochemicals derived from natural fats and oils by splitting or *trans*-esterification, such as fatty acids, methyl esters, and glycerine are termed basic oleochemicals. Fatty alcohols and fatty amines may also be counted as basic oleochemicals, because of their importance in the manufacture of derivatives (8). Further processing of the basic oleochemicals by different routes, such as esterification, ethoxylation, sulfation, and amidation (Figure 1), produces other oleochemical products, which are termed oleochemical derivatives.

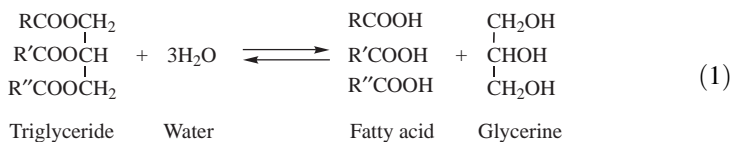
The succeeding discussions detail the processing methods in the manufacture of the basic oleochemicals and their derivatives.

## 4. FATTY ACIDS

Fatty acids and fatty acid methyl esters are probably the most important basic oleochemicals in the oleochemical industry. Fatty acids are used as starting materials for soaps, medium-chain triglycerides, polyol esters, alkanolamides, and many more.

#### 4.1. Chemistry of Fat Splitting

Coconut oil, like any other fat or oil, can be hydrolyzed or split into its corresponding fatty acids and glycerine. The reaction is represented by the following equation:



In the case of coconut oil, the fatty acid fractions are C8 to C18 with a trace amount of C6.

Fat splitting is essentially a homogeneous reaction that proceeds in stages. The fatty acid radicals are displaced from the triglyceride one at a time from tri to di to mono. An incomplete splitting will thus contain monoglycerides and diglycerides as well as triglycerides. During the initial stage, the reaction proceeds slowly, limited by the low solubility of the water in the oil phase. In the second stage, the reaction proceeds fairly rapidly brought about by the greater solubility of water in the fatty acids. The final stage is characterized by a diminishing reaction rate as the fatty acids liberated and the glycerine byproduct reach equilibrium conditions.

Fat splitting is a reversible reaction. At the point of equilibrium, the rates of hydrolysis and re-esterification are equal. The glycerine byproduct must be withdrawn continuously to force the reaction to completion.

Increasing the temperature and pressure accelerates the reaction because of the increased solubility of the water in the oil phase and to its higher activation energy. Temperature, in particular, exerts a significant effect. An increase in temperature from 150°C to 220°C increases water solubility by two to three times.

The presence of small amounts of mineral acids, such as sulfuric acid or certain metal oxides, such as zinc or magnesium oxide, accelerates the splitting reaction. These metal oxides are true catalysts. They also assist in the formation of emulsions.

#### 4.2. Fat-Splitting Processes

There are at least four known methods of fat splitting. These are (1) Twitchell process, (2) batch autoclave process, (3) continuous process, and (4) enzymatic process.

*Twitchell Process.* The Twitchell Process (9) is one of the earliest processes developed for fat splitting. It is still used in a small way because of its low initial cost and simplicity of installation and operation. However, it is no longer of great commercial importance, due to its high-energy consumption and poor product quality. The process makes use of the Twitchell reagent and sulfuric acid to catalyze the hydrolysis. The reagent is a sulfonated mixture of oleic or other fatty acid and naphthalene.

The operation is carried out in a wooden, lead-lined, or acid-resistant vat where the fat, water amounting to approximately half the fat, 1–2% sulfuric acid, and 0.75–1.25% Twitchell reagent are boiled at atmospheric pressure for 36–48 h, using open steam. The process is usually repeated two to four times, drawing off the glycerine–water solution after each stage. At the last stage, water is added and the mixture boiled to wash off any remaining acid.

The long reaction period, high-steam consumption, and discoloration of the fatty acids are disadvantages of the process and are the reasons for its limited use today.

*Batch Autoclave Process.* The batch autoclave process is the oldest commercial method used for splitting higher grade stock to produce light-colored fatty acids. It is also more rapid than the Twitchell process, taking about 6–10 h to complete. Distillation is normally performed to remove glyceride esters.

This process uses a catalyst, usually zinc, magnesium, or calcium oxides. Of these, zinc is the most active. About 2–4% catalyst is used, and a small amount of zinc dust is added to improve the color of the fatty acids.

The autoclaves are tall cylinders, 1220–1829 mm in diameter and 6–12 m high (10) made of corrosion-resistant alloy and fully insulated. An injection of live steam provides the agitation, although some, in addition, use mechanical agitators.

In operation, the autoclave is charged with the fat, water amounting to about half the fat, and the catalyst. Steam is blown through to displace any dissolved air, and the autoclave is closed. Steam is admitted to raise the pressure to 1135 kPa and is injected continuously at the bottom while venting a small amount to maintain the desired agitation and operating pressure. More than 95% conversion is achieved after 6–10 h. The contents of the autoclave are transferred to a settling tank where two layers are formed: the fatty acid upper layer and the glycerine (sweetwater) lower layer. The fatty acid layer is drawn off, treated with mineral acid to split the soap formed, and finally washed to remove traces of the mineral acid.

*Continuous Process.* The continuous countercurrent, high-pressure fat-splitting process, more popularly known as the Colgate–Emery process, is the most efficient of the current methods of fat hydrolysis. The high temperature and pressure used permit short reaction time. Full countercurrent flow of oil and water produces a high degree of splitting without the need of a catalyst. However, a catalyst may be used to increase reaction rate further.

The splitting tower is the heart of the process. Most splitting towers have the same configuration and basically operate the same way. Depending on the capacity, the tower can be 508–1220 mm in diameter and 18–25 m high and is made of corrosion-resistant material such as stainless steel 316 or Inconel alloy designed to operate at a pressure of about 5000 kPa.

Figure 2 shows a Lurgi single-stage countercurrent splitting plant. The deaerated fat is introduced by means of a sparge ring, around 1 m from the bottom with a high-pressure pump. Water is introduced near the top at a ratio of 40–50% of the weight of the fat. The high splitting temperature (250–260°C) ensures adequate dissolution of the water phase into the fat so that mechanical means for bringing the two phases into contact are not required.

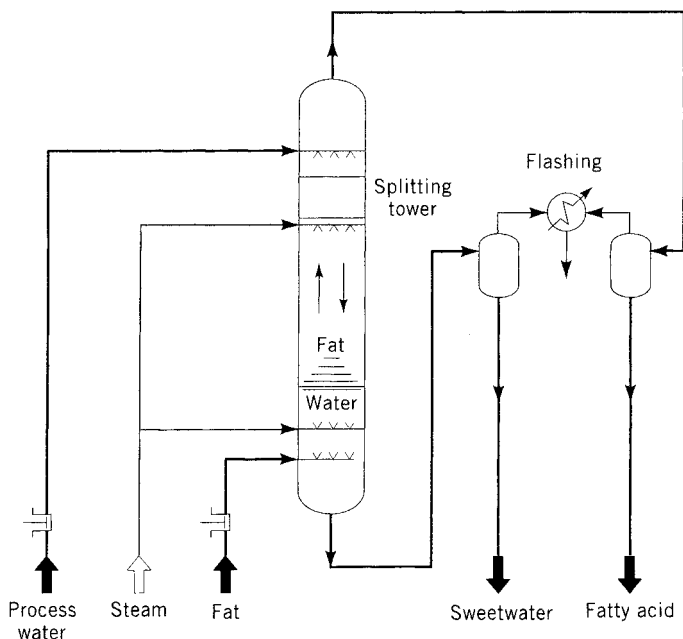


Figure 2. Single-stage countercurrent splitting (11).

The empty volume of the tower is used as the reaction compartment. The crude fat passes as a coherent phase from the bottom to the top through the tower, whereas the heavier splitting water travels downward as a dispersed phase through the mixture of fat and fatty acid. Degrees of splitting up to 99% can be reached (11).

The continuous countercurrent high-pressure process splits fats and oils more efficiently than other processes in a reaction time of only 2–3 h. Little discoloration of the fatty acids occur. As a result of the efficient internal heat exchange, this process affords high steam economy.

The utilities consumption per ton of feed is as follows (11).

Steam (6000 kPa)	190 kg
Cooling water (20°C)	3 m <sup>3</sup>
Electrical energy	10 kWh
Process water	0.6 m <sup>3</sup>

*Enzymatic Splitting.* Fats and oils can hydrolyze in the presence of natural enzymes. Fat splitting through the use of lipolytic enzymes had been carried out in experimental trials. However, at present, this process is of doubtful importance because of its high cost and long reaction time.

The enzymatic splitting of fats and oils by lipase from *Candida Rugosa*, *Aspergillus niger*, and *Rhizopus arrhizus* had been studied at a temperature range of 26–46°C for a period of 48–72 h. About 98% splitting is possible (12). There are

still major problems to be solved in the further development of this process before it becomes commercially viable.

### 4.3. Fatty Acid Distillation and Fractionation Operations

The fatty acids produced from the various fat-splitting processes are purified and separated into fractions or even individual fatty acids by distillation and fractionation.

*Fatty Acid Distillation.* Distillation of crude fatty acids removes both the low and high boiling impurities as well as odor bodies. Fatty acids are extremely sensitive to heat, oxidation, and corrosion effects. This is due to the reactive acid group at the end of the long carbon chain. These factors are taken into consideration in the design of the distillation unit and its operating parameters. Distillation is carried out under high vacuum and lower temperatures and with the shortest residence time allowable.

Technical design of most distillation units features high vacuum with no allowance for air leakage, effective heating to achieve short contact time, good circulation for effective mass transfer between vapor and condensate, and steam economy. Internal arrangements of the column vary among suppliers with the ultimate purpose of achieving the design objectives. The basic steps in processing are about the same. Figures 3 and 4 show Badger's and Lurgi's continuous fatty acid distillation plants.

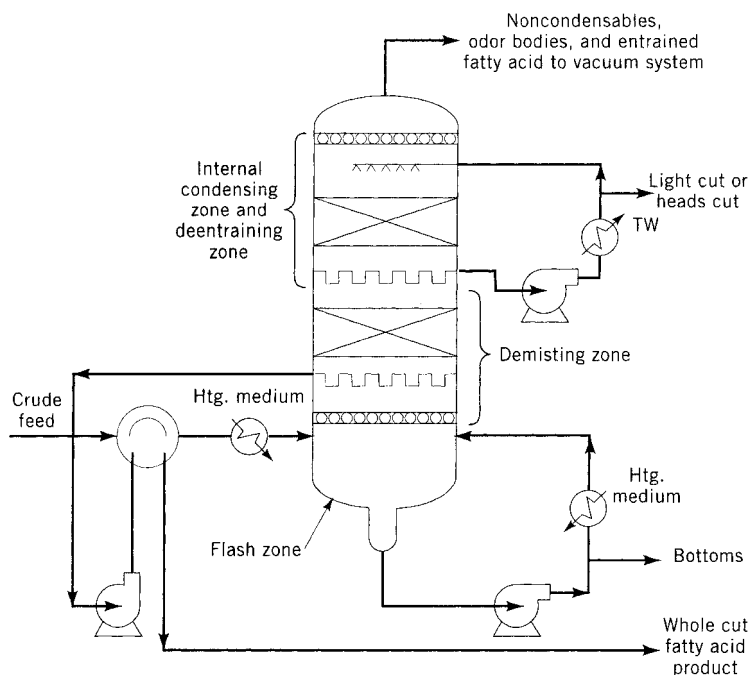


Figure 3. Badger's continuous fatty acid distillation (13).

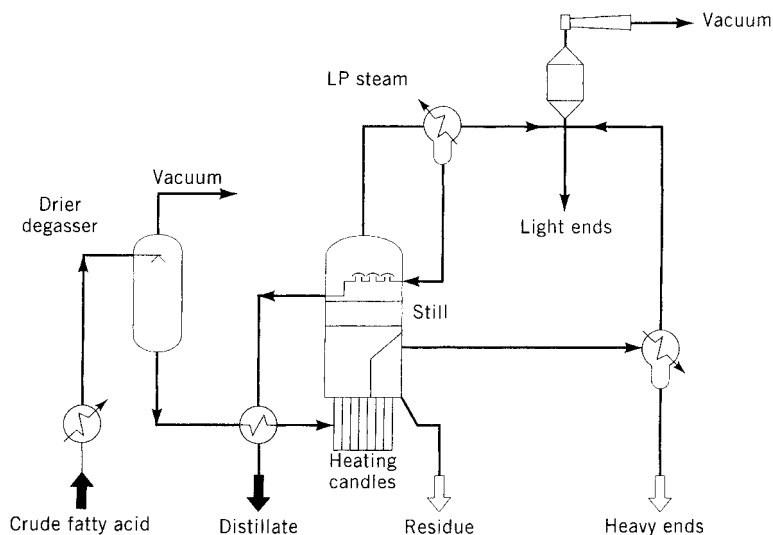


Figure 4. Lurgi's continuous fatty acid distillation (11).

Crude fatty acid is predried and degassed under vacuum and fed to the distillation unit, which is operated at a vacuum of 1.2 kPa or less and a temperature of approximately 200°C. Modern stills use thermal oil or high-pressure steam as the heat source. Stripping steam is provided to improve circulation and reduce partial pressure, thus lowering the temperature and reducing degradation losses. The steam facilitates the removal of low boiling impurities as well as odor and color bodies from the vapor exiting the system and from the light ends. The distilled fatty acid has an almost water white color and is free of the major impurities. The heavy ends consist of the higher boiling components, usually of lower quality, which can either be withdrawn separately or recycled directly for redistillation (11). The bottoms or residue are charred viscous polymerized material, which is disposed of by blending with heavy residual oil and used as boiler fuel. It may also find use as an asphalt additive.

Lurgi (11) establishes an approximate utility consumption per ton of crude fatty acid feed for plant sizes from 50 t to 200 t per day as follows:

Heating steam (5000 kPa)	370 kg
Steam (300–1000 kPa)	150 kg
Cooling water (20°C)	15 m <sup>3</sup>
Electrical energy	5 kWh
Export steam (300 kPa)	120 kg

*Fatty Acid Fractionation.* Today's market is becoming more and more demanding. Specific fractions with purity in excess of 99% are in demand for special



products. Fortunately, developments in fractionation technology can now readily meet this challenge. Purities of 99.5% can be achieved for pure cuts of C12 or C14 fraction.

Fractionation makes it possible to separate the fatty acid mixtures into narrower cuts or even individual components. The detergent-grade feedstock C12–C18 fraction is separated from the whole cut by topping off the C8–C10 fraction. The middle cut, C12–C14, can be further fractionated from the C12–C18 cut through the use of multistage fractionation employing two or more columns.

All commercially available fractionation processes can give comparable performances. Basically, each process uses a deaerator, heat source, fractionation column, condensing system, and vacuum source. These processes may differ in the internals of the column, in the manner of evaporation and condensation, and in the piping arrangement for better heat recovery, but they can give equally good results.

The column internals are designed to provide intimate contact of the vapor and the distillate, together with the lowest pressure drop possible through the column. Various arrangements are employed, such as bubble caps, exchange trays, regular packings, or structured packings. Lurgi uses the Thorman tray, which claims high-separation efficiency, flexible loading, and long-term trouble-free operation. Structured packings, such as the Mellapak or Sulzer or the Glitch, are finding favor in modern columns. These packings are claimed to have high-separation efficiencies and minimal pressure drops of 10–50 Pa mbar per stage. As a result of their structure, there is little liquid holdup. Slight changes in operating parameters can easily upset the equilibrium conditions, and it is thus best to operate the column at steady conditions.

The vacuum system is provided independently for each column and usually consists of a mechanical vacuum pump and a steam ejector to achieve the highest vacuum. A centralized vacuum system is not recommended, as a process upset in one stage can readily affect the other stages. The heat source can be a shell-and-tube heat exchanger using thermal oil. Figure 5 shows a Lurgi fractionation system that has two columns to produce three separate fractions.

## 5. METHYL ESTERS

Fatty acid methyl esters play a major role in the oleochemical industry. Methyl esters have increasingly replaced fatty acids as starting materials for many oleochemicals. They are used as chemical intermediates for a number of oleochemicals, such as fatty alcohols, alkanolamides,  $\alpha$ -sulfonated methyl esters, and many more. One other potential use of methyl esters is as a substitute for diesel oil (14). Methyl esters are clean burning with no sulfur dioxide emission. Although the heat of combustion is slightly lower, there is no engine adjustment necessary and there is no loss in efficiency.

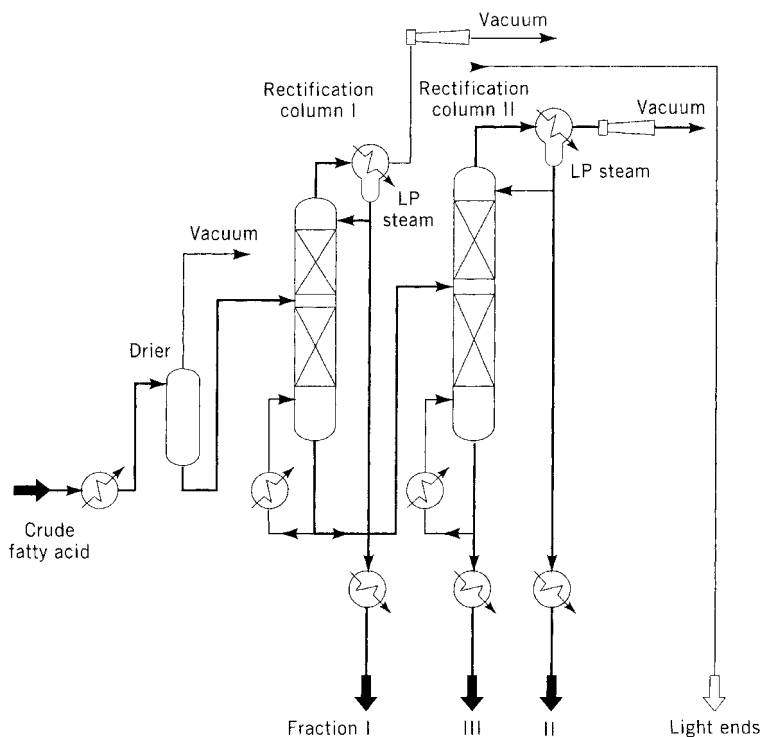


Figure 5. Fatty acid fractionation (11).

### 5.1. Advantages of Methyl Esters

The use of methyl esters instead of fatty acids as starting materials for many oleochemicals is rapidly gaining ground because of the following advantages (14):

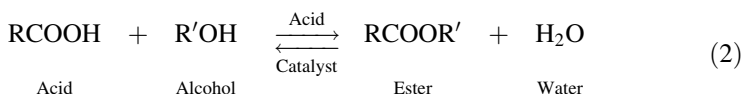
1. *Lower energy consumption.* The production of methyl esters requires much lower reactor temperatures and pressures than the splitting of fats and oils to obtain fatty acids.
2. *Less expensive equipment.* Methyl esters are noncorrosive and are produced at lower operating pressure and temperature conditions, hence they can be processed in carbon steel equipment; fatty acids are corrosive and require heavy-duty stainless steel equipment.
3. *More concentrated glycerine byproduct.* Transesterification is a dry reaction and yields concentrated glycerine, while fat splitting produces glycerine water, which has more than 80% water; thus, recovery of the latter uses more energy.
4. *Easier to distill-fractionate.* Esters are more easily distilled because of their lower boiling points and are more heat stable than the corresponding fatty acids.

5. *Superior to fatty acids as chemical intermediates in some applications.* In the production of alkanolamides, esters can produce superamides, with more than 90% purity against fatty acids, which can only produce amides with a purity of 65–70% amides.
6. *Easier to transport.* As a result of their chemical stability and noncorrosive property, esters are far easier to transport than fatty acids.

One major consideration is the need to recover and recycle the methanol. As methanol is a toxic and an explosive material, use of explosion proof equipment and extra safety precautions are mandatory.

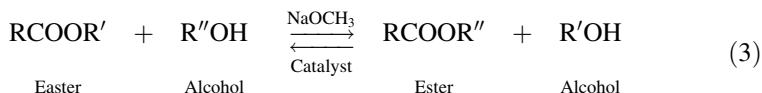
## 5.2. Chemistry of Esterification and Transesterification

*Esterification.* Esterification is the reaction of an acid with an alcohol in the presence of a catalyst to form an ester. The reaction is expressed by the general equation:



Generally, acid catalysts like sulfuric acid are employed. Esterification is a reversible reaction. Thus, water must be removed to drive the reaction to the right and obtain a high-ester yield.

*Transesterification.* Transesterification, on the other hand, is the displacement of the alcohol from an ester by another alcohol in a process similar to hydrolysis, except that an alcohol is used instead of water. This reaction, cleavage of an ester by an alcohol, is more specifically called alcoholysis and is represented by the general equation:

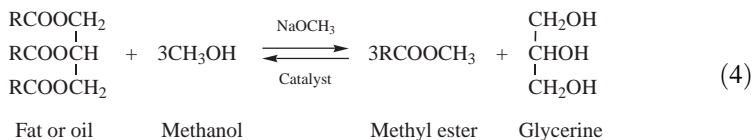


In this case, a new ester is formed. Generally, alkaline catalysts are used with sodium methylate said to be the most effective, although sodium hydroxide can also be used.

Transesterification is an equilibrium reaction. To shift the reaction to the right, it is necessary to use a large excess of alcohol or to remove one of the products from the reaction mixture. The second option is preferred where feasible, as in this way, the reaction can be driven to completion.

*Transesterification* is a general term. More specifically, if methanol is used, the reaction is termed *methanolysis*. Methanol is generally used because it is cheap, but other alcohols can be used.

The reaction with fats and oils and using methanol is represented by the general equation:



Although the equation reflects the overall reaction, the reaction usually consists of a series of consecutive reversible steps. The sequence of steps is triglyceride to diglyceride to monoglyceride with 1 mole of methyl ester formed at each cleavage (14).

The stoichiometry of the reaction requires 3 moles of methanol for each mole of triglyceride. When up to 100% excess methanol is used, the conversion rate is at its highest (14). The catalysts used are alkaline catalysts. Sodium methyllate is commonly used, although KOH or NaOH are also used.

The conversion rate is also strongly influenced by the reaction temperature. However, given enough time, the reaction will also proceed to near completion even at room temperature. Generally, the reaction is conducted close to the boiling point of methanol (14).

Impurities in the oil used also affect conversion rates. Under the same conditions, 67–84% conversion using crude vegetable oils can be obtained, compared with 94–97% when using refined oils (14). The free fatty acids in the original oils interfere with the catalyst. However, under conditions of high temperature and pressure, this problem can be overcome.

### 5.3. Methods of Manufacture

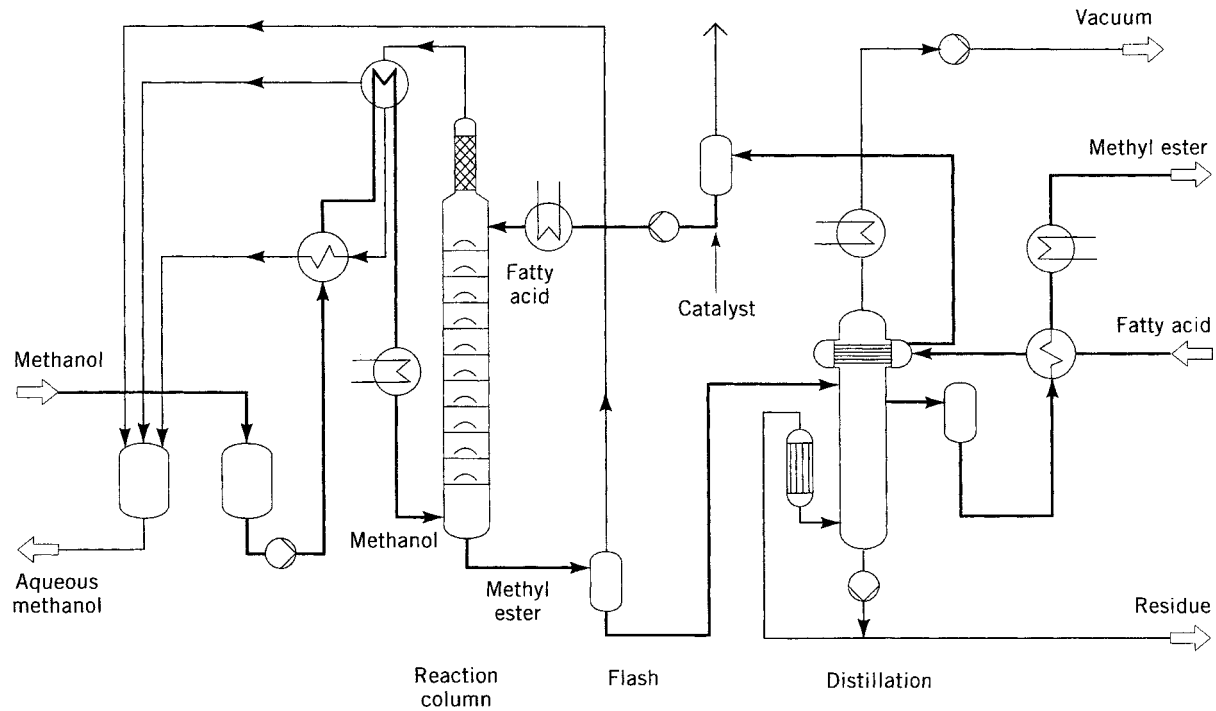
Methyl esters of fatty acids can be made either by esterification of the fatty acids or transesterification of the triglycerides using methanol.

*Esterification Processes.* There are two general methods used for esterification: the batch process and the continuous process. Esterification can be done batchwise under pressure at a temperature of 200–250°C. As it is an equilibrium reaction, the water is removed continuously to obtain a high-ester yield (15).

Henkel has developed a continuous countercurrent esterification using a double-plate reaction column. The technology is based on the principle of an esterification reaction with the simultaneous absorption of the superheated methanol vapor and desorption of the methanol–water mixture (15).

Figure 6 shows the Henkel continuous process of fatty acid esterification. The reaction is carried out at a pressure of approximately 1000 kPa and a temperature of 240°C. One advantage of this process is the excess methanol can be kept significantly lower at 1.5:1 molar ratio of methanol:fatty acid versus the batch process at 3–4:1 molar ratio. The methyl ester, having undergone distillation, does not require further refining. The excess methanol is rectified and reused (15).

The continuous esterification process is superior to the batch process, in that the same high yield can be obtained in a much shorter dwell time and with substantially



**Figure 6.** Manufacture of methyl esters by esterification (15).

less excess methanol. The esterification process is a preferred method for the production of esters from specific fatty acids (15).

*Transesterification Processes.* Transesterification of fats and oils is the most commonly used process for the manufacture of methyl esters, except in cases where methyl esters of specific fatty acids are needed.

Triglycerides can readily be transesterified batchwise at atmospheric pressure and at a slightly elevated temperature of approximately 60–70°C with an excess of methanol and in the presence of an alkaline catalyst. The mild reaction conditions, however, requires the removal of free fatty acids from the oil by refining or pre-esterification before transesterification. This pretreatment is not required if the reaction is carried out under high pressure (9000 kPa) and high temperature (240°C). Under these conditions, simultaneous esterification and transesterification take place (15). The mixture at the end of the reaction is allowed to settle. The lower glycerine layer is drawn off whereas the upper methyl ester layer is washed to remove entrained glycerine and is then processed further. The excess methanol is recovered in the condenser, sent to a rectifying column for purification, and recycled.

Continuous transesterification is well suited for large capacity requirements. Depending on the quality of the feedstock, the unit can be designed to operate at high pressure and high temperature or at atmospheric pressure and slightly elevated temperature.

Figure 7 shows a Henkel process flow diagram operated at 9000 kPa pressure and 240°C using unrefined oil as feedstock. Unrefined oil, methanol in excess, and catalyst are metered and heated to 240°C before feeding into the reactor. The bulk of the excess methanol is flashed off as it leaves the reactor and is fed to a bubble tray column for purification. The recovered methanol is recycled into the system.

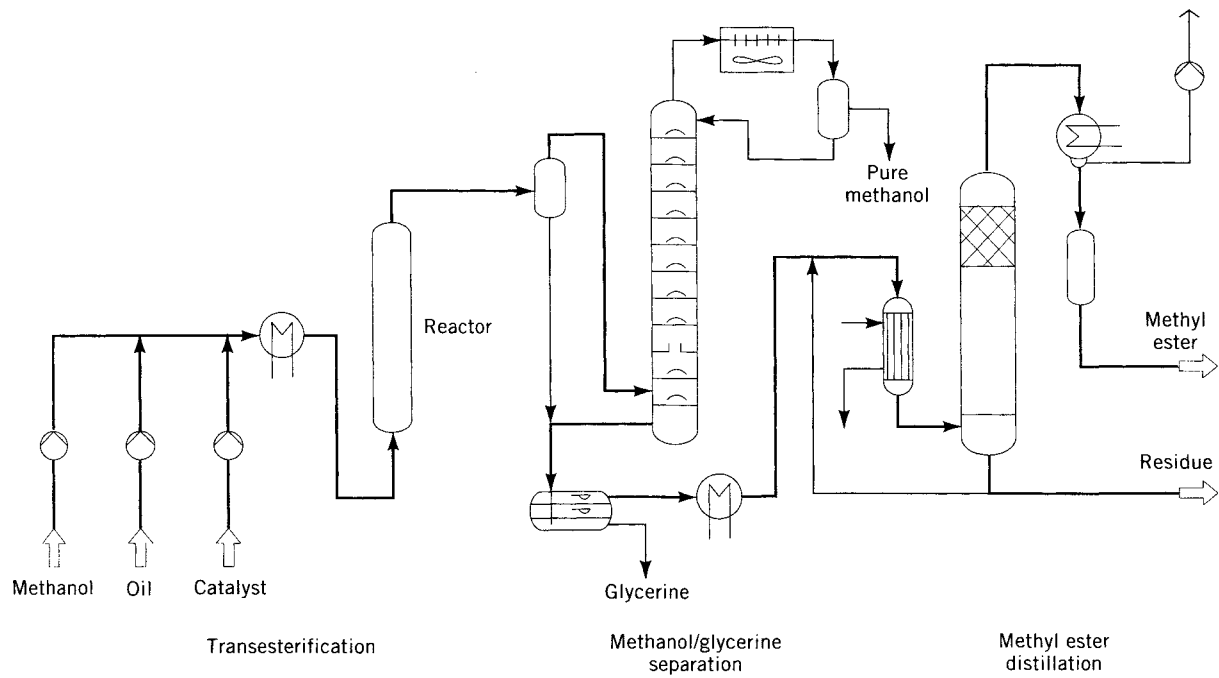
The mixture from the reactor enters a separator where the glycerine in excess of 90% concentration is removed. The methyl ester is subsequently fed to a distillation column for purification. Further fractionation into special cuts may follow if desired (15).

Figure 8 shows a Lurgi process flow diagram operating at normal pressure. The process requires the use of a degummed and deacidified feedstock. The refined vegetable oil and methanol are reacted in a two-stage mixer-settler arrangement in the presence of a catalyst. The glycerine produced in the reaction, dissolved in the surplus methanol, is recovered in the rectification column. Most of the entrained methanol and glycerine are recovered from the methyl ester in the countercurrent scrubber. The methyl ester can be further purified by distillation (11).

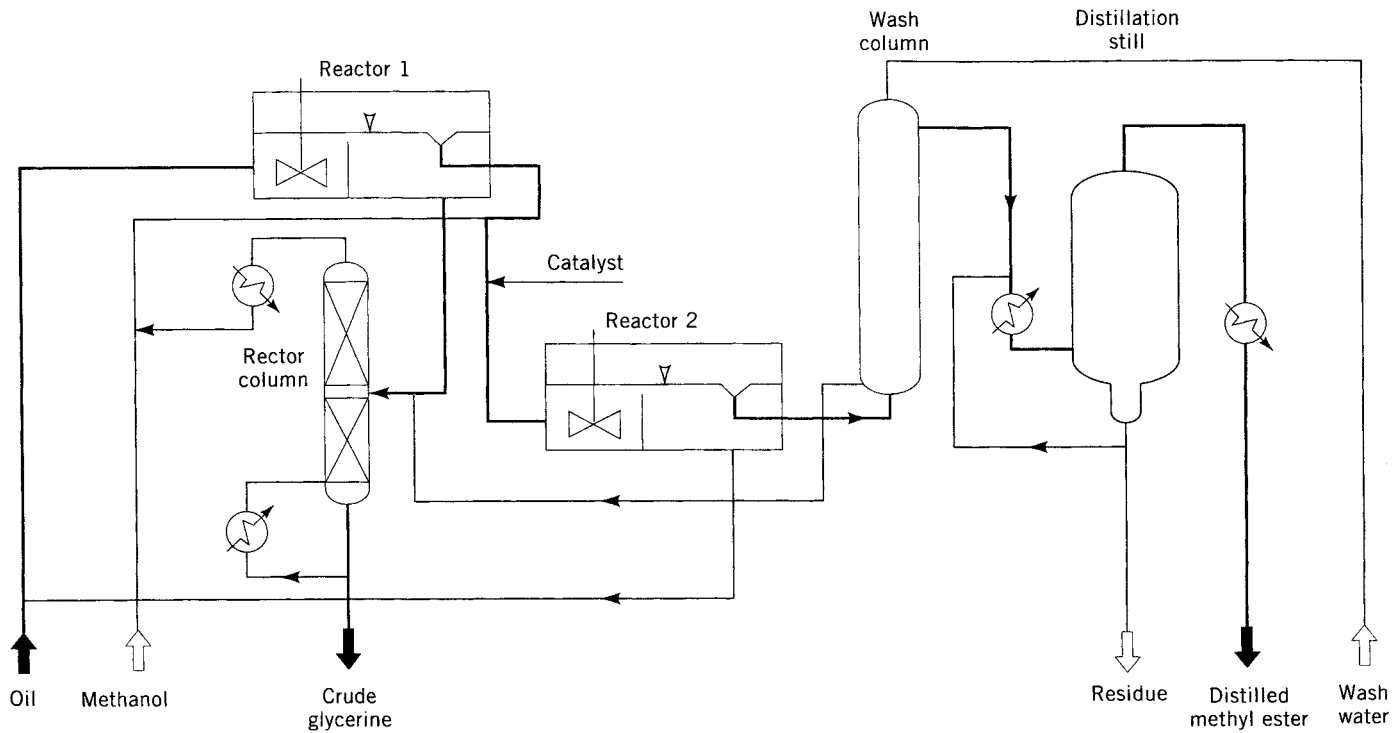
#### 5.4. Materials and Utilities Consumption Per Ton of Ester

Lurgi's (11) technical data for plant sizes of 30–250 tpd are as follows:

Methanol	142 kg
Refined PKO or CNO	995 kg



**Figure 7.** Manufacture of methyl ester by transesterification (15).



**Figure 8.** Manufacture of methyl esters by transesterification (11).



Steam (400 and 1000 kPa)	250 kg
Heat energy (thermal oil)	420,000 kJ
Cooling water (20°C)	25 m <sup>3</sup>
Electrical energy	10 kWh

Lion Corporation of Japan developed a continuous transesterification process using unrefined feedstock known as the ES process. The free fatty acid in the oil is pre-esterified by passing the feedstock and methanol through a packed column of a special catalyst resin, after which transesterification is conducted through a two-stage reactor. A high conversion rate of more than 99% is claimed (16).

## 6. FATTY ALCOHOLS

Fatty alcohols make up one of the major basic oleochemicals enjoying a continuing growth rate. As a primary raw material for surfactants, its growth parallels increasing economic prosperity and improvement in the standard of living. Fatty alcohols are increasingly gaining favor as the surfactant raw materials of choice because of their biodegradability as well as availability from renewable resources.

Fatty alcohols can be produced from natural oils, or synthesized from petrochemicals. The current world supply of fatty alcohols is equally divided between natural and synthetic. However, the use ratio of natural: synthetics varies with each region. The overall world ratio is projected to go in favor of natural fatty alcohols. This can be attributed to the increasing supply and price stability of the lauric oils. The primary source of lauric oil is in the Southeast Asian region where most processing plants have been established.

### 6.1. Methods of Manufacture

The different methods used in the production of fatty alcohols from various sources are (1) hydrolysis of wax esters using animal fats, (2) sodium reduction process using fats and oils, (3) Ziegler process using ethylene, (4) oxo process using olefins, (5) catalytic hydrogenation of fatty acids and methyl esters from fats and oils, and (6) direct hydrogenation of fats and oils.

Although not applicable to coconut oil, a brief description of the first method is included for historical reference; brief descriptions of the second and sixth methods are also included. The third and fourth methods use raw materials of petrochemical origin and will not be discussed here. However, it should be mentioned again that approximately 50% of the world's supply of fatty alcohols is produced from these two methods. A more detailed discussion of the fifth method is given later.

*Hydrolysis of Wax Esters.* Fatty alcohol was first obtained from the hydrolysis of wax esters of animal origin, primarily spermaceti from sperm whale. With the worldwide ban on whale hunting, however, this source is no longer available.

Spermaceti wax is split by heating with concentrated sodium hydroxide above 300°C. The liberated alcohol, approximately 35% yield, is separated by vacuum

distillation from the soap and water formed. The product consists essentially of cetyl, oceyl, and arachidyl alcohols (5).

*Sodium Reduction Process.* In 1909, Beauvaut and Blanc discovered the sodium reduction process of manufacturing fatty alcohols from coconut esters. Fatty alcohol plants established in the 1930s used this process. Although the basic process is relatively simple, actual plant operations in handling the reactants and products are much more complex. The hazards of handling metallic sodium is an additional factor that caused this process to gradually lose out to the catalytic hydrogenation process.

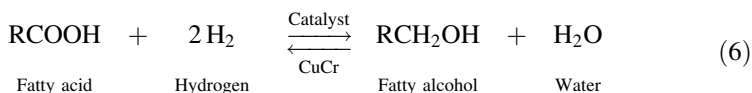
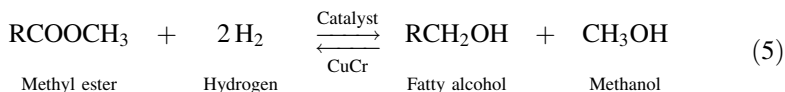
*Direct Hydrogenation.* A more recent process, developed and patented by Henkel KGaA, is the direct hydrogenation of natural oils or triglycerides. However, because of the severe conditions employed, a major portion of the valuable glycerine byproduct is also hydrogenated to lower valued propylene glycol and propyl alcohol. The loss of glycerine, the higher consumption of hydrogen gas, and increased use of catalyst do not justify commercial production as yet (15).

## 6.2. Fatty Alcohols from Natural Fats and Oils

The manufacture of fatty alcohols from natural oils can be made either through the methyl ester route or fatty acid route. These two methods are both well established and are strongly competitive with each other. Commercial plants around the world use either route. Figure 9 shows the manufacturing routes of fatty alcohols from natural fats and oils.

## 6.3. Chemistry of Hydrogenation

The hydrogenation of methyl esters and of fatty acids to form fatty alcohols is given by the following general equations.



The direct hydrogenation of fatty acids as shown in Equation 6 is not used in a large commercial scale due to the need for higher reaction temperatures resulting in lower yields, and to the damaging effects on the catalyst. Traditionally, the fatty acids are first converted to esters before hydrogenation (15, 17).

Lurgi (17) has managed to overcome this problem by simultaneous esterification of the fatty acid with the alcohol and the hydrogenation of the ester in the same reactor as given by the following equations:

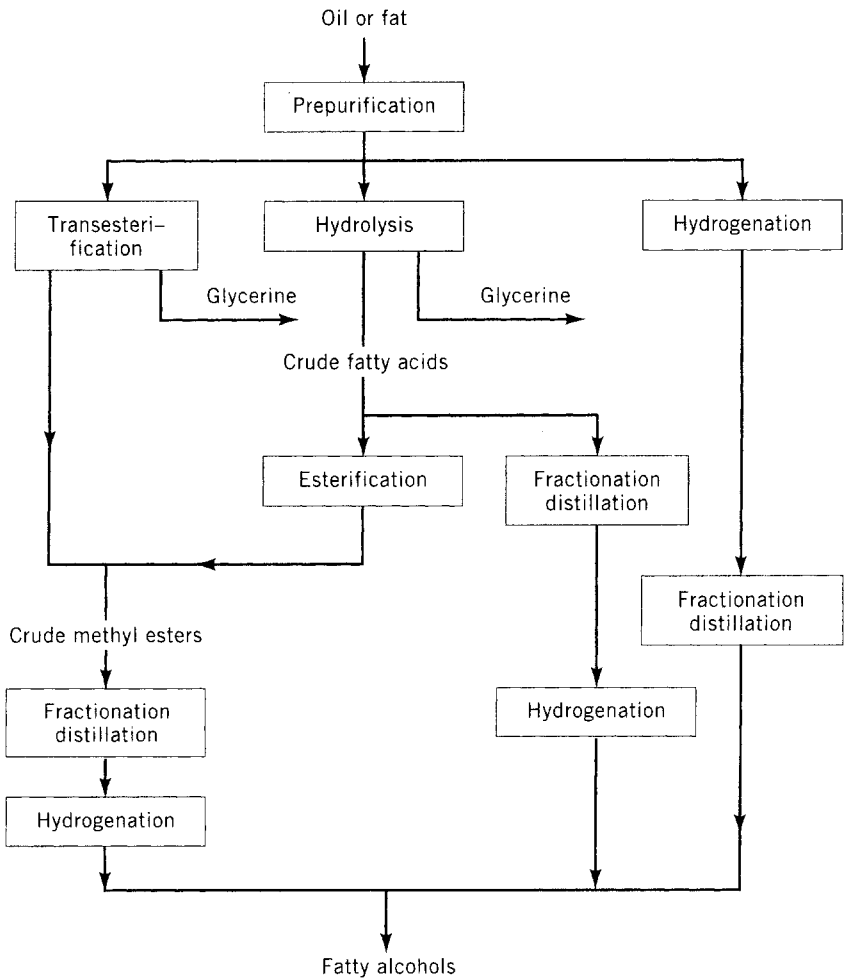
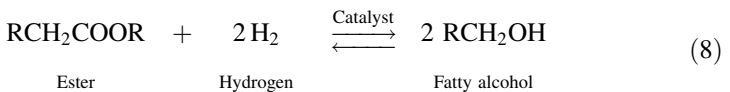
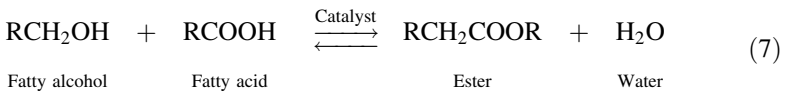


Figure 9. Manufacturing routes of fatty alcohols from natural fats and oils (15).



The fatty acids are introduced into a large volume of circulating fatty alcohols, more than 250 times the volume of the acids, so that the esterification is effected rapidly without the damaging effect of the fatty acids on the catalyst (17).

## 6.4. High-Pressure Hydrogenation Processes

The fractionated methyl esters can be converted into fatty alcohols by the high-pressure hydrogenation process in the presence of a catalyst. Usually, copper chromite catalyst is used. Copper chromite catalyst also converts any unsaturated carbon double bonds so that only saturated fatty alcohols are formed. If unsaturated fatty alcohols are desired, a special zinc-bearing catalyst is employed.

The hydrogenation process is carried out at 25,000–30,000 kPa and a temperature of 250–300°C in a tubular column. Depending on the method by which the catalyst is employed, the hydrogenation can be conducted using the suspension process or the fixed bed process.

*Suspension Process.* In the suspension process, the catalyst is slurried with a small amount of the methyl ester and then fed into the reactor together with the rest of the ester.

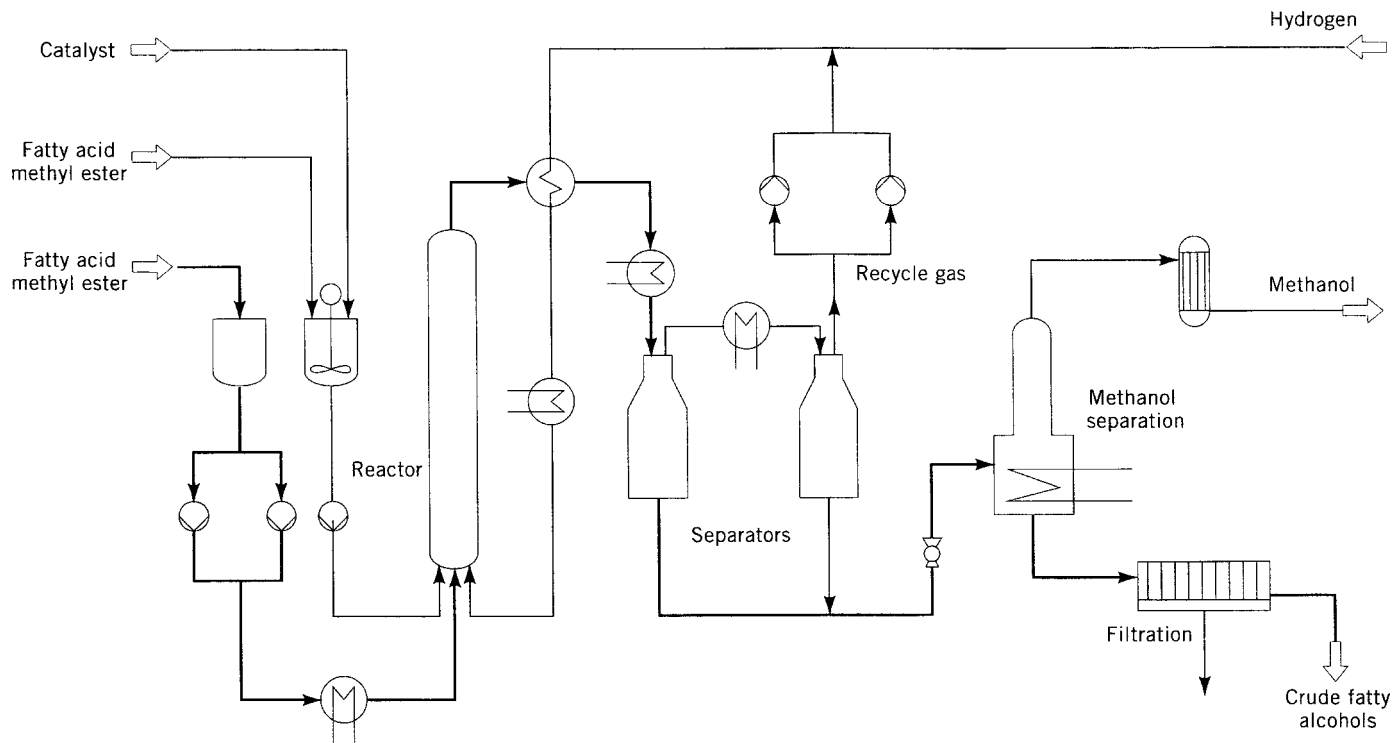
Figure 10 shows a simplified process flow diagram of the hydrogenation of methyl ester using the suspension process. The methyl ester and hydrogen gas are preheated separately. The copper chromite catalyst is pre-slurried in a small amount of methyl ester and is introduced simultaneously with the preheated methyl ester and hydrogen gas into the base of a tubular reactor (without packing). The catalyst is metered into the system in an amount of at least 2.0%. Approximately 20 moles of hydrogen gas per mole of ester feed are used. The hydrogen gas, bubbling through, serves also to agitate the reactants.

The reaction is conducted at approximately 25,000–30,000 kPa and 250–300°C. As hydrogenation is an exothermic reaction, care must be taken to control the reaction temperature to minimize side reactions leading to the formation of undesirable hydrocarbons. From the column, the reaction mixture is cooled, separating the hydrogen gas from the alcohol–methanol mixture. The hydrogen gas is recycled, and the alcohol–methanol mixture goes to the methanol-stripping unit where, at lower pressure, the methanol is stripped off, recovered, and recycled to the esterification or *trans*-esterification section. The crude fatty alcohol is filtered to separate the catalyst. A major portion of the catalyst is recycled, so that the consumption averages 0.5–0.7% of the alcohol produced.

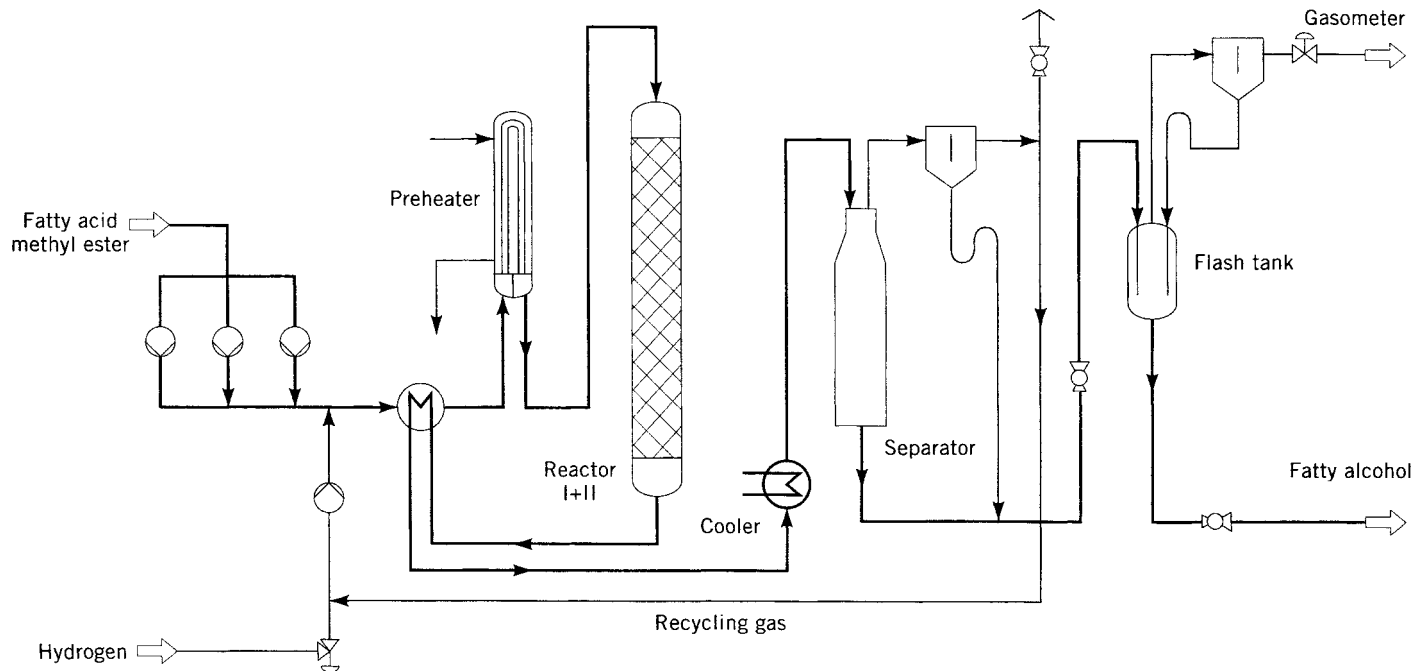
The filtered alcohol is subsequently treated with caustic soda to form soap with any unreacted ester. The alcohol is finally distilled to strip off any hydrocarbons formed, which amount to about 2–3%. The soap remains in the still bottoms (15).

*Fixed Bed Process.* The fixed bed process, as differentiated from the suspension process, has the catalyst “fixed” as a bed inside the reactor. The catalyst is either compact pelletized or supported on a carrier. Figure 11 is a simplified process flow diagram for the hydrogenation of methyl esters using the fixed bed process.

The reaction is conducted in the vapor phase where a part of the organic feed is vaporized in an excess of hydrogen gas (20–25 moles) through a peak heater before passing through the fixed catalyst bed. The hydrogenation is carried out at 20,000–30,000 kPa and 200–250°C. The reaction mixture leaving the reactor is cooled and is separated into the gas and liquid phases. The gas phase, mostly excess hydrogen,



**Figure 10.** High-pressure hydrogenation of fatty acid methyl esters—suspension process (15).



**Figure 11.** High-pressure hydrogenation of fatty acid methyl esters—fixed bed process (15).

is recycled. The liquid phase is expanded into a flash tank to strip off the methanol from the fatty alcohol.

The operating conditions are comparatively mild, so that the fatty alcohol produced does not require further processing. The overall yield is 99% with hydrocarbons and unreacted ester not exceeding 1.0%. Catalyst consumption is claimed to be below 0.3% (15).

*The Suspension Process Versus the Fixed Bed Process.* The fixed bed process requires a somewhat higher capital investment, as it needs larger reaction vessels, gas recirculation pumps, and pipe work due to the higher volume of hydrogen gas used. The suspension process, on the other hand, requires additional equipment for the separation of catalyst, distillation of the crude fatty alcohol, and rework of the unreacted methyl ester.

In terms of raw materials consumption, the fixed bed process has a higher yield and the catalyst consumption is only half as much. The fatty alcohol via the fixed bed process has a higher quality. However, the quality of the fatty alcohol via the suspension process can also be brought up to the same level by further distillation (15).

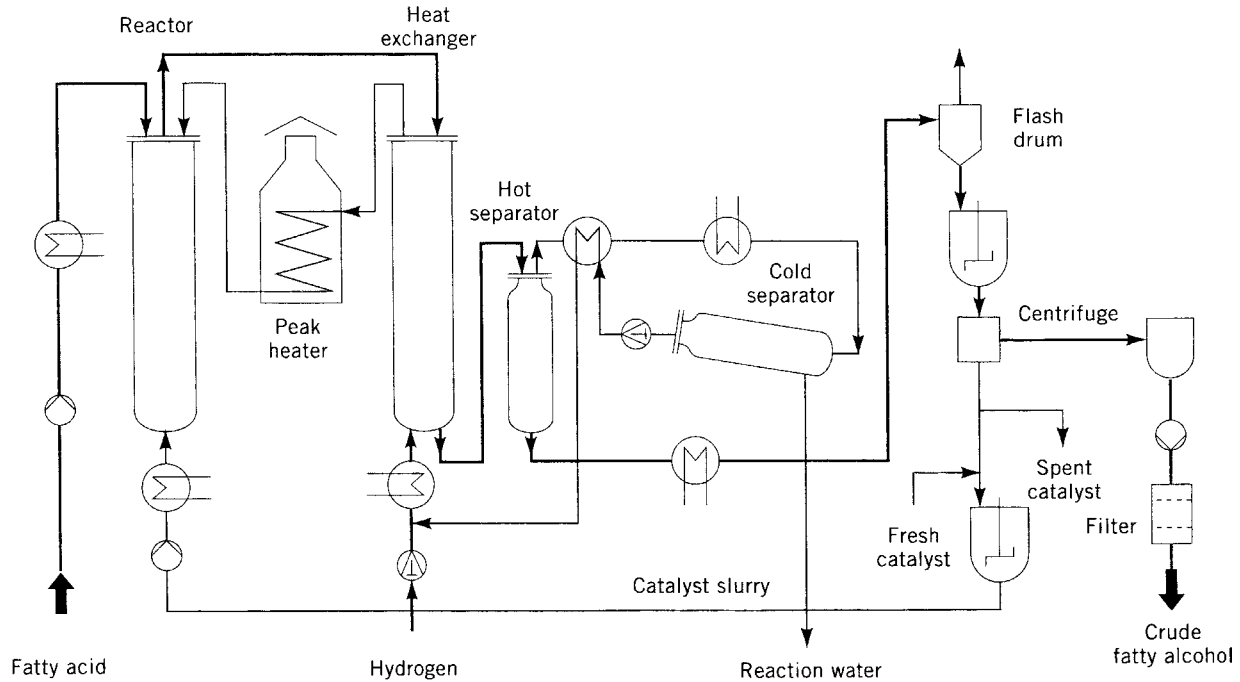
## 6.5. Lurgi Fatty Acid Hydrogenation Method

The Lurgi method, which is a suspension process, makes possible the direct hydrogenation of fatty acids to fatty alcohols overcoming the damaging effects of the fatty acids on the copper-bearing catalyst. This is achieved by a two-step reaction. The first reaction is the esterification of the fatty acid with the fatty alcohol to yield the ester and water. The second reaction is the hydrogenation of the ester formed to produce 2 moles of the alcohol. Both reactions occur simultaneously in the same reactor. The large volume of the fatty alcohol recirculated, more than 250 times the fatty acid feed, effectively dilutes the feed, thus providing for an optimum condition for rapid and complete esterification (17). Figure 12 is a simplified process flow diagram of the Lurgi method of converting fatty acids to fatty alcohols.

The hydrogenation takes place inside the high-pressure reactor where the preheated materials—fatty acid feed, recirculated fatty alcohol with the slurried catalyst, and hydrogen gas—are fed continuously. The reaction is carried out at approximately 30,000 kPa and 280°C. The heat of the product mixture leaving the reactor is recovered by the recirculating hydrogen gas through the heat exchanger, after which the products are separated through a two-stage cooling–expansion system.

The gas phase (essentially excess hydrogen gas, some low boiling alcohols, and reaction water) is separated from the liquid alcohols in the hot separator. This mixture is cooled further in the cold separator, where the low boiling alcohols and reaction water are condensed and separated. The excess hydrogen gas is recycled into the system.

The liquid alcohols from the hot separator are pumped into a flash drum where the dissolved hydrogen is released and recycled with the make up hydrogen. The catalyst is separated from the crude fatty alcohol using a centrifugal separator. Part of the catalyst is replaced with fresh catalyst to maintain the activity and is



**Figure 12.** Fatty alcohol synthesis via hydrogenation of fatty acids (18).



recirculated with the fatty alcohol. The clear phase portion from the centrifugal separator is passed through a polishing filter to remove all traces of suspended solids. The resulting crude alcohol undergoes further distillation to remove hydrocarbons and may undergo fractionation if desired (18).

## 6.6. Materials and Utilities Consumption Per Ton of Crude Fatty Alcohol

Technical data for plant capacities of 50+ t/day are (11) as follows:

Distilled coco fatty acid	1050–1100 kg
Steam (ca. 15 bar)	170 kg
Cooling water (20°C)	27 m <sup>3</sup>
Electric energy	130 kWh
Fuel gas	1.1 × 10 <sup>6</sup> kJ
Catalyst (copper chromite)	5 kg
Hydrogen (0°C, 100 kPa, depending on hydrogen purity and fatty acid specification)	230–300 m <sup>3</sup>
Boiler feed water	185 kg
Export steam (ca. 4 bar)	120 kg

*Material Balance.* Figure 13 shows the overall material balance for the manufacture of 1000 MT of distilled fatty alcohol starting from distilled coco fatty acids.

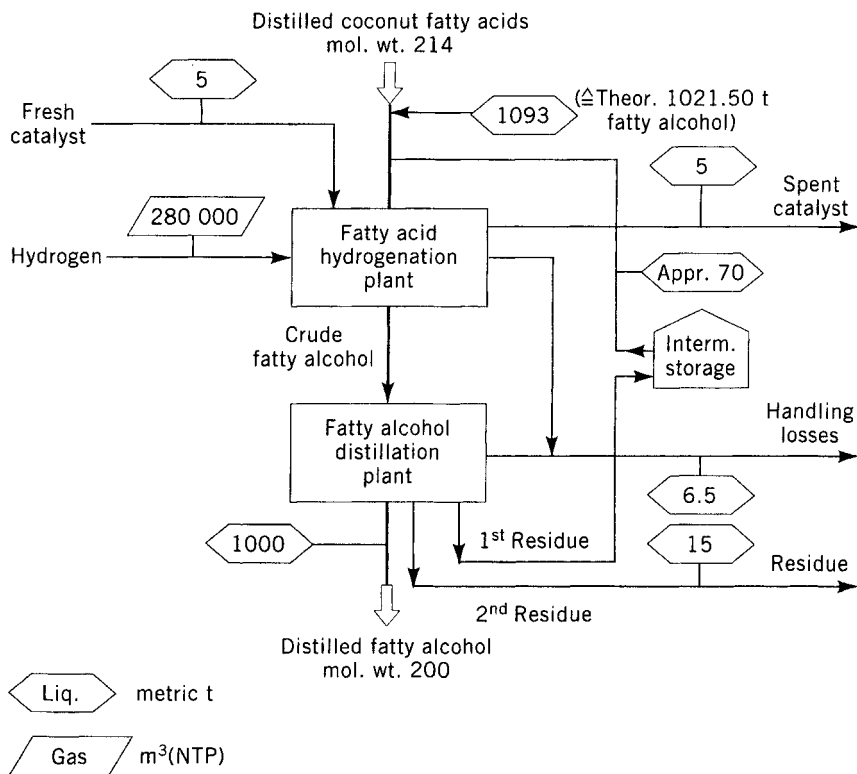
## 6.7. Fractionation of Fatty Alcohols

Crude fatty alcohol resulting from the various hydrogenation processes described previously may undergo fractionation to produce different cuts of alcohol. Figure 14 shows a flow diagram for the fractionation of crude fatty alcohols.

## 7. GLYCERINE

Glycerine is a tribasic alcohol occurring in nature in the form of triglycerides, which are glyceryl esters of fatty acids. All fats and oils are made up of triglycerides. Coconut oil contains approximately 13.5% glycerine, the highest among commercial fats and oils. The glycerine contents of other fats and oils range from 9 to 12%.

Glycerine is an important high-value byproduct obtained from the processing of fats and oils. It can also be synthesized from petrochemicals. However, with the increasing output of vegetable fats and oils worldwide, glycerine, from this latter source, plays a significant role in the world supply of this material. The production of glycerine from petrochemicals will not be discussed here.



**Figure 13.** Overall material balance for the manufacture of 1000 MT distilled fatty alcohol from distilled coconut fatty acids (18).

## 7.1. Glycerine from Fats and Oils

Glycerine is obtained by cleavage of triglycerides using several methods:

1. Saponification of fats and oils using caustic soda to form soap and glycerine.
2. Splitting or hydrolysis of fats and oils in the presence of a catalyst to produce fatty acids and glycerine; the sweetwater formed contains 16–20% glycerine.
3. Transesterification, which results in the displacement of the glycerine from triglycerides when fats and oils are reacted with methanol in the presence of a catalyst to produce methyl esters; in this process, glycerine concentration in excess of 90% can be obtained because this is basically a dry reaction.

Both splitting or hydrolysis and transesterification were discussed earlier; therefore, only saponification will be covered briefly in this section.

*Saponification.* When fats and oils are saponified with caustic soda, the reaction is represented by the following equation:

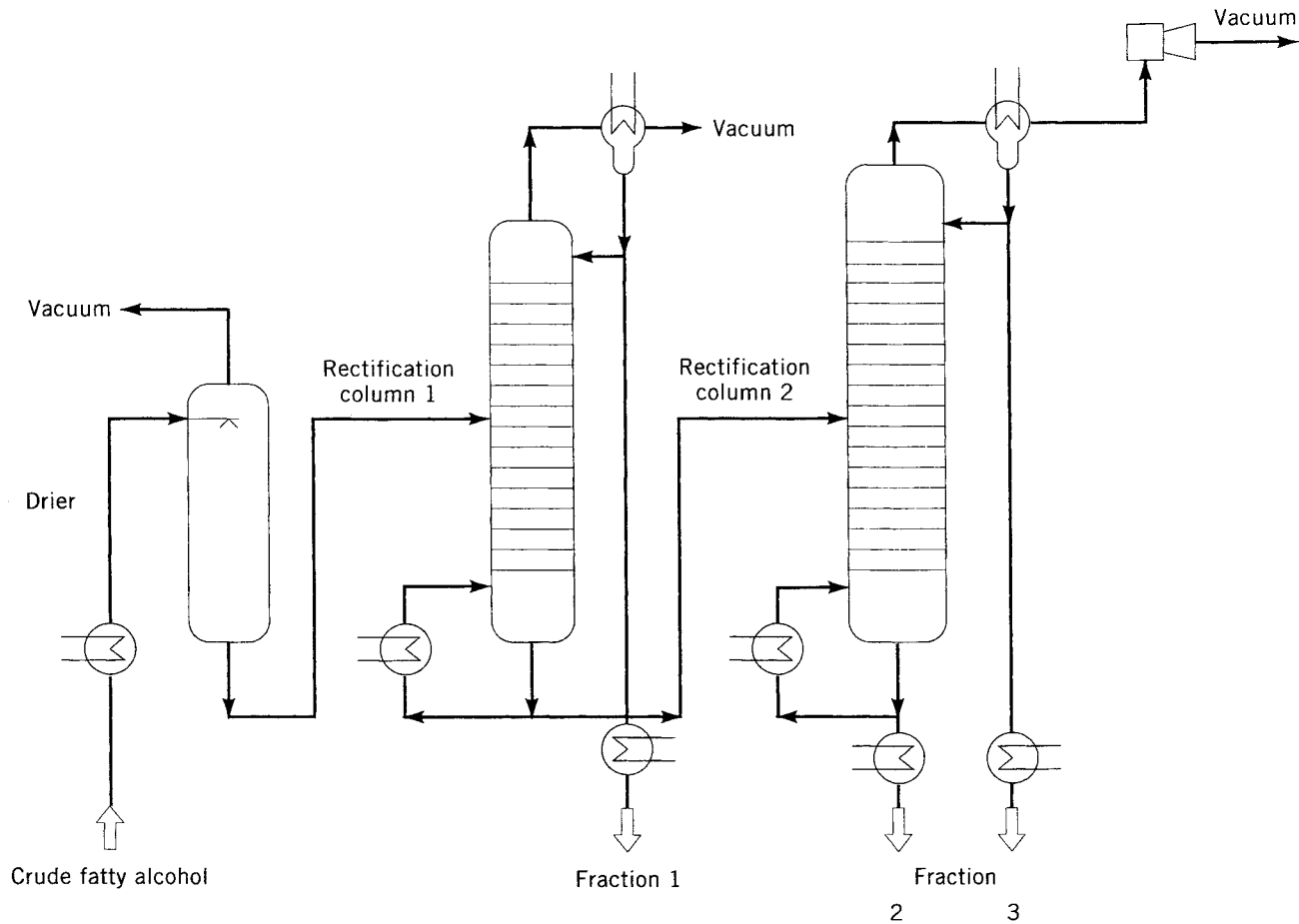
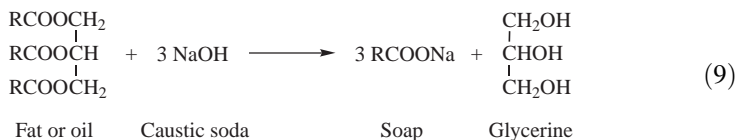


Figure 14. Fractionation of fatty alcohol (18).



Saponification yields soap and spent soap lye containing 8–12% glycerine.

Fats and oils can be saponified via the full-boiled process. The saponification process can be briefly described as follows. A refined blend of fats and oils is charged into a kettle and a predetermined amount of soap lye with sufficient concentrations of caustic soda, and salt is added. The mixture is boiled vigorously, using closed steam coils, until saponification is almost complete. The amount of caustic soda added is deliberately made less than the stoichiometric requirement, to ensure that the spent soap lye containing the glycerine has minimum alkalinity. The caustic soda in the spent soap lye is neutralized during subsequent treatment of the latter.

The salt used in the lye is necessary to keep the soap in the grained state and to facilitate the separation of the soap and the spent lye. The latter is drawn off after settling and transferred to the glycerine-processing section. Meanwhile, the soap undergoes further boiling and countercurrent multiple washing to complete the saponification and recover as much glycerine as possible before being finished to neat soap.

Continuous soap boiling, which is widely practiced, makes use of multiple washing columns or centrifuges. The objective is to effect optimum glycerine recovery with less lye bulk.

## 7.2. Methods of Purification

The glycerine obtained as a byproduct from the three processes noted above contains impurities and must undergo further processing to purify and concentrate it. Commercially, there are two known processes in use:

1. The conventional method of purification of the spent soap lye or sweetwater by acid–alum or ferric chloride treatment followed by evaporation, distillation, deodorization, and bleaching.
2. The ion exchange method of purification followed by evaporation and polishing.

*Conventional Method.* The conventional method is widely popular. Several manufacturers of equipment using this method are established worldwide. Among these are Lurgi and Feld & Hahn from Germany, Wurster & Sanger and Badger from the United States, and C.M.B. (Ballestra) and Mecchaniche Moderne from Italy. Figures 15–18 show some glycerine processing flow diagrams.

Basically, the steps used to produce high-grade glycerine with up to 99% purity are essentially the same. The spent soap lye, or sweetwater, is acidified with mineral

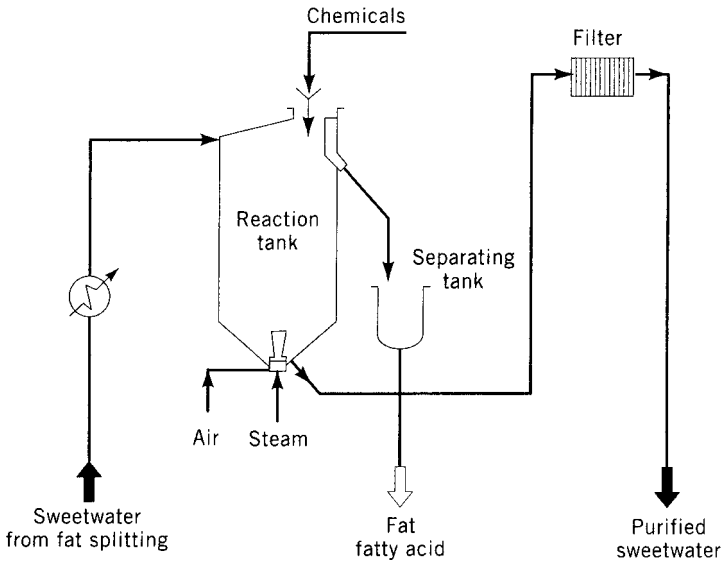


Figure 15. Sweetwater purification (batch) (11).

acid to split any dissolved soap and release it as fatty acid, which is skimmed off. The pH is adjusted, and alum or ferric chloride as a flocculant is then added to entrap the impurities, after which the mixture is filtered. The filtered lye is adjusted to a pH of 6.5 or higher before it is fed to the evaporator.

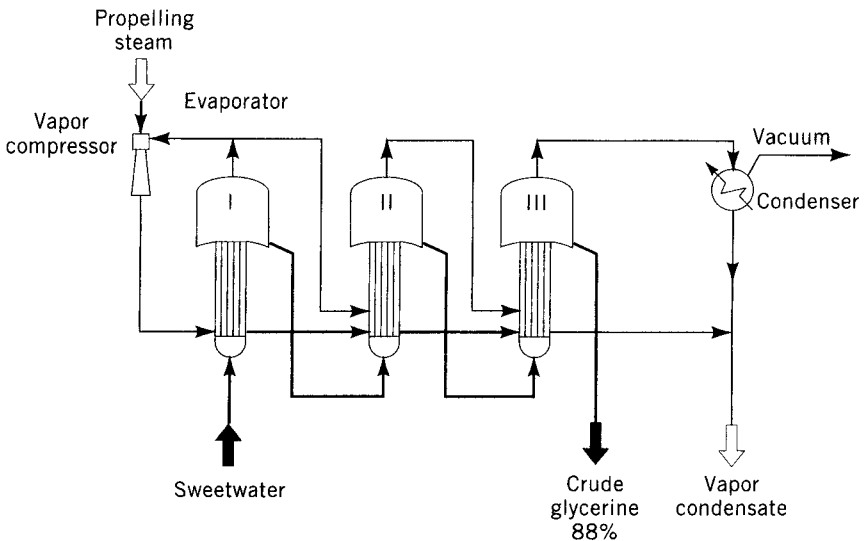
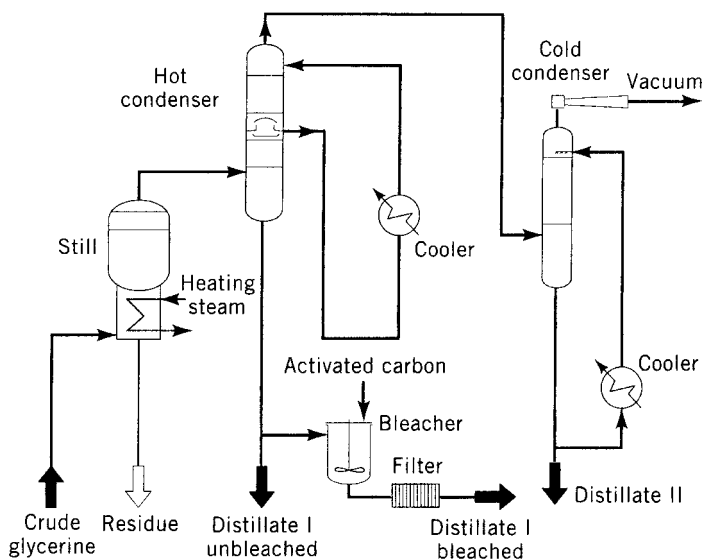


Figure 16. Sweetwater evaporation (triple effect with vapor compression) (11).



**Figure 17.** Glycerine distillation and bleaching (11).

The type of evaporator, either single or multiple effect, depends on the volume of materials to be processed. The crude glycerine obtained after evaporation has a concentration of 80–88% and undergoes further processing. Salt, which separates out during the evaporation of treated glycerine soap lye, accumulates in a salt pot positioned underneath the evaporator. The salt is recovered and recycled to the soap-making section.

The crude glycerine from the evaporator is distilled under a high vacuum of 660–1330 Pa absolute. Live steam is injected during distillation to keep the distilling temperature below 200°C. This is done to prevent the polymerization and decomposition of glycerine, which start at 204°C. Controlled condensation of the vapor separates the glycerine from the water vapor.

The condensed glycerine, with up to 99% purity, undergoes deodorization by blowing steam into it in a deodorization vessel under high vacuum. The glycerine is finally bleached with activated carbon and filtered to yield pharma-copoeia-grade glycerine with a purity of 99+%. Lower quality distillate near the tail end run is collected and sold separately as technical-grade glycerine.

**Ion Exchange Method.** The ion exchange method of glycerine purification is gaining wide acceptance because of the simplicity of operations and low energy consumption. This method is made possible by the availability of suitable ion exchange resins and is particularly suitable for refining of glycerine obtained from fat splitting or from transesterification, which is practically salt free. When salt is high, as in the spent lye from saponification, pretreatment to remove the salt is necessary.

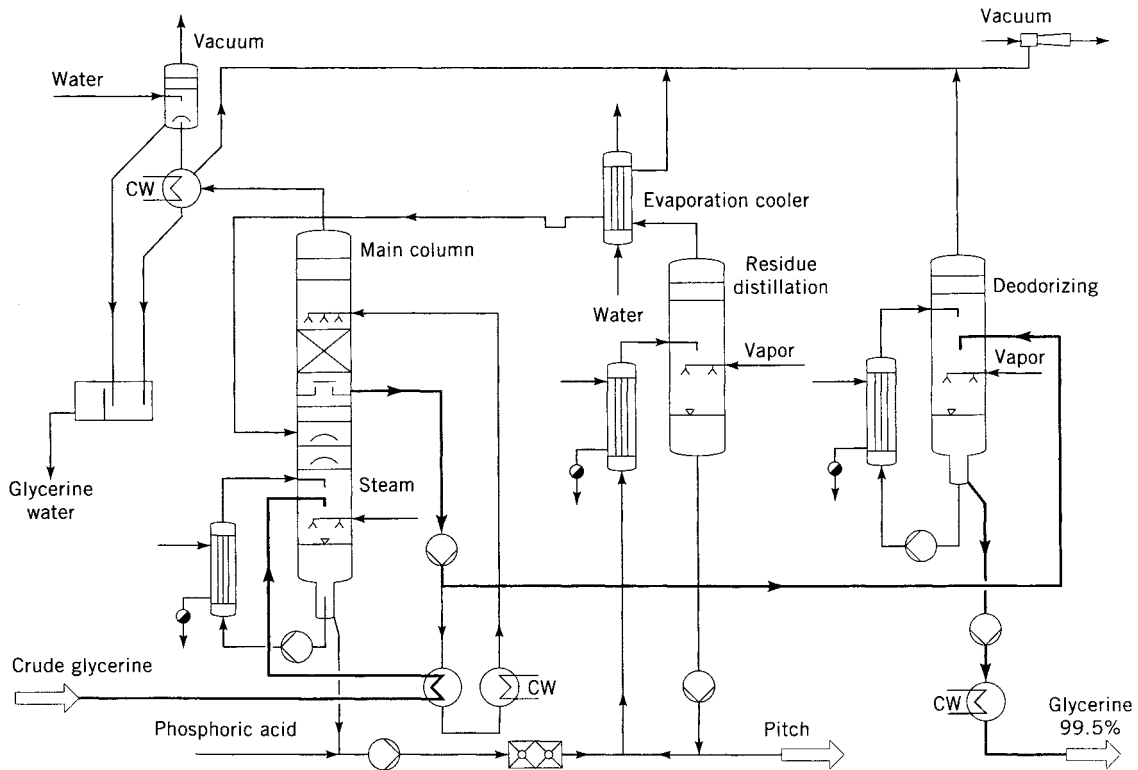


Figure 18. Glycerine distillation, Wurster & Sanger.

Purification by ion exchange involves the passage of the prefiltered material through successive beds of a strong cation, a weak anion, and a mixed bed of strong cation–anion resins. The ion exchange units operate efficiently with dilute solutions containing 25–40% glycerine.

Several resins are available commercially. Rohm and Haas has Amberlite 1R 120 or IRA 900 or Duolite C20 as strong cation; IRA 93SP or Duolite A 378 as weak anion; and the mixed bed polishing resins C20 MB and A 101D as strong cation–strong anion (19). Bayer, Germany supplies Lewatit S100, MP 64, and MP 500 resins for glycerine purification.

Passage through the resin beds eliminates traces of free fatty acids, color, odor bodies, and other mineral impurities present. Subsequent concentration of the purified glycerine solution is through evaporation using multiple-effect evaporators to produce glycerine with a purity of more than 99%. Final decolorization by passage through an activated carbon bed or treatment with activated carbon followed by filtration yields pharmaceutical- or C.P.-grade glycerine. Figure 19 shows a flow sheet for the ion exchange method of purifying glycerine.

*Conventional Method Versus Ion Exchange Method.* The conventional method offers greater flexibility but uses more energy, considering that much water must be evaporated and that the glycerine is distilled at a higher temperature. The ion exchange method requires less energy but cannot be used for sweetwater containing high chlorides. Chlorides foul up the ion exchange resin.

## 8. MONOALKYL PHOSPHATES

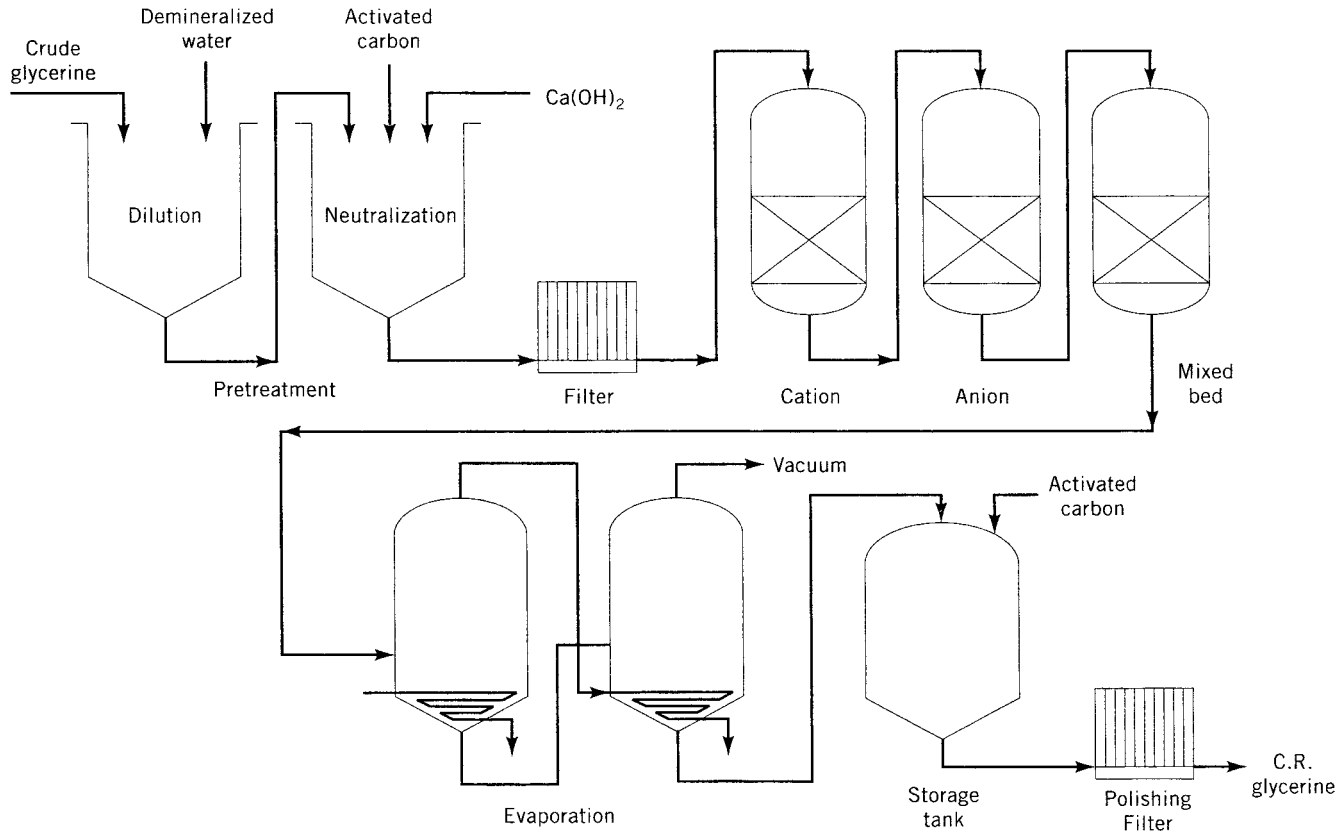
Monoalkyl phosphate and phosphate esters are special types of phosphorus-containing anionic surfactants that are of great industrial importance. They are used for flameproofing, as antistatic for textiles, for foam inhibition, as an extreme pressure (EP) lubricant additive, as a surfactant component for alkaline, and as acid cleaners and for special cosmetic preparations (5). The commercially available phosphate ester products are complex mixtures of monoester and diester, free phosphoric acid, and free nonionic.

### 8.1. Chemistry of the Reaction

Phosphate esters are prepared by the partial esterification of fatty alcohol with phosphorous oxychloride followed by hydrolysis (20). This method yields a product that is a mixture of monoalkyl, dialkyl, and trialkyl phosphates.

Another method of preparing alkyl phosphate is by reacting the fatty alcohol either with phosphorous pentoxide or with polyphosphoric acid. As shown by analysis of experimental results, the composition of alkyl acid phosphates differs significantly, depending on which of the two phosphating agents is used. The use of polyphosphoric acid leads to a higher monoester: diester ratio than that obtained with phosphorous pentoxide. Typical composition of the reaction products are given





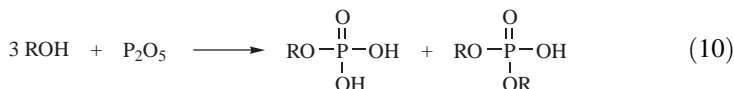
**Figure 19.** Ion exchange method of glycerine purification.

TABLE 3. Typical Composition of the Phosphate Ester Reaction Products (21)

Route	Components	Formula	Quantity (%)
Polyphosphoric acid	Monoalkyl phosphates	$O=P(OH)_2 OR$	74
	Dialkyl phosphate	$O=P(OR)_2 OH$	2
	Orthophosphoric acid	$O=P(OH)_3$	10
	Free alcohol	$ROH$	14
Phosphorous pentoxide	Monoalkyl phosphate	$O=P(OH)_2 OR$	35
	Dialkyl phosphate	$O=P(OH)_2 OH$	42
	Orthophosphoric acid	$O=P(OH)_3$	1
	Free alcohol	$ROH$	22

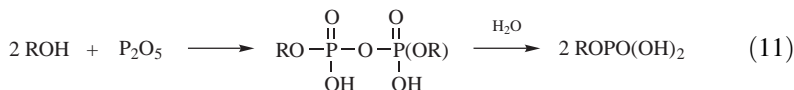
in Table 3. The difference in composition of the phosphate product also results in different properties and industrial applications.

Alkyl phosphates are more easily prepared by the addition of phosphorous pentoxide to fatty alcohols or fatty alcohol ethoxylates.



The resulting product is a mixture of dialkyl and monoalkyl phosphate esters. These products also contain small amounts of condensed phosphates and phosphoric acid. Neutralization of the acids with bases like alkali hydroxides, ammonia, or amines produces water-soluble anionic surfactants and emulsifiers.

The presence of water in the fatty alcohol or in the pentoxide increases the percent of monoalkyl acid phosphate. The use of higher molecular-weight fatty alcohols also increases the monoalkyl acid phosphate content. Products containing higher amounts of monoalkyl acid phosphate can also be derived from the reaction of 2 moles of fatty alcohol with 1 mole of phosphorous pentoxide. This reaction gives a high-yielding dialkyl pyrophosphate product, which is readily hydrolyzed to give an end product with a higher monoalkyl acid phosphate content than that obtained from the 3 : 1 ratio of alcohol to pentoxide (20).



## 8.2. Method of Manufacture

Phosphate esters are prepared at reaction temperatures from 80°C to 120°C at essentially atmospheric pressure. It has been claimed that temperature may be maintained at 30–80°C. Lower temperature favors lower color of the product (22), whereas, at temperatures near 100°C, noticeable decomposition occurs (23).

The phosphorous pentoxide is added portionwise to the alcohol at a rate such that the pentoxide dissolves and reacts without lumping. Lumping makes complete dissolution difficult and the  $P_2O_5$  unreactive. The reaction between the alcohol and the  $P_2O_5$  is carried out in the liquid phase and is basically exothermic. No catalyst is required. The addition of a small amount of hypophosphorous acid or its salt gives a colorless, color-stable product.

### 8.3. Properties and Uses

Monoalkyl phosphates inhibit the foam generation of other anionic and nonionic surfactants. The composition of the phosphate ester greatly affects the functional properties of the product.

Most phosphate esters are pale yellow to amber in color, sweet smelling, viscous liquid, or pastes. Depending on the type of phosphate esters, they have many important properties, among which are wetting, detergency, solubilizing, emulsification, surface tension reduction, foaming, dedusting, lubricity, antistatic, corrosion inhibition, chelating, dispersing, and antisoil redepositing agent (23). They have been proven to be extremely useful in a variety of applications where alternative anionic, nonionic, or amphoteric surfactants fail to give the desired functional properties.

Phosphate esters possess strong tolerance to electrolytes and alkalis. Their wetting properties are independent of the pH and, therefore, are used as surfactant components in both alkaline and acid cleaners (5). Their detergent properties are considered equal to nonionic surfactants, and they have excellent temperature stability. In their acid form, phosphate esters are moderately strong acids and, when neutralized to pH 5–8, have low order of toxicity. Phosphate esters are considered to be hydrotrope agents and are biodegradable.

## 9. ALKANOLAMIDES

Alkanolamides are condensation products of the reaction of a primary or a secondary alkanolamine with a fatty acid, a methyl ester, or a triglyceride such as coconut oil. The composition and functional properties of alkanolamides vary considerably, depending on the reactants employed and the reaction conditions. Such properties include superfatting, wetting, foam boosting, foam stabilizing, thickening, lubricating, emolliency, skin protection, emulsifying, and corrosion inhibition.

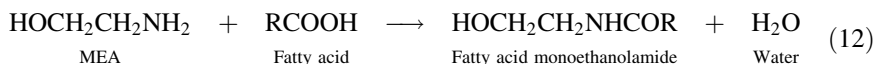
Alkanolamides are nonionic surface active agents that find application in a multitude of uses. There are four major groups of fatty alkanolamides: (1) monoethanolamides (MEA), (2) diethanolamides (DEA), (3) monoisopropanolamides (MIPA), and (4) ethoxylated or PEG alkanolamides. Each group has its own specific functions and uses in formulations.

Early experiments in the preparation of alkanolamides were begun by Kritchevsky (24, 25). It involves condensation reactions of fatty acids, triglycerides, esters, amides, anhydrides, and halides with an alkanolamine. The reaction was carried out at 100–300°C at atmospheric pressure. An important improvement was made by

Meade (26), who made use of an alkali metal alkoxide as a catalyst at 100°C at atmospheric and slightly above atmospheric pressure. Further refinement was made by Tesoro (27), who conducted the reaction at 55–75°C and a vacuum of 4–8 kPa. Schurman (28) patented a continuous process for making alkanolamide, which makes use of a thin film reactor. It is claimed the short contact time in the reactor produces a high-purity alkanolamide (29).

### 9.1. Chemistry of the Reaction

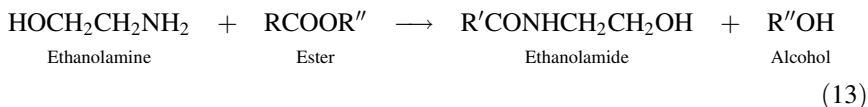
*Reaction with Fatty Acids.* When alkanolamines react with fatty acids in the ratio of 1 : 1 at 140–160°C, *N*-alkylolamides are formed.



The reaction is a two-stage reaction with the initial formation of the alkanolamine soap followed by dehydration to form the alkanolamide. At the same time, significant quantities of amine esters and amide esters are formed by the side reaction. If a dialkanolamine is used as the starting material, smaller amounts of amine diester and amide diester are formed as well as some morpholine and piperazine derivatives.

Reaction of dialkanolamines with fatty acids in a 2 : 1 ratio at 140–160°C gives a second major type of alkanolamide. This product, in contrast to 1 : 1 alkanolamide, contains a considerable amount of unreacted dialkanolamine, which accounts for the aqueous solubility of the product.

*Reaction with Esters.* The reaction of an ester, such as cocomethyl ester, with an alkanolamine at 110°C produces an *N*-alkylolamide.



The reaction of methyl esters of fatty acids with alkanolamines is used commercially to prepare high-purity 1 : 1 alkanolamide. This product, in contrast to the 1 : 1 alkanolamides prepared from the parent fatty acids, contains only a small quantity of the byproducts or unreacted starting material.

There are two competing reactions: amide formation and transesterification. The most desirable reaction is one that gives the highest amide ester byproduct. For the yield of the amide to exceed 90%, reduced pressure, lower temperature, and a slightly higher catalyst concentration were found to be necessary (29). Plant operations employ 0.3–0.5% catalyst, a temperature of 70–75°C, and a vacuum of 4 kPa or less.

Another variation is the use of triglycerides, such as coconut oil, as the starting material. This involves the reaction of 1 mole of triglycerides with 6 moles of the alkanolamine. The reaction can be catalyzed by sodium methylate at 60°C.

Glycerine is a byproduct that usually remains with the final product. The typical composition of the product consists of 80–85% alkanolamide, 5–7% or less free amine, and traces of fatty acid soap and other byproducts.

The reaction is relatively mild and proceeds readily with the use of 0.2–0.3% sodium methylate catalyst at an operating temperature of 70–75°C and at atmospheric pressure.

## 9.2. Types of Alkanolamides

There are at least four types of alkanolamide commercially available. The first is formed by reacting monoalkanolamine or dialkanolamine with fatty acids in a 1 : 1 ratio at elevated temperatures of 140–160°C. Significant quantities of the byproducts—amine esters and amide esters—are also formed.

A second major type is the Kritchevsky type made from alkanolamine and fatty acids in the ratio of 2 : 1. Such product contains 60–70% alkanolamide plus byproducts. If diethanolamine is used in addition to the mentioned byproducts, some morpholine and piperazine derivatives are also obtained. The excess alkanolamine renders the product water soluble (29). Both are of commercial importance as detergent and detergent additives.

A third type is the so-called superamide prepared from the methyl esters in a 1 : 1 mole ratio. They are generally products that have an alkanolamide content in excess of 90%. Some of the same byproducts as in the first type are formed but in much smaller quantities. As a result of the relatively small amounts of free alkanolamine, superamides have poor water solubility. They are used in conjunction with a small amount of anionic or nonionic surfactant, which acts as a solubilizer to form a clear viscous solution (29).

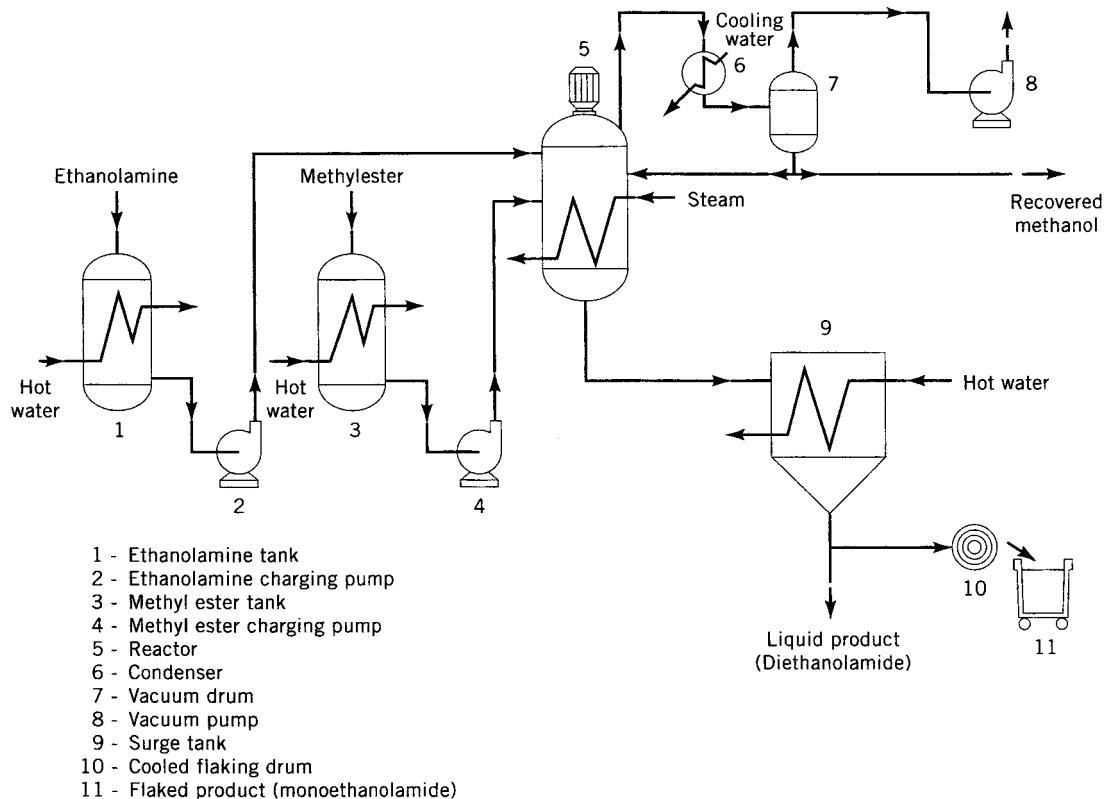
The fourth variant is the product of the direct reaction of a triglyceride with an alkanolamine. The glycerine byproduct is retained with the other byproducts. It is also used as a detergent additive in light-duty liquid detergents.

Other applications of alkanolamides include dry cleaning soaps, fuel oil additives, rust inhibitors, textile scouring, and dye leveling agents.

## 9.3. Method of Manufacture

Depending on which of the four types of alkanolamide is to be produced, the starting material could be either fatty acid, methyl ester, or triglyceride (coconut oil) and monoethanolamine or diethanolamine. Usually, a batch system of manufacturing is employed, as shown in Figure 20.

*Alkanolamide from Coconut Oil.* A 2 : 1 cocodiethanolamide (CDEA) can be produced using 6 moles of diethanolamine and 1 mole of refined and bleached coconut oil. The materials are charged with the reactor and a small amount of catalyst (0.25–0.3% sodium methylate or sodium hydroxide) is added. The temperature of the batch is increased to 70–75°C at normal pressure. After 90 min, the reaction is completed. For a 10-t batch, total cycle time from charging the materials, heating them up, allowing the reaction to proceed to completion, and transferring the finished product takes at least 4 h.



**Figure 20.** Manufacture of normal and super alkylolamides (30).

If fatty acid is the starting material, a different operating parameter is used to drive off the water formed during the reaction. If superamide is to be produced, coco-methyl ester and diethanolamine can be used as the starting materials in a mole ratio of 1 : 1. These materials are charged to the reactor with 0.3–0.5% sodium methylate as the catalyst. The reaction is carried at around 100°C and a vacuum of 4–5.3 kPa for a period of 90–120 min. The reaction temperature can be lowered to 70–75°C by employing a vacuum of less than 4 kPa. The reaction time takes longer to enable the maximum vaporization of the methanol byproduct. The methanol is rectified and recycled for use in the transesterification of the coconut oil to produce the methyl ester.

The final product in the liquid form is transferred into the storage tank. Postreaction can be done by keeping the product in another vessel, preferably at slightly elevated temperature for a few days. This allows further conversion of the ester byproduct to amide, thus further increasing the amide concentration.

If monoethanolamine is used as the starting material, the monoethanolamide product (CMEA) is a waxy solid. Accordingly, the finished product after the reaction is passed through a cooled flaking drum to convert it into flakes. It is subsequently packed in bags.

## 10. SURFACTANTS

Surfactants or surface-active agents make up a special class of chemicals used in practically all industries. Surfactants may be of petrochemical or natural oils origin. This chapter discusses only those of vegetable oil origin, specifically those from coconut oil.

Surfactants are chemical compounds that possess great surface activity. They act so diversely because of the unbalanced molecular structure. A surfactant molecule may be visualized as a tadpole or a mini-racquet. The “head” is the hydrophilic (water-loving), strongly polar portion and the “tail” is the hydrophobic (oil-loving) nonpolar portion. The head can be an anion, a cation, or nonion. The tail is a linear or branched hydrocarbon chain. It is this characteristic configuration that makes surfactants perform such diverse function in industry.

Surfactants find broad application in practically all industries as, for example, the main ingredients of detergents and cleaners, foaming agents and emulsifiers in cosmetics and pharmaceuticals, emulsifiers for paints, scouring agents for textiles, flotation agents for the mining industry, and emulsifiers and sanitizing agents for the food industry.

### 10.1. Types of Surfactants

Surfactants are classified into four categories: anionic, cationic, nonionic, and amphoteric, each with its own molecular structure and behavior.

*Anionic Surfactants.* Anionic surfactants are surface-active agents in which the hydrophobic portion is connected to an anion or negatively charged ion. In an

aqueous medium, an anionic surfactant dissociates into a positively charged cation and a negatively charged anion. The latter is the carrier of the surface-active properties. Typical examples are the alcohol sulfates and the ester sulfonates.

*Cationic Surfactants.* Like the anionic surfactants, cationic surfactants also dissociate in an aqueous medium. However, the head (hydrophilic portion) is a cation, which is the carrier of the surface-active properties. Examples are the quaternary ammonium compounds.

*Nonionic Surfactants.* The nonionic surfactants do not dissociate in an aqueous medium. Their solubility is provided by the polar group, such as a polyglycol ether or a polyol. The most important types of fatty nonionic surfactants are the polyglycol ethers of fatty alcohols, fatty acids, amines, and amides.

*Amphoteric Surfactants.* Amphoteric surfactants in aqueous solution contain both positive and negative charges in the same molecule. Thus, a hydrophobic fatty chain is attached to a hydrophilic group that contains both positive and negative charges. Its behavior depends on the condition of the medium or its pH value. Examples of this type are the alkyl betaines.

*Coco-Based Surfactants.* The most important coconut oil-based surfactants are fatty alcohol sulfate, fatty alcohol ether sulfate, and fatty alcohol polyglycol ether. Two relatively new coco-based surfactants are fatty acid methyl ester sulfonate and alkyl polyglycoside, which is produced from fatty alcohol and starch or sugar, both renewable materials.

*Fatty Alcohol Sulfates.* Sodium salt of fatty alcohol sulfate of the C12–C18 range is one of the more common active ingredients of choice in the formulation of laundry detergent products, due to its good detergency, wetting and foaming properties, and biodegradability. The C12–C14 fatty alcohol sulfate, known in the trade as sodium lauryl sulfate (SLS), provides optimum foam and is used extensively as the foaming agent in most toothpastes. The ammonium and alkanolamine salts of the C12–C14 and C12–C16 alcohol sulfates possess high solubility and are well suited for liquid shampoos and bubble bath preparations. In combination with other surfactants and alkanolamides, they deliver desirable qualities, such as fine structured foam, low irritancy, and smooth hair feel (5).

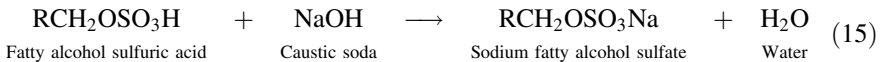
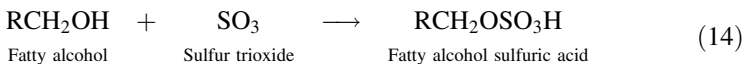
*Fatty Alcohol Polyglycol Ethers.* The fatty alcohol polyglycol ethers belong to the most important class of nonionic surfactants. They are characterized by having excellent wetting properties, having low foam, and being effective even at low temperatures. They are produced by the reaction of fatty alcohol with ethylene oxide (EO) or propylene oxide (PO), using alkaline catalyst such as sodium methylate or potassium hydroxide. The degree of ethoxylation can vary, depending on the chain length of the fatty alcohol and the purpose for which it will be used. The C12–C14 and C12–C16 alcohols used as raw material for the ether sulfates can have 2–3 moles of EO. The C12–C18 alcohol, with 7–10 moles of EO, are used primarily as wetting agents. Higher saturated alcohols, with up to 30 moles of EO, are used as emulsifiers for waxes and ointments bases. Fatty alcohol polyglycol ethers have replaced the alkylphenol polyglycol ether in most applications. In the United States, however, the latter is still in use for institutional and industrial products because of cost considerations.



*Fatty Alcohol Ether Sulfates.* Probably the most important derivatives of fatty alcohol in the C12–C14 and C12–C16 ranges are the fatty alcohol ether sulfates. They are produced by the sulfation of the fatty alcohol, containing 2–3 moles of ethylene oxide, with sulfur trioxide or chlorosulfonic acid and subsequently neutralized with caustic soda, ammonia, or an alkanolamine. The ether sulfates possess superior properties over the fatty alcohol sulfates. They have unlimited water solubility, are unaffected by water hardness, and possess superior skin compatibility. Accordingly, they are used in liquid shampoos and bath preparations. One characteristic of this material is its ability to increase its viscosity by the addition of an electrolyte such as salt (5).

## 10.2. Chemistry of the Reaction

Fatty alcohol sulfate of commerce is actually a neutralized salt primarily as sodium coco fatty alcohol sulfate. It is produced by the direct reaction of fatty alcohol with sulfur trioxide and subsequently neutralized with caustic soda, according to the following reactions:



The intermediate product is a half ester of sulfuric acid. It is quite unstable and must be neutralized immediately. The final product contains approximately 1.5% of sodium sulfate, 1.0–1.5% unreacted alcohol, and less than 0.5% of free alkali.

In sulfation, other sulfating agents may be used, such as oleum or chlorosulfonic acid, as the source of  $\text{SO}_3$ . However, oleum generates a large amount of waste sulfuric acid. It is also not recommended for the sulfation of fatty alcohol ethoxylate.

Chlorosulfonic acid is an excellent sulfating agent, but it generates unwanted hydrochloric acid as a byproduct. As a result of the corrosive nature of the hydrochloric acid vapor, special equipment must be used.

In neutralization, other alkali can be used such as ammonia, monoethanolamine, or triethanolamine. The same chemical reaction and equipment applies to the production of fatty alcohol ether sulfate, except that the raw material is fatty alcohol ethoxylate.

## 10.3. Method of Manufacture

The latest technology in the manufacture of fatty alcohol sulfate or fatty alcohol ether sulfate is the direct use of  $\text{SO}_3$  gas as the sulfating agent. This process not only produces a high-purity product but is also much more economical and generates minimum waste.

The heart of the process is the reactor. Reactors can be batch, cascade, or falling-film type. Industry favors the falling-film type because the reaction is much more controllable and highly efficient. Falling-film reactors can be multitube, monotube, or annular (Figure 21).

The manufacture of fatty alcohol sulfate or ether sulfate can be divided into five stages: process air preparation, sulfur trioxide generation, sulfation, neutralization, and exhaust gas treatment.

*Process Air Preparation.* Process air must be totally dry, preferably with a dew-point below  $-50^{\circ}\text{C}$ . The presence of moisture not only makes it corrosive (due to its reaction with  $\text{SO}_3$  gas) but also increases the color of the product.

Briefly, air is introduced by a large compressor to a cooling system, where it is cooled down to  $3\text{--}5^{\circ}\text{C}$  and most of the moisture is condensed. From there, the air is passed through a dehumidifier, such as silica gel, where the last traces of moisture are retained. Figure 22 shows Ballestra's air drying system.

*Sulfur Trioxide Generation.* For sulfur trioxide generation, high-purity sulfur (99.5% pure) is melted in a tank and then kept at  $145\text{--}150^{\circ}\text{C}$  to maintain its viscosity at the minimum and constant value. The molten sulfur is introduced into the sulfur burner by a special metering pump and then burned to  $\text{SO}_2$ , using the dried air. The diluted  $\text{SO}_2$  gas (6–7%) leaves the burner at around  $650^{\circ}\text{C}$  and is cooled to  $430^{\circ}\text{C}$  before feeding to the converter.

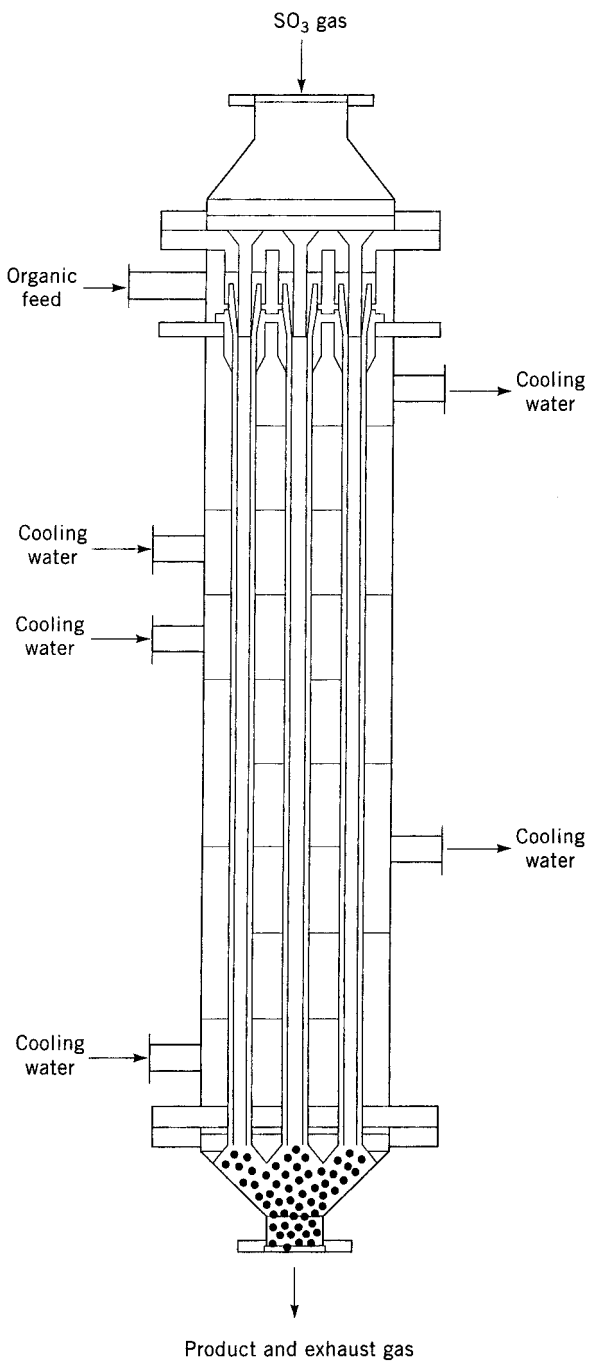
The catalytic converter, with three to four beds of vanadium pentoxide catalysts, converts  $\text{SO}_2$  to  $\text{SO}_3$  with up to 98% conversion efficiency. The  $\text{SO}_3$  gas is cooled to below  $60^{\circ}\text{C}$ , diluted to 4% by volume, and is passed through a mist eliminator to remove traces of oleum formed before it is fed to the reactor. Figure 23 shows a typical system for the generation of  $\text{SO}_3$  gas.

*Sulfation.* The sulfation is carried out in a multitube film reactor, especially designed to control accurately the mole ratios between the  $\text{SO}_3$  and the organic feed in each tube. The materials enter the top section and flow cocurrently downward inside the tube. As the reaction is almost instantaneous and exothermic, cooling water at controlled flow is introduced into the jacket to maintain the reaction temperature at  $45\text{--}50^{\circ}\text{C}$  maximum. A reaction yield of 97% can easily be achieved (see Figure 21).

*Neutralization.* The intermediate product from the reactor must be neutralized immediately, as hydrolysis may occur and badly affect the process and the product quality. This is achieved in a double-step continuous neutralization unit. The multi-bladed mixer gives exceptionally homogeneous mixing (30).

It is important that the neutralized paste be kept slightly alkaline to ensure processability and product stability. An average concentration of 72% active matter is readily achievable. A much higher concentration is not recommended, as processing difficulties may be encountered. If a dried product is desired, the paste can be further processed through a wiped film evaporator. Figure 24 shows Ballestra's double-step neutralization.

*Exhaust Gas Treatment.* The gas effluent must be treated to comply with environmental regulations. The exhaust gas from the reactor contains traces of organic matter and unreacted  $\text{SO}_3$  and  $\text{SO}_2$  gases. The first two impurities are removed by



**Figure 21.** Multitube film reactor (30).

- 1 - Process air filter
- 2 - Process air compressor
- 3 - Chiller
- 4 - Air cooling group
- 5 - Circulating pump
- 6 - Glycol recycle vessel
- 7 - Circulating pump
- 8 - Silicagel regeneration unit
- 9 - Regeneration air fan
- 10 - Silicagel air dehumidifier

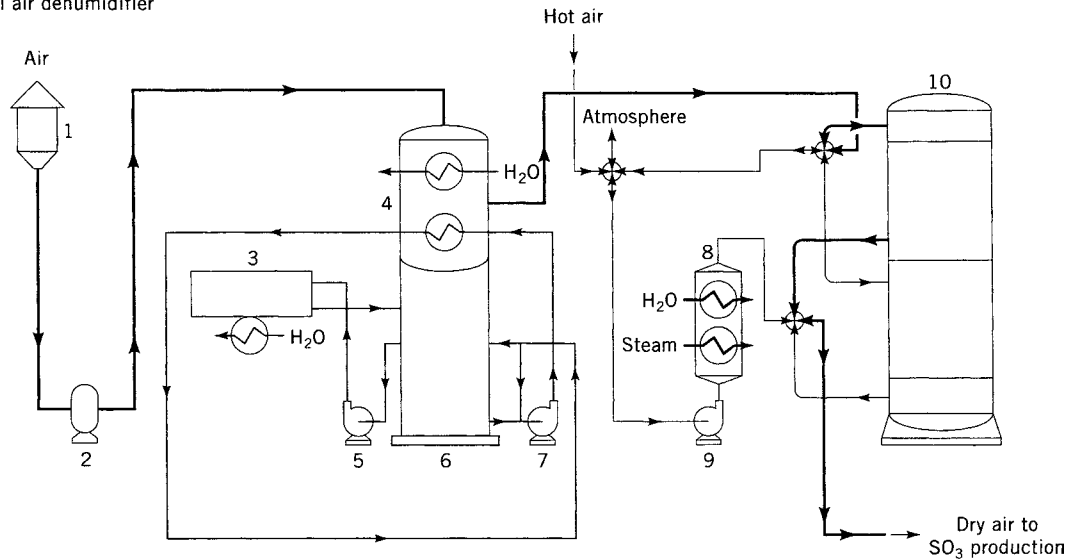


Figure 22. Ballestra's air drying system (30).

- 1 - Sulfur melting vessel
- 2 - Molten sulfur filter
- 3 - Molten sulfur dosing unit
- 4 - Sulfur combustion furnace
- 5 - Preheating air generator
- 6 - SO<sub>2</sub> cooler-preheater
- 7 - Catalysis tower
- 8 - Interstage heat exchangers
- 9 - Electric igniter
- 10 - SO<sub>3</sub> coolers
- 11 - Cooling fan

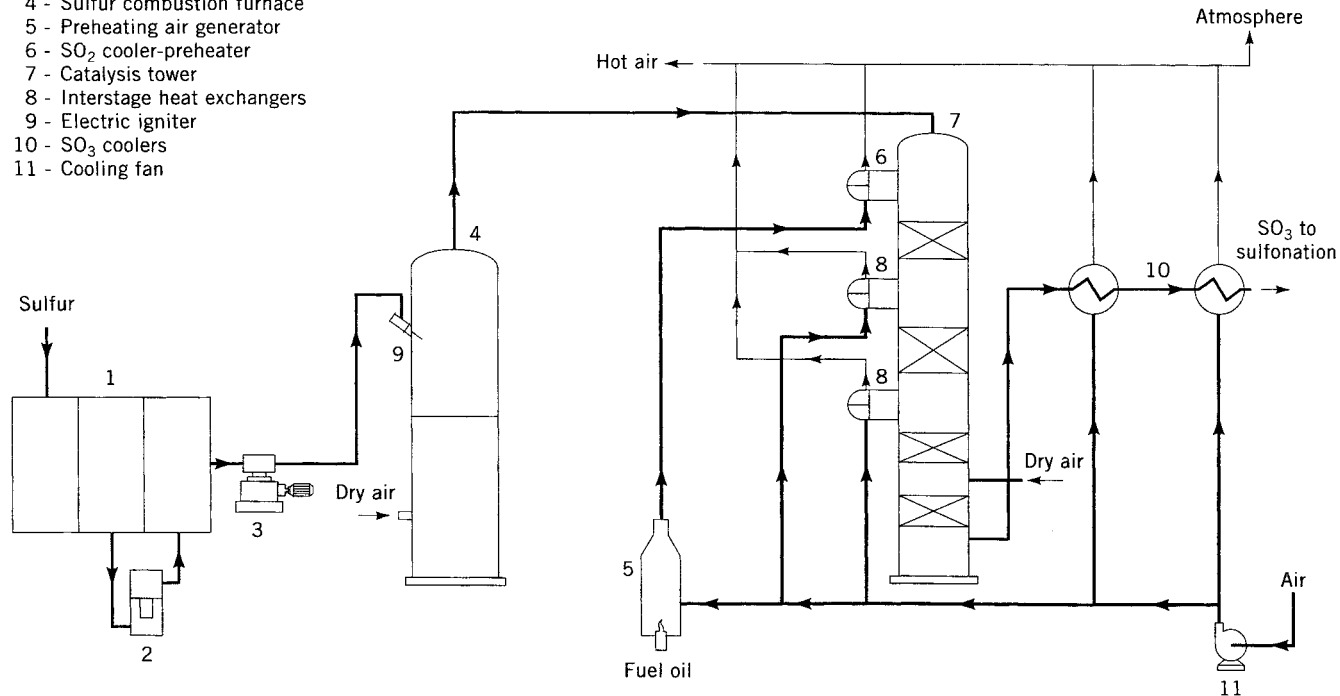
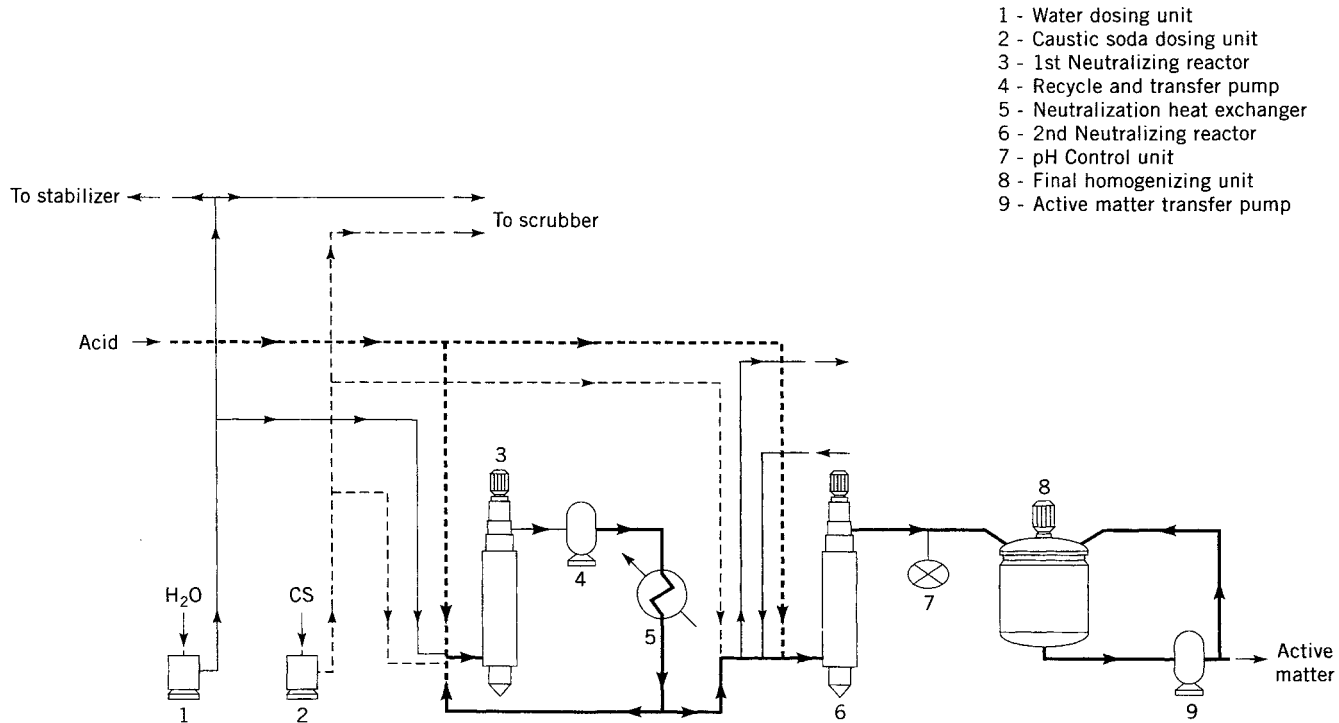
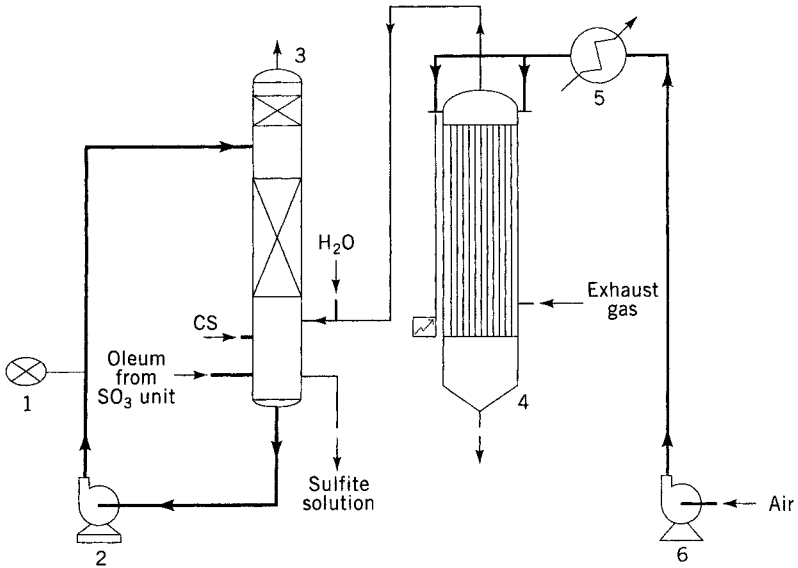


Figure 23. Ballestra's SO<sub>2</sub>-SO<sub>3</sub> production (30).



- 1 - Water dosing unit
- 2 - Caustic soda dosing unit
- 3 - 1st Neutralizing reactor
- 4 - Recycle and transfer pump
- 5 - Neutralization heat exchanger
- 6 - 2nd Neutralizing reactor
- 7 - pH Control unit
- 8 - Final homogenizing unit
- 9 - Active matter transfer pump

**Figure 24.** Ballestra's double-step neutralization (30).



- 1 - pH Control unit
- 2 - Scrubbing solution circulation pump
- 3 - Gas scrubbing column
- 4 - Electrostatic precipitator
- 5 - Air heater
- 6 - Air fan

**Figure 25.** Ballestra's gas-scrubbing system (30).

the electrostatic precipitator. The remaining  $\text{SO}_2$  gas is removed by the reaction with caustic soda solution flowing countercurrently inside the scrubbing column. The concentration of the residual  $\text{SO}_2$  in the exhaust gas vented into atmosphere is kept at 5 ppm maximum, well below the allowable standards (Figure 25).

#### 10.4. Sulfonation of Fatty Acid Methyl Esters

Fatty acid methyl esters sulfonate may be considered a material with a great potential. Its superior sequestering effect on water hardness gives it an advantage over fatty alcohol sulfate. The sulfonation of fatty acid methyl ester is different from that of fatty alcohol. The reaction mechanism involves two steps (31). In the first reaction, the  $\text{SO}_3$  gas reacts quickly to form sulfoanhydride. In the second step (which takes 40–90 min), the sulfoanhydride becomes the sulfonating agent, reacting with the still-unreacted ester.

The reaction requires an excess of  $\text{SO}_3$  in the range of 20–30 mol % to get initiated. With such an excess, the formation of disalt during neutralization is unavoidable. This is somehow minimized by subsequent re-esterification after the second step.

The neutralization step can be carried out in much the same way as in the neutralization step in the production of fatty alcohol sulfate. As a result of the nature of the reaction and conditions during sulfonation, a dark-colored product is produced, which requires bleaching. Postreaction treatment with  $H_2O_2$  and  $NaOCl$  yields a product with an acceptable color (30).

### 10.5. Other Surfactants

Three highly successful types of coco-based surfactants used in toilet soap formulations that are mass marketed in the United States are worth mentioning. They impart superior hardwater lather performance while retaining the mildness aspect of toilet soap. These are (1) sodium cocoyl isethionate (SCI) used in Lever's Dove and their other brands, (2) sodium cocoglyceryl ether sulfate (LGES) used in Procter & Gamble's Zest, and (3) sodium cocomonoglycerides sulfate (CMGS) used in Colgate's Vel Bar (4).

Other coco-based surfactants are sulfosuccinates formed by the reaction of coco fatty alcohol with maleic anhydride and further reaction with sodium sulfite or bisulfite. This product possesses good foaming properties, is compatible with soap, and is a good lime dispersant. It is used in toilet soap formulation, shampoos, hand cleaning pastes, and for scouring raw wool. Its ether variant, with 2–4 moles ethylene oxide, forms intense, finely structured foam and is used in combination with ether sulfate in baby shampoos and bath preparations.

Alkyl phosphates, also coco based, are formed by the reaction of fatty alcohol with phosphorous pentoxide. The product is a mixture of monoesters and diesters. Its sodium salt (MAP) is reportedly mild and is used in facial wash, such as Kao's Biore. Other uses are as antistatics in textile auxiliaries, corrosion inhibitors, surfactant additives for extreme pressure (EP) lubricants, and surfactant components in alkali and acid cleaners. Coco-based alkyl polyglycosides (APG) have been successfully produced and marketed by Henkel in the United States. The alkyl polyglycoside fully satisfies the demand for a mild surfactant and is completely biodegradable.

## 11. TERTIARY AMINES

Fatty amines and their derivatives represent the most important nitrogen compounds of fatty acids. They possess great ionization constants compared with other alkyl derivatives of ammonia. They are cationic, basic, biologically active, and strongly adsorbed on many surfaces due to their high adsorption potential. They are indispensable in many surface-related physicochemical processes. They are the starting materials for the manufacture of quaternary ammonium compounds and various cationic and amphoteric substances. These derivatives find wide application in many industries as biocides, sanitizing agents for algae control in water treatment, ore flotation agents in mining, effective corrosion inhibitors, and lubricants in drilling and formulation. Fatty amines derived from tallow or palm oil fatty acid fractions are widely used in the production of fabric softeners.



The main raw material is the fatty nitrile derived from the reaction of fatty acid with ammonia. Catalytic hydrogenation of the nitrile produces the amines. The amines are available as primary, secondary, and tertiary amine, depending on the number of the alkyl groups attached to the nitrogen atom.

### 11.1. Types of Tertiary Amines

Of the three fatty amines, the tertiary fatty amines, with three alkyl groups, find the most application in industries. They are further classified into three types: (1) symmetrical trifatty amine ( $R_3N$ ), in which all three alkyl chains are identical; (2) asymmetrical dimethyl fatty amine  $[RN(CH_3)_2]$  or methyl difatty amine ( $R_2NCH_3$ ); and (3) those derived from primary or secondary amine by the reaction with ethylene oxide. These asymmetrical fatty amines are the bases for the manufacture of organomodified clay for the petroleum industry, biocides, and algicides.

### 11.2. Methods of Preparation

Several methods are available for the preparation of tertiary amines from fatty acids. One of the oldest is the classical Leuckart reaction (32), which uses formaldehyde and formic acid for the reductive alkylation of a secondary amine.

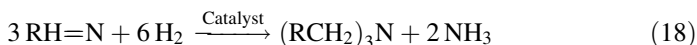


Modification to this reaction had been reported. Shapiro (33) developed the reductive alkylation of primary and secondary amine with formaldehyde in the presence of nickel catalyst.

Of commercial importance is the manufacture of fatty amines via the nitrile route. Fatty acid is first converted to nitrile by the reaction with ammonia at elevated temperatures of 280–360°C at atmospheric pressure.



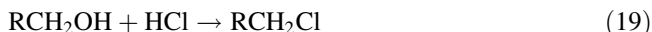
The reaction that produces the nitrile is essentially a dehydration reaction of the intermediate product. The nitrile is then catalytically hydrogenated to produce the fatty amines.



Depending on the process conditions, primary, secondary, or tertiary amines can be produced. If more tertiary amines are desired, continuous removal of the  $NH_3$  is necessary to shift the reaction forward. The presence of ammonia favors the formation of primary and secondary amines.

Fatty alcohols are also available raw materials in the commercial preparation of tertiary amines. The fatty alcohol may be converted directly to an alkyl halide by

the reaction with a concentrated hydrogen halide (5). The alkyl halide can then be reacted with dimethylamine to produce the tertiary amine (33).



Long-chain alcohols can be converted directly to *N,N*-dimethylalkylamines by the reaction with dimethylamine at 36°C in the presence of  $\text{Th}(\text{SO}_4)_2$  or in the presence of Cu–Cr catalyst and hydrogen at elevated temperatures and pressure (33, 34).



Ethoxylated tertiary amines can be produced by the reaction of primary or secondary amines with ethylene oxide. The water solubility of this amine increases with the increasing degree of ethoxylation (33).

### 11.3. Uses

The asymmetrical tertiary amines are used exclusively as starting materials for the manufacture of quaternary ammonium compounds, cationic and amphoteric surfactants, and amine oxides. Quaternary ammonium compounds used as bactericides and algicides are produced by the reaction of tertiary amines with benzyl chloride, methyl chloride, or dimethyl sulfate. Of these, the benzyl ammonium chloride salt is the most widely used.

Probably the largest volume use of fatty amines is in the manufacture of fabric softeners. The latter is distearyldimethyl ammonium chloride produced by the reaction of distearylamine and methylchloride with continuous addition of alkali. However, as stated earlier, the starting materials for this product are the C16–C18 fatty acid fractions from tallow or palm oil.

Tertiary amines are also used in the manufacture of amine oxides by the oxidation with hydrogen peroxide. The amine oxides are used in cosmetic preparations for its good foaming properties and mildness to the skin. Another raw material used in the cosmetic industry is the betaines produced by the reaction of a tertiary fatty amine with sodium chloroacetate. The betaines exhibit good foam stability over a wide range of pH, are insensitive to water hardness, and are mild to the skin (33).

## REFERENCES

1. S. Marash, R. Gubler, and K. Yagi, "Fats and Oils Industry Overview," *Chemical Economics Handbook*, SRI, Menlo Park, California, 2001.
2. R. Modler, R. Gubler, and K. Yagi, "Natural Fatty Acids," *Chemical Economics Handbook*, SRI, Menlo Park, California, 2002.

3. "Alcohols, Higher Aliphatic Synthetic Processes," in *Kirk-Othmer Encyclopedia of Chemical Technology*, vol. 2, 5th ed., Wiley-Interscience, Hoboken, New Jersey, 2004, p. 28.
4. L. Spitz, *Soap Technology for the 1990's*, AOCS, Champaign, Illinois, 1990.
5. H. Schutt, H. D. Kamp, H. P. Kubersky, and J. Glasl, *Fatty Alcohols*, Henkel KGaA, Dusseldorf, 1982.
6. B. Brackman, J. Knaut, and P. Wallscheid, *Oleochemicals*, Henkel KGaA, Dusseldorf, 1984.
7. *Fatty Acid, Product Specifications 1993*, United Coconut Chemicals, Inc., Metro Manila, Philippines, 1993.
8. H. J. Richtler, *Challenges to a Mature Industry*, Henkel KGaA, Dusseldorf, 1983.
9. U.S. Patent 601,603, 1897.
10. D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, vol. 2, 4th ed., John Wiley & Sons, Inc., New York, 1982, p. 107.
11. *Fatty Acid Technology*, Technical brochure no. 197e/3.91/30, Lurgi AG, Frankfurt, 1991.
12. W. M. Linfield, R. A. Barauskas, L. Sivieri, S. Serota, and R. W. Stevenson Jr., *J. Amer. Oil Chem. Soc.*, **61**(2), 191–195 (1984).
13. *Advanced Technology and Services for Oleochemical and Tall Oil Processing*, Technical brochure Badger, Cambridge, 1990.
14. B. Freedman, *Methyl Ester—Manufacture and Utilization*, U.S. Department of Agriculture, Peoria, Illinois, .
15. U. R. Kreutzer, *J. Amer. Oil Chem. Soc.*, **61**(2), 343–348 (1984).
16. T. Ogoshi and Y. Miyawaki, *J. Amer. Oil Chem. Soc.*, **62**(2), 331–335 (1985).
17. T. Voeste and H. Buchold, *J. Amer. Oil Chem. Soc.*, **61**(2), 350–352 (1984).
18. *Fatty Alcohol Process*, Technical brochure no. 1480 e/2.91/2.7, Lurgi AG, Frankfurt, 1991.
19. F. de Dardel, *Glycerine Purification*, Rohm & Haas, Paris, 1989.
20. D. H. Chadwick and R. S. Watt, *Phosphorus and Its Compounds*, vol. 11, Interscience Publishers, New York, 1961.
21. M. Dahanayake and W. Kristoff, *Phosphate Ester Hydrotropes*, HAPPI, Ramsey, New Jersey, 1992.
22. H. Adler and W. H. Woodstock, *Chem. Ind.*, **51**, 516 (1942).
23. J. M. H. Daemer and W. Dalkelman, *J. Chromatogr.*, **78**, 281–291 (1973).
24. W. Kritchevsky, U.S. Patent 2,089,212, August 10, 1937.
25. W. Kritchevsky, U.S. Patent 2,096,749, October 26, 1937.
26. E. Meade (to Lankro Chemicals, Ltd.), U.S. Patent 2,464,094, March 8, 1949.
27. G. Tesoro (to Onyx Oil and Chemical Co.), U.S. Patent 2,844,609, July 22, 1958.
28. J. Schurman (to Colgate Palmolive Co.), U.S. Patent 2,863,888, December 9, 1958.
29. *Alkanolamines handbook no. 111-1159-88 R*, SAI, Dow Chemical Co., Midland, Michigan, 1988.

30. A. Davidsohn and G. Moretti, paper presented at the International Symposium on Natural Base Cleaning Agents and Personal Care Products, Marseille, 1985.
31. A. J. Stirton, *J. Amer. Oil Chem. Soc.*, **39**, 490 (1962).
32. H. Maag, *J. Amer. Oil Chem. Soc.*, **61**(2), 259–267 (1984).
33. S. H. Shapiro, *Fatty Acids and Their Industrial Applications*, Marcel Dekker, Inc., New York, 1968, pp. 123–128.
34. S. Billenstein and G. Blaschke, paper presented at the World Conference on Oleochemicals, 1984.

# 2

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## *Rendering*

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### **1. INTRODUCTION**

The production of animal byproducts into useful items is not a recent event. For example, early Eskimos and Native Americans used the parts of the animals that could not be eaten. Hides and skins were used for clothing and shelter, bones provided utensils, and crude soap was made from melted fat and wood ash. However, it took the development of other industries (i.e., soap and candle making) before rendering became an established industry.

The production of soap played a large part in the early development of rendering. The first record of rendering was the melting down of animal fat to obtain tallow. One of the earliest accounts of rendering dates back to 79 A.D. in Rome. Historians are not sure whether the Romans adopted their tallow-making process from the chance discoveries of wandering Bedouins or from the Celts who are known to have made soap from boiling animal fats and plant ashes together. Following the decline and fall of Rome, historians find little reference to the use of soap. Its manufacture and popular use appears to have been developed largely in Italy, France, and England during the Middle Ages.

Candle making also played a major role in the development of the rendering industry. The method of making candles using tallow goes back to the Roman days. They used tallow as the combustible material surrounding a flax or cotton

wick. As the candles became available to the “common” people, the need for tallow increased.

Rendering was a process developed over the better part of 1800 years as a direct offshoot of the candle-making and soap industries. During this period, tallow was derived primarily from the fat of sheep and goats because recovered fat from goat and sheep was harder and made better candle fat. Pork fat, although used in soap making, made poor candles. As the population of cattle increased, more beef tallow was produced. However, it took the United States until the late 1800s to move the rendering industry into its present place of prominence.

During the 1800s, there was an explosive development in the cattle industry in California that had little to do with beef as a food. Cattle were raised for their hides and tallow with little of the beef consumed. Most of the meat went to the coyotes and other wild animals. During the Mexican-Spanish regime, the chief source of wealth in California was raising stock. Trading hides and tallow to foreign vessels gave California nearly all of the manufactured articles and most of the luxuries it could not produce.

Development of the cattle industry in California was different from what took place in Texas and mid-America. However, as the nation became involved in the Industrial Revolution, immigrants from abroad moved into eastern cities. These immigrants developed a greater taste for beef, which increased the demand from the cattle industry. The nation’s first combined slaughter and meatpacking operation was established in 1838 at Alton, Illinois, and in 1865, the Chicago Stockyard became the nation’s leading livestock market.

As cattle slaughter made the transition from the farm and ranch to the city, so did the rendering operation. Until this time, rendering existed in concert with soap manufacturing. Once the rendering companies discovered it was more profitable to produce tallow and sell it to the soap manufacturer than making it into soap themselves, the rendering industry, as it is known today, came into being. A more complete history of the rendering industry has been published (1).

The nutritional benefits of the rendered byproducts were not fully understood until the nutritional needs of the domesticated animals produced for food were known. Until these nutritional benefits were understood, little fat was used in animal rations (not until the 1950s), and the protein meal was applied to the land as a fertilizer. Once the breakthrough in using rendered animal proteins and fats in feed occurred, the contribution of rendered products to the total value of the slaughtered animals was recognized. Processing methods also changed to improve the quality of the byproducts made through rendering. Optimization of byproduct use reduces the ultimate cost to the consumer of the primary product, meat.

## **2. MODERN DAY RENDERING**

The rendering industry processes or “recycles” animal and poultry byproducts such as animal fat, bone, hide, offal, feathers, hoofs, horns, hair, and blood. For example,

using basic approximates, byproducts represent about 50% of the live weight of cattle, 42% of the live weight of pigs, 37% of the live weight of broilers, and 57% of the live weight of most fish species. Currently, factors exist that have resulted in even higher inedible raw material quantities being generated, which include further processing, prepacked/table ready meat products, which leave increasing amounts of the inedible portions at the processing locations. Additionally, the removal of nonambulatory cattle and specific tissues from slaughtered cattle from foods because of recent and impending regulations contribute to the raw material quantity increases. The current annual amount of raw material generated in the United States alone is expected to exceed 27 million tons in 2004.

At the present time, over 200 rendering facilities operate in North America, and they serve the animal industries by using more than half the volume of its total annual production. The rendering industry represents a major force in maintaining a clean environment by the annual disposal of approximately 25–30 million tons of perishable waste material generated by the beef, pork, and poultry industries. The rendering process provides these same industries with high-energy and high-quality protein ingredients to supplement the feed grains that are used to produce the beef, veal, pork, poultry, and milk in the United States. The industry also supplies a large portion of its products to the pet food industry. It is also significant that tallow, grease, and animal protein are renewable resources.

Rendering is a global industry with markets throughout the world. Table 1 outlines the global import and export of meat meal by continent for the period 1992–2002. The data is taken from the FAO database and is only available through 2002 (2). As the United States dominates the North and Central American region, U.S. data has been shown separately from the rest of North and Central America. Table 2 shows similar data for rendered animal fats over the same period of time (2).

**TABLE 1. World Trade in Meat Meal<sup>a</sup>, 1992–2002 Average (2).**

LOCATION	IMPORTS	EXPORTS
Africa	1279567	6988
Asia	569258	21062
Europe	719613	829411
North and C. America Minus USA	138504	54487
USA	59573	384202
Oceania	9845	247881
South America	25915	97585
World	1650664	1641616

<sup>a</sup>In metric tons

**TABLE 2. World Trade in Animal Fats<sup>a</sup>, 1992–2002 Average (2).**

LOCATION	IMPORTS	EXPORTS
Africa	288559	14242
Asia	793188	35087
Europe	977231	802050
North & C. America Minus USA	531363	232503
USA	45830	1240858
Oceania	13338	484570
South America	172829	62731
World	2822338	2872041

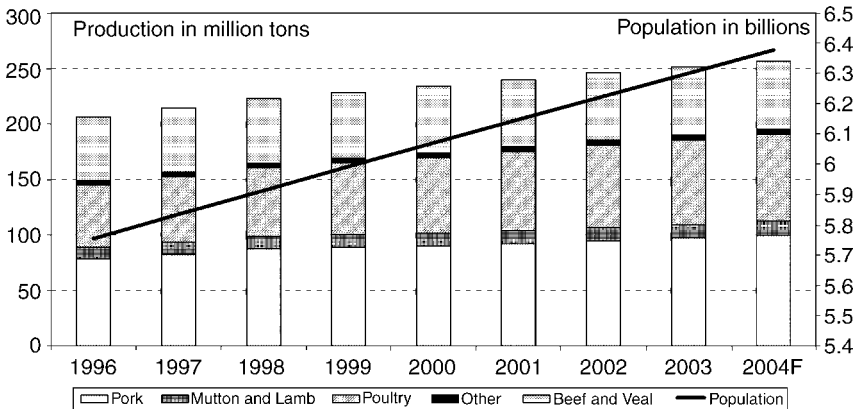
<sup>a</sup>In metric tons.

### 3. BYPRODUCTS

The rendering industry performs the important job of recycling inedible tissues of animals into protein meals, fats, and oils that are valuable animal feed and industrial ingredients. According to the publication *Oil World Annual*, the world produces about 250 million tons of red meat and poultry meat per year. Data for the period 1996–2004 forecast is shown in Figure 1 (3). The production of meat generally follows the growth in the world population. Poultry meat, pork, beef, and veal account for most of the meat production, which is shown in the Figure 2 (3). If approximately 50% of the red meat and poultry meat was waste, this would amount to a world availability of about 125 million metric tons of raw material suitable for rendering. Rendering, however, is not done in many areas of the world.

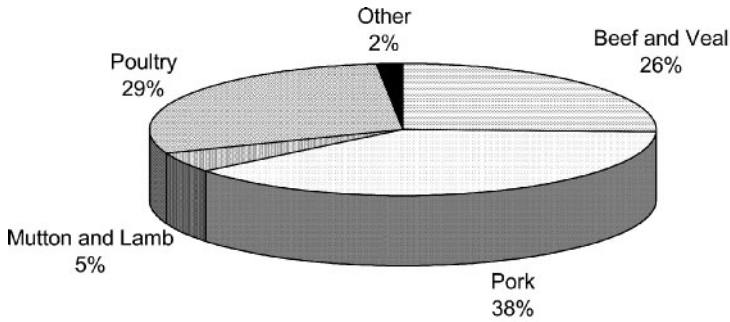
#### 3.1. Protein Meals

Animal byproduct meals help fulfill protein and mineral needs in animal feeds. These meals are incorporated into rations for livestock, fish, and crustaceans. The



**Figure 1. World production of red meat and poultry meat (3).**





9 Year Average Production 233 Million Metric Tons.

**Figure 2.** Nine year average world production of red meat and poultry (3).

Association of American Feed Control Officials (AAFCO) defines various feed ingredients so that no issue exists with identity or composition. According to their latest publication, the following is a description of the byproduct meals.

- *Blood Meal* is produced from clean, fresh animal blood exclusive of all extraneous materials such as hair, stomach belchings, and urine, except as might occur unavoidably in good processing practices. The process used must be listed as a part of the product name such as conventional cooker dried, steam, spray, or hydrolyzed.
- *Hydrolyzed Poultry Feathers* is the product resulting from the treatment, under pressure, of clean, undecomposed feathers from slaughtered poultry, free of additives or accelerators. Not less than 75% of its crude protein content must be digestible by the pepsin digestibility method.
- *Meat and Bone Meal* is the rendered product from mammal tissues, including bone, exclusive of any added blood, hair, hoof, horn, hide trimmings, manure, stomach, and rumen contents, except in such amounts as may occur unavoidably in good processing practices. It shall contain a minimum of 4.0% phosphorous (P), and the calcium (Ca) level shall not be more than 2.2 times the actual phosphorous (P) level.
- *Poultry Byproduct Meal* consists of the ground, rendered, clean parts of the carcass of slaughtered poultry, such as necks, feet, undeveloped eggs, and intestines, exclusive of feathers, except in such amounts as might occur unavoidably in good processing practices.
- *Meat Meal* is the rendered product from mammal tissues, exclusive of any added blood, hair, hoof, horn, hide trimmings, manure, stomach, and rumen contents, except in such amounts as may occur unavoidably in good processing practices. The calcium (Ca) level shall not exceed the actual level of phosphorous (P) by more than 2.2 times.
- *Meat Meal and Tankage* is the rendered product from mammal tissues exclusive of any added hair, hoof, horn, hide trimmings, manure, stomach, and

rumen contents, except in such amounts as may occur unavoidably in processing factory practices. It may contain added blood or blood meal; however, it shall not contain any other added extraneous materials not provided for by this definition. The calcium (Ca) level shall not exceed the actual level of phosphorous (P) by more than 2.2 times.

- *Fish Meal* is the clean, dried, ground tissue of undecomposed whole fish or fish cuttings, either or both, with or without the extraction of part of the oil. It must contain not more than 10% moisture. If it contains more than 3% salt, the amount of salt must constitute a part of the product name, provided that the salt content of this product not exceed 7% (4).

As with any dry feed ingredient, if stored in the proper environment, byproduct meals can be kept for long periods of time. The rendering process kills bacteria. However, the meals can be recontaminated through various environmental sources. As animal proteins provide a favorable climate for the growth of bacteria, extra care must be taken during storage and handling to prevent bacterial growth. The biggest factor is moisture; therefore, meals should be kept in a dry environment. The fat in the meal, especially fishmeal, can become rancid because of oxidation. Antioxidants can be added to the meal, either during the cooking process or during the blending of various meals to increase the shelf life of the meals.

The global production of animal proteins is not listed by Oil World, although they do carry data on tallow/grease, lard, and fish oil, which could be because, in most countries, the rendered animal proteins are consumed locally and not generally exported. With an estimated conversion ratio from raw material to finished product of 5:1 for both the protein and fat fractions, the global potential production of meat and bone meal would be in the range of about 25 million metric tons each for the protein and fat products. According to data taken from the FAO databases, the European Union, Australia, New Zealand, the United States, Canada, and South America account for most of the exports of meat meals. FAO does not distinguish the types of meals that make up meat meal, but it appears to include all the red meats including poultry. Within South America, Argentina and Uruguay account for most of the meat meal exports. Figure 3 shows the exports of meat meal for the period 1992 through 2002, the last year available in the FAO database (2).

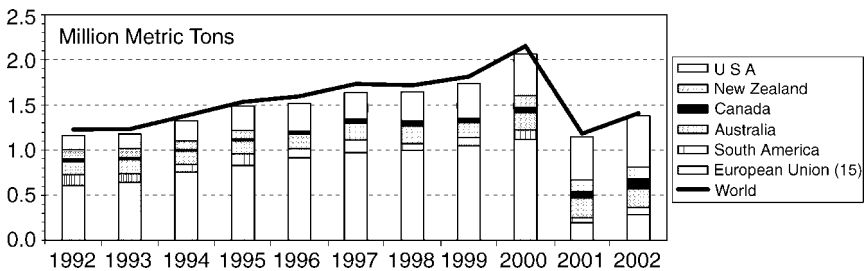


Figure 3. Major meat meal exporters (2).

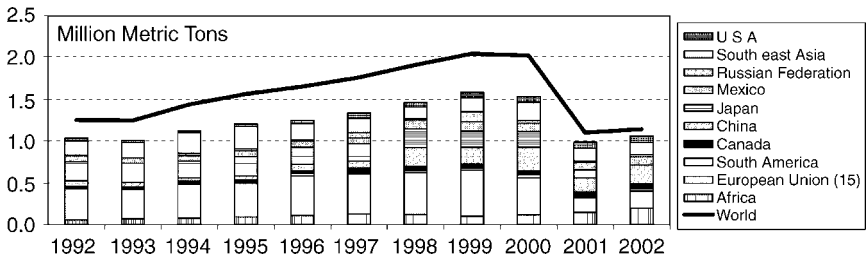


Figure 4. Major importers of meat meal (2).

China, Japan, Mexico, the European Union, South East Asia, and Africa are major importers of meat meal, which can be seen in Figure 4. The data covers 1992–2002, the most current year in the FAO database (2). Without specific production data, it is difficult to understand why some regions are both major importers and exporters of the meat meal category. One would assume that because this category includes a number of products, some products are not consumed domestically and are exported and replaced by a different product in that category, for example, exporting ruminant meals and importing poultry meals. The data in Figures 3 and 4 clearly show the dramatic effect that the global bovine spongiform encephalopathy (BSE) crisis (discussed later) has had on exports of meat and bone meal.

Within the United States, four major categories of rendered animal protein products exist. The production and export of these products over the period 1998–2002 is shown in Table 3 (5).

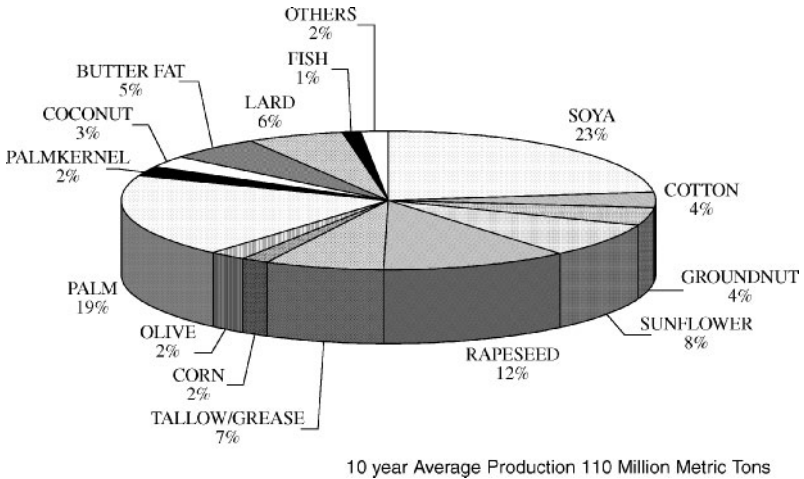
### 3.2. Fats

Oil World Annual lists tallow/grease, lard, and crude fish oil in their statistical database. However, no listings for the other fat products that are produced by the

TABLE 3. U.S. Production and Export of Rendered Protein Products<sup>a</sup> 1998–2002 (5).

	1998	1999	2000	2001	2002
<b>PRODUCTION</b>					
Meat Meal & Tankage	2,511	2,748	2,612	2,509	2,514
Meat and Bone Meal	1,921	2,138	1,996	1,962	2,025
Dry Rendered Tankage	572	583	595	542	490
Feather Meal	366	379	368	354	362
All Other Inedible Products	1,038	1,148	1,235	1,258	1,319
Total Production	6,408	6,996	6,806	6,625	6,710
<b>EXPORTS</b>					
Meat & Bone Meal	313	382	435	452	565
Feather Meal	30	21	25	42	39
Sub-total	343	403	460	494	604
Bone & Bone Products	21	26	36	37	24
Total Exports	707	832	956	1,025	1,232

<sup>a</sup>In Thousand Metric Tons.



**Figure 5.** Global 10-year average production of oils and fats (3).

rendering industry exist. Based on Oil World Annual, Figure 5 shows the 10-year average production of the various global fats and oils (3).

AAFCO defines a number of animal fats in its latest official publication.

- *Animal Fat* is obtained from the tissues of mammals or poultry in the commercial processes of rendering or extraction. It consists predominantly of glycerol esters of fatty acids and contains no additions of free fatty acids or other materials obtained from fats. It must contain, and be guaranteed for, not less than 90% total fatty acids, not more than 2.5% unsaponifiable matter, and not more than 1% insoluble impurities.
- *Hydrolyzed Fat or Oil (Feed Grade)* is obtained in the fat processing procedures commonly used in edible fat processing or soap making. It consists predominantly of fatty acids and must contain, and be guaranteed for, not less than 85% total fatty acids, not more than 6% unsaponifiable matter, and not more than 1% insoluble impurities.
- *Fat Product (Feed Grade)* is any fat product that does not meet the definitions for animal fat, vegetable fat or oil, hydrolyzed fat, or fat ester.
- *Lard* is rendered fat of swine.
- *Tallow* is animal fat with a titer above 40°C.
- *Grease* is animal fat with a titer below 40°C. Choice white grease is derived primarily from the rendering of pork offal whereas yellow grease is derived from restaurant grease (4).

The fats and oils produced from the rendering process provide a concentrated source of energy for use in animal feeds. Addition of fat in feeds also reduces dustiness of the feed and makes it more palatable. Fats and oils also provide important

ingredients for further processing for the making of many products such as soap, fatty acids, and glycerol. Edible fats produced in the rendering industry may be used for commercial baked goods, fast-food restaurants, margarine production, or medicinal applications such as omega-3 fatty acids in marine oils. Table 4 shows the U.S. production and export of rendered fat products for the period 1998–2002 (5).

Animal fats are not difficult to handle, and if kept in good condition, the fat can be kept for long periods of time. Several factors will determine how long animal fats can be kept. Fats should be stored and handled in steel or iron tanks and piping, avoiding any contact with brass or copper. Even short exposure to brass and copper will cause the fat to go rancid. Dry, clean fat will not be damaged by moderate heat; however, excess moisture, insoluble impurities, and unsaponifiables (MIU) with moderate heat will increase the free fatty acids (FFA) and instability of the fat while increasing the probability of rancidity. Peroxide value (PV) is a measure of stability. When fats are handled properly, and stabilized with a proper amount and type of stabilizer such as an antioxidant, the fats will remain stable with a low peroxide value for long periods. The fats and oils go through many processes before they reach their finished form. These processes are described in more detail in Sections 8 through 10 this chapter.

The major use of edible tallow and lard is for cooking and frying. Until recently, edible tallow was used quite extensively by the fast-food industry for frying foods such as French fries. However, because of the perceived health concerns regarding cholesterol, tallow has been replaced by vegetable oils, and human consumption of tallow has declined. Nonetheless, the use of edible tallow in other markets has expanded, including the manufacturing of foods such as bakery products and margarine.

The majority of the rendered fat produced is inedible tallow and grease. A large portion of the market for inedible tallow and grease is accounted for by animal

**TABLE 4. U.S. Production and Export of Rendered Fat Products<sup>a</sup> for 1998–2002 (5).**

	1998	1999	2000	2001	2002
<b>PRODUCTION</b>					
Inedible Tallow & Greases	2,982.4	3,209.5	3,242.9	3,116.2	3,272.6
Inedible Tallow	1,638.2	1,750.5	1,764.4	1,704.2	1,711.3
Greases	1,327.9	1,438.7	1,461.1	1,441.0	1,561.4
Edible Tallow	697.1	784.4	834.8	836.9	892.7
Lard	246.9	243.1	234.9	182.9	175.1
Total	3,926	4,237	4,313	4,136	4,340
<b>EXPORTS</b>					
Inedible Tallow	1,028.1	875.8	789.4	605.4	779.4
Yellow Grease	210.3	183.4	182.6	184.3	287.5
Other Inedible Fats & Oils	189.9	247.6	229.7	190.3	206.7
Edible Tallow	111.8	143.8	110.8	165.3	209.3
Lard	59.6	66.9	78.9	46.9	38.0
Total	1,600	1,518	1,391	1,192	1,521

<sup>a</sup>In thousand metric tons.

feeds. However, nonfeed uses exist for inedible fats, which will also be discussed in Sections 11 through 14 in this chapter.

## 4. PROCESSING

Rendering is accomplished via a variety of equipment and processes. It is a process of both physical and chemical transformation, using trimmings from the production and processing of food animals for meat. All of the rendering processes involve the application of heat, the extraction of moisture, and the separation of fat when it is part of the raw material.

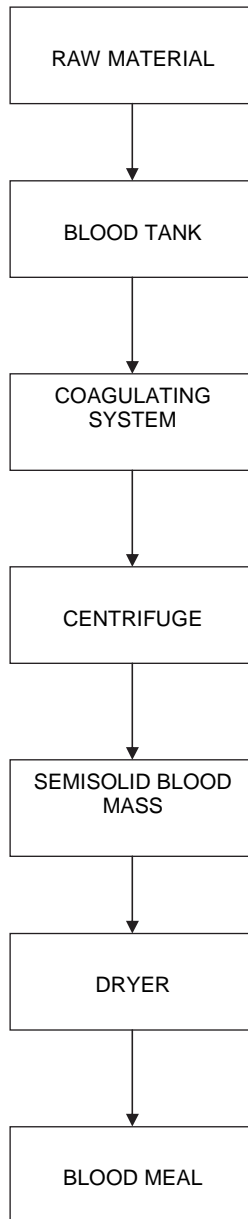
### 4.1. Protein Meals

**4.1.1. Blood Meal** Blood meal may be prepared by several different processes. Cooker-dried blood is produced in a conventional batch cooker. Spray-dried blood is a two-step procedure by which moisture is first removed by a low-temperature evaporator under vacuum until it contains approximately 30% solids and is then spray dried. Flash-dried blood meal is also a two-step process and is described in Figure 6.

The blood is first put into a coagulating system. The raw blood is pumped through a heated pipe (live steam injected into a pipe), which causes coagulation. The coagulated blood is then transferred to a dewatering device (such as a centrifuge), and a large portion of the moisture is removed, yielding a semisolid protein. The coagulated blood may also have the water removed by cooking in a condenser to a semisolid state. The semisolid blood mass is then transferred to a rapid drying facility, such as a flash dryer, where the more tightly bound water is rapidly removed. Ring-dried blood is similar to flash-dried blood meal, except that the rapid drying facility is a ring dryer. Ring drying the blood produces a better quality product than the conventional method, because it uses warm air to dry the blood mass and not warm metal. The blood is not exposed to a direct heat surface and, consequently, the sensitive amino acids such as lysine are not destroyed.

Care must be taken in the processing and purchasing of blood meal. These different processing methods produce different types of blood meal, which may vary in crude protein and amount and bioavailability of lysine and other amino acids (6, 7).

**4.1.2. Feather Meal** Feathers are rendered either using a batch or a continuous hydrolyzer. Poultry feathers consist of the protein keratin, which is essentially indigestible. To make the protein digestible and available for the domesticated animal, the feathers must be hydrolyzed, which is done in a hydrolyzer under pressure at approximately 30–50 psi for 45–60 minutes to break the bonds that hold the protein together (such as amino acid bonds, sulfur-sulfur bonds of cystine, and hydrogen bonds between nonadjacent amino acids). Figure 7 shows the basic flow diagram for rendering feathers. The raw material may first go through a spreader to cut up any large particles (such as heads or feet) that may have entered the system. The



**Figure 6.** Flow diagram of blood meal production.

raw feathers are then fed into the hydrolyzer and the protein bonds are broken down. The hydrolyzed feather is then fed into a dryer to remove excess moisture to yield feather meal. At this point, the meal can then be ground to produce a finer ground feather meal. Hoof, horn, and hair meal are produced in much the same way.

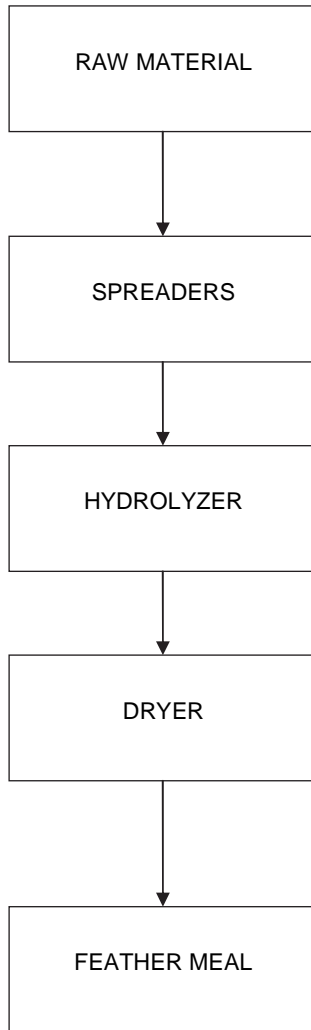
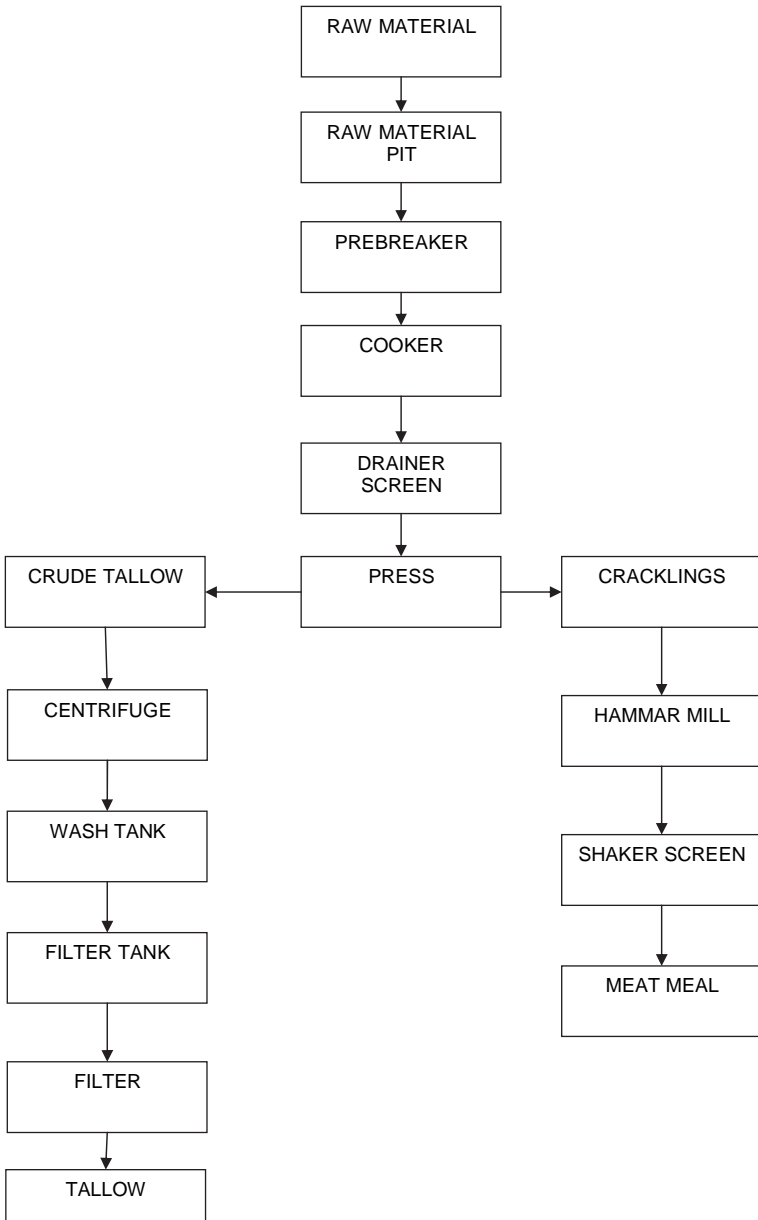


Figure 7. Flow diagram of feather meal production.

**4.1.3. Meat and Bone Meal and Poultry Byproducts Meal** Understanding that beef, pork, and poultry processing procedures are similar, for simplification, only the rendering process of ruminant meat and bone meal will be discussed. Raw material collection is the first, and an important, step in the rendering process. The timely and efficient collection of raw materials is critical to the industry. The raw material must be clean and fresh to yield the highest quality products. Dirty and decayed offal will lead to a darker colored fat, which is undesirable. Figure 8 shows a typical flow diagram of the production of meat and bone meal and tallow.

The raw material is first collected by the trucks and then discharged into a receiving bin. The material is then screw-conveyed to a crusher, prebreaker, or





**Figure 8.** Flow diagram for the production of meat meal and inedible tallow.

similar device for size reduction to about 2.5 to 5.0 cm (1–2 inches). It is important to reduce the size of the raw material before processing to increase the production rate, decrease energy costs, and lead to uniform cooking, which enhances the quality of the finished product.

At this point, the raw material goes into either a batch or continuous cooking system. Within the cooking system, the heating and grinding action breaks down the fat cells in the animal tissue to release the fat. The rendering process consists essentially of two steps. First, the raw material is heated or “cooked” to evaporate the moisture. The second step is the removal of fat.

The batch cooking system is the process by which batches of raw material are cooked according to a repetitive cycle. Two methods of batch cooking exist: wet rendering and dry rendering. For the wet rendering method, the material is cooked by steam under pressure in large closed tanks. The material is boiled with open steam under pressure (25–75 psi internal pressure) from the bottom of the cooker. After approximately 1.5–2.5 hours, the pressure is then reversed and applied from the top, which causes the water and sludge to settle to the bottom of the cooker, where it is drained off. After the water is drained off, the fat is removed from the bottom and diverted to another tank for further processing. The tankage then goes to the press for further fat removal.

The dry rendering process is the newer and more efficient method of cooking. All the material is cooked in its own grease by dry heat in open steam jacketed drums until the moisture has evaporated. Efficient cookers usually range in temperature from 115–120°C and take approximately 1.5–4 hours. Agitators in the cooker reduce the cooking time. However, care must be taken because increased agitation speed usually increases the amount of fines or particulate matter in the fat. After the cooking process is complete, the material goes across a screen that allows the free fat to run off. The tankage is then conveyed to a press where the residual fat is removed, resulting in a product that contains 6–10% fat.

The continuous cooking system is similar to the dry rendering batch method except the raw material is fed into the system in a semicontinuous fashion, which is the most modern and efficient cooking method. A continuous system normally consists of a single cooker, whereas the batch system consists of multiple cooker units. As a result, continuous cooking systems offer many advantages over batch cooking methods, including reduced labor requirements, less floor space required (one cooker versus several), lower maintenance and operating costs (less time, temperature, and fuel consumption with one cooker), and easier control of air and water pollution (more enclosed environment with the one cooker). The continuous system produces a more consistent product than the batch system. As the flow is essentially constant, process variables are more easily controlled. However, care must be taken because some raw material could be short circuited without sufficient contact time to adequately destroy the bacteria in the raw material. Temperature of the continuous process usually falls into two categories: low temperature (100–115°C) and high temperature (130–150°C). The time the material stays in the cooker ranges from 30 to 60 minutes (depending on temperature; the lower the temperature, the longer it stays in the cooker) and the material is continuously discharged. The product usually has 4–6% moisture.

After the cooking process is completed, the cooked material is drained and pressed to remove the fat using either a hydraulic or screw-press. The solid protein (cracklings) is then normally ground with a hammer mill. After the hammer mill,

the meal passes over a shaker screen to remove any coarse material and to produce a uniform protein meal. The fat discharged from the press normally contains fine solid particles, which are removed by centrifugation or filtration (7).

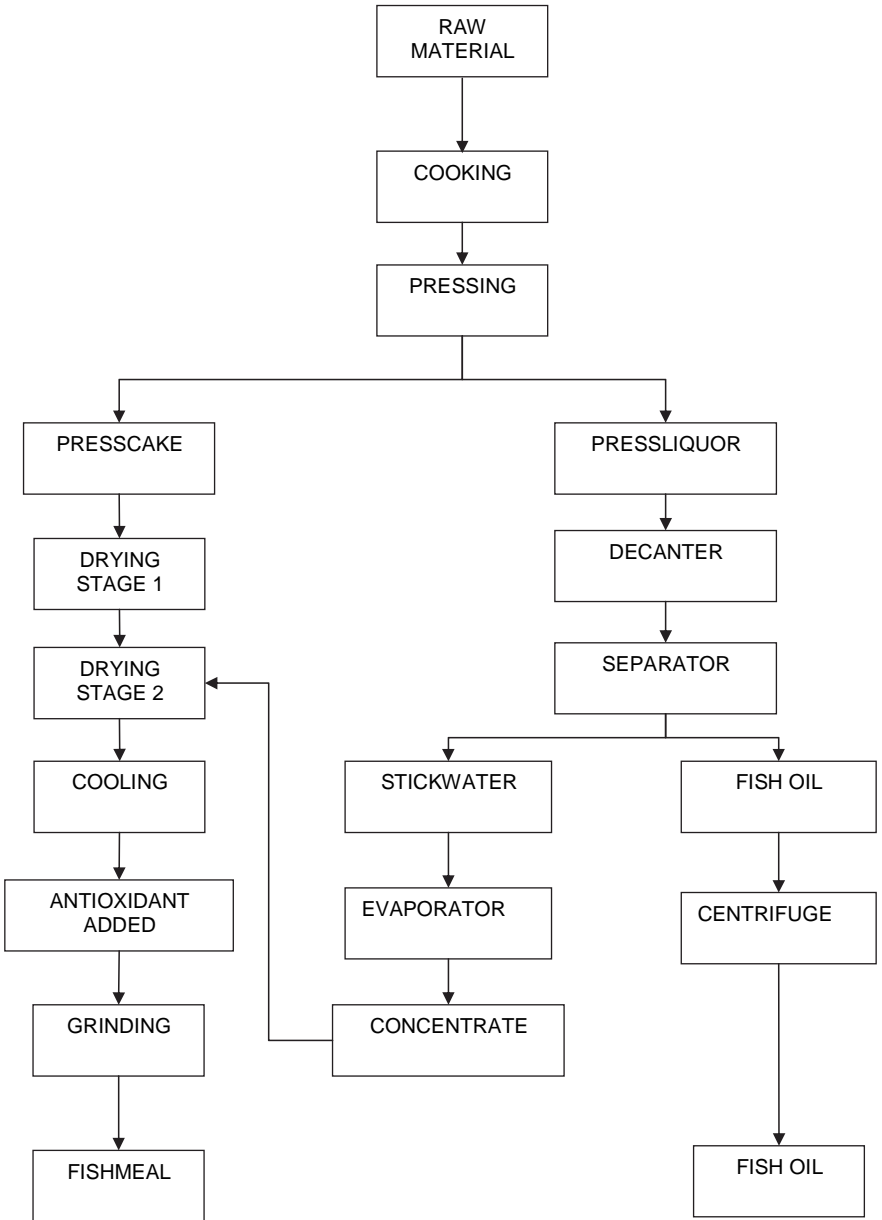
**4.1.4. Fish Meal** Two general methods of manufacturing fishmeal exist: the dry rendering process and the wet rendering process. In the dry process, the oil produced during cooking is not removed. This process can be used when the starting material is white fish or fish with low oil content, and the finished product is called white fishmeal. A flow diagram of the wet rendering process used for fishmeal production is shown in Figure 9.

In the wet process, a portion of the fish oil in the product is removed during pressing. Fishmeal made from fish with a high oil content, such as menhaden and anchovy, must have the oil removed to produce a stable product that can be easily handled. Most fishmeal is produced using the wet method, which is usually a continuous cooker process similar to meat and bone meal production. The raw material is sometimes ground and fed into a cooker. Unlike the rendering process, the cooking step is designed to break down the fat cells to release oil. The cooked material is then hydraulically pressed to remove water, soluble protein, and oil. What is left is called presscake. The presscake is then dried and ground to produce fishmeal. The aqueous phase from the pressing process after removal of the oil is called stickwater. The stickwater is concentrated and added back to the presscake to form what is called wholemeal. The concentrated stickwater is called fish solubles and is sometimes sold as a separate product (8).

## 4.2. Fat Products

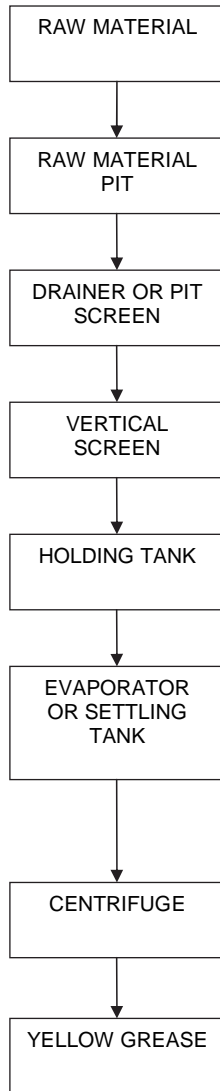
**4.2.1. Greases** Several steps are taken to process the liquid phase from the rendering process once it is released by the presses or from the street collection of restaurant grease. A typical flow diagram of a grease processing system is shown in Figure 10. The grease is brought into the rendering plant in either 50-gallon drums or bulk containers. The raw grease is dumped into a receiving pit; then it is conveyed over a drainer screen followed by a vertical screen to remove any foreign material such as rags, plastics, etc. The grease can then go to a vertical settling tank (holding tank) and is held while the water and more impurities are settled out or the grease goes to an evaporator system, which evaporates the water from the grease. Once the water is removed, the grease goes through a centrifuge to remove more impurities. After the moisture and impurities are reduced to an acceptable level, the grease is pumped into a storage tank (9).

**4.2.2. Tallow** A flow diagram of a typical edible tallow system is shown in Figure 11. In this process, edible fat trimmings removed from edible beef carcasses are rendered into the edible tallow product. The inedible tallow process was shown in Figure 8. After the press, the crude tallow is pumped to a centrifuge, where small proteinaceous fines are removed, and then to a holding tank for washing or filtering



**Figure 9.** Flow diagram of the production of fishmeal and fish oil.

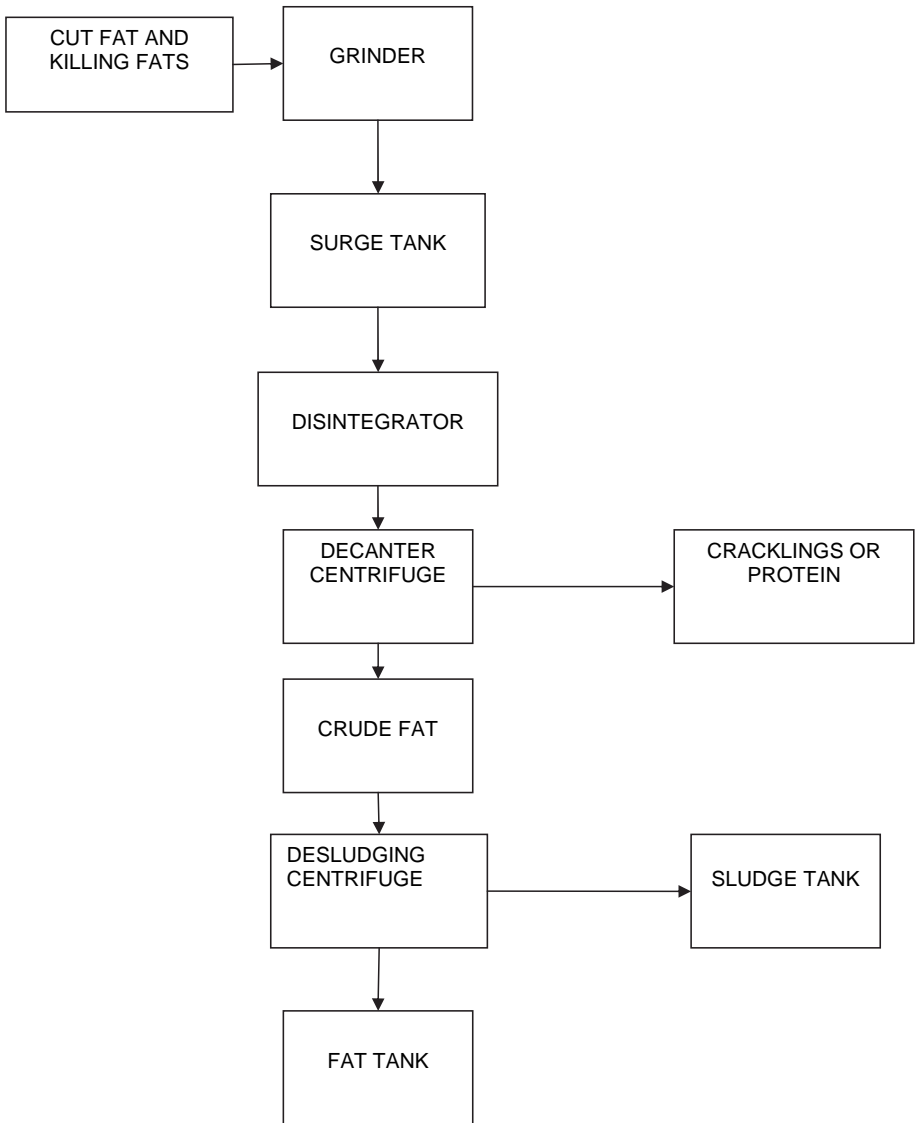
to remove any impurities. After filtering, tallow can undergo further processing, including refining, bleaching, deodorizing, fatty acid splitting, and hydrogenation. In the edible tallow process, the fat cuttings are ground up and heated to melt the fat. The melted mix is then sent to a decanter centrifuge where the fat is separated



**Figure 10.** Flow diagram of the production of yellow grease.

from the solids. After further cleaning in another centrifuge, the edible tallow can be further processed or refined.

The term *refining* refers to any purifying treatment to remove impurities in the tallow. These impurities may include free fatty acids, phosphatides, or other material found undesirable in the fat. The terms exclude *bleaching* and *deodorizing*. Bleaching refers to the treatment of the tallow solely to reduce the color of the oil. *Deodorization* is the term used for the removal of volatile fractions in the fat



**Figure 11.** Flow diagram for the production of edible tallow.

(e.g., free fatty acids and peroxide/degradation products), that may cause off-flavors and off-odors.

Individual fatty acids are produced by “splitting” the triacylglycerol to form fatty acids and glycerol. The glycerol is then separated and sold as a byproduct. Distillation is used to separate and purify the fatty acids for a wide range of industrial applications, including surfactants, soaps, plastics, resins, rubber, paints, lubricants, textiles, and cosmetics.

Hydrogenation is the addition of hydrogen to the carbon-carbon double bonds in the presence of a metal catalyst, which results in the conversion of liquid oils to hard or plastic fats and soft fats to firmer products. It also results in improved resistance of the fats and oils to deterioration through oxidation.

**4.2.3. Fish Oil** The liquid removed from the fish presscake is separated by centrifugation into an oil fraction and a water-soluble protein fraction called solubles. The solubles are often concentrated by evaporation and either added back to the presscake before drying, sold separately as “fish solubles,” or discarded. Fishmeal plants operating onboard fish processing ships usually discard the solubles.

The fish oil produced after the solubles have been removed can be further processed and used in various applications, such as components of paint, feed ingredients for aquaculture diets, edible oil used in preparing a variety of human foods, and pharmaceutical uses. Currently, the primary pharmaceutical application is in the use of fish oil for its high omega-3 fatty acids. Fish oil was always considered as an edible oil outside the United States and as an industrial oil within the United States. The primary use for the oil in foods was in the hydrogenated form until the mid-1990s when the health issues related to *trans*-fatty acids caused a major shift in the market. The shift moved the market away from hydrogenation and toward use in aquaculture diets where the omega-3 fatty acids in the oil are considered essential for carnivorous fish. The US FDA GRAS (generally recognized as safe) affirmation of hydrogenated menhaden oil in 1989 and nonhydrogenated menhaden oil in 1995 opened the U.S. market to fish oil, especially in the nonhydrogenated form where the omega-3 fatty acids were considered beneficial to health. The approval of a qualified health claim (QHC) for omega-3 fatty acids and their effect on cardiovascular disease in late 2004 will further open up the food market to fish oils (8).

## 5. PRODUCT COMPOSITION

The rendering process converts raw animal or fish tissue into various protein, fat, and mineral products, all of which are transformed into rich granular-type powders and fats with specific nutritional and chemical components. The animal byproduct raw material has approximately 60%+ of water and yields about 20% protein and 20% fat. These protein, fat, and mineral fractions are then available for a variety of uses. The annual volume in the United States approximates 4.2 million metric tons of animal protein and 4.2 million tons of rendered fat.

### 5.1. Proteins

The major animal protein ingredients are all important feed ingredients for livestock, poultry, aquaculture, and pet food diets throughout the world. Meat and bone meal (MBM), meat meal, and poultry byproduct meal (PBM) comprise the largest available quantities. Animal proteins contribute not only protein but are also excellent sources of amino acids, fat, essential fatty acids, minerals, and vitamins. Thus, when compared with other protein ingredients, multiple nutrients are derived from animal protein ingredient sources in addition to the protein and amino acids.

**TABLE 5. Composition of Various Rendered Animal Proteins<sup>a</sup> and Menhaden Fishmeal<sup>b</sup>.**

	Meat and Bone Meal	Blood Meal	Feather Meal	Poultry By-Product Meal	Menhaden Fishmeal
Crude Protein, %	50.4	88.9	81.0	60.0	61.25
Fat, %	10.0	1.0	7.0	13.0	9.13
Calcium, %	10.2	0.4	0.3	3.0	4.87
Phosphorous, %	5.1	0.3	0.5	1.7	2.93
TME <sub>N</sub> , kcal/kg	2666	3625	3276	3120	3025
<b>Amino Acids, % of Sample</b>					
Methionine, %	0.7	0.6	0.6	1.0	1.61
Cystine, %	0.7	0.5	4.3	1.0	0.55
Lysine, %	2.6	7.1	2.3	3.1	4.55
Threonine, %	1.7	3.2	3.8	2.2	2.44
Isoleucine, %	1.5	1.0	3.9	2.2	2.32
Valine, %	2.4	7.3	5.9	2.9	2.83
Tryptophan, %	0.3	1.3	0.6	0.4	0.48
Arginine, %	3.3	3.6	5.6	3.9	3.68
Histidine, %	1.0	3.5	0.9	1.1	1.52
Leucine, %	3.3	10.5	6.9	4.0	4.32
Phenylalanine, %	1.8	5.7	3.9	2.3	2.17
Tyrosine, %	1.2	2.1	2.5	1.7	1.83
Glycine, %	6.7	4.6	6.1	6.2	4.49
Serine, %	2.2	4.3	8.5	2.7	2.51

<sup>a</sup>Reference (10), <sup>b</sup> Reference (8).

## 5.2. Protein Product Specifications

The typical nutrient composition of the four most common animal proteins and fishmeal is shown in Table 5 (8, 10).

## 5.3. Fats

The animal feed industry is a major user of rendered animal fats, recycled restaurant grease, and cooking oils. Fats are the highest caloric dense feedstuff and foodstuff. In addition, fats and certain of their component fatty acids are essential and indispensable for body functions in addition to their caloric function. The U.S. rendering industry processes about 5.3 million metric tons (MMT) of the following fats on an annual basis:

- Edible tallow—0.74 MMT
- Inedible tallow—1.75 MMT
- Lard and grease—0.59 MMT
- Yellow Grease—1.20 MMT
- Poultry fat—1.01 MMT



This annual volume is about one-third of the total U.S. production of fats and oils (10). No data exists on the global production of these rendered animal fats.

### 5.4. Fat Product Specifications

The typical fatty acid composition of these rendered animal fats and fish oil is shown in Table 6 (11, 12). Rendered animal fats can also be characterized by their

**TABLE 6. Comparison of the Fatty Acid Composition % of Various Rendered Fats<sup>a</sup> and Fish Oil.**

	Chicken Fat	Yellow Grease	Choice White Grease	Tallow	Gulf Menhaden Fish Oil <sup>b</sup>
C8:0	<0.10	<0.10	<0.10	<0.10	
C10:0	<0.10	<0.10	<0.10	<0.10	
C11:0	<0.10	<0.10	<0.10	<0.10	
C12:0	<0.10	<0.10	<0.10	<0.10	
C14:0	0.57	0.70	1.57	2.73	9.65
C14:1	0.26	0.14	0.36	0.50	
C15:0	<0.10	0.11	0.26	0.43	1.78
C15:1	<0.10	<0.10	<0.10	0.16	
C16:0	22.76	14.26	22.04	22.99	13.33
C16:1	8.37	1.43	5.03	2.86	12.67
C16:2	<0.10	<0.10	<0.10	<0.10	2.18
C16:3	<0.10	<0.10	<0.10	<0.10	2.92
C16:4	<0.10	<0.10	<0.10	<0.10	1.74
C17:0	0.11	0.33	0.63	1.35	2.54
C17:1	0.12	0.23	0.43	0.75	
C18:0	5.36	8.23	9.95	19.44	3.06
C18:1	42.07	43.34	42.45	41.60	10.75
C18:2	17.14	26.25	13.17	3.91	1.91
C18:3	1.07	2.51	0.97	0.49	1.5
C18:4	0.22	0.47	0.29	0.36	2.30
C20:0	<0.10	0.33	0.14	0.14	0.19
C20:1	0.45	0.48	0.56	0.33	1.03
C20:2	0.20	<0.10	0.19	<0.10	0.15
C20:3	0.19	<0.10	0.12	<0.10	0.30
C20:4	0.45	<0.10	0.34	<0.10	1.84
C20:5	<0.10	<0.10	0.11	<0.10	13.07
C21:5	<0.10	<0.10	<0.10	<0.10	0.76
C22	<0.10	3.50	<0.10	<0.10	0.11
C22:1	<0.10	<0.10	<0.10	<0.10	0.25
C22:2	<0.10	<0.10	<0.10	<0.10	
C22:3	<0.10	<0.10	<0.10	<0.10	
C22:4	0.10	<0.10	<0.10	<0.10	0.21
C22:5	<0.10	<0.10	0.14	<0.10	2.56
C22:6	<0.10	<0.10	0.22	<0.10	6.84
C24	<0.10	0.12	<0.10	<0.10	0.29
C24:1	<0.10	<0.10	<0.10	<0.10	0.56
Unknown	0.56	0.72	1.03	1.96	5.51
IV	80	75	65	50	165

<sup>a</sup> Reference (11), <sup>b</sup> Reference (12).

**TABLE 7. Titer and Iodine Values of Fat from Various Livestock Species<sup>a</sup> Including Menhaden Fish Oil.**

Species	Titer, °C (°F)	Iodine Value
Sheep	44–48 (111–118)	42–43
Cattle	42–45 (108–113)	43–45
Hogs	36–40 (97–104)	64–65
Horses	35–38 (95–100)	80–85
Poultry	31–35 (89–95)	77–80
Menhaden <sup>b</sup>	Not available	150–200

<sup>a</sup>Reference (10), <sup>b</sup>Reference (12).

degree of hardness or melting point. A comparison of the different animal fats is given in Table 7 (10, 12).

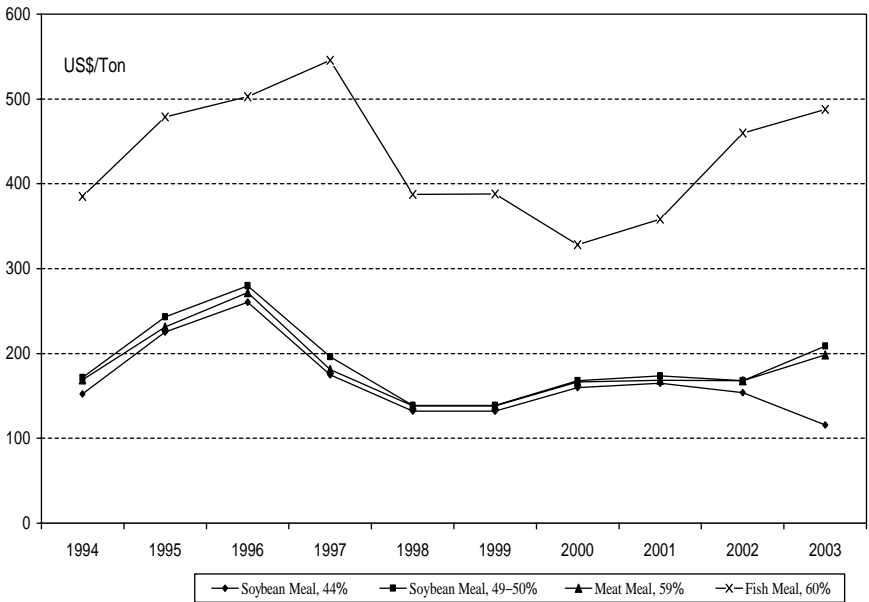
## 5.5. End Uses

The U.S. livestock sector slaughters 139 million head of cattle, calves, sheep, hogs, and horses and nearly 36 billion pounds of poultry annually. In addition to protein for human consumption, the system produces a large amount of byproducts that are, in turn, transformed into highly valuable feed and industrial products. The task of transforming byproducts from slaughterhouses and packing plants into safe and valuable products lies with the renderers. Animal offal (including viscera, heads, bone, blood, and other waste) is the primary raw material for rendering operations, along with waste generated from restaurants, grocery stores, and butcher shops. From this raw material, renderers manufacture meat and bone meal (MBM) for use in livestock feed, as well as tallow, greases, and various other products of value to the feed, industrial, and food processing industries (13).

The rendering industry performs functions vitally important to the livestock and poultry sectors: It provides an outlet for over 23 million tons of byproducts from meat packers, poultry processors, restaurants, retail meat stores, and other entities in the United States alone. From this waste, the rendering industry produces about 5 million tons of protein ingredients, highly valued by the feed industry. Also produced is a wide range of other lipid materials used in various feed and industrial applications, which amounts to over 4.5 million tons in the United States.

## 6. PROTEINS

Rendered animal proteins are especially valuable to the livestock and feed industry because of their high protein content, digestible amino acid levels (especially lysine), mineral availability (especially calcium and phosphorous), and relatively low cost in relation to their nutrient value. They have few close substitutes in most rations, although specific feeding characteristics of animal products can be



**Figure 12.** Commodity prices of several protein sources (5).

reproduced to some degree using vegetable protein sources (e.g., soybean meal) supplemented with minerals and synthetic amino acids where necessary, but often at significantly higher cost. For most feeding applications, animal proteins provide one of the lowest cost balanced nutrients compared with plant-based or synthetic alternatives. For other applications, the natural forms of amino acids (e.g., lysine in blood meal) are the only effective source (10).

## 6.1. Protein Prices

Rendered animal proteins are commodities and compete on the world market with other sources of protein. A comparison of meat meal, fishmeal, and soybean meal prices is shown in Figure 12. In this case, the soybean meal with 49–50% protein is basis Decatur, the soybean meal with 44% protein is also basis Decatur, the meat meal with 59% protein is basis Kansas City, and the fishmeal with 60% protein is basis U.S. Gulf Coast (5).

## 7. FATS

Rendered fats have many nonfood and nonfeed uses. In the United States, most of the rendered fats are derived from beef animals (tallow), swine (lard), and poultry (poultry grease or poultry fat). Some minor amounts of fats are derived from other

species such as sheep (mutton tallow) and fish. Some users prefer to use the term fats to describe rendered products with titers of more than 39°C and the term oils to describe those products with titers of less than 39°C. Rendered fats with lower titers may also be referred to as greases, as shown previously in Table 7.

The industrial uses of the different fats depend on the characteristics of such fats as determined by analyses such as titer, fatty acid profile, free fatty acid content (or sometimes acid value), saponification value, refined and bleached (R&B) color, peroxide value, and by the absence of impurities, such as moisture and unsaponifiable matter. Some abbreviations may be used, such as FFA for free fatty acids and IV for iodine value.

Rendered fats may be further processed by refining, bleaching, and deodorizing, and these may be used as raw materials in hydrogenation, hydrolysis to fatty acids, and *trans*-esterification to fatty esters. Many factors affect the suitability of rendered fats for such use; these include

- The types and blends of animal byproducts to be rendered.
- The storage conditions of the animal raw material byproducts before processing.
- The methods and procedures of the rendering process used.
- The storage conditions of the rendered fats after processing.

Rendering plants in the United States produce a wide variety and types of fats with widely varying characteristics. For example, in the southeastern United States, the swine and poultry meat-processing industries generate a large part of the total meat byproducts available, and many rendering facilities process a blend of these swine and poultry raw materials along with beef raw materials.

The inclusion of different species in rendering can generate quite different characteristics for rendered fat products. The inclusion of poultry byproduct raw material and swine byproduct raw material will generally lower the titer of a “tallow” product, because of a change in fatty acid profile toward more unsaturation. The standard grades, specifications, and quality tolerances for tallow and greases can be found in the American Fats and Oils Association (AFOA) trading and arbitration rules. Some of these specifications include titer, iodine value, free fatty acids, refined and bleached color, moisture, impurities, and unsaponifiables. These AFOA specifications are shown in Table 8 (14). Titer and iodine value (IV), both being a measurement of fat hardness, are important because the hardness of fat varies with different animal species. Hardfats make firm soaps, whereas soft fat makes soft soap. The harder fats resist oxidation more than the softer fats, so that the firmer soaps do not go rancid as fast as the softer soaps. Free fatty acids (FFA) provide a measure of the amount of hydrolysis that has taken place within the fat molecule. Time, temperature, and the presence of moisture all favor the hydrolysis of fat into free fatty acids and glycerol. Refined and bleached color (R&B) is a factor that is determined by the degree of damage done to the fat. A low R&B color indicates the fat was rendered from high-quality raw materials by using good rendering techniques, which is important for finished soap quality. Moisture, impurities,

**TABLE 8. Rendered Fats Specifications (14).**

Grade	Titer, °C min	FFA, % max	FAC Color max	R&B Color, max	MIU <sup>1</sup> , % max
Edible Tallow	41.0	0.75	3	None	<sup>a</sup>
Edible Lard	38.0	0.50	<sup>b</sup>	None	<sup>a</sup>
Top White Tallow	41.0	2	5	0.5	1
All Beef Packer Tallow	42.0	2	None	0.5	1
Extra-fancy Tallow	41.0	3	5	None	1
Fancy Tallow	40.5	4	7	None	1
Bleachable Fancy Tallow	40.5	4	None	1.5	1
Prime Tallow	40.5	6	13-11B	None	1
Special Tallow	40.0	10	21	None	1
No. 2 Tallow	40.0	35	None	None	2
"A" Tallow	39.0	15	39	None	2
Choice White Grease	36.0	4	13-11 B	None	1
Yellow Grease	<sup>c</sup>	15	39	None	2

<sup>1</sup> MIU: Moisture, Insoluble Impurities and Unsaponifiables.

<sup>a</sup> Moisture maximum 0.20%. Insoluble impurities maximum 0.05%.

<sup>b</sup> Lovibond color for 5.25 inch cell: maximum 1.5 red. Lard peroxide value: 4.0 Meq/Kg maximum.

<sup>c</sup> Titer minimum, when required, to be negotiated between buyer and seller on a contract-by-contract basis.

and unsaponifiables (MIU) are important determinants of fat quality. Excess MIU will cause deterioration of the fat during storage; therefore, the lower the MIU, the better the quality. Sterols are generally the major portion of the unsaponifiable fraction.

The major use of rendered fats is in the production of tallow soaps and in the area of fat hydrolysis, splitting triglycerides into fatty acids, and byproduct glycerine. As the soap-making industry grew and became more refined, the quality standards and specifications for animal fat became more precise to meet the soap industry's need. The specifications and terminology, from the soap industry, form the basis for the quality standards and characteristics used today.

Rendered fats have many other industrial uses. In these other uses, more than 70% require such processes as refining, bleaching, filtration, hydrogenation, *trans*-esterification, and winterization before they can be converted to more useful products. All of these products and processes fall under the general category of oleochemistry.

## 7.1. Fat and Oil Prices

Rendered animal fats are commodities and compete with other fats and oils on the world market. A comparison of some of these products is shown in Figure 13. In this figure, the fish oil price is for crude oil any origin cost + insurance + freight (CIF) N.W. Europe, the lard is EU unrefined 0.5%, the tallow is U.S. bleachable fancy, CIF Rotterdam, and the palm oil is crude oil CIF N.W. Europe (3).

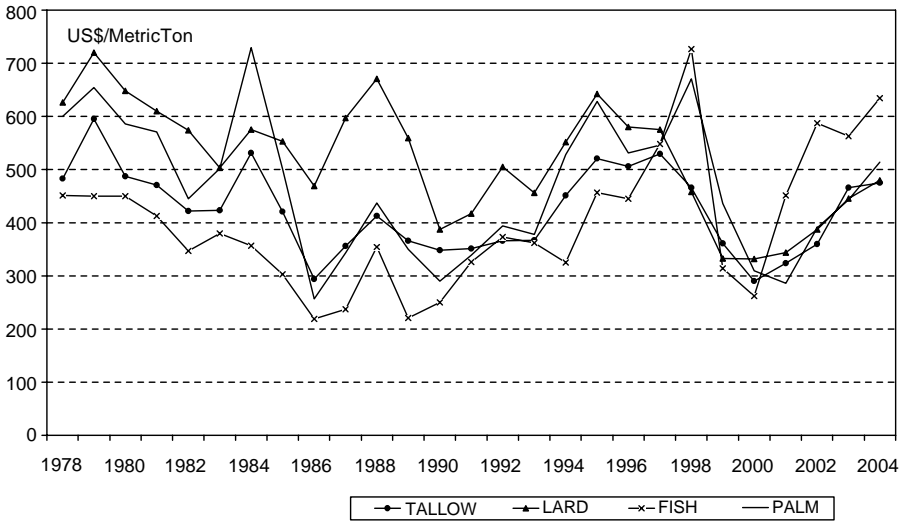


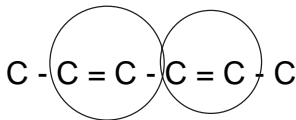
Figure 13. Commodity prices of selected fats and oils (3).

## 8. OLEOCHEMISTRY

Basic oleochemicals are produced by splitting oils and fats and then reacting the split materials to produce glycerine, fatty acids, esters, amines, amides, alcohols, and other compounds (15). Any reaction that can take place at the carboxyl end or the double bond (see Figure 14) offers the opportunity for a potential product

### OLEOCHEMISTRY

Any reaction that takes place at the double bond



Any reaction that takes place at the carboxyl group

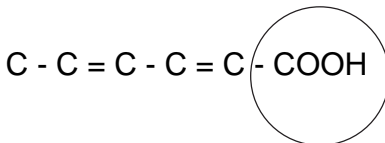


Figure 14. Oleochemistry definition.

or group of products, but in actuality, about 95% of the reactions take place at the carboxyl end and 5% at the double bond (16). These products are classified as natural oleochemicals to distinguish them from synthetic oleochemicals, which are derived from petrochemicals such as ethylene, paraffin, and propylene. Traditionally, tallow, coconut, palm, and palm kernel oil are the major oleochemical raw materials, and only about 20% of the total world production of fats and oils goes into the nonedible market. Other oils such as tung, castor, and linseed have special uses in the industrial market, but their relative volume is small and their prices high because they have specialized properties. Oleochemicals are used across all markets including technical, food, personal care, and agricultural uses; therefore, when one begins to look at quantities, the potential for overlap exists. A tendency to overlook these oleochemical uses also exists, especially when more lucrative markets for the oil or fat exist. For example, the industrial applications for fish oils have never received much attention outside the United States because of the general edible use of the oil throughout the world and the overwhelming interest in the omega-3 fatty acids and their possible incorporation into foods. However, marine oils have a long and respectable history in technical (industrial) products. This history developed within the United States where marine oils were not considered edible until the affirmation as GRAS for menhaden oil was approved in 1989 for hydrogenated menhaden oil and 1995 for refined or nonhydrogenated menhaden oil. Some of these industrial uses are shown in Figure 15 (17).

As a result of the current demand for biodegradable and environmentally friendly sources of raw materials, these uses have a place in the marketing of marine oils well into the twenty-first century and will command premium prices if special niches for the oil can be developed. The competitiveness of the oleochemicals

ATTRACTANTS AND LURES	AUTOMOTIVE GASKETS	CAULKING COMPOUNDS	
CERAMIC DEFLOCCULANTS	CORE OILS	CUTTING OILS	FATTY ACIDS
OLEOCHEMICALS	FERMENTATION SUBSTRATES	FIRE RETARDANTS	
FUEL OILS	GLAZING COMPOUNDS	ILLUMINATING OILS	INSECTICIDAL COMPOUNDS
LEATHER TANNING	LINOLEUM	LUBRICANTS AND GREASES	MOLD RELEASE AGENTS
MUSHROOM CULTURE	OIL-FIELD CHEMICALS	ORE FLOATION	
PLASTICIZERS	OILED FABRICS	PRESSWOOD FIBER BOARDS	
PRINTING INKS	PROTECTIVE COATINGS	REFRACTORY COMPOUNDS	
RUBBER MANUFACTURE	RUSTPROOFING	SOAPS AND DETERGENTS	
SURFACTANTS AND EMULSIFIERS	TIN-PLATING	FUNGICIDE COMPOUNDS	

**Figure 15.** Some of the industrial applications of marine oils (17).

and their opportunities usually lie with economics, but today, a universal awareness exists of protection of the environment, sometimes called the “green” revolution, and in these cases, economics becomes less important. The same would hold true for rendered animal fats and oils.

Much of the current interest in oleochemicals centers on the lauric type oils, coconut, palm, and palm kernel oil because of the physical properties that these medium-chain fatty acids impart to the finished products. So the demand for the longer chain fatty acids, such as found in fish oils and animal fats and oils, is not as great in some markets, but might be essential in others.

### 8.1. Production of Oleochemicals

Oleochemicals are derivatives of fats and oils that are used in the chemical industry to produce a wide variety of products that are then used in numerous applications. The chemical reactions that are used require clean, relatively high-quality raw materials in most, but not all, cases. Some of these markets purchase raw materials on price alone, so they usually get the lowest quality oils. The successful operation can take the cheapest waste oils and convert them into high-value end-products. A good example of this is the conversion of spent restaurant grease into bio-diesel fuel.

The flow diagram for the production of oleochemicals and some of the derivatives produced is shown in Figure 16 (18). Fatty acids and methyl esters are the main products from which a variety of other derivatives can be manufactured.

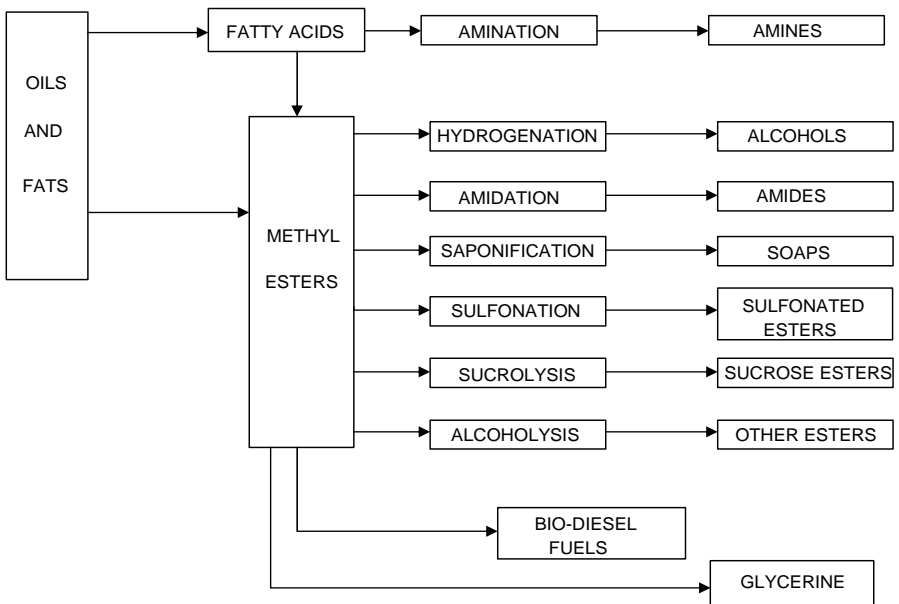


Figure 16. Flow chart of oleochemistry (18).



Glycerine, a byproduct, is a major part of the economics. Other reactions also exist that are not listed here, for example, the heat bodying or blowing (oxidation) of polyunsaturated oils forms polymers that have unusual properties and are used in a variety of markets. Boiled or bodied linseed oil is an example of this type of product.

## 9. REFINING

In general, crude fish oils and animal fats cannot be used directly to produce oleochemical compounds; they must first be refined or further processed. Free fatty acids are usually thought of as impurities in commercially available rendered fats. Consequently, manufacturers of purer grades of tallow will “refine” tallow and other fats by adding a caustic material compound such as sodium hydroxide to form soaps of fatty acids, which are washed out with water.

Steam refining and deodorization are sometimes used in place of caustic refining. New equipment designs have improved refining yields and product quality, and most modern vegetable oil refiners use some form of these more efficient separation methods. However, a large percentage of rendered fats processors have not invested in these technologies and continue to use caustic refining as a means of reducing the level of free fatty acids and color (19).

During caustic refining, the soaps are either gravity or centrifugally separated from the neutral fats; then they are typically acidulated to reform free fatty acids, properly known as acidulated soapstocks, which can be used in animal feeds. This soapstock acidulation is essentially the same process used for the processing of vegetable oil soapstocks. Costs of manufacturing are affected by refining losses, because the acidulated soapstocks are sold for a price somewhat less than the original rendered fat. Acidulated soapstocks contain some proteinaceous material, gums, and some unsaponifiable matter washed out with the soaps in the refining process. Consequently, soapstocks are usually darker than the original fat, and they have a lower nutritional value when used in animal feeds.

A major item in the efficiency of processing rendered fats is the neutral oil loss associated with caustic refining. In tallow-derived chemicals, a general rule of thumb is used that suggests that an average loss of fatty acids and neutral oil will be approximately equal to 1.5 multiplied by the initial free fatty acid content of the fat, assuming that the initial FFA is less than 5% (20). Consequently, a refiner who buys tallow from a renderer that typically supplies a product with 3% FFA should expect a loss of approximately 3% multiplied by 1.5; that is 4.5%. Compared with gravity separation of the soap water in a tank, centrifugal separation of soaps and water from the neutral fat may lead to better yields during the refining process.

Any new developments by equipment manufacturers in vegetable oil refining are transferable to rendered fat processing. In-line monitoring of the free fatty acid (FFA) content with automatic addition of the caustic solution followed by mixing and separation by centrifuge has improved refining yields. Some processors have

been able to improve profitability by improving yields as they switch from batch processing to a continuous operation. The equipment and procedural advances that can improve refining yields mentioned earlier have been slow to be implemented in most instances, however, because of the initial costs of the equipment compared with the yearly savings in chemicals and fat yields. Silica refining has been used in the refining and bleaching of vegetable oils to improve efficiency and quality of the finished oil (21).

Poultry grease or fat is a rendered fat product that presents special problems in refining because of the presence of a significant amount of gums (phospholipids). During conventional caustic refining and washing, these gums can emulsify neutral poultry grease with the soap and water wash, leading to drastic reductions in refining yields. Some improvements in poultry grease refining yields have been seen when prerefining steps are used such as saltwater washing as a degumming step.

Although not a well-known fat in the industrial area, poultry grease has some characteristics that make it potentially valuable. Poultry grease has a low titer, usually within the range of 32–36°C, which causes it to be a liquid oil at room temperature (22). If left to settle at room temperature, poultry grease separates into two fractions: one clear and liquid and the other opaque but still fairly liquid. Either or both of these layers might be used as a cheaper substitute for certain grades of neatsfoot or lard oils or might also be used as a raw material for low cloud point esters such as low cloud, low fallout substitute for methyl tallowate and methyl lardate. Improvements in the refining characteristics of poultry grease may lead to improved economy in the production of refined poultry grease, which would make it more price competitive as a substitute for other fats and oils.

## 10. BLEACHING OR COLOR REDUCTION

The bleaching of rendered fats, typically using commercially available bleaching clays, is done to remove color bodies and other contaminants that darken the color of the fat. Raw rendered fats are usually described by FAC (the Fat Analysis Committee of the American Oil Chemists Society) colors as outlined by the American Oil Chemists' Society (AOCS) Method Cc13a-43. The AOCS notes that the 1992 version was declared obsolete and was removed from the book of methods in 1993. However, its use is not precluded and its official method status is not changed.

After the bleaching process, the reduced color fats are usually described using the Lovibond 5.25-inch scale, correctly known as the AOCS Wesson color, but also as AOCS Lovibond color or just Lovibond color. The method is outlined as Method Cc 13b-45 by the AOCS (23). In bleaching rendered fats, bleaching earth, commonly known as bleaching clay, and possibly a filtration aid such as diatomaceous earth, may be added to the fat, which is agitated then filtered. Adequate filtration has been obtained by using both plate and frame presses and pressure leaf filters. Advantages to each type exist, but for larger operations, a pressure leaf filter is typical.

Bleaching and color reduction are important in removing contaminants and also in improving the perception of quality in textile sizing applications. For example, the application of bleached tallow or bleached hydrogenated tallow on textile fabrics is preferred over the use of darker fats. When color requirements become important in the use of rendered fats, processors must pay careful attention to raw material selection. An important analysis of the rendered fat is R&B color, or refined and bleached color.

As a result of processing methods, condition of the original byproduct raw material, storage conditions, and other factors, rendered fats may have a minimum color that can be achieved by refining and bleaching. This “best attainable color” can be determined by the AOCS Method Cc 8d-55, refined and bleached color (tallow and greases for soaps). The various grades of rendered fats reflect different R&B color values as outlined by the AFOA (14).

## 11. USES OF REFINED AND BLEACHED FATS

Refined and bleached fats may be described as such or may bear the product name *acidless*, such as acidless tallow. These fats are primarily used as raw materials for further processing steps or as a raw material for reactions, but also may be used as finished products in areas where their reduced FFA content and lighter color make them more suitable than the original rendered fat. Uses to be noted are as textile sizing compounds, additives, and lubricants in metalworking and leather processing. Cloth soaked in acidless tallow has been placed along the edge of cardboard tube manufacturing lines to apply a thin coating of fat to the ends of the tube to assist the tubes in sliding down a metal track.

## 12. HYDROGENATION AND HYDROGENATED PRODUCTS

Hydrogenation reduces the level of unsaturation, which increases the titer and melting point of the rendered fat. For example, fully hydrogenated tallow will have a titer of 59–61°C. Fully hydrogenated lard can have a titer 1–2°C higher, because of its slightly higher stearic content and slightly lower palmitic content (24). Industrial users of hydrogenated fats can make serious errors when trying to use melting point as an analytical measure rather than titer. Triacylglycerols of varying amounts of long-chain fatty acids are known to exhibit a high degree of polymorphism (25). Different crystallization conditions can lead to varying melt points for the same product, and the misuse of melting points in analytical work or product selection may give the user the false impression that the fully hydrogenated fat product does not meet specifications. This problem can be remedied by the use of titer to describe the joint effect of chain length and unsaturation as it relates to melting point. If an industrial user is concerned about levels of unsaturation only, iodine value should be used. A fatty acid composition analysis using gas chromatography, however, is the most accurate measurement.

Hydrogenated fats find major uses in the textile industry as lubricants and softener additives in sizing compounds. *Sizing* is a term used to denote the thin film that is used to coat fibers that are to be woven together. Sizing compounds can consist of tallow, hydrogenated tallow, plant starches (such as corn starch), polyvinyl alcohol, and adjuvants (such as emulsifiers, defoamers, and other materials). The blend of all of these materials is known as a sizing compound, or informally as “size” (26). The hydrogenated tallow used as an ingredient in sizing compounds is referred to as sizing wax. The term *wax* refers to the white, waxy appearance of the solid particles of hydrogenated tallow in flake or prill form. The purpose of using fat-derived products as fiber lubricants is to impart a thin film coating on the yarns, which reduces friction and abrasion between the yarns and the textile weaving equipment and between the yarns themselves. Some fat products act as softeners, giving a softer feel to the fabric and decreasing the brittleness of the size film through a plasticizing effect. Many different formulations exist for sizing compounds. The “feel” of the resultant fabric, or hand, can be changed with a specific formulation of the main ingredients or with the addition of additives. In some instances, unhydrogenated or bleached tallow is added to reduce the melting point of the sizing wax to provide different hand properties to the fabric and to aid in the removal of the sizing film from the fabric (26). In some cases, other triacylglycerols are added that impart different sizing properties to the formula. Examples are vegetable oils such as canola and other rendered fats such as hydrogenated menhaden oil and unhydrogenated tallow.

Compared with hydrogenated tallow, the use of hydrogenated menhaden oil gives improved lubricity per pound of “wax” used while also reducing the melting point of the formula. However, the high cost of hydrogenated menhaden oil compared with the cost of hydrogenated tallow keeps it from being more fully used.

The characteristics of hydrogenated fats can be modified with the addition of unhydrogenated fats. In metal buffing and polishing compounds, a blend of hydrogenated fat and unhydrogenated fats or oils may form a paste with a lower melting point than the hydrogenated material alone. This paste can be used as a base for the abrasive material in the compound. At times, these products are impregnated in buffing pads, which can be used with mechanical polishers.

Industrial products consisting of, or containing blends of, two different melting point fats may exhibit properties that reflect the mixture of triacylglycerols. When melting points are used as quality assurance measures, blends tend to exhibit melting ranges as opposed to melting points. For example, Wax 725 is a brand name for a textile wax made of approximately 75% hydrogenated tallow (titer 60°C) and 25% bleached tallow (titer 42°C). The resultant product has a titer of about 54°C. In melting point determination tests, Wax 725 tends to begin melting at about 45°C and completes melting at about 59°C. If Wax 725 is produced by the partial hydrogenations of tallow, i.e., to an iodine value of 10–12 or a titer of 54°C, this melting range becomes more of a melting point of approximately 56°C. Oleostearine is another product that can consist of varying percentages of hydrogenated fats and unhydrogenated fats.

In an effort to satisfy varied customer requirements, manufacturers have made different grades available, each differing only in the titer or melting point. The melting point can be changed by varying the percentages and types of hydrogenated fat and unhydrogenated fat used. Users of these oleostearine products include manufacturers of the metal polishing compounds, paste lubricants, and oils for leather processing.

In leather processing, natural oils from the hides are lost or “washed out.” The resultant leather products would be cracked and dry without a process step that replaces those natural oils. Leather processors can use a variety of fats: neatsfoot oil, lard oil, tallow, lard, poultry “oil”, different types of fish oils, hydrogenated or partially hydrogenated fats, and some vegetable oils. After processing, the finished leather products must be flexible and somewhat soft, depending on the application, but must not have an oily surface. The fat also gives some degree of water repellency. Relatively saturated oils are best for this purpose and the high natural level of saturation of rendered fats make them more suitable than other less saturated oils. Unsaturated oils such as fish are first oxidized with air (blowing) to reduce the unsaturation and increase the viscosity.

In leather processing, the fats are melted, and the “fat liquor” is pumped or poured to a long, narrow vat. Hides are soaked in the melting fat or oil at a temperature and time that allows them to absorb the fat. The hides are removed from the vat and allowed to drip “dry” before the further processing of the leather. In dyed leather products, such as horse reins, shoes, or fashion accessories, the fat deposited inside the leather should not migrate from the inner areas to the surface. Less saturated fats flow more freely through the leather, can leave an oily film on the surface, and are quickly dissipated from the inside and from the surface. Flexibility of the leather fibers is lost, leading to cracking and a general weakening of the leather. More highly saturated fats can still migrate through the leather, especially at higher temperatures, and after many cycles of heating and cooling, the solid fats can be seen on the leather surface as a dusty, waxy powder called “spew.”

Oleostearine is an important fat in the leather-processing industry because it is unsaturated enough to provide good lubricating qualities and does not leave a powdery residue on the surface, yet it is saturated to the degree that migration through the leather is reduced. There have been recent advances in the use of fat liquors that are adsorbed by the leather, then polymerized in place to reduce the possibility of fat migration to the surface.

### 13. TRANS-ESTERIFICATION AND FATTY ACID ESTERS

Many fatty chemicals are manufactured from the fatty acids produced by the hydrolysis of natural fats and oils. However, some fatty chemicals can be produced without the need for this step, and the fatty acids can be *trans*-esterified from the base glycerine molecule to the lower chain length alcohols such as methanol or butanol. *Trans*-esterification of rendered fat triacylglycerols with these lower chain length

alcohols produce esters of the alcohols and glycerine as a byproduct. Examples are methyl lardate and butyl tallowate.

Usually, a rendered fat with a low free fatty acid content is required, although some continuing work exists in reacting yellow grease with a high FFA (15%) by first reacting the free fatty acids, then reacting the acylglycerols. Hydrogenated tallow is approximately 67% stearic acid and its reaction with methanol produces a high-quality methyl stearate used as textile and metalworking lubricants and a textile softener.

The reaction of hydrogenated tallow with butanol produces butyl stearate, which is used in some metalworking formulations and as a lubricant for threads in the coning process. As thread or yarn is being spun from the original fibers, cotton for example, a thin coating of the butyl ester can be sprayed on the thread as it is being wound on the cardboard cone. This thin film of butyl ester lubricant reduces friction and abrasion when the yarns are used later in the weaving process.

As rendered fats are composites of different fatty acid chain lengths with different levels of saturation, raw material selection is guided by the fatty acid profiles of the rendered fats. Butyl stearate made from triple-pressed stearic acid has a higher palmitic to stearic ratio (ca. 1:1) than butyl stearate made from hydrogenated tallow (ca. 0.5). Although this gives the hydrogenated tallow butyl stearate a higher stearic content, it also gives it a higher cloud point, which may make it less desirable as a lubricant. An official method exists for determination of cloud point, AOCS Method Cc 6-25. Generally, a clear, heated sample is cooled in an ice water bath until the product turns cloudy. Differences in cloud point methods have led to other confusing descriptions such as haze point, fallout, and fallout percent.

The term *haze point* has been used to describe the temperature at which cloudy material begins to form in a cooled sample. *Fallout* is another confusing term. In the metalworking industry, a 4-oz. glass bottle containing the sample is allowed to sit at room temperature for 48 hours. The fallout percent can be estimated volumetrically. It is suggested that suppliers and users abide by industry standard methods when describing this aspect of rendered fats (23).

Some butyl stearate manufacturers distill unreacted acylglycerols from the reacted butyl stearate and, in doing so, selectively maximize the butyl palmitate fraction while leaving the butyl stearate fraction. These “distilled” or fractionated butyl stearates from hydrogenated tallow can approach the low cloud points and fatty acid profiles of the butyl stearate derived from triple-pressed stearic acid, sometimes with much improved economics. In most applications, complete removal of any free butanol by postreaction stripping is important. Heating during the industrial use of butyl stearate can lead to the release of alcohol vapors from the lubricant creating odor and safety problems.

Methyl esters of rendered fats have many important uses as industrial chemicals. Methyl esters of hydrogenated tallow are also known as methyl stearate because of the high stearic content. A similar product is known as hydrogenated methyl tallowate. In this case, the tallow is methylated first, and then hydrogenated. Except for fractionation steps, these products exhibit identical fatty acid profiles, which qualify

them as industrial-grade methyl stearate. Methyl tallowate and methyl lardate are used extensively as lubricant bases and as additives in metalworking formulations with applications in the processes of polishing, grinding, honing, cutting, and wire forming. Two important elements in the area of quality assurance analysis in methyl ester use are cloud point and fallout, but as mentioned earlier, precautions should be taken that standard methods for those analyses are used.

Most methyl ester manufacturers use cloud point as an indication of quality. The completion of the reaction can be gauged by reacting an in-process sample with excess methanol and then determining whether additional glycerine is produced. Cloud point determination gives a quicker method to determine how far the reaction has progressed because the unreacted acylglycerols will have a higher cloud point than the methyl ester. Methyl lardate has cloud points ranging from 9–11°C, whereas methyl tallowate is marginally higher, 10–12°C, depending on raw material sourcing.

Haze point is another quality assurance tool that can be used in conjunction with cloud point. Haze point is defined as the point at which the sample starts to become hazy as it is cooled to the cloud point. In a study of partially reacted versus fully reacted methyl esters of tallow and lard, it was found that fully reacted methyl esters exhibit haze points much higher; on esters in which approximately 95% of the original fatty acids as acylglycerols had been reacted to methyl ester, the haze points ranged from 5–8°C higher than the cloud points. As measured in this manner, haze points are not approved methods by AOCS standards; however, the manufacturer or user of methyl esters may find these data useful.

Again, it is important to note that methyl esters of lard and tallow are the sum of the methyl esters of fatty acids found in the original fat. Methyl lard for example, will contain methyl myristate, methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, and methyl linolenate in the same percentages as the lard used as the raw material, unless the manufacturer fractionates the material, thereby selecting methyl esters of certain fatty acids over others.

Some metalworking formulators have noted “fallout” problems with methyl lardate and methyl tallowate if their products are stored in drums or tanks without agitation, especially in cooler weather. Over time, the saturated fatty acid esters tend to crystallize and settle, leaving a cloudy or opaque substance in the bottom of the storage container. In sample bottles, this settled opaque material can appear to be as much as 50% of the volume of the total sample.

It should be noted that the saturated fractions are a natural portion of the product and that this fallout is a natural characteristic of the product. Some users have requested methyl lardate with lower levels of fallout or no fallout. In an attempt to provide this product, manufacturers have blended certain crystal inhibitors. Up to 15% methyl esters of less saturated vegetable oils have been blended in to reduce cloud point and fallout, but some problems may result as the unsaturated fatty esters perform differently in high heat metalworking processes. However, vegetable oils are typically more expensive than the rendered fats, and the practice of blending in methyl esters of vegetable oils makes the resultant product more expensive.

Isopropanol or other alcohols may be added in the range of 0.5–2% to reduce cloud point, but this reduces the lubrication value of the esters. In addition, the ester will exhibit a lower flash point, and if the fatty ester application involves heating, the heated alcohol may vaporize leading to safety or odor problems. In storage tanks, a more practical solution has been for metalworking formulators to circulate the product periodically to make sure that settled esters are mixed well with the top layers. Appearance problems with endusers of metalworking compounds have been created when the fatty ester was not agitated or recirculated to provide a thoroughly mixed product before its use in the formulation. A layer of cloudy material was pumped from the bottom of the tank leading to the appearance of haze in the final product. Samples can be taken from the top and bottom of storage tanks and then compared, either visually or by running a cloud point, to determine whether the fatty ester has settled. The settling rate depends on the relative levels of saturation of the fatty esters, the temperature, and the settling time.

Butyl ester of lard, or butyl lardate, has been used as a textile softener and lubricant to replace butyl oleate. Butyl oleate raw material sources have ranged from oleic acid to canola oil. Butyl lardate gives a much more saturated product, which imparts certain stability advantages in this area. Yet it is still somewhat unsaturated with the butyl oleate percentage reaching as high as 50%. The major advantage of butyl lardate is its lower cost compared with other substitute products.

An industrial grade glyceryl monostearate (GMS) is made by *trans*-esterification of hydrogenated tallow with methanol to produce methyl stearate and the byproduct glycerine; the resultant methyl stearate reacted with excess glycerine to produce a glyceryl monostearate with an  $\alpha$ -mono number of approximately 35. GMS made in this manner is used as a waxy paste in metal lubricants and polishing compounds and as textile softeners and lubricants. The two hydroxyl groups lead to a greater water solubility than triacylglycerols, although GMS cannot be described as water soluble. Higher grades of GMS with  $\alpha$ -mono values of greater than 80% can be produced using fatty acids as raw materials. Some grades are described as GMS-SE, or self-emulsifying.

In the textile chemical industry, GMS is sometimes modified by the addition of hydrogenated tallow, which changes the  $\alpha$ -mono number, the melting point, and the emulsification properties of GMS giving the fabric a different feel or “hand.” GMS produced with hydrogenated tallow as a raw material will contain the same fatty acid composition as the original hydrogenated tallow; consequently, GMS will also have a fairly high amount of glyceryl monopalmitate.

### 13.1. Markets

The oleochemicals and their derivatives are used in a variety of products across different market segments. Figure 17 outlines a flow diagram showing the fat and oil producer as the origin through the final finished product for some of the uses (18).



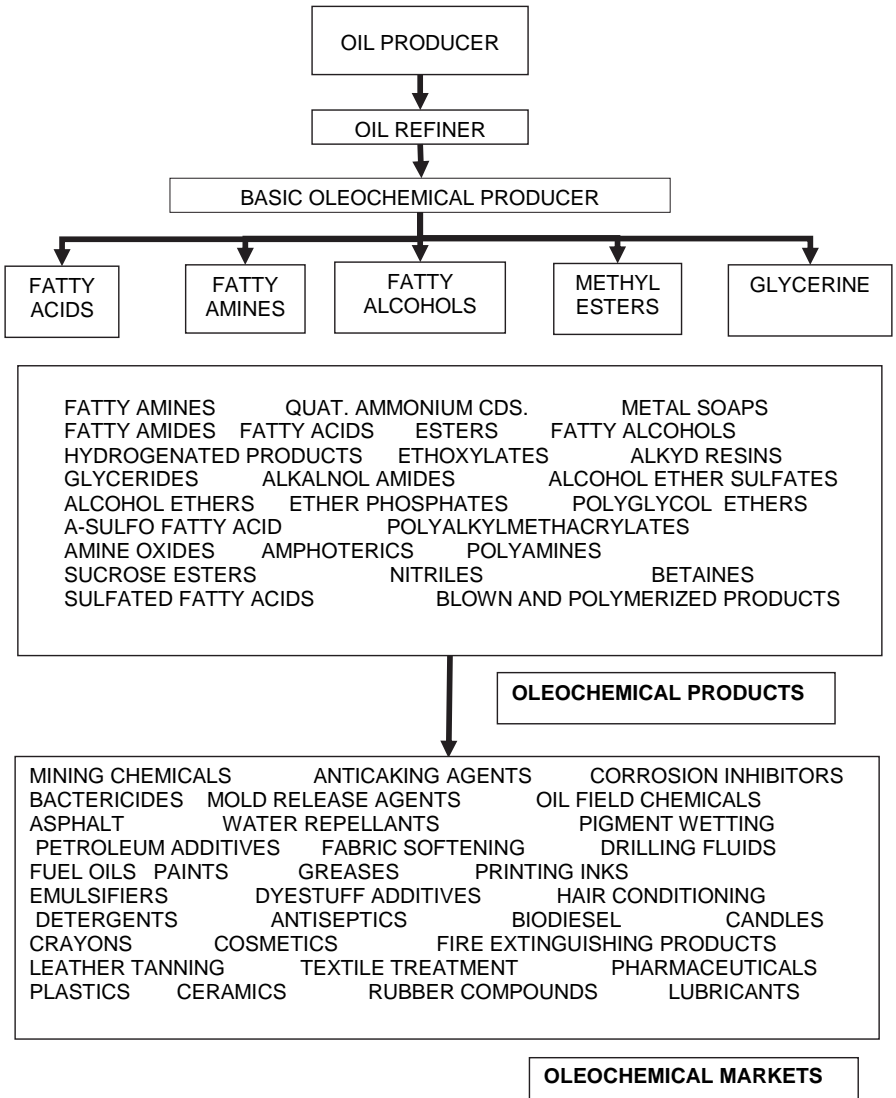


Figure 17. Flow chart leading to basic oleochemicals and their potential markets (18).

### 14. BIO-FUEL AND BIO-DIESEL PRODUCTS

Chicken fat, yellow grease, choice white grease, and beef tallow are readily available from meat, poultry, and other food-processing operations and are competitively priced relative to No. 2 fuel oil. They represent a potential source of energy at 1.2 therms per gallon. At times, the price of fish oil is so low that producers use the oil as an extender for the fuel oil that is being burned in the plants and some companies

**TABLE 9. Fuel Energy Content of Various Rendered Fats and Oils (11).**

Fuel	Energy Content <i>Btu/Lb</i>	Ash	Carbon	Hydrogen	Nitrogen	Oxygen	Sulfur
Chicken Fat	16873	0.14	75.3	11.4	0.04	13.1	0.006
Yellow Grease	16899	0.02	76.4	11.6	0.03	12.1	0.005
Choice White Grease	16893	0.08	76.5	11.5	0.05	11.6	0.007
Tallow	16920	0.03	76.6	11.9	0.02	11.4	0.003
Fish Oil <sup>a b</sup>	17112	0.032	78.92	11.59	na	10.58	0.004
No. 2 Fuel Oil	19237	0.02	84.0	11.9	0.01	3.78	0.35

na=not available

<sup>a</sup> Reference (27), <sup>b</sup>Reference (28).

use the fish oil blended into diesel fuel for the fishing vessels. In these cases, the oil is not modified; that is, it is in the natural triacylglycerol form and has not been converted to methyl esters.

In 2002 a report was issued by the University of Georgia Engineering Outreach Service under the Georgia Environmental Partnership Program. In this report, chicken fat, yellow grease, choice white grease, and tallow were thoroughly evaluated for use as an industrial boiler fuel. The results looked very good and the analysis of the various fats compared favorably with No. 2 fuel oil. In Alaska, fish oil has been burned as a boiler fuel for many years because the cost of shipping the fish oil to markets in the lower forty-eight States makes the oil noncompetitive. Data from the fish oil work in Alaska appears along with a comparison of the composition of the various rendered animal fats in Table 9 (11, 27, 28).

Bio-diesel differs from the term bio-fuel in that bio-diesel is conventionally defined as a bio-fuel that is produced through *trans*-esterification of the oil or fat with methyl alcohol resulting in the formation of methyl esters (bio-diesel feed-stock) and the byproduct glycerine. Bio-diesel can be made from animal fats, recycled cooking oils, restaurant greases, vegetable oils, marine oils, and others. The product has been produced in Europe for many years, and European development is more advanced than in the United States. Table 10 shows the relative production of bio-diesel in five European countries compared with the United States for three different years, 1997, 2000, and 2003 (29).

**TABLE 10. Global Bio-Diesel Capacity (2a).**

Country	1997	2000	2003
	million	gallons	per year
France	21.0	76.4	98.3
Germany	21.6	61.4	73.7
U S A	1.0	7.0	25.0
Italy	0.4	10.5	23.4
Austria	5.1	6.0	8.3
Belgium	4.5	5.1	6.0
Total	53.6	166.4	234.7

Most bio-diesel is produced from vegetable oils by a two-step process of acid-catalyzed esterification to convert the free fatty acids to methyl esters followed by an alkaline-catalyzed *trans*-esterification step to convert the acylglycerols to methyl esters. If the free fatty acid content is high, the process becomes more complex and yields are lower. Reports in the United States indicate that enough feedstock exists to produce 1.6 billion gallons of pure bio-diesel annually, which represents excess production and exports that can be diverted to domestic use for fats and oils (30). The properties and attributes of bio-diesel have been well defined and summarized by the U.S. Environmental Protection Agency (EPA) in 2002. According to the EPA report, which evaluated 39 different studies, when comparing bio-diesel with petroleum diesel, the bio-diesel

- Reduced total unburned hydrocarbons by 67%
- Reduced carbon monoxide emissions by 47%
- Reduced particulate matter emissions by 48%
- Increased NO<sub>x</sub> by 10%

Although the report did not form an official rulemaking by the EPA, it does provide a government-validated reference for federal, state, and local pollution strategies to reduce, emissions that are harmful to human health and the environment (31, 32).

## 15. GOVERNMENTAL REGULATIONS

Two categories of tallow and grease exist: edible and inedible. Edible tallow and lard are considered to be of the highest quality and are produced exclusively in rendering plants operated by meat packers or independent edible renderers. The edible rendering plants fall under the same inspection and processing standards set by the U.S. Department of Agriculture for table meats. The rendering plants producing inedible tallow, greases, and protein byproducts are governed by the U.S. Food and Drug Administration, using the same standards set for the feed industry. Similar types of regulations exist in the EU and other countries where rendered products are produced.

### 15.1. BSE (Mad Cow Disease)

In an era of globalization, a disease incident anywhere becomes a disease incident everywhere (33). Bovine spongiform encephalopathy (BSE) is a transmissible, neurodegenerative, fatal brain disease of cattle. The disease has a long incubation period of four to five years, but ultimately is fatal for cattle within weeks to months of its onset. BSE first came to the attention of the scientific community in November 1986 with the appearance in cattle of a newly recognized form of neurological disease in the United Kingdom (UK). Epidemiological studies conducted in the UK suggested that the source of BSE was cattle feed prepared from bovine tissues, such as brain and spinal cord that was contaminated by the BSE agent. Speculation as to the cause of the appearance of the agent causing the disease has ranged from

spontaneous occurrence in cattle, the carcasses of which then entered the cattle food chain, to entry into the cattle food chain from the carcasses of sheep with a similar disease, scrapie. BSE in the brain affects the brain and spinal cord of cattle. Lesions are characterized by sponge-like changes visible with an ordinary microscope. The agent is highly stable, resisting freezing, drying, and heating at normal cooking temperatures, even those used for pasteurization and sterilization. The nature of the BSE agent is still a matter of debate. According to the prion theory, the agent is composed largely, if not entirely, of a self-replicating protein, referred to as a prion. Another theory argues that the agent is virus-like and possesses nucleic acids that carry genetic information. Strong evidence collected over the past decade supports the prion theory, but the ability of the BSE agent to form multiple strains is more easily explained by a virus-like agent (34).

Until the advent of BSE in the UK in 1986, the rendering industry operated globally on the premise of relative safety assurance based on a historical record of producing safe feed ingredients. This all changed in 1988 based on the epidemiological hypothesis that meat and bone meal of ruminant origin was the most likely cause of transmitting the disease. This linkage of an animal protein that has been used as a nutritional supplement in livestock rations for at least a century to a new disease created initial alarm and anxiety within the UK with concurrent reverberations throughout the global agricultural and livestock communities that exist to this day, approximately 18 years since the confirmatory diagnosis of the first two reported cases in the UK (33).

The whole issue of BSE or mad cow disease has had a profound effect on the global rendering industry. For example, the North American exports of tallow into the European Union dropped from about 350,000 tons per year to less than 70,000 tons prior to 2003 and were expected to be further reduced after the EU's Animal Byproducts Regulations (ABPR) were put into effect in late 2003. The EU has maintained that feed-borne food crises such as BSE all point to control deficiencies in the animal feed sector. The new regulation builds on previous EU measures that required the exclusion of dead animals, specified risk materials (SRMs), and other condemned materials from feed, and the pressure treatment of mammalian materials intended for use in feed. It also bans intraspecies feeding of farmed animals. It classifies animal byproducts into three categories based on their potential risk to animals, the public, or the environment, and sets out how each category must or may be disposed of.

- Category 1 materials (animal byproducts presenting highest risk, e.g., transmissible spongiform encephalopathies or scrapie; residues of prohibited substance, e.g., hormone used for growth promotion; or environmental contaminants, e.g., dioxins, PCBs) must be completely disposed of as waste by incineration or landfill after appropriate heat treatment.
- Category 2 materials include animal byproducts presenting a risk of contamination with other animal diseases, e.g., dead stock or animals killed in the context of disease control measures or where there is risk of residues of veterinary drugs. Products produced from these tissues may be recycled for

uses other than food and feeds after appropriate treatment. Approved disposal includes biogas production, composting, and production of oleochemical products to be used in the technical industry.

- Category 3 materials, tissues derived from healthy animals slaughtered for human consumption, are the only materials that may be used in the production of feeds following appropriate treatment in approved processing plants.

The ABPR approval does not affect the current EU total ban on the feeding of animal protein meals to farmed animals, which is a separate issue and remains in force until all the meat and bone meal in storage (approximately 400,000–500,000 tons) at present, awaiting incineration, does not find its way into the feed chain. The EU has also developed tests to differentiate the different types of animal protein meals. Such tests are needed in order to lift the ban for fishmeal and allow for traceability. However, the ABPR establishes clear safety rules for the production of meat and bone meal in case it is ever reauthorized for inclusion in feed for nonruminant species. Concurrent with the feed ban has been a ban against the export of animal protein meals. The United States was once the world's largest exporter with over a million tons exported per year; now that figure is less than 300,000 tons (35). The timeline of the BSE situation follows:

- **1986** Bovine spongiform encephalopathy (BSE) is identified as a new disease in cattle.
- **1988** The United Kingdom requires new cases of BSE to be reported and investigated, bans the feeding of ruminant-derived meat and bone meal protein (MBM) to ruminants, and introduces a mandatory slaughter and compensation plan for detected cases.
- **1989** The European Union bans the export of UK cattle born before the July 1988 feed ban. The first case of BSE outside the UK is detected in a native herd in Ireland. The UK government bans the use of certain high-risk specified bovine offal in human food.
- **1990** The UK establishes surveillance for Creutzfeldt–Jakob disease (CJD) following concern about possible spread to humans.
- **1993** An EU-funded surveillance system for CJD is introduced in 10 countries in Europe, Australia, and Canada.
- **1994** The feeding of mammalian protein to ruminants is banned throughout the EU.
- **1997** A new human transmissible spongiform encephalopathy, variant CJD or vCJD, is identified and distinguished from classic CJD. The UK bans the use of mammalian MBM in feed for all farm animals. The UK introduces slaughter scheme to keep cattle older than 30 months out of food and feed chains. Cattle “passports” are made mandatory for all cattle born beginning July 1, 1996. The United States FDA imposes a ban on feeding MBM protein to ruminants (36).

- **1998** The UK prohibits specified risk materials (SRM) in feed, cosmetics, pharmaceuticals, and medical products.
- **2000** The EU implements an SRM ban. CJD surveillance is extended to cover eight more countries.
- **2001** BSE is reported in Japan. It's the first native-born case reported outside Europe. CJD surveillance expands to include countries in Central and Eastern Europe and China.
- **2002** The first confirmed case of vCJD in North America is reported in a man in Saskatchewan, Canada. EU issues regulations under Directive 1774/2002 setting out a system for handling Animal Byproducts (ABPR).
- **2003** The first BSE-positive cow in Canada in a decade is discovered. Several countries ban beef from Canada. The first BSE case in the United States is found in a dairy cow imported from Canada. Several countries ban beef and other products of U.S. cattle. USDA imposes new restrictions to prevent BSE cattle from entering the food supply.
- **2004** USDA begins expanded BSE surveillance aimed at testing almost 268,000 cattle. Of the 8,500 cattle tested to date, none has tested positive. EU issues Directive 780/2004 setting out the transition into regulation 1774/2002 over the period 2004–2005 (37).

The various feed bans, regulations, and restrictions on feeding animal byproducts including fishmeal have had a dramatic effect on the rendering industry, the fishmeal industry, and the animal agriculture industry. In the case of fishmeal, the feeding ban in the EU was designed to prevent the deliberate or accidental adulteration of fishmeal with animal byproduct meals until such time as an analytical method could be devised that would detect the presence of mammalian protein in fishmeal, which has now been done but a lifting of the ban on the use of fishmeal in ruminant diets is not expected before late 2005 or early 2006.

## 16. ENVIRONMENTAL ISSUES

Environmental issues are important to the rendering industry; they are the original recyclers. Without rendering, byproducts from the meat packing industry would fill up the landfills. Many modern rendering plants are using new techniques to help conserve energy and water. Although the rendering industry can be viewed positively for its role in recycling organic material, it has the potential to create environmental problems in the air, water, and on the land.

Air pollution is typically in the form of odors. During the rendering process, numerous breakdown products are formed or released from the raw materials. These compounds travel with the water being evaporated in the cooking process. If released directly to the air, though not hazardous, they would be quite objectionable. For this reason, condensers are placed after the cooking unit to cool the gas flow. This condenses the steam back into water as well as condensing some of the low boiling

point organics. Many of the odorous compounds are soluble and remain with the condensed liquid, which is sent to a wastewater treatment plant. Any materials that remain in the gas phase from the condenser are either incinerated (using a steam boiler or a designated burner) or scrubbed by passing them through an oxidizing media.

As rendering processes are not tightly sealed, some odorous vapors will escape into the surrounding room. This room air is also scrubbed through oxidizing media. Large volumes of air are drawn through the scrubber to keep the building at a negative pressure so that any leaks in the structure will be drawing fresh air in.

Water is produced in large quantities during the rendering process. The two main areas of wastewater production are from condensers (mentioned above) and plant wash up. Washwater is high in fat, whereas condenser water can have a BOD<sub>5</sub> (a measure of organic material) of 10,000 parts per million (ppm) and ammonia of 750 ppm. For this reason, rendering waste water cannot be discharged directly to a stream, and most municipal plants will require some sort of treatment before discharge to their facilities.

A typical rendering waste water plant will contain a skimmer or dissolved air floatation (DAF) unit, which floats fatty and lightweight solids for recovery, which is followed by an extended aeration biological treatment system. In this system, bacteria are used to digest organic material (BOD<sub>5</sub> and ammonia, as well as others) that remains in the water. These bacteria are settled out in clarifiers so that only clear water continues through the process. High-quality streams may require phosphorous removal, filtration, or chlorination before discharge.

Excess bacteria generated in the waste water treatment plant, as well as other solids generated in various areas of the rendering process, are often applied to the land either as a disposal option or as a beneficial soil conditioner. Care must be taken to ensure that nutrient loadings do not exceed vegetative demand, or they will migrate off-site and create potential pollution problems (including elevated nitrates in groundwater).

Dissolved solids or salts in land-applied material can also create problems. They chemically collapse the clay structure, which normally allows soil to hold water. Plants will exhibit drought-like symptoms even with adequate rainfall. Applying sludge in liquid form greatly increases the potential for salt overload.

## 17. FUTURE OUTLOOK

The rendering industry is constantly changing. Only those companies that are able to adapt to this ever-changing industry will survive. The primary changes will continue to occur in the area of environmental issues, governmental regulations, raw material, and market conditions.

Environmental considerations, particularly in terms of water and air pollution control, will continue to have an impact on the economics of the business. As government regulations in odor control and water pollution get stricter, production

costs will increase. As with other industries, smaller operations that do not have the financial ability to make substantial capital investments may be forced to close.

Another major environmental concern will be of dwindling energy sources. Large amounts of energy are used during the rendering process, in the cooking process, and by the fleet of trucks needed to haul the raw and finished material to the plant or to customers. As energy sources dwindle and become more expensive, the renderer will be forced to find more efficient and economical rendering equipment. The renderer will also be forced to supply fuel to the truck fleet that will be cleaner burning and more abundant. One consideration is that of bio-diesel discussed previously. The bio-diesel is cleaner burning than today's diesel and is made from a renewable source, fats and oils.

As with any business, the renderer will have to abide by all governmental regulations within the country where they operate. Like other businesses, the smaller rendering companies will find it harder and harder to stay competitive and still maintain a profit.

Changes in consumer preference of meat cuts will change the face of the meat-packing industry. As a result of consumer demand, meatpackers now must trim the fat on the meat cuts closer, leaving less to be trimmed when the meat is prepared for retail sale at the local supermarket level, which could lead to more fat and bone staying in the packing plant and less at the supermarket. This change will increase the need for the rendering plant to be located next to the packing plant and could result in a shortage of material in the areas served by metropolitan renderers. These urban renderers will have to rely more on profits from processing restaurant grease and less from rendering fat and bone from butcher shops and supermarkets.

## REFERENCES

1. F. A. Burnham, "The Rendering Industry: A Historical Perspective," in D. A. Franco and W. Swanson, eds., *The Original Recyclers*, The Animal Protein Producers Industry, The Fats and Proteins Research Foundation and The National Renderers Association, Alexandria, Virginia, 1996.
2. FAOSTAT. 2004. Available: <http://apps.fao.org/faostat/form?collection=Trade.CropsLivestockProducts&Domain=Trade&servlet=1&hasbulk=0&version=ext&language=EN>.
3. T. Mielke, ed., *Oil World Annual*, ISTA Mielke GmbH, Hamburg, Germany, 2004.
4. Association of American Feed Control Officials. Official Publication Manual, AAFCO, Oxford, Indiana, 2004. ISBN 1-878341-15-4.
5. U. S. Department of Agriculture. (2004). *Agricultural Statistics*. Available: <http://www.usda.gov/nass/pubs/agr04/acro04.htm>.
6. W. R. Boehme, *New Drying Techniques Preserve Lysine in Blood Meals*, Digest No. 116, Fats and Proteins Research Foundation, Ft. Myers, Florida, 1974.
7. W. H. Prokop, "Technological and Environmental Impacts on the Rendering Industry," in D. A. Franco and W. Swanson, eds., *The Original Recyclers*, The Animal Protein



- Producers Industry, The Fats and Proteins Research Foundation and The National Renderers Association, 1996.
8. A. P. Bimbo, "Fish Meal and Oil," in R. E. Martin, E. P. Carter, G. J. Flick, Jr., and L. M. Davis, eds., *Marine & Freshwater Products Handbook*, Technomic Publishing Co., Lancaster, Pennsylvania, 2000.
  9. W. Schottelcotte, *Render Mag.*, **XXIV**(3), 10–21 (1996).
  10. G. G. Pearl, *Render Mag.*, **XXXIII**(4), 30–39 (2004).
  11. T. T. Adams, J. Walsh, M. Brown, J. Goodrum, J. Sellers, and K. Das, "A Demonstration of Fat and Grease as an Industrial Boiler Fuel," the Univ. of Georgia Engineering Outreach Service, The Georgia Environmental Partnership, Athens, Georgia, 2002. Available: [http://www.engr.uga.edu/service/outreach/Publications %20\(Articles%20and%20Reports\).htm](http://www.engr.uga.edu/service/outreach/Publications%20(Articles%20and%20Reports).htm).
  12. A. P. Bimbo, *JAOCs*, **66**(12), 1717–1726 (1989).
  13. Sparks Companies Inc., "The Rendering Industry: Economic Impact of Future Feeding Regulations," report prepared for the National Renderers Association (2001). Available: [http://www.renderers.org/economic\\_impact/index.htm](http://www.renderers.org/economic_impact/index.htm).
  14. American Fats and Oils Association, Trading and Arbitrations Rules, AFOA, New York, 1994, p. 8.
  15. E. C. Leonard, and S. L. Kopald, Jr., *JAOCs*, **61**(2), 176–179, (1984).
  16. J. F. Heidrich, *JAOCs*, **61**(2), 271–275 (1984).
  17. A. P. Bimbo and J. B. Crowther, *JAOCs*, **69**(3), 221–227 (1992).
  18. H. J. Richtler and J. Knaut, *JAOCs*, **61**(2), 160–175 (1984).
  19. I. Tauskey, personal communication, 1989.
  20. T. Hamme, personal communication, 1985.
  21. P. M. Parker, "Commercial Experience with Modified Caustic Refining (MCR) Using TriSyl Silicas," paper presented at the 84th Annual Meeting of the American Oil Chemists' Society, Anaheim, California, May 1993.
  22. D. Smith, personal communication, 1994.
  23. American Oil Chemists' Society, Official Methods and Recommended Practices of the AOCS, 5th ed. 2nd Printing, American Oil Chemists Society, Champaign, Illinois, 2004.
  24. P. Smith, personal communication, 1994
  25. H. Murdoch, personal communication, 1994.
  26. P. V. Seydel and J. R. Hunt, *Textile Warp Sizing*, Long & Clopton, Inc., Atlanta, Georgia, 1972, pp. 91–93.
  27. J. A. Steigers, "Demonstrating the Use of Fish Oil as Fuel in a Large Stationary Diesel Engine," in Peter J. Bechtel, ed., *Advances in Seafood By-Products 2002 Conference Proceedings*, Alaska Sea Grant College Program, Univ. of Alaska Fairbanks, Fairbanks, Alaska, 2003, pp. 187–199.
  28. N. X. Blythe, "Fish Oil as an Alternative Fuel for Internal Combustion Engines," paper presented at the Spring Technical Conference, *ASME/ICE*, **26**, (1996).
  29. D. Talley, *Render Mag.*, **XXXIII**(2), 16–191 (2004).
  30. G. G. Pearl, *Render Mag.*, **XXXII**(4), 10–14 (2003).
  31. National Bio-Diesel Board, *Render Mag.*, **XXXI**(6), 28–29 (2002).

32. US EPA, "A Comprehensive Analysis of Bio-diesel Impacts on Exhaust Emissions," Draft Technical Report EPA420-P-02-001 (2002). Available: <http://www.epa.gov/otaq/models/biodsl.htm>.
33. D. A. Franco, *Render Mag.*, **XXX**(6), 26–29 (2001).
34. World Health Organization, "Bovine Spongiform Encephalopathy Fact Sheet No.113," revised November 2002. Available: <http://www.who.int/mediacentre/factsheets/fs113/en/>.
35. National Renderers Association, *Render Mag.*, **XXXI**(6), 30–31 (2002).
36. Food and Drug Administration (FDA), "Animal Proteins Prohibited From Use in Ruminant Feed," Title 21, Code of Federal Regulations, Part 589.2000, 1997.
37. American Farm Bureau Federation, *Farm Facts Today*, **83**(14), (2004). Available: [http://www.fb.org/news/fbn/04/07\\_12/html/bsetimeline.html](http://www.fb.org/news/fbn/04/07_12/html/bsetimeline.html).
38. W. H. Prokop, "Rendering Plants," in W. T. Davis, ed., *Air Pollution Engineering Manual*, 2nd ed. Air & Waste Management Association, Wiley, New York, 2000, pp. 497–510.
39. A. P. Bimbo, "Fish Processing," in W. T. Davis, ed., *Air Pollution Engineering Manual*, 2nd ed. Air & Waste Management Association, Wiley, New York, 2000, pp. 481–488.
40. A. P. Bimbo, "Technology of Production and Industrial Utilization of Marine Oils," in R. G. Ackman, ed., *Marine Biogenic Lipids, Fats and Oils*, Vol. 2., CRC Press, Boca Raton, Florida, 1989, pp. 401–433.

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## *Soaps*

Michael R. Burke

### 1. INTRODUCTION

Soap is one of the oldest known manufactured chemical substances and was first produced thousands of years ago through the reaction of animal fats with the ashes from plants (1). Early soaps were used primarily for the cleansing of clothing, not for personal hygiene, because of cultural as well as practical reasons. For instance, these animal fat soaps possessed almost unbearable odors and probably contained excessive amounts of unreacted caustics. Today soaps are produced using a variety of processes, including kettle boiling, continuous saponification, and hydrolysis/neutralization, as well as different fats and oils feedstocks, yielding finished materials that possess specifically desired properties for application as personal cleansing products.

Soap is one example of a broader class of materials known as surface-active agents, or surfactants. Surfactant molecules contain both a hydrophilic or water-liking portion and a separate hydrophobic or water-repelling portion. The hydrophilic portion of a soap molecule is the carboxylate head group and the hydrophobic portion is the aliphatic chain. This class of materials is simultaneously soluble in both aqueous and organic phases or preferential aggregate at air–water interfaces. It is this special chemical structure that leads to the ability of surfactants to clean dirt and oil from surfaces and produce lather.

Although soaps have many physical properties in common with the broader class of surfactants, they also have several distinguishing factors. First, soaps are most

often derived directly from natural sources of fats and oils. Fats and oils are triglycerides, i.e., molecules comprising a glycerol backbone and three ester-linked fatty oils. Other synthetic surfactants may use fats and oils or petrochemicals as initial building blocks, but generally they require additional chemical manipulations such as sulfonation, esterification, sulfation, and amidation.

Second, soaps form insoluble complexes, commonly referred to as curd, in the presence of calcium and magnesium ions in solution. Calcium and magnesium ions are the principal metal ions found in water and their level is commonly referred to as the hardness of the water; hard water has high levels of both of these ions, soft water has very low levels. This curd reduces the effectiveness of soap as a surfactant and gives rise to other undesirable properties during use, e.g., precipitation on surfaces. Many synthetic surfactants are considerably less susceptible to water hardness. This water hardness insensitivity has led to the replacement of soap by synthetic surfactants in a variety of applications, such as dish and laundry detergents and shampoos. Although soap is still the predominant material used in personal cleansing products, e.g., facial, body, and hand cleansing, soap-based personal cleansing products are being rapidly replaced by products that contain increasing amounts of synthetic surfactants to meet changing consumer needs, such as rinsing and lather in hard water and improved mildness to the skin.

## 2. PHYSICAL PROPERTIES OF SURFACTANTS

Surfactants, including soap, possess a bipolar structure, composed of both a hydrophobic tail and a hydrophilic head group. As a result of this bifunctional structure, surfactants possess many unique physical properties. In solution, surfactants preferentially concentrate as monolayers at the interfacial region between any two phases of dissimilar dielectric constants or polarity. Examples of interfacial regions are the interfaces between oil and water or air and water. The hydrophilic portion preferentially solubilizes in the polar or higher polarity phase, whereas the hydrophobic portion preferentially solubilizes in the nonpolar or lower polarity phase. The interface between dissimilar polarity phases provides an ideal location to satisfy both preferences. The presence of surfactants at the interface provides stability to the interface by lowering the total energy associated with maintaining the boundary. Thus, surfactants facilitate stabilization of intermixed, normally immiscible phases, such as oil in water, by decreasing the energy necessary to maintain the large interfacial region associated with mixing. For example, in the absence of surfactants, an oil-in-water mixture, commonly referred to as an emulsion, rapidly separates into two distinct layers to minimize the surface or contact area between the two phases. The ability of surfactants to lower this interfacial energy between the oil and water allows for the formation and stabilization of smaller oil droplets dispersed throughout the water. In this case, the decrease in interfacial energy offsets the increase in total surface area of the system.

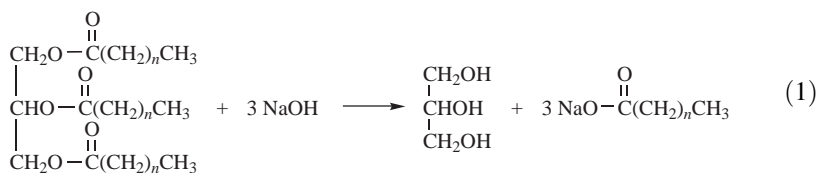
Another property of surfactants is their ability to aggregate in solution to form various composite structures or phase states, such as micelles and liquid crystals, as

a function of concentration and temperature. At very low surfactant levels, the surfactant exists as individual molecules in solution associating primarily with water molecules. It also concentrates or partitions at the interfacial regions as described above. However, as the concentration of surfactant in solution is increased, a point is reached where the molecules aggregate to form micelles. This concentration is defined as the critical micelle concentration (CMC). The micellar structure minimizes energy through surfactant self-association; the micelle in water is typically characterized with the hydrophobic tails pointing to the center and the head groups pointing out toward the water in spherical superstructures. As the concentration of surfactant in solution is further increased, the micelles elongate into long tubules that align with each other to form a hexagonal arrangement when viewed end-on. These structures are commonly referred to as hexagonal liquid crystals. As the surfactant concentration is further increased, the tubules expand in a second direction to form large, stacked lamellar sheets of surfactants, commonly referred to as lamellar liquid crystals. These liquid crystals are very important in soap making.

As the core of an aqueous micelle is extremely hydrophobic, it has the ability to solubilize oil within it, as well as to stabilize a dispersion. These solubilization and suspension properties of surfactants are the basis for the cleansing ability of soaps and other surfactants. Furthermore, the ability of surfactants to stabilize interfacial regions, particularly the air–water interface, is the basis for lathering, foaming, and sudsing.

### 3. SOAP RAW MATERIALS AND THEIR PROCESSING

Carboxylate soaps are most commonly formed through either direct or indirect reaction of aqueous caustic soda, i.e., alkali earth metal hydroxides such as NaOH, with fats and oils from natural sources, i.e., triglycerides. Fats and oils are typically composed of both saturated and unsaturated fatty acid molecules containing between 8 and 20 carbons randomly linked through ester bonds to a glycerol backbone. Overall, the reaction of caustic with triglyceride yields glycerol and soap in a reaction known as saponification. The reaction is shown in Equation 1.



Saponification can proceed directly as a one-step reaction as shown above, or it can be achieved indirectly by a two-step reaction where the intermediate step generates fatty acids through simple hydrolysis of the fats and oils and the finishing step forms soap through the neutralization of the fatty acid with caustic soda. There

TABLE 1. Fatty Acid Compositions of Common Fats and Oils.<sup>a</sup>

Common Name	Chemical Name	Chemical Formula	Symbol	Animal Fats, %		Vegetable Oils, %		
				Tallow	Lard	Coconut	Palm kernel	Soybean
<i>Saturated Fatty Acids</i>								
caprylic	octanoic	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	C 8			7	3	
capric	decanoic	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	C10			6	3	
lauric	dodecanoic	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	C12			50	50	0.5
myristic	tetradecanoic	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	C14	3	1.5	18	18	0.5
palmitic	hexadecanoic	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	C16	24	27	8.5	8	12
margaric	heptadecanoic	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	C17	1.5	0.5			
stearic	octadecanoic	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	C18	20	13.5	3	2	4
<i>Unsaturated Fatty Acids</i>								
myristoleic	tetradecenoic	C <sub>14</sub> H <sub>26</sub> O <sub>2</sub>	C14:1	1				
palmitoleic	hexadecenoic	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	C16:1	2.5	3			
oleic	octadecenoic	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	C18:1	43	43.5	6	14	25
linoleic	octadecadienic	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	C18:2	4	10.5	1	2	52
linolenic	octadecatrienic	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	C18:3	0.5	0.5	0.5		6

<sup>a</sup>From historical data and Procter & Gamble analyses.

are practical considerations that must be addressed when performing this reaction on a commercial scale.

Compositional differences in the fats and oils give rise to their significantly different physical properties and those of the resulting fatty acids and soaps. Fats and oils are triglycerides composed of glycerol ester linked to three fatty acids. The main compositional difference is the chain-length distribution of the fatty acids associated with the fats or oils. The compositions found in some commercially important fats and oils are summarized in Table 1. High levels of unsaturated (containing double bonds) or short-chain-length components produce fatty acids that are liquid and soaps that have high water solubilities at room temperature. Conversely, high levels of saturated, long-chain-length components produce waxy and hard fatty acids, e.g., candle wax, and soaps that are essentially insoluble at room temperature. Furthermore, unsaturated components are more susceptible to oxidative degradation, i.e., the oxidation of the double bond to form a number of shorter chain components. This gives rise to undesirable odors and darker colors. A key to producing soaps with acceptable qualities is the proper blending of these fats and oils.

The quality, i.e., level of impurities, of the fats and oils used in the manufacture of soap is important in the production of commercial products. Fats and oils are isolated from various animal and vegetable sources and contain different intrinsic impurities. These impurities may include hydrolysis products of the triglyceride, e.g., fatty acid and mono/diglycerides; proteinaceous materials and particulate dirt, e.g., bone meal; and various vitamins, pigments, phosphatides, and sterols,

i.e., cholesterol and tocopherol; as well as less desirable odor and color bodies. These impurities affect the physical properties, such as odor and color, of the fats and oils and can cause additional degradation of the fats and oils upon storage. For commercial soaps, it is desirable to keep these impurities at the absolute minimum for both storage stability and finished product quality considerations.

There are a number of processing steps that can be used to improve the quality and stability of the fats and oils raw material. These include water washing, alkali refining, physical (steam) refining, deodorization, bleaching, and hydrogenation. Water washing, also called degumming when dealing with vegetable oils, is an effective means of improving the color of fats and oils through the elimination of proteinaceous solids, phosphatides, and other water-soluble impurities. Hot water, possibly containing some phosphoric acid or sodium phosphate, is mixed with the fats or oils. The water layer is allowed to separate either statically or by using centrifugal force. Many of the solids and other impurities become either solubilized or suspended in the water and removed. Alkali and physical (steam) refining can be used to decrease the amount of fatty acid and other color bodies present in fats and oils. Alkali refining washes the fats and oils with alkaline water and converts fatty acids into soap. The resulting soap is removed with the alkaline aqueous phase through settling or centrifugation. In physical refining, volatile impurities, including low-boiling fatty acids, are vaporized and removed from the fats and oils by steam-heating the material. Deodorization, also called steam stripping, is another steam distillation process. For deodorization, however, the distillation is performed under a vacuum that allows for more efficient removal of the less volatile odor bodies. Bleaching is most commonly done using a physical adsorption process in which an activated clay, e.g., bentonite, is slurried with the oil at temperatures around 100°C. The color bodies adsorb onto the clay, which is subsequently removed through a filtration process. Hydrogenation is also frequently used in the processing of fats and oils to improve their storage stability through reduction in the amount of polyunsaturates and unsaturates present. This is achieved by passing the fats and oils through a heated column containing a catalyst, e.g., Ni or Pt, and hydrogen gas under pressure so that hydrogen adds across the double bonds.

Industry uses a number of analytical methods to characterize fats and oils, which include moisture, titre (solidification point), free fatty acid, unsaponifiable material, iodine value, peroxide value, and color. Moisture content of the fats and oils is an important measure for storage stability at elevated temperature because it facilitates hydrolysis, which, in turn, impacts odor and color quality. Titre is a measure of the temperature at which the material begins to solidify, signifying the minimum temperature at which the material can be stored or pumped as a fluid. Free fatty acid is a measure of the level of hydrolysis the fats and oils have undergone. Increased fatty acid content usually negatively impacts product color stability because fatty acids are more susceptible to oxidation. Unsaponifiable material is a measure of the nontriglyceride fatty material present, which affects the soap yield of the material. The iodine value is a measure of the amount of unsaturation present in the fats and oils. Peroxide value is a measure of the amount of oxidation the fats and oils have undergone and indicates the potential for further degradation.

Fats and oils are treated as commodities in the open market and are purchased in bulk. As commodities, their prices fluctuate with supply and demand. Furthermore, fats and oils come in different grades that reflect different levels of processing and have industry-standardized specifications, such as the American Fats and Oil Association. In the manufacture of soap in the United States, the source of animal fats is domestic whereas the vegetable oils are frequently obtained from Southeast Asia, primarily Malaysia and the Philippines.

*Tallow* is the fat obtained as a byproduct of beef, and, to a lesser degree, sheep processing, and is the most commonly used animal fat in the manufacturing of soaps. The high content of longer chain-length fatty acids present in tallow fat necessitates the addition of other oils, such as coconut oil, in order to produce a bar with acceptable performance.

*Coconut oil* is one of the primary vegetable oils used in the manufacture of soap products. Coconut oil is obtained from the dried fruit (copra) of the coconut palm tree. The fruit is dried either in the sun or over open fires from burning the husks of the fruit, with the oil pressed out of the dried fruit.

*Palm kernel oil*, obtained from the nuts of the palm tree, is another frequently used vegetable oil and is somewhat similar in properties and composition to coconut oil (see Table 1).

*Palm oil* is derived from the fleshy fruit of the palm tree rather than the nut, as with palm kernel oil. Palm oil has a longer chain-length distribution than palm kernel oil and provides properties and compositions more similar to tallow than to other vegetable oils (see Table 1).

### 3.1. Other Sources

The four oils named above are the most commonly used fats and oils in the soap-making industry in the United States, but other sources are also used throughout the world, including lard or hog fat, Babassu oil, rice brand oil, and soybean oil.

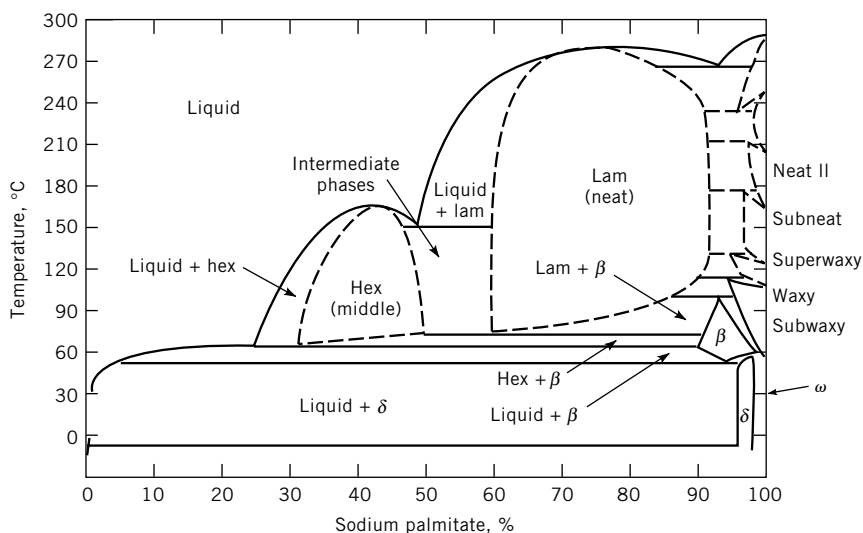
## 4. SOAP SOLUTION-PHASE PROPERTIES

Commercially, soap is most commonly produced through either the direct saponification of fats and oils with caustic or the hydrolysis of fats and oils to fatty acids followed by stoichiometric (equal molar) neutralization with caustic. Both of these approaches yield workable soap in the form of concentrated soap solutions (~70% soap). This concentration of soap is the target on account of the aqueous-phase properties of soap as well as practical limitations resulting from these properties. Hence, before discussing the commercial manufacturing of soap, it is imperative to understand the phase properties of soap.

### 4.1. The Binary Soap–Water System

Mixtures of soap in water exhibit a rich variety of phase structures, depending on temperature and concentration of the mixture (2). Phase diagrams chart the phase





**Figure 1.** Binary soap–water phase diagram for sodium palmitate (3). Courtesy of Academic Press, Ltd.

structures, or simply phases, as a function of temperature (on the y-axis) and concentration (on the x-axis). Figure 1 shows a typical soap–water binary phase diagram, in this case for sodium palmitate–water. Sodium palmitate is a fully saturated, 16-carbon chain-length soap. At lower temperatures, soap crystals coexist with a dilute isotropic soap solution. Upon heating, liquid crystalline phases may form, depending on the relative concentration of soap in water. In dilute solutions, the crystals disproportionate and form simple micellar isotropic solutions (nigre phase). As the soap concentration is increased, hexagonal (hex) liquid crystal (middle phase) is formed. At even higher soap levels, lamellar (lam) liquid crystal (neat phase) is formed. With other chain-length distributions of soap, the position of the phase boundaries with respect to temperature and composition varies. However, the general features and phase progressions are similar.

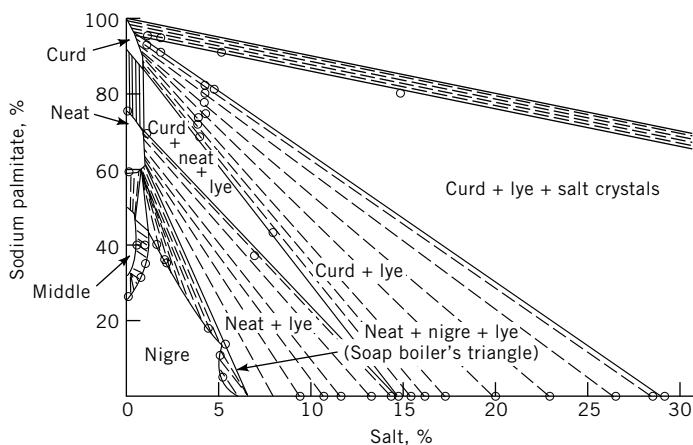
At typical soap-processing temperatures (80–95°C), three liquid soap phases are possible: nigre (sometimes called isotropic), middle, and neat soap (3). Nigre soap is observed in dilute soap solutions and is characterized as very fluid. However, because of the dilute nature of this phase, it is not of practical use in the manufacture of soap on account of high-energy requirements for drying. Middle soap is a liquid crystalline phase that is extremely viscous and difficult to handle and work. In commercial soap-making processes, care must be taken to avoid the middle-phase region on account of the physical problems associated with it; neat phase is always approached from the more concentrated soap direction. Neat soap is considerably more fluid than middle phase and is readily pumped and mixed. This is the phase most commonly desired for soap making. Neat soap is generally found in the concentration range of 60–90% soap, with commercial processes typically targeting ~70% soap as the optimal concentration. Higher soap concentrations

require increased temperatures to maintain the fully liquid crystalline properties (note the curved boundary in Figure 1) and exhibit increased viscosities that become difficult to manage.

## 4.2. Ternary Soap–Water–Salt Systems

A variety of components, such as salt (4), fatty acid (5), and glycerol (6), can alter the general phase characteristics of the soap–water system. Ternary phase diagrams are constructed to account for the presence of a third material. These diagrams are displayed as triangles where each of the vertices defines one of the three components and each of the three sides defines the relative concentrations of the two components contained by the two vertices associated with the side. Although temperature continues to be another important variable, these ternary diagrams are often drawn for a defined temperature because of the difficulties in representing an additional dimension. Sometimes the ternary triangle is modified by increasing the angle of one of the vertices to  $90^\circ$ , emphasizing the most important components.

The soap–water–salt diagram is typically shown graphically with the  $90^\circ$  vertices (Figure 2). At 0% salt, the phases along the axis present a slice of the binary soap–water-phase diagram at  $90^\circ\text{C}$  (sodium palmitate in this case). The addition of salt to the system greatly reduces the concentration ranges for the liquid crystalline phases and increases the ranges for the isotropic phases: nigre and lye (a caustic-rich aqueous phase). Further increase in the salt concentration drives the system into a biphasic region in which both a concentrated soap and a nigre (or lye) phase coexist. This ability of salt to drive the system into a biphasic, neat soap–nigre/lye phase structure is the basis for the direct saponification approach to soap making. The soap can be separated at a controlled concentration from an aqueous lye or salt phase. The aqueous phase can be used to wash out the excess lye, impurities, and, most importantly, the glycerol, a valuable byproduct of soap making.



**Figure 2.** Ternary soap–NaCl–water-phase diagram for sodium palmitate (4). Courtesy of Academic Press, Ltd.

## 5. SOAP SOLID-PHASE PROPERTIES AND CRYSTALLIZATION

Soap crystallizes into bilayer structures composed of stacks of soap layers arranged head-to-head and tail-to-tail, with water of hydration present in the head-to-head intralamellar region (7). There have been a variety of reports regarding a large number of crystalline phase structures for soap, but it is generally accepted that only four common, distinct pure sodium soap crystalline phases exist (8). These phases are designated a, b, d, and w and were originally identified through powder x-ray diffraction. Only w, b, and d are observed in conventional soaps; a-phase is found only when crystallized out of ethanol. In a strict sense, these crystalline sodium soap phases are not polymorphs, i.e., different crystal arrangement of same composition; rather, they are different phase compounds, i.e., compositionally different. This distinction occurs not only because of the differences in crystal arrangement but also because of the different levels of hydration present in the various crystalline states (9). This fact is emphasized in Figure 1.

For sodium palmitate, d-phase is the thermodynamically preferred, or equilibrium state, at room temperature and up to  $\sim 60^{\circ}\text{C}$ ; b-phase contains a higher level of hydration and forms at higher temperatures; and w-phase is an anhydrous crystal that forms at temperatures comparable with b-phase. Most soap in the solid state exists in one or a combination of these three phases. The phase diagram refers to equilibrium states. In practice, the drying routes and other mechanical manipulation used in the formation of solid soap can result in the formation of nonequilibrium phase structure. This point is important when dealing with the manufacturing of soap bars and their performance.

X-ray diffraction (xrd) is the simplest and most reliable method for distinguishing between the different crystalline phases, including sodium soap (10). Each phase has a different crystal-packing arrangement and thus produces different x-ray diffraction scattering patterns that can be used to identify and quantify the soap phase composition. The most common means of phase identification is through the measurement of the chain-to-chain packing lengths (0.25–0.4 nm), which are insensitive to the chain length of the soap being examined (8).

When processing a hot mixture of soap, no crystals are present. However, crystals form when the hot mixture is cooled to a critical temperature usually referred to as the Krafft boundary (11). The type of crystalline structure that develops initially is dependent on both the mixture composition and temperature. However, the remaining processing of the soap can further alter the crystal structure of the soap in terms of both colloidal structure, i.e., distribution and size of crystals, and crystalline phase composition. The temperatures at which crystals form differ for different chain-length soaps and different distributions, as well as water content and counter cation. Shorter chain length and unsaturated soaps exhibit significantly lower Krafft boundaries than longer, saturated chain-length soaps. Potassium soaps, as well as ammonium soaps, possess considerably lower crystallization temperatures, whereas heavy metal and alkali earth metal soaps possess higher crystallization temperatures.

There are some general guidelines that allow the prediction of which crystal structure will form upon cooling of a hot soap mixture. The most pronounced is

the fatty acid chain-length distribution. Those mixtures that have more shorter or unsaturated chain lengths (the more soluble soaps) typically result in w- or b-phase soap crystals. The *cis*-configuration unsaturated isomers have a greater impact than the *trans*-configuration isomers. Those hot soap mixtures enriched in the longer saturated chain-length (palmitate and stearate) soaps typically result in the formation of d-phase soap crystals.

Other factors also impact the type of crystals formed upon cooling of hot soap. Water activity or moisture content contribute to the final crystal state as a result of the different phases containing different levels of hydration. Any additive that changes the water activity changes the crystallization pathway. For example, the addition of salt reduces the water activity of the mixture and pushes the equilibrium state toward the lower moisture crystal structure. Additionally, the replacement of sodium with other counter cations influences the crystallization. For example, the replacement of sodium with potassium drives toward the formation of d-phase.

Phase diagrams can be used to help understand the resulting crystal formation. Upon definition of the starting conditions (temperature and composition), the crystal phases can be estimated by following a line down in temperature at a constant composition. The first crystals to form may not be the equilibrium crystals at room temperature. As soap phases are phase compounds and not simple polymorphs, once they form, they must first disproportionate to reform into a different crystal of another phase structure. This process is inherently slow and requires energy, e.g., work or additional heat, so the first crystals encountered upon cooling are likely to be frozen into the cooled, solid soap. Although, in theory, the crystal phase structure of soap should be predictable from knowledge of composition and temperature, in practice, the use of various drying approaches, e.g., chill rolls, vacuum dryers, and spray towers, often leads to the formation of a nonequilibrium, room temperature phase. It is the rapid cooling of the hot soap through these various drying techniques that, in essence, traps the soap in nonequilibrium phases. These nonequilibrium phases give rise to the phase changes that occur during processing into finished bar soap products. For the milled bar process, work and heat is put into the soap during additional processing, which results in microheating and physical mixing of the soap. Work tends to transform soap from w-phase to either b- or d-phase. But, in addition to the work, appropriate temperature control must be maintained to allow the formation of the desired phase. For example, d-phase is most readily achieved through the cold milling of soap; w-phase can be maintained through the hotter milling of the soap. The common phase progression is from w to b to d, with the reverse progression only observed at high mill temperatures. For framed products, conditioning of the finished soap at various temperatures can result in enough energy input to facilitate the disproportionation reaction to the more thermodynamically stable crystal.

The phase of the soap can have a dramatic impact on both the in-process properties and finished product performance. Soap phase can change the ability of the soap to weld together during finishing, and induce soap stickiness, thus creating problems in milling and conveying soap to and from the various unit operations. In terms of finished product performance, phase can influence a variety of

attributes. These attributes include lathering (or solubility), wet cracking, smear, and firmness. Lathering includes both the amount and type, e.g., creaminess, of lather a product imparts during use. Wet cracking is a measure of the degree of cracking or fissures found in a bar after it has adsorbed water and subsequently dried out. Smear measures the total amount of partially solubilized soap that forms on the bar surface during long duration contact with water. Firmness is a measure of the physical rigidity of the bar. Simple correlation between the soap phase and these performance attributes have been noted as far back as 1929 (8). For example, b- and w-phase soaps possess comparable firmness grades, but have significantly different lathering abilities (b much greater). However, b-phase also creates more smear or loss of soap to the water and formation of surface gel than w-phase soap. Therefore, bar soaps are specifically designed and manufactured to create a soap phase structure that is a good balance of these properties.

In the presence of excess fatty acid, different soap crystalline phase compounds can form, commonly referred to as acid-soaps. Acid-soap crystals are composed of stoichiometric amounts of soap and fatty acid and associate in similar bilayer structures as pure soap crystals. There are a number of different documented acid-soap crystals. The existence of crystals of the composition 2 acid-1 soap, 1 acid-1 soap, and 1 acid-2 soap has been reported (12-14). The presence of the acid-soaps can also have a dramatic impact on the physical and performance properties of the finished soap. The presence of acid-soaps increases the plasticity of the soap during processing and decreases product firmness, potentially to the point of stickiness during processing. Furthermore, the presence of the acid-soap changes the character of the lather, decreasing the bubble size and subsequently increasing lather stability and creaminess.

It would be incomplete for any discussion of soap crystal phase properties to ignore the colloidal aspects of soap and its impact. At room temperature, the soap-water phase diagram suggests that the soap crystals should be surrounded by an isotropic liquid phase. The colloidal properties are defined by the size, geometry, and interconnectiveness of the soap crystals. Correlations between the colloid structure of the soap bar and the performance of the product are somewhat qualitative, as there is little hard data presented in the literature. However, it might be anticipated that smaller crystals would lead to a softer product. Furthermore, these smaller crystals might also be expected to dissolve more readily, leading to more lather. Translucent and transparent products rely on the formation of extremely small crystals to impart optical clarity.

## 6. COMMERCIAL PROCESSING

### 6.1. Direct Saponification

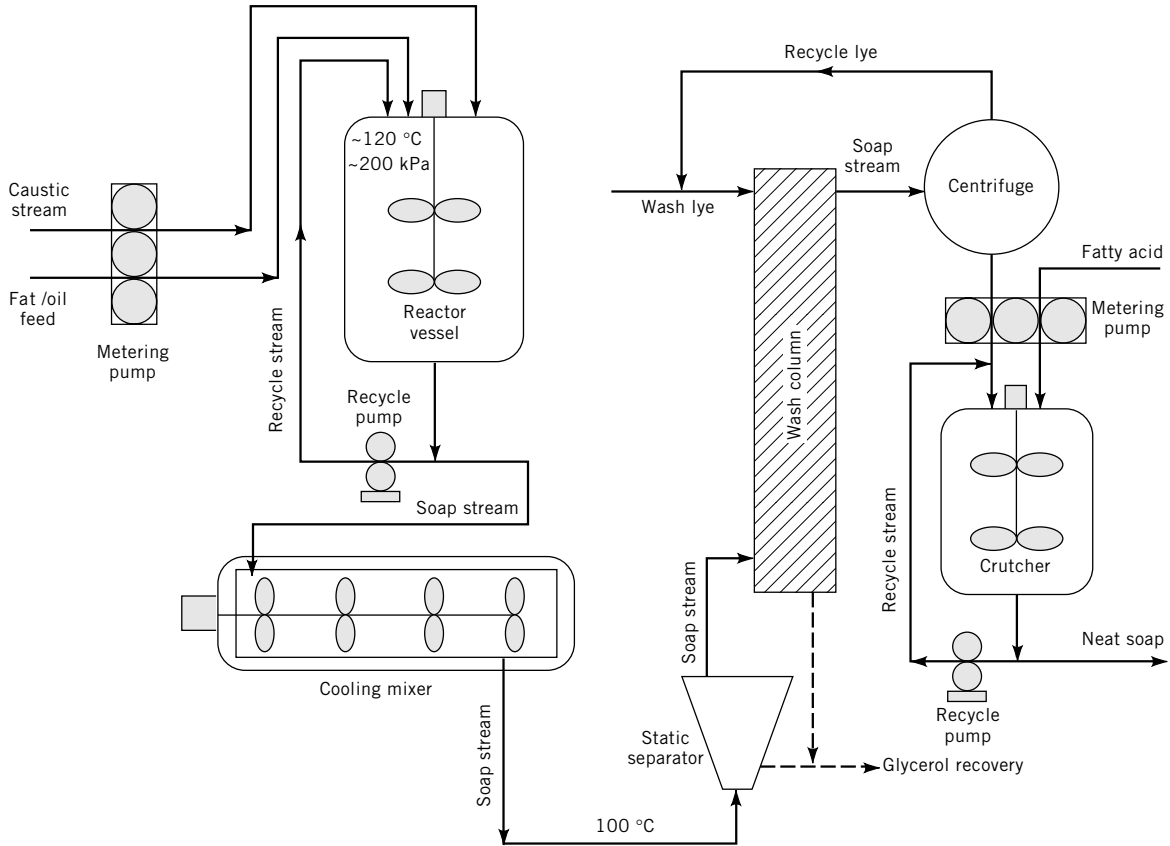
Direct saponification of fats and oils is the traditional process used for the manufacturing of soap. Commercially, this is done through either a kettle boiling batch process or a continuous process.

**6.1.1. Kettle Boiled Batch Process** This process produces soap in large, open steel tanks known as kettles, which can hold up to 130,000 kg of material. Kettles are cylindrical tanks with conical bottoms, which contain open steam coils for heating and agitation. To make soap by this process, fats and oils, caustic soda, salt, and water are simultaneously added to the kettle. Effective mixing is important in this process because of the low miscibilities of the fats and oils and caustics. The addition of steam to the system facilitates mixing and the saponification reaction. In some systems, the mixing is enhanced through the use of specially designed saponification jets, which allow for intimate mixing of the two components during the charging of the kettle. Care must be taken when blending the fats and oils with caustic soda, salt, and water to ensure a consistent reaction rate for forming the desired neat soap. It is common practice to leave some previously formed soap in the kettle before charging the kettle with the new saponification starting materials. This soap, through its surfactant properties, helps disperse the fats and oils and water phases through better emulsification, thus increasing the reaction rate. To complete the saponification process, the soap batch is boiled for a period of time using steam sparging.

Upon completion of the saponification reaction, additional salt is added to the kettle while boiling with steam to convert the mixture from a pure neat-soap phase composition into the curd soap-lye seat biphasic composition. This process is commonly called opening the grain of the soap. The lye seat is a phase having extremely high electrolytic strength (high salt and lye levels), glycerol, and small amounts of soap, which has minimal solubility in saturated salt solutions. The mixture is allowed to separate for several hours, after which the aqueous solution or lye seat is removed from the bottom of the kettle. The lye seat is usually transferred to a glycerol recovery system, where the glycerol is recovered, purified, and used for other purposes. The curd soap remaining in the kettle is typically washed a few times by adding water, converting it back into neat soap and repeating the salt addition, boiling, and separation process. This washing process provides a more complete removal of glycerol and other impurities from the soap. After the final wash, the water level in the curd soap remaining in the kettle is adjusted to achieve the proper physical properties for additional processing. This process, referred to as fitting, results in the formation of a neat soap-nigre-phase mixture, which facilitates further removal of impurities through the settling of the nigre phase. What remains in the kettle is pure neat soap at ~70% concentration with low levels of salt and glycerol. This process is time-consuming and requires several days to complete.

**6.1.2. Continuous Saponification Systems** A relatively recent innovation in the production of soap, these systems have led to improved manufacturing efficiency and considerably shorter processing times. There are a number of commercial systems available; even though these systems are different in design aspects or specific operations, they all saponify fats and oils to finished soap using the same general process (Figure 3).

Blended fat and oil feedstocks are continuously and accurately metered into a pressurized, heated vessel, commonly referred to as an autoclave, along with the



**Figure 3.** Process stream diagram for a continuous saponification soap manufacturing facility. To convert kPa to psi, multiply by 0.145.

appropriate amount of caustic, water, and salt. The concentrations of these ingredients are adjusted to yield a mixture of neat soap and a lye seat. At the temperatures ( $\sim 120^\circ\text{C}$ ) and pressures ( $\sim 200\text{ kPa}$ ) used, the saponification reaction proceeds quickly ( $<30\text{ min}$ ). A recirculation system ensures a residual level of soap in the autoclave to improve contact between the oil and water phases and provides additional mixing. After a relatively short resident time in the autoclave, the neat soap and lye seat reaction blend is pumped into a cooling mixer where the saponification reaction is completed and the reaction product is cooled to below  $100^\circ\text{C}$ . The reaction product is pumped next into a static separator, where the lye phase containing a high level of glycerol (25–30%) is separated from the neat soap through gravitational force or settling.

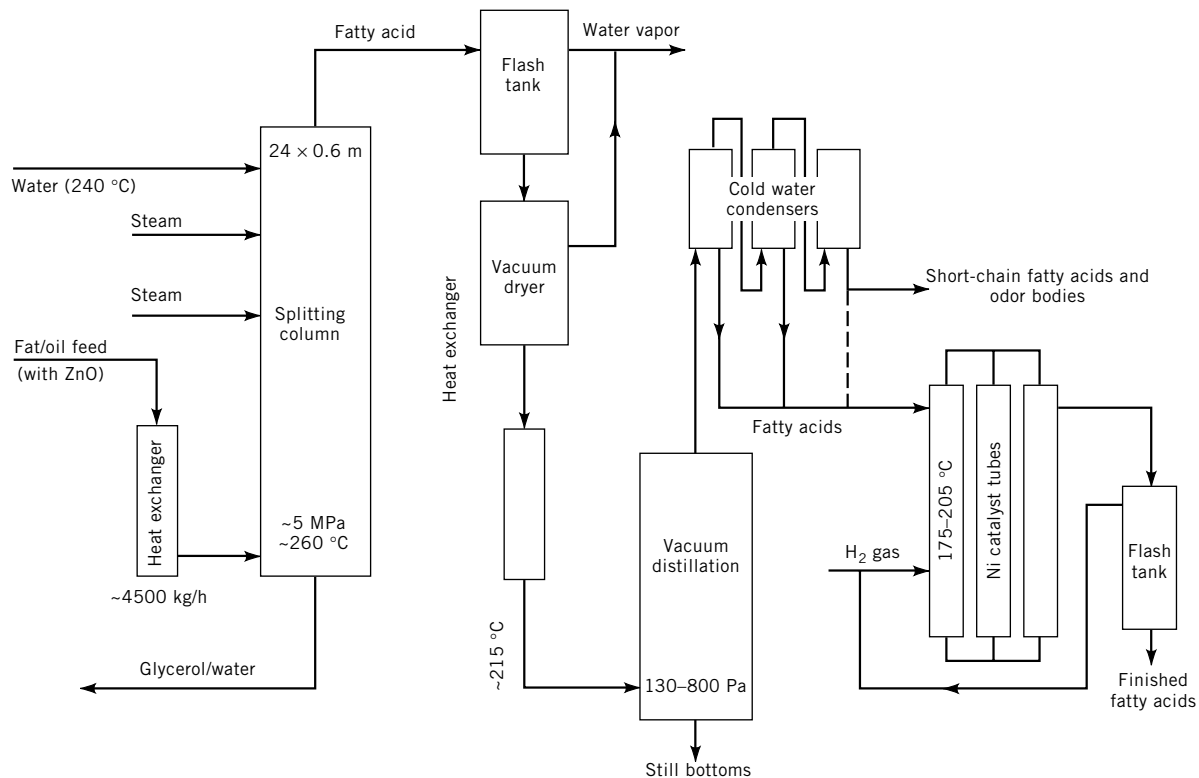
The neat soap is then washed with a lye and salt solution using a countercurrent flow process. This is often done in a vertical column, which might be an open tube or contain mixing or baffle stages. The neat soap is introduced into the bottom of the column and the lye/salt (washing) solution is pumped into the top. The less dense neat soap rises up in the column while the lye/salt solution falls to the bottom. The washing solution removes impurities and allows for further collection of the glycerol. As with the kettle process, it is important to have a proper level of electrolyte (salt and lye) for effective removal of the glycerol. Final separation of the lye seat from the neat soap is commonly achieved using centrifugation. After centrifugation, the remaining caustic or residual alkalinity in the separated neat soap is neutralized through the accurate addition of fatty acid in a steam-jacketed mixing vessel (crutcher). The soap is now ready for use in the manufacturing of soap bars.

## 6.2. Fatty Acid Neutralization

Another approach to produce soap is through the neutralization of fatty acids with caustic. This approach requires a stepwise process where fatty acids are produced through the hydrolysis of fats and oils by water, followed by subsequent neutralization with appropriate caustics. This approach has a number of inherent benefits over the saponification process.

**6.2.1. Hydrolysis Step** The hydrolysis of fats and oils by water requires intimate mixing of these two normally immiscible phases. The reaction is carried out under conditions where water possesses appreciable solubility (10–25%) in fats and oils. In practice, this is achieved under high pressure 4–5.5 MPa (580–800 psi) and with high temperatures ( $\sim 240\text{--}270^\circ\text{C}$ ) in stainless steel columns of around 24–31 m in height and 50–130 cm in diameter (Figure 4). ZnO is sometimes added as a catalyst to the feedstock fats and oils to facilitate the reaction. The fat and oil feedstock is injected at the bottom and water is injected at the top of the column. The columns may be either open in design, or contain baffles to ensure better mixing through turbulent flow. High-pressure steam inlets are placed at three or four different heights in the column for heating. This design establishes a countercurrent flow pattern with the water moving through the column from top to bottom and the fats and oils the opposite direction. As these materials





**Figure 4.** Process stream diagram for the production of fatty acids through hydrolysis of fats and oils. Steam is at 5.2–6.2 MPa (750–900 psi). To convert MPa to psi, multiply by 145. To convert Pa to mm Hg, multiply by 0.0075.

intermix at the high temperatures and pressures employed, the ester linkages in the fats and oils hydrolyze to create fatty acids and glycerol. The newly formed fatty acids continue to rise up the column, while the resulting glycerol is carried (washed out) downward with the water phase. As this is a reversible reaction, it is important to remove the glycerin from the mixture through the countercurrent washing process. The concentrations of glycerol and glycerides (mono, di, and tri) are lowest and the concentration of fatty acid is highest toward the top of the column.

The rate-limiting step in the reaction is the removal of glycerol from the fatty acids. This removal relies on interaction with the washwater falling through the column. The Zn-soap formed by the reaction of ZnO and fatty acid acts as a phase-transfer catalyst, improving the transfer of glycerol from the oil to the water phase. The separation of the glycerol and fatty acid in the column prevents the reverse reaction from occurring. The hydrolyzer process provides around 99% efficiency for the conversion of the fats and oils to fatty acids and glycerol, and requires around 90-min residence time.

The fatty acids that emerge from the top of the column contain entrained water, partially hydrolyzed fat, and the Zn-soap catalyst. This product stream is passed into a vacuum dryer stage where the water is removed through vaporization and the fatty acid cooled as a result of this vaporization process. The dried product stream is then passed to a distillation system.

The distillation system allows for improved fatty acid quality, i.e., odor and color, through the separation of the fatty acid from partially saponified fats and oils, the Zn catalyst, and odor and color bodies. This is achieved by heating the product stream in a heat exchanger to around 205–232°C and introducing it into a vacuum chamber (flash still) at 0.13–0.8 kPa (1–6 mm Hg) absolute pressure. The fatty acids are vaporized under these conditions and removed from the undesired materials such as the partially hydrolyzed triglyceride. The vaporized fatty acids are then passed through a series of cold water condensers for fractionation and collection. Systems vary in the number of condensers but a three-condenser system is common. The fatty acids are typically separated into a heavy cut, a mid-cut, and a very light cut. Depending on the end uses for the fatty acid, these fractions may be used as separated for specialty fats such as stearic acid or rebled for desired fat ratios such as 80:20% tallow:coconut soap. The light cut is often removed from the other condensates because it contains many of the odor bodies present in the fatty acid.

The fatty acids obtained from the process can be used directly or further manipulated for improved or modified performance and stability. Hardening is an operation in which some fraction of the unsaturated bonds present in the fatty acids are eliminated through hydrogenation or the addition of H<sub>2</sub> across a carbon-carbon double bond. This process was initially intended to improve the odor and color stability of fatty acids through elimination of the polyunsaturated species. However, with the growth in the use of specialty fatty acids, hydrogenation is a commercially important process to modify the physical properties of the fatty acids.

Hardening is typically achieved by passing the preheated fatty acid through a series of tubes packed with catalyst in the presence of hydrogen gas. The most

commonly used catalyst is Ni, but other catalysts are available. The amount of hardening is governed by the amount of hydrogen, the reaction temperature, the pressure, and the residence time. The hardened fatty acids are filtered to remove any residual catalyst and then cooled in a flash tank where the excess hydrogen gas is removed. In addition to the reduction of the unsaturate level in the fatty acid, the process may also convert some of the naturally occurring *cis*-configuration unsaturated fatty acids into the corresponding *trans*-configuration. The conversion can affect the finished product properties and is typically controlled to within desired specifications.

**6.2.2. Neutralization Step** The formation of soap from fatty acids is achieved through the reaction of the fatty acid with the appropriate caustic. This reaction is extremely rapid for most common caustics, e.g., NaOH or KOH, and requires proper stoichiometry and rigorous mixing to ensure processing effectiveness. Although this appears relatively straightforward, in practice, there are a number of processing considerations that must be addressed. First, an exact ratio of fatty acids, caustic, water, and salt must be maintained to ensure formation of the desired neat soap phase. The process is controlled to avoid the formation of middle soap, which has a high viscosity and does not dissipate rapidly, or the formation of a neat soap–nigre biphasic mixture, which may separate upon storage. Second, intimate mixing of the oil and aqueous reactants is necessary to ensure uniform neat soap phase composition. Third, because of the heat liberated by the reaction, temperature control must be maintained within certain limits to prevent overheating and boiling/foaming.

There are a variety of commercial systems for achieving neutralization. Generally, a heated fatty acid blend ( $\sim 50\text{--}70^\circ\text{C}$ ) and caustic–salt–water ( $\sim 25\text{--}30^\circ\text{C}$ ) streams are metered into some form of a high shear mixing system, commonly referred to as a neutralizer. The mixed stream heats to between  $85^\circ\text{C}$  and  $95^\circ\text{C}$  on account of the latent heat of reaction and is pumped into a receiver tank that effectively mixes the soap through both a recirculation system and agitation. After a short residence time in the receiver tank to ensure a uniform composition, the resulting neat soap is pumped into storage tanks or to the finishing operations.

### 6.3. Glycerol Recovery

The spent lyes resulting from soapmaking processes generally contain 8–15% glycerol; sweet waters from hydrolysis of fats contain as much as 20% glycerol; crude glycerol from esterification contains 80% or more glycerol. The grade of fat used directly affects the treatment required to produce glycerol of an acceptable commercial quality. The chemicals most commonly used to remove impurities from spent lye and sweet water are hydrochloric acid and caustic soda.

The treatment of spent lye consists of a series of operations designed to remove nearly all of the organic impurities (15, 16). The spent lye commonly is treated with mineral or fatty acids to reduce the content of free caustic and soda ash and to reduce the pH to 4.6–4.8 (17). Sulfates are to be avoided because they are

associated with foaming and heat exchanger fouling during subsequent refining. After cooling, the solid soap is skimmed, and an acid and a coagulant are added, followed by filtration. Addition of caustic soda removes the balance of coagulant in solution and adjusts the pH to a point at which the liquor is least corrosive to subsequent process treatment. Spent lyes from modern liquid-liquid countercurrent extraction used with continuous saponification systems require little treatment other than reduction of free alkali by neutralization with hydrochloric acid. The dilute glycerol is now ready for concentration to 80% soap lye crude glycerol.

The sweet water from continuous and batch autoclave processes for splitting fats contains little or no mineral acids and salts and requires very little in the way of purification, as compared with spent lye from kettle soapmaking (8). The sweet water should be processed promptly after splitting to avoid degradation and loss of glycerol by fermentation. Any fatty acids that rise to the top of the sweet water are skimmed. A small amount of alkali is added to precipitate the dissolved fatty acids and neutralize the liquor. The alkaline liquor is then filtered and evaporated to an 88% crude glycerol. Sweet water from modern noncatalytic, continuous hydrolysis may be evaporated to ca 88% without chemical treatment.

Ester crude glycerol is usually of high quality; however, salt residue from the esterification catalyst is typically present at a concentration of one percent or higher. Crude glycerol originating from esterification or splitting of 100% vegetable oils is segregated from other glycerols throughout processing to produce kosher glycerin.

**6.3.1. Concentration** The quality of crude glycerols directly affects the refining operation and glycerin yield. Specifications for crude glycerols usually limit ash content, i.e., a measure of salt and mineral residue; matter organic nonglycerol (MONG), which includes fatty acids and esters; trimethylene glycol (TMG), i.e., propane-1,3-diol; water; arsenic; and sugars (7).

Dilute glycerol liquors, after purification, are concentrated to crude glycerol by evaporation. This process is carried out in conventional evaporation under vacuum heated by low-pressure steam. In the case of soap-lye glycerol, means are supplied for recovery of the salt that forms as the spent lye is concentrated. Multiple effect evaporators are typically used to conserve energy while concentrating to a glycerol content of 85–90%.

**6.3.2. Refining** The refining of natural glycerol is generally accomplished by distillation, followed by treatment with active carbon. In some cases, refining is accomplished by ion exchange.

**6.3.3. Distillation** In the case of spent-lye crude, the composition is ca 80% glycerol, 7% water, 2% organic residue, and less than 10% ash. Hydrolysis crudes are generally of a better quality than soap-lye crudes with a composition of ca 88% glycerol, <1% ash (little or no salt), and <1.5% organic residue.

Distillation equipment for soap-lye and esterification crude requires salt-resistant metallurgy. The solid salt that results when glycerol is vaporized is removed by filtration or as bottoms from a wiped film evaporator. The Luwa scraped wall

evaporator is capable of vaporizing glycerol very rapidly and almost completely, such that a dry, powdery residue is discharged from the base of the unit (7). Distillation of glycerol under atmospheric pressure is not practicable because it polymerizes and decomposes glycerol to some extent at the normal boiling point of 204°C. A combination of vacuum and steam distillation is used in which the vapors are passed from the still through a series of condensers or a packed fractionation section in the upper section of the still. Relatively pure glycerol is condensed. High vacuum conditions in modern stills minimize glycerol losses due to polymerization and decomposition.

**6.3.4. Bleaching and Deodorizing** The extensive use of glycerol and glycerol derivatives in the food industry stresses the importance of the removal of both color and odor (also necessary requirements of USP and extra-quality grades). Activated carbon (1–2%) and diatomite filter aid are added to the glycerol in the bleach tank at 74–79°C, stirred for 1–2 h, and then filtered at the same temperature, which is high enough to ensure easy filtering and yet not so high as to lead to darkening of the glycerol.

**6.3.5. Grades** Two grades of crude glycerol are marketed: (1) soap-lye crude glycerol obtained by concentration of lyes from kettle or continuous soapmaking processes contains ca 80% glycerol; and (2) hydrolysis crude glycerol resulting from hydrolysis of fats contains ca 88–91% glycerol and a small amount of organic salts. As glycerol from methyl ester production contains salt, it is usually marketed as soap-lye crude.

Several grades of refined glycerol, such as high gravity, dynamite, and USP, are marketed; specifications vary depending on the consumer and the intended use. USP-grade glycerol is water-white, and meets the requirements of the USP. It is classified as GRAS by the FDA, and is suitable for use in foods, pharmaceuticals, and cosmetics, or when the highest quality is demanded or the product is designed for human consumption. It has a minimum specific gravity (25°C/25°C) of 1.249, corresponding to no less than 95% glycerol. Kosher glycerin meets all USP requirements and is produced synthetically or from 100% vegetable glycerides. The *European Pharmacopoeia* (PH.EUR.) grade is similar to the USP, but the common PH.EUR. grade has a minimum glycerol content of 99.5%. The chemically pure (CP) grade designates a grade of glycerol that is about the same as the USP but with the specifications varying slightly as agreed by buyer and seller. The high-gravity grade is a pale-yellow glycerol for industrial use with a minimum specific gravity (25°C/25°C) of 1.2595. The dynamite grade has the same specific gravity but is more yellow (see Explosives and propellants, explosives). All these grades satisfy the federal specifications for glycerol (0-G491B-2).

## 6.4. Comparison of Base Soap Manufacturing Routes

Direct saponification of fats and oils is well known, characterized, and straightforward; requires little equipment; and is relatively energy-efficient. However, it is not

very effective with regard to changes in the fats and oils ratio desired for finished soap bar formulations. Furthermore, direct saponification has the drawbacks of lower glycerol yields, limited flexibility toward formation of mixed counterion soaps, and requires higher quality feedstock for good quality soaps. In contrast, the hydrolyzer/neutralizer system is more flexible with regard to formation of mixed counterions and formula changes, allows for posthardening of fatty acids, and provides better glycerol recovery. Furthermore, the ability for both distillation and posthardening provides greater flexibility in fats and oils feedstock selection; lesser grades can be used to yield comparable quality base soap. However, this process is extremely energy-intensive and requires more specialized process equipment, e.g., hydrolyzer columns, stills, a hydrogenation system, and neutralizer, and necessitates the use of stainless steel on account of the corrosive nature of fatty acid.

## 7. BAR SOAP MANUFACTURING

The conversion of wet base soap into consumer-acceptable bar soaps can be achieved using one of three commonly used manufacturing processes: framing, milling, and hot extrusion, all of which use a variety of processing unit operations or finishing steps. These steps include wet mixing or crutching, drying, dry mixing or compounding, and bar forming.

### 7.1. Framing

The framed bar process is by far the oldest and the most straightforward process used in the production of bar soaps. The wet base soap is pumped into a heated, agitated vessel commonly referred to as a crutcher. The minor ingredients used in soap bars, such as fragrance or preservative, are added to the wet soap in the crutcher or injected in-line after reduction of product stream temperature. The hot mixture is then pumped into molds and allowed to cool.

These molds can be either finished bar-shape molds or large blocks. Finished bar-shape molds can be either a mated two-piece design or a five-sided, open-top design. Upon cooling, the solid bar is removed from the mold and packaged as desired. For the large blocks, the mold is pulled apart and the block of solid soap is removed. Wire cutters are employed to cut the blocks first into slabs, then into stripes, and finally into rectangular bricks representing the finished size of the bar. The rectangular brick is finished by a final stamping step that typically embosses the logo and any shape modifications into the brick. This large-block approach is only suitable for brick-like shapes, whereas the finished bar-shape molds allow for the production of much more complex shapes.

Traditionally, this process has been used primarily for simple soap bars because it tends to be time consuming and thus somewhat limited for large-scale bar production. However, advances have been reported in automating this approach (18). Furthermore, the process requires fluid crutcher compositions for flow into the molds. This typically requires the formulation to contain either a high level of

solvents, including water, glycerol, and alcohol, and be at elevated temperatures ( $>80^{\circ}\text{C}$ ) when poured into the frames. Despite these limitations, it has proven to be the preferred route to producing certain specialty products, for example, transparent bars.

## 7.2. Milled Bar Process

The process used in the 1990s for the production of most bar soaps is the milled bar process. The milled soap process produces high-quality soap bars. The process requires drying as well as milling and plodding (extrusion) of low-moisture soap and is capable of high efficiency and throughput ( $\sim 300\text{--}400$  bars/min for a given packing line). This process, however, is also equipment-intensive on account of the number of process unit operations required.

For this process, the wet soap is pumped into a mixing vessel (crutcher) where the addition and mixing of minor ingredients may be achieved. Minor ingredients include excess fatty acids, preservatives, and potentially other synthetic surfactants. Alternatively, mixing can be achieved through the use of in-line static mixers, with the accurate addition of the minors into a flowing stream of the wet soap.

The wet soap is put into the drying operation where moldable solid soap is created by reducing the water content in the wet soap from around 30% to between 7% and 15%. This drying step can be attained through three typical approaches: atmospheric flash drying, vacuum drying, and chilled surface drying. For all three approaches, the wet soap stream is first superheated in a high-pressure steam-heat exchanger of either a plate-and-frame or tube-and-shell design. The amount of heating the soap undergoes depends on the drying needs.

Atmospheric flash drying is similar to spray tower drying used for the formation of detergent granules. The superheated soap ( $\sim 190\text{--}220^{\circ}\text{C}$ ) is sprayed at pressures of about 2.8 MPa (400 psi) (with specially designed nozzles) as small particles into the upper part of a tower (at atmospheric pressures). The high-pressure spraying process causes a rapid loss of moisture from the superheated soap in the form of steam. Cooling of the hot, dry soap particles is achieved using cooling air that is blown into the bottom of the tower. The air cools the soap as it falls to the bottom of the tower. The cooling air is usually humidified to prevent overdrying of the soap. The soap is removed from the bottom of the tower.

Vacuum drying is similar to atmospheric drying but does not require as high a temperature to drive the moisture loss; it can be performed in a considerably smaller tower. Wet soap, heated to around  $130\text{--}150^{\circ}\text{C}$  in a low-pressure heat exchanger, is sprayed onto the walls of an evacuated tower using a nozzle. The nozzle can either be unidirectional, mounted on a rotating shaft, or statically mounted and multidirectional. Cooling and drying is achieved in one step through the rapid release of moisture as vapor, which occurs on introduction of superheated soap into the vacuum chamber (Joule-Thompson cooling). The dried, cooled soap is scraped off the tower wall with a scraper blade mounted on a rotating shaft. The moisture in the dried soap is dependent on the flow rate, the temperature of the soap, and the pressure in the vacuum chamber; the last also controls the final

temperature of the soap. The dried soap is obtained at the bottom of the tower in the form of small pellets through an airlock created by the screw extrusion of the soap through a multiholed orifice plate. Vacuum drying has a number of advantages over atmospheric flash drying, including the lower pressure steam requirements, lower overall temperatures, and the more compact drying system/tower.

The chilled surface drying process is similar to atmospheric drying, with the cooling process being driven by a chilled surface as opposed to air flow through a tall tower. The wet soap is superheated in high-pressure, nonboiling heat exchangers. Drying is achieved by the release of steam when this superheated soap is introduced into a chamber with a slight negative pressure, which is commonly referred to as a flash chamber. The resulting hot, dry soap melt is cooled through the formation of a thin film on a chilled surface, commonly in the form of a roll (rotating cylinder). The hot, dry soap falls into the small gap ( $\sim 10\text{--}50\ \mu\text{m}$ ) formed at the interface between a large chilled roll and a smaller, temperature-controlled (may be heated or cooled) applicator roll that aids in uniform film formation. As the chilled roll rotates, the dry, cold soap is removed via scraping with a doctor blade and emerges in the form of flat flakes. The amount of soap drying is governed by the temperature that the soap is introduced and the air flow in the flash chamber. This process is exceptionally good for modern synthetic surfactant-containing formulations because it is amenable to more sticky in-process materials. This drying approach can also be achieved using a chilled belt in place of the chilled roll.

Additional minor ingredients, e.g., pigments, fragrances, dyes, preservatives, and antibacterial actives, or some cosurfactants in modern-day bar soaps are introduced into the dried soap in either a batch or continuous fashion in a unit operation referred to as amalgamation. An amalgamator is a paddle mixer where both solids and liquids can be effectively pulverized and mixed on a macroscopic scale. In a batch process, the materials are individually weighed and placed into the amalgamator. The batch process is quite labor intensive and time consuming. The continuous process is more commonly used because it allows for higher efficiencies and throughput. In a continuous mixer, solids are typically introduced into the amalgamator through weighbelts, whereas liquid components are delivered using metering pumps. The materials are mixed and moved through the amalgamator using a helical agitator.

More intimate mixing of the soap and minor ingredients is achieved using controlled-temperature milling. Milling is an operation in which the soap is passed through a series of closely spaced, temperature-controlled steel rolls that dictates product temperature, inputs work into the soap mixture, and provides efficient micromixing. Four-roll mills are very common but three- and five-rolls are also used. Mills are designed such that successive rolls rotate in opposite directions and at slightly increased rates than the previous roll. Therefore, at the point of contact between two mill rolls, the two surfaces are moving in the same direction but with different speeds. A zone, called a bead, is created at this point of contact where material is micromixed through the high-shear nature of the zone. This high-shear mixing also causes heating of the product stream. The spacing between the rolls is set to generate a final product of around 1.0–1.5 mm thickness. At the top roll, the



soap is scraped off using a knife blade into ribbons of less than 5 cm in width. A typical four-roll mill can handle around 3600 kg/h of material, and inputs enough work and temperature to raise the dried soap temperature from 25°C to 40°C.

Milling not only provides intimate mixing, but also eliminates variation in ribbon thickness and crushes lumpy materials, e.g., overdried soap, which might impact finished bar texture. Milling is also used for the formation of the proper bar soap crystalline phase, which plays a critical role in both the performance properties of the soap bar and the handling characteristics of the in-process soap. For example, too hot a milling temperature can create sticky soap that is difficult to process further.

The formation of finished bar soaps is accomplished through the continuous extrusion of a shaped plug of soap. This extrusion is commonly referred to as plodding and is achieved using a two-stage single- or twin-worm-screw extruder. The purpose of plodding is to compact the soap noodles into a solid mass of soap that is in a manageable form and devoid of air. The first stage of the plodder pushes the soap through a multiholed orifice plate that acts as an airlock for the second stage. The second stage is under vacuum to ensure the removal of entrained air that impacts final bar appearance. The second stage also pushes the soap through a temperature-controlled barrel that terminates in a cone having a shaped orifice plate. The orifice plate yields a soap plug with proper dimensions for cutting and stamping into the desired bar shape. During this plodding step, heat may be added or removed. The worm screw and conical termination of the barrel force the soap into a plastic mass (at appropriate temperatures), which is welded together and emerges as a smoothly surfaced, continuous plug of soap.

The plug at the exit of the plodder is cut into the appropriate length and directed into the stamping and packaging operations. Product can be stamped into the desired shape on account of its intrinsic plasticity using either fixed capacity or box dies. Capacity dies are a pair of casts pressed together to form the desired shape of the bar. The dies possess a fixed capacity and excess material is pushed outside the mated die pair. The excess, on the order of 20% of the original plug mass, is recycled back to the plodder. Box dies are an arrangement of two dies that, in conjunction with a cavity referred to as a box, form the shape of the bar. The plug is placed into the cavity and the two dies push the soap to fill in the shape confined by the two dies and the box. There is very little excess because this design uses the total mass of material to fill out the shape. However, the resultant bar soap has a band around its perimeter on account of the box. Capacity dies provide greater flexibility in bar shape design, whereas box dies have the advantage of producing much lower amounts of recycle material. Dies are typically produced out of brass, highly polished to produce a high gloss, smooth bar surface, and cooled ( $\sim 0$ –15°C) to eliminate product sticking during stamping. To further eliminate the sticking of the final bar to the die, a liquor of concentrated brine solution or glycerin is often applied to the die surface. Stamped bars are then either wrapped or placed in cartons and bundled for sale. The entire bar-finishing operation from the plodder to cases of finished product operate at rates of between 150 bar/min to 400 bar/min, depending on the stamp design and packaging equipment.

### 7.3. Hot Extrusion Process

The hot extrusion process was originally developed as a replacement of the traditional framed process used for the manufacturing of floating soap bars, i.e., Ivory (19). This process uses a scraped-wall heat exchanger (SWHX) to provide controlled crystallization and plug forming in one step. Wet soap is first partially dried from  $\sim 30\%$  to  $\sim 20\%$  moisture using a high-pressure heat exchanger, operating around  $180\text{--}190^\circ\text{C}$ , and an atmospheric flash tank to drive off the water. The high temperature and pressure drop causes the flashing. The reduced moisture soap, at  $\sim 85\text{--}95^\circ\text{C}$ , is then pumped through a high-shear mixer where minor ingredients such as fragrance and preservative are added. For a floating bar, air is also injected into the product stream at this point.

The product stream is then pumped into the SWHX for cooling and crystallization. The SWHX is an open tube and is jacketed for cooling using  $<0^\circ\text{C}$  brine circulated at a high rate. Inside the open tube is a rotating shaft containing scrapers. This arrangement provides efficient cooling of the product stream as it transverses the 1–2 m of the SWHX by scraping cooled product off the wall and effectively mixing it back into the bulk product. At the end of the SWHX is a cone that causes compression of the product stream into a rectangular plug. Inside the cone are a series of rotating arms that help to ensure a homogenized product stream. At the outlet of the SWHX emerges a partially crystallized strip at  $\sim 55\text{--}70^\circ\text{C}$ ; this partial crystallization makes the strip firm enough to maintain its shape once extruded. The extruded strip is taken away from the outlet of the SWHX on a conveyor belt, cut to appropriate dimensions, and allowed to cool further under specific temperature and humidity conditions to ensure proper crystallization. The cut strips are then finished into packaged bars using processes similar to those described previously.

## 8. FORMULATION OF SOAPS

The formulation of bar soaps has become increasingly complex with changing consumer bathing habits and expectations. In the past, consumers' bathing habits were such, e.g., once-a-week baths, that simple lye soaps were acceptable. However, today it is not uncommon to shower everyday, which puts greater demands on the performance properties of soap bar, for example, mildness to skin and formation of bathtub ring (20). Manufacturers of bar soaps have developed a variety of formulation approaches to deliver products that better meet the consumer needs of the 1990s. This is achieved through the proper balancing of soap components, inclusion of various additives, or the blending of synthetic surfactants into the formula. In addition, new forms of cleansing products have been introduced to address these changing habits and consumer needs, e.g., liquid handsoaps and shower gels or body washes. For personal cleansing products, including bar soaps, performance is measured by such tests as lather, wet cracking, smear, firmness, rinsability (the amount of residue left on surfaces after rinsing with hard water), and mildness to skin (21, 22). It is through use of these measures and exhaustive consumer research that modern soapmakers develop better products for consumer needs.

## 8.1. Soap Bars

In soap bars the primary surfactant is predominantly sodium salts of fatty acids. These products typically contain between 70% and 85% soap. Occasionally, potassium soap (~5–30%) is included in the formulation to increase the solubility of the soap and, hence, the bar's lathering properties. The low Krafft temperatures for potassium soap are the basis for the lather enhancement, but also limits their content in bars.

Soap performance can be controlled through the proper blending of fats and oils to specific ratios, and the formation of the proper phase and colloidal structure. It is common to produce soap using a blend of tallow and coconut or palm kernel oils, generally in a ratio of between 85:15 and 50:50. As the amount of coconut oil is increased in the bar, the lathering profile of the product typically increases as a result of the inherent higher solubility of the soaps formed from coconut (shorter chain-length soaps). However, this higher lather comes at the expense of bar smear, which increases for the same solubility reasons. Furthermore, the higher content of sodium laurate in these high coconut soaps can negatively impact the mildness of the product, because laurate soaps are intrinsically more irritating to skin than other chain lengths (23). High-lathering, pure coconut oil soaps are still marketed in the United States as Castille soaps.

Additionally, soap bars typically contain between 8% and 20% water, 0.5% to 1% NaCl, and low levels of glycerol. Salt modifies the processibility of soap during plodding and milling, as well as being a carryover ingredient from the manufacture of the base soap. Glycerol is also an impurity remaining from the base soap production, but in some bars it is actually added for rinsing or skin-feel purposes.

## 8.2. Bar Soap Additives

There are a variety of additives that may be formulated into soap bars to provide additional consumer benefits or modify the performance of the products.

**8.2.1. Free Fatty Acid** Soap bars are intrinsically alkaline in nature on account of the physical properties of soap in water and the process used in its manufacture, which yields base soap having a very slight excess of free caustic. Free fatty acid, commonly either coconut or palm kernel, is added into the formulation to neutralize this slight excess of caustic. Often higher fatty acid levels (1–8%) are incorporated into the formula to modify the performance of the product, referred to as superfatting. The free fatty acid associates with soap to form acid–soap crystals. The formation of acid–soap crystals changes both the texture and plasticity of the bar, as well as the lather performance. Superfatted bars are smoother and longer lasting. They also yield lathers that are more stable, creamier, and more dense than non-superfatted products. However, superfatting can also decrease the odor and color stability of the final product because oxidative degradation of fatty acids is faster than for the analogous soaps. Other materials can be used to achieve similar characteristics as superfatted bars, for example, waxes and triglycerides. These types of superfatting agents may also serve as emollients.

**8.2.2. Glycerol** This common skin care ingredient is formulated in bar soaps because of its humectant properties. Glycerol, at levels of 10%, has been shown to change significantly the consumer skin softness and smoothness perception (24). Even at low levels, glycerol can alter the skin-rinsing profile of the bar. Unfortunately, high levels of glycerol can negatively impact bar processibility through increasing product softness and stickiness.

**8.2.3. Colorants, Dyes, and Pigments** It is quite common to modify the appearance or aesthetic properties of bar soaps through the incorporation of various colorants and opacifiers. The most commonly used material is titanium dioxide, which at low levels (<0.8%) is an effective whitener and opacifier. Most marketed bar soaps contain some level of  $\text{TiO}_2$  as either an opacifier in conjunction with other colorants or as a whitener. A variety of dyes are also used in addition to  $\text{TiO}_2$  to generate desired product colors. The dyes used are almost exclusively dyestuffs of Drug and Cosmetic or Food, Drug, and Cosmetic grades. Some producers also use inert, inorganic pigments for product coloration. Pigments have an advantage over dyes; they are inherently more color-stable and not water soluble. The latter attribute is important for striped or two-toned products, because water-soluble dyes can migrate in the product and eventually lessen the contrast between the two tones present.

**8.2.4. Fragrance** A key aesthetic for consumer acceptance of personal cleansing products is the product fragrance. Fragrance is used by manufacturers of soaps as one of the primary means of targeting products for specific user groups and connoting different product marketing positions. A secondary purpose of fragrance is to mask the fatty base odor of the soap. Commonly, fragrance development is performed by perfume houses who focus their development on product appeal needs. For example, fragrances used for deodorant products tend to be impactful and residual to skin to provide long-lived fragrance on skin. A number of products are appearing on the market that are designed for individuals with sensitive skin. The fragrance types and levels used in these sensitive-skin products are such that they mask the base odors of the soap while providing some soft perfume notes during use, reinforcing their mildness to or compatibility with skin. Fragrance levels are typically in the range of 0.7% to 1.5%, but sensitive-skin products contain much lower levels. The level of fragrance used is a function of the target audience for the product and the odor stability of the fragrance in the product. Product odor instability results from both the loss of fragrance during storage and the propensity for oxidation of fragrance and soap components. Hence, a product may change from an acceptable to an unacceptable odor profile during its lifetime if not properly formulated.

**8.2.5. Chelants and Antioxidants** Soaps, fatty acids, and fragrances are susceptible to oxidation during aging (25). The oxidation process is quite complex but typically results from the reaction of the unsaturated bonds in these components with oxygen in the air, resulting in the formation of shorter chain-length acids,

aldehydes, and ketones that are extremely odoriferous. In the case of fragrance components, oxidation can produce a change in product odor character and cause discoloration of the bar. To minimize the oxidation of the base soap and other minor ingredients in soap bars, both chelants and antioxidants are commonly used.

Chelants at concentrations of 0.1% to 0.2% improve the oxidative stability through the complexation of the trace metal ions, e.g., iron, which catalyze the oxidative processes. Examples of the chelants commonly used are pentasodium diethylenetriaminepentaacetic acid (DTPA), tetrasodium ethylenediaminetetraacetic acid (EDTA), sodium etidronate (EHDP), and citric acid. Magnesium silicate, formed in wet soap through the reaction of magnesium and silicate ions, is another chelant commonly used in simple soap bars.

Antioxidants are also used in conjunction with chelants to further improve product odor and color stability. Antioxidants work by chemically trapping the free radicals formed during the oxidation process, significantly decreasing the rate of the degradation reaction. This is particularly important for fragrance components. Butylated hydroxytoluene (BHT), one of the most commonly used antioxidants, is usually incorporated at levels of 100–200 ppm in the formulation. BHT is frequently added directly to the fragrance to improve the storage stability of the neat material.

**8.2.6. Mildness and Skin Additives** The increased frequency of bathing and the changing consumer need has necessitated the development of products having skin care benefits. In addition to the two most common additives, fatty acid and glycerol, there are a wealth of other additives that are frequently used. Examples include lanolin, Vitamin E, aloe vera gel, mineral oil, and baking soda.

Inert materials are sometimes used in soap bars as a means of improving the skin mildness of the product by decreasing the level of soap and surfactant in the bar. The cleansing agents at high concentrations can sometime dry and irritate skin. A variety of inert materials, both inorganic and organic, have been reported in the literature, including oatmeal, dextrin, starch, wax, and talc (26). These materials may also deposit on the skin during washing, further modifying the rinsing properties of the soap bar and impacting the consumer perception of the product and its aesthetic properties.

Newer technologies have been used in the manufacture of bar soaps, which truly improve the clinical mildness-to-skin of these products. One approach relies on minimizing the overall levels of the more irritating soap species, such as the laurates and unsaturated species, through appropriate balancing of feedstocks (27, 28). Another approach is the incorporation of quaternary amine compounds into the formula, which effectively complexes the soap during the wash–rinse process, reducing its potential to remove oils from or interact with the skin. The amines commonly take the form of cationic polymers based on natural materials such as cellulose, guar gums, and proteins (29).

**8.2.7. Antimicrobial Agents** Antimicrobial agents have been used for a number of years in soap bars as a means of providing additional deodorant protection

through their residual effectiveness on suppressing the growth of odor-causing bacteria. These materials actually deposit on skin during the washing process and provide a reservoir of active ingredient that is effective at suppressing bacterial growth between washings. It is widely believed that these soaps may provide additional benefits on account of their ability to control the microflora on the skin surface. One such benefit may be the reduction in the level or frequency of minor skin infections by controlling the *Staphylococcus aureus* level on the skin surface (30). Only two active ingredients are commonly used in bar soaps: trichlorocarbanalide or TCC (Triclocarban) and trichlorohydroxydiphenyl ether or TCS (Triclosan). These compounds are typically used at concentrations of 0.25% to 1.5% in the final product and have activity against a wide range of micro-organisms.

**8.2.8. Specialty Soaps** There are a variety of specialty soaps that require certain additives to deliver the special consumer needs for which they were developed. Scouring soaps contain an abrasive agent homogeneously distributed throughout the soap to aid in the cleaning properties of the product. The abrasives are extremely small particles of insoluble material such as pumice. Striped bar soaps are commonly produced using two soap streams with different colorant systems that are intentionally poorly mixed during extrusion through the plodder. Transparent soap bars, often called glycerin soap, are formed through the quiescent cooling of a high solubility soap system containing a high level of solvent (31). The solvent aids in the formation of a clear gel through retardation of large crystal formation. Common solvents include glycerol, triethanolamine, ethyl alcohol, and sugars. A common type of soap used in these products is triethanolamine soaps, e.g., Neutrogena, which have relatively low crystallization temperatures.

**8.2.9. Synthetic Surfactant** Much of the development of new soap bar technologies has been focused on products containing some level of synthetic surfactants. The primary benefits of synthetic surfactants over soaps are their intrinsic lower sensitivity to water hardness, which improves their rinsing profiles, their lathering ability, and their effects on skin feel and mildness. Anionic, nonionic, and amphoteric surfactants have all been formulated into bar soaps. In most bar soaps, the synthetic surfactant serves the purpose of a secondary surfactant (at levels of 1–20%), modifying the lathering, rinsing, or skin effects profile. However, in some skin care or beauty bars, these surfactants represent the primary cleansing agent (up to 50% or 60% of formulation), with soap taking the role as the secondary surfactant, present primarily for structural purposes.

Anionic surfactants are the most commonly used class of surfactant. Anionic surfactants include sulfates such as sodium alkylsulfate and the homologous ethoxylated versions and sulfonates, e.g., sodium alkylglycerol ether sulfonate and sodium cocoyl isethionate. Nonionic surfactants are commonly used at low levels (~1–2%) to reduce soap scum formation of the product, especially in hard water. These nonionic surfactants are usually ethoxylated fatty materials, such as  $\text{HOCH}_2\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_n\text{R}$ . These are commonly based on triglycerides or fatty alcohols. Amphoteric surfactants, such as cocamidopropyl betaine and cocoam-

phoacetate, are more recent surfactants in the bar soap area and are typically used at low levels (<2%) as secondary surfactants. These materials can have a dramatic impact on both the lathering and mildness of products (32).

These surfactants, in conjunction with soap, produce bars that may possess superior lathering and rinsing in hard water, greater lather stability, and improved skin effects. Beauty and skin care bars are becoming very complex formulations. A review of the literature clearly demonstrates the complexity of these very mild formulations, where it is not uncommon to find a mixture of synthetic surfactants, each of which is specifically added to modify various properties of the product. For example, one approach commonly reported is to blend a low level of soap (for product firmness), a mild primary surfactant (such as sodium cocoyl isethionate), a high lathering or lather-boosting cosurfactant, e.g., cocamidopropyl betaine or AGS, and potentially an emollient-like stearic acid (33). Such benefits come at a cost to the consumer because these materials are considerably more expensive than simple soaps.

### 8.3. Liquid Soaps and Body Washes

In the late 1970s and early 1980s a new form of soap product emerged, commonly referred to as liquid handsoaps. These liquid soaps were offered as a practical replacement of soap bars for use at sinks in the bathroom and kitchen. Manufacturers have taken two basic approaches to the formulation of these products: soap-based and synthetic-based formulations. Soap-based formulas use potassium or ammonium salts to yield soaps that are highly soluble at room temperature. These soaps have typically been of either short chain lengths, such as coconut soap, or a blend of short chain lengths and unsaturated soaps, such as oleic. More recently, these soap-based formulations have been replaced by synthetic surfactant-based formulations. Synthetic surfactant formulations have the advantages of being milder-to-skin, cleaner rinsing, higher lathering, and less sensitive to water hardness. A typical synthetic surfactant formulation is around 80% water and may contain sodium alkyl sulfate and sodium alkylethoxy sulfate as the primary surfactant, a nonionic surfactant such as lauramide DEA, and potentially a lather-building amphoteric surfactant such as cocamidopropylbetaine (34). Other commonly used surfactants include sodium cocoyl isethionate and sodium olefin sulfonate. The U.S. market has evolved significantly since the initial introduction of liquid handsoaps, and most products in the 1990s possess both antibacterial agents (usually TCS) and moisturizers. The latter are used to protect hands from drying and germs. The most commonly used moisturizing agents are cationic polymers such as those previously discussed.

Body washes are another more recent introduction into the marketplace. These products have become a mainstay in the European market and, in only a few years, have grown to be a significant fraction of the U.S. market. Body washes can be simple formulas similar to those used for liquid handsoaps or complex 2-in-1 oil-in-water emulsion, moisturizing formulations. These products contain a wide range of synthetic surfactants not typically found in bar soaps or liquid handsoaps,

**TABLE 2. Primary Manufacturers of Bar Soaps, Liquid Handsoaps, and Body Washes.**

Producer	Product
Procter & Gamble Co.	Ivory, Oil of Olay, Zest, Coast, Safeguard, Camay
Lever Brothers Co.	Dove, Lever 2000, Caress, Shield, Lux
Colgate-Palmolive Co.	Irish Spring, Cashmere Bouquet, Softsoap, Palmolive
Dial Corp.	Dial, Pure & Natural, Tone, Spirit
Andew Jergens Co.	Jergens Mild, Naturals

such as sodium monoalkyl phosphate. It is not uncommon to find over 20 different components in these formulations, with no less than six or seven different surfactants. The 2-in-1 products also contain skin-conditioning agents, such as cationic polymers, and emollients or beauty oils to provide even milder-to-skin cleansing and in-use moisturization.

## 9. ECONOMIC ASPECTS

The personal cleansing market in the United States represents nearly 2 billion in consumer sales divided among the three primary categories: bar soaps (~77%), liquid handsoaps (~13%), and body washes (10%) (35, 36). A few large manufacturers produce the majority of these products, as indicated in Table 2. However, a number of smaller producers also manufacture bars for both general consumption and specialty uses.

## 10. ANALYTICAL CHARACTERIZATION OF SOAP

There are a variety of analytical methods commonly used for the characterization of neat soap and bar soaps. Many of these methods have been published as official methods by the American Oil Chemists' Society (23). Additionally, many analysts choose *United States Pharmacopoeia* (USP), *British Pharmacopoeia* (BP), or *Food Chemical Codex* (FCC) methods. These methods tend to be colorimetric, potentiometric, or titrametric procedures. However, a variety of instrumental techniques are also frequently used, e.g., gas chromatography, high-performance liquid chromatography, nuclear magnetic resonance spectroscopy, infrared spectroscopy, and mass spectrometry.

Some of the traditional methods used for the characterization of bar soaps are moisture, free acid or free alkalinity, total soap, and chloride (salt). Moisture determinations are typically run by Karl Fischer titration. However, under certain circumstances, moisture can be measured gravimetrically. The measure of free fatty acid or free alkalinity is typically done via a simple pH titration using phenolphthalein or potentiometric end point. Total soap is generally done using an extraction/gravimetric procedure: the soap sample is acidified in an ethanol–water mixture, the fatty acids are extracted into a hydrocarbon solvent, and the amount of extracted



fatty acid is measured by gravimetric analysis after elimination of the solvent. The measurement of sodium chloride is accomplished commonly using an ion-selective electrode titration. Glycerol is analyzed using gas chromatography. The analysis of finished bar soaps include, in addition to those stated above, color and odor analysis; analysis of active ingredients, e.g., antimicrobial actives; and quantitation of synthetic surfactants.

## 11. HEALTH, SAFETY, AND TOXICOLOGY

The manufacture of soap poses some material handling concerns because of the reaction of strong caustics with either neutral fats and oils or fatty acids at relatively high temperatures. The caustics, i.e., sodium hydroxide and potassium hydroxide, represent the primary hazard. At around 50% concentrations, these caustics are extremely corrosive and may cause serious body burns and eye injuries if not removed quickly through rinsing with copious amounts of water. Appropriate protective clothing is strongly urged when handling these materials.

Soap, as used in personal cleansing products, has a long, safe history of use. Modern soaps have been specifically formulated to be compatible with skin and to be used on a daily basis with minimal side effects. Excessive use of soap for skin cleansing can disrupt the natural barrier function of skin through the removal of skin oils and disruption of the lipid bilayer in skin. This can result in imperfect desquamation or a dry appearance to skin and cause an irritation response or erythema, i.e., reddening of the skin. Neither of these is a permanent response; and the elicitation of this type of skin reaction depends on the individual's skin type, the product formulation, and the frequency of use.

There is a considerable amount of research into the compatibility of cleansing products with skin (20, 38, 39). Modern soap manufacturers improve the skin compatibility of their products through a variety of chemical testing methods. These methods are often used to evaluate the mildness (irritation potential) of test formulations in comparison with other formulations on the basis of the dry skin and irritated (red) appearance of skin. There are many reports of comparative studies of various formulations and their mildness-to-skin; however, the results of these overly exaggerated test methods may not reflect consumer experience with products (20). On direct contact with other sensitive membranes, such as eyes, soap may also cause irritation in the form of stinging. Again, this is a temporary response that can be rectified through rinsing with water. Ingestion of soap poses little risk at the levels of materials usually ingested. Typically, temporary minor irritation of mucous membranes and gastrointestinal disorders, e.g., nausea, vomiting, and diarrhea, may be experienced.

## 12. ADDITIONAL USES OF SOAP

The primary use of carboxylate soaps is in the manufacture of personal cleansing products, principally bar soaps. Liquid soaps comprise a small percentage of total

usage. There are also a number of other applications for both consumer and industrial use. Soluble soaps, such as potassium and sodium, are used in cleansing applications where their detergent and emulsification properties can be leveraged, for example, in the textile industry where the cleansing of various in-process fibers and leather (defatting) is desired. Soaps are also used in emulsion polymerization. Furthermore, soaps are used in a variety of cosmetic products. Sodium stearate provides the structure of many modern antiperspirant and deodorant sticks (40, 41). Amine soaps, typically triethanolamine and ammonium stearate, are used in products where high-volume, stable lathers are desired, such as cleansing and shaving creams (42).

The lower solubility metal and alkaline-earth metal soaps are also widely used in a variety of different applications. For example, calcium oleate is used in the waterproofing of cement. Metal soaps, such as calcium, magnesium, and aluminum soaps, are commonly used for the thickening of hydrocarbon lubricating greases, mold release, and suspending agents in paints (43–47). Magnesium stearate is frequently used as a filler material and binder in drug tablets and as either inert binder or emulsification agent in cleansing products and cosmetics, respectively (48, 49). Aluminum soap has been used in the manufacture of weapons of war, applying its emulsification properties to napalm. Zinc soaps have been studied and applied to specialty cleansing products because of their antibacterial and antifungal properties.

## REFERENCES

1. L. Spitz, ed., *Soap Technology for the 1990's*, American Oil Chemists' Society, Champaign, Illinois, 1990.
2. D. Small, ed., *Handbook of Lipid Research 4, The Physical Chemistry of Lipids from Alkanes to Phospholipids*, Plenum Press, New York, 1986, Chs. 3 and 9.
3. R. G. Laughlin, *The Aqueous Phase Behaviour of Surfactants*, Academic Press, Ltd., London, 1994, pp. 448–451.
4. Ref. 3, pp. 377–383.
5. P. Ekwall and L. Mandell, *Kolloid Z.*, **233**, 938–944 (1969).
6. P. Ekwall, L. Mandell, and K. Fontell, *J. Coll. Interfacial Sci.*, **28**, 219–226 (1968).
7. D. Chapman, *The Structure of Lipids by Spectroscopic and X-ray Techniques*, John Wiley & Sons, New York, 1965.
8. R. H. Ferguson, F. B. Rosevear, and R. C. Stillman, *Ind. Eng. Chem.*, **35**, 1005 (1943).
9. M. J. Buerger, L. B. Smith, A. deBretteville, and F. V. Ryer, *Proc. Nat. Acad. Sci.*, **28**, 529 (1942).
10. N. Garti and K. Sato, *Crystallization and Polymorphism of Fats and Fatty Acids*, Marcel Dekker, New York, 1988.
11. Ref. 3, Chapt. 5.
12. J. W. McBain and M. C. Field, *J. Chem. Soc.*, 920 (1933).
13. P. Z. Ekwall, *Anorg. Allem. Chem.*, **210**, 337 (1933).
14. M. L. Lynch, Y. Pan, and R. G. Laughlin, *J. Phys. Chem.*, **100**, 357 (1996).

15. T. M. Patrick, Jr., E. T. McBee, and H. B. Haas, *J. Am. Chem. Soc.*, **68**, 1009 (1946).
16. W. E. Sanger, *Chem. Met. Eng.*, **26**, 1211 (1922).
17. E. Woollatt, *The Manufacture of Soaps, Other Detergents and Glycerin*, John Wiley & Sons, New York, 1985, pp. 296–357.
18. E. Jungerman, T. Hassapis, R. A. Scott, and M. S. Wortzman U.S. Patent 4,758,370, 1988 (to Neutrogena Corp.).
19. V. Mills, U.S. Patents 2,295,594; 2,295,595; 2,295,596, September 15, 1942 (to Procter & Gamble Co.).
20. R. Wolf, *Dermatology*, **189**, 217 (1994).
21. K. D. Ertel, B. H. Keswick, and P. B. Bryant, *J. Soc. Cos. Chem.*, **46**, 67 (1995).
22. B. H. Keswick, K. D. Ertel, and M. O. Visscher, *J. Soc. Cos. Chem.*, **43**, 187 (1992).
23. C. Prottey, P. J. Hartop, and T. F. M. Ferguson, *J. Soc. Cosmet. Chem.*, **24**, 473–492 (1972).
24. R. M. Dahlgren, M. F. Lukacovic, S. E. Michaels, and M. O. Visscher, in A. R. Baldwin, ed., *Proceedings of the Second World Conference on Detergents*, American Oil Chemists' Society, Champaign, Illinois, 1987.
25. H. W. S. Chan, *Autoxidation of Unsaturated Lipids*, Academic Press, Inc., Orlando, Florida, 1987.
26. J. R. O'Roark U.S. Patent 4,151,105, April 24, 1979 (to Hewitt Soap Co., Inc.).
27. F. R. Tollens, P. J. Kefauver, and S. W. Syfert U.S. Patent 5,387,362, February 7, 1995 (to Procter & Gamble Co.).
28. N. M. Moroney et al. U.S. Patent 5,264,144, November 23, 1993 (to Procter & Gamble Co.).
29. D. B. Wilson et al. U.S. Patent 5,296,159, March 22, 1994 (to Procter & Gamble Co.).
30. M. B. Finkey, N. C. Corbin, L. B. Aust, R. Aly, and H. I. Maibach, *J. Soc. Cosmet. Chem.*, **35**, 351 (1984).
31. T. Instone and M. Bottarelli U.S. Patent 5,041,234, August 20, 1991 (to Lever Brothers Co.).
32. J. F. Ashley, A. C. Coxon, and R. S. Lee Eur. Patent 0,472,320,A1, February 26, 1991 (to Unilever).
33. K. J. Rys, A. P. Greene, F. S. Osmer, and J. J. Podgorsky U.S. Patent 4,954,282, September 4, 1990 (to Lever Brothers Co.).
34. M. Fujiwara, C. Vincent, K. Villa Anathapadmanabhan, and V. Virgilio V. World Patent 9532705, December 7, 1995 (to Unilever PLC).
35. 1987 Census of Manufacturers, Vol. II, U.S. Government Printing Office, Washington, D.C., Product Statistics, Table 6A.
36. S. Colwell, *Soap Cps. Chem. Spec.*, **71**, 32 (1995).
37. *Official and Tentative Methods of the American Oil Chemist's Society*, American Oil Chemist's Society, Chicago, Illinois, annual publication.
38. F. R. Bettley and E. Donoghue, *Brit. J. Derm.*, **72**, 67 (1960).
39. D. D. Strube and G. Nicoll, *Cutis*, **39**, 544 (1987).
40. D. E. Katsoulis and J. M. Smith, U.S. Patent 5,232,689, August 3, 1993 (to Dow Corning Corp.).
41. P. B. Katsat and B. D. Moghe, U.S. Patent 5,424,070, June 13, 1995 (to Mennen Co.).
42. A. G. Barnet and M. R. Mezikofsky, U.S. Patent 5,326,556, July 5, 1994 (to Gillette Co.).

43. T. Hutchings and K. M. Pilgrim, U.S. Patent 5,385,682, January 31, 1994 (to Exxon Res. & Eng. Co.).
44. P. D. Maples U.S. Patent 5,472,625, December 5, 1995.
45. J. A. Waynick U.S. Patent 5,096,605, March 17, 1992 (to Amoco Corp.).
46. J. A. Waynick U.S. Patent 5,102,565, April 7, 1992 (to Amoco Corp.).
47. T. Hanano, U.S. Patent 5,279,750, January 18, 1994 (to Hanano Commercial Co., Ltd.).
48. B. L. Redd and co-workers U.S. Patent 5,328,632, July 12, 1994 (to Procter & Gamble Co.).
49. G. W. Radebaugh, R. Glinecke, and T. N. Julian, U.S. Patent 4,806,359, February 21, 1989 (to McNeilab, Inc.).

## GENERAL REFERENCES

- E. Woollatt, *The Manufacture of Soaps, Other Detergents and Glycerine*, John Wiley & Sons, New York, 1985.
- L. Spitz, ed., *Soap Technology for the 1990's*, American Oil Chemists' Society, Champaign, Illinois, 1990.

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## *Detergents and Detergency*

Jesse L. Lynn, Jr.

### 1. INTRODUCTION

The cleaning of a solid object, i.e., the removal of unwanted foreign matter from its surface, is done by methods ranging from simple mechanical separation such as blotting or abrasion to removal by solution or selective chemical action. The term *detergency* is limited to systems in which a liquid bath is present and is the main cleaning component of the system. The action of the bath involves more than simple solution or simple hydraulic dislodging of soil, although both will occur and contribute to the cleaning. The cleaning is enhanced primarily by the presence in the bath of a special solute, the surfactant, that alters interfacial effects at the various phase boundaries within the system. Thus, a typical deterative system consists of a solid object to be cleaned, called the substrate, soil or dirt attached to it that is to be removed in the washing process, and a liquid bath that is applied to the soiled substrate. In turn, each of these elements can vary widely in properties and composition. The final cleaning benefit results from interaction of these elements and the conditions used, i.e., temperature, time, mechanical energy input (agitation), and, in the case of aqueous baths, the presence of hardness ions in the water.

In the cleaning or washing process in a typical deterative system, the soiled substrate is immersed in or brought into contact with a large excess of the bath liquor. Enough bath is used to provide a thick layer over the whole surface of the substrate. During this stage, air is displaced from soil and substrate surfaces; i.e., they are wetted by the bath. The system is subjected to mechanical agitation, either rubbing or shaking, which provides the necessary shearing action to separate the soil from

**TABLE 1. Deterasive Systems.**

System	Soil	Mechanical Action	Bath Ratio	Detergent
<i>Fabric and Fibrous</i>				
Textile and allied manufacturing				
Raw wool scouring	Liquid at operating temperature	Very gentle	High	Organic surfactant
Wool yarn and piece goods	Liquid	Gentle	Moderately high	Organic surfactant
Scouring gray cottons	Mostly solid; waxy and starchy	Vigorous	Low	Built surfactant
Scouring synthetic fabrics	Mixed; oily lubricant and sizing residues	Moderate	Medium	Built surfactant
De-inking paper	Mixed; oily and pigment	Vigorous	High	Heavily built surfactant
Laundering				
Household	Mixed and variable	Moderate	High	Unbuilt or built surfactant
Commercial and industrial	Mixed and variable; heavier than household soil	Vigorous	High	Built surfactant
Rug cleaning in plant	Heavy solid	Moderate gentle	Medium to low	Organic surfactant, may be built
On location	Heavy solid	Vigorous; superficial brushing	Very low; foam bath	Organic surfactant foam
<i>Hard Surfaces</i>				
Glass and ceramics				
Hand dishwashing	Mixed oily and solid organic	Moderate to vigorous	High	Organic surfactant
Machine dishwashing	Mixed oily and solid	Vigorous hydraulic	High	Inorganic <sup>a</sup>
Commercial bottle washing	Light solid organic	Vigorous hydraulic	High	Inorganic <sup>a</sup>
Metals				
Prefinishing cleaning	Oily	Moderate to gentle hydraulic	High	Inorganic <sup>a</sup>
Postforming emulsion cleaning	Oily and mixed	Gentle hydraulic	High	Mixed and inorganic and surfactant

**TABLE .1. (Continued)**

System	Soil	Mechanical Action	Bath Ratio	Detergent
Cleaning metal structures and equipment, tanks, dairy equipment, etc.	Variable; mostly oily or organic solid	Usually vigorous rubbing, sometimes hydraulic	Usually low in wash cycle; may be high in rinse cycle	Inorganic <sup>a</sup> , built surfactant
Organic surfaces, paint, linoleum, plastic tile	Mixed solid, organic solid, and oily	Usually vigorous low rubbing <i>Cosmetic and Personal Cleaning</i>	Low	Lightly to moderately built surfactant
Shampooing	Oily	Vigorous rubbing in rinse cycle	Low in wash cycle; high	Organic surfactant
Bathing and washing	Mixed, mostly oily	Mild to vigorous rubbing	High; sometimes low in wash cycle	Organic surfactant

<sup>a</sup> Organic surfactant frequently added to aid wetting and draining.

substrate and disperse it in the bath. Agitation also promotes mass transfer in the system, just as in a heterogeneous chemical reaction. The bath carrying the removed soil is drained, wiped, squeezed, or otherwise removed from the substrate. The substrate is rinsed free of the remaining soiled bath. This rinsing step determines the final cleanliness of the substrate. The cleaned substrate is dried or otherwise finished.

A meaningful discussion of detergency requires a definition of clean. In the physicochemical sense, a surface is clean if it contains no molecular species other than those in the interior of the two adjoining phases. It is difficult to achieve such a state even under the most exacting laboratory conditions. Practically, a surface is clean if it has been brought to a desired state with regard to foreign matter present upon it, as judged by agreed-upon criteria. Household linen, for example, is considered clean when it is free of visible soil even though it may carry a starch and a softening finish. In the dyehouse of a textile mill, a piece of goods such as this would be rejected as dirty and returned for scouring because these finishes interfere with dyeing. Most standards for cleanness involve a visual or optical judgment for the presence of foreign matter. In some systems, for example, the desizing of cotton, the degree of cleanness may be specified by weight percentage of soil on the substrate. In other systems, such as the degreasing of metal, it is the weight of soil per unit area of substrate surface that specifies cleanness. In washing dishes or glassware, cleanness is often specified by complete water wettability or freedom from water break, as well as by appearance.

Although it is impossible to list all the practical deterative systems that might be encountered, a large proportion fall in a small number of classes. This classification disregards surfactant structure and type of substrate (fibrous or hard surface) and is restricted to a consideration of the soil present on the substrate, the mechanical action employed, the bath ratio, and the detergent used. Some of the more commonly encountered deterative systems are classified on this basis in Table 1.

## 2. COMPONENTS OF DETERSIVE SYSTEMS

### 2.1. Substrates

Solid objects to be cleaned vary widely in chemical composition and surface configuration. With few exceptions, however, they can be divided into fabrics and fibrous materials and hard surfaces. Fabrics present a highly complex configuration and can entrap soil even though it may be physicochemically removed from the surface. Most fabrics are organic polymeric materials that may be swellable by water or permeable to small molecules and ions dissolved in the bath. Many common polymeric fabrics, cotton, polyester, rayon, nylon, wool, and cellulose esters contain ionogenic or polar centers capable of localizing (generally anionic) electric charges. Hard surfaces, on the other hand, are relatively flat and smooth. They cannot entrap soil that has been detached by physicochemical action. In general, they are impermeable to water and water-soluble materials although they vary widely in their wettability and polarity. The most important types are glass, metal, and organic polymeric materials including painted surfaces, linoleum, and plastic tile.



## 2.2. Soils

Soils vary greatly. They may be a single solid or liquid phase but usually are two or more phases, intimately and randomly mixed and irregularly disposed over the substrate. In a large number of important detergent systems, the nature of soil and the quantity present are well known. This is the case, for example, in most textile mill operations such as raw wool scouring, the boil-off and scouring of loom-state woven goods, and the soaping of printed cottons. In the cleaning of fabricated metal parts to remove forming and drawing lubricants, buffing compounds, and so on, the nature of the soil is well known. The behavior of soils encountered in dishwashing is well characterized.

As a result of many painstaking investigations, the soils on apparel encountered in laundering have been shown to be complex mixtures containing both oily and finely divided solid material (1–3). The oily material consists largely of fatty acids and polar fatty material, but a considerable proportion of neutral nonpolar oil is also present. The solid components vary widely with the locale in which samples are taken, and they resemble local street dust in composition.

Particle size is one of the most important factors in determining the ease with which solid soil can be removed from a substrate. Particles of  $>5 \mu\text{m}$  diameter are generally easily removed. Particles of  $<10 \text{ nm}$  diameter cannot be removed by ordinary detergent processes once they are attached to a typical textile fabric. Such particles are responsible for the gradual irreversible graying of white goods with continued wear and laundering. Particles in this size range tend to form clumps and clusters before they reach the fiber surface. These clusters behave like individual large particles. Particles or clusters in the range of  $100 \text{ nm}$  diameter resist removal by simple agitation in liquids that are not surface active, but these particles are removable by normal detergent processes. This is the size range of greatest interest.

Soil may include material that is soluble in the bath, such as encrusted sugar residues and molecularly dispersed material such as fruit juice stains. Removal of these soils is an important aspect of cleaning but is not generally considered in discussions of detergency.

## 2.3. Baths

The baths discussed here are aqueous solutions. Most nonaqueous cleaning systems, such as metal degreasing operations, depend entirely on solvent action and therefore cannot be considered examples of detergent systems. Some nonaqueous systems, however, are true detergent systems. Modern dry cleaning baths, for example, contain solutes that are surface active in the conventional hydrocarbon or chlorinated hydrocarbon medium and aid soil removal. The physical chemistry of such systems differs considerably from that of aqueous systems. Among bath components, the solute that is effective in cleaning, usually a mixture of several components, is called the detergent. The term *detergent* is also used frequently in the restricted sense of a surfactant of high detergent power. In many hard-surface

systems, however, nonsurfactants such as alkaline silicates and phosphates exert a true deterative effect. They are, in fact, the principal detergents in these systems even in the complete absence of any surfactants. In the cleaning of fabric systems, the most important deterative component in the bath is the surfactant. Nonsurfactant components that augment the cleaning effect of surfactants are called builders. Many materials that act as builders in fabric systems, e.g., phosphates and silicates, are the primary detergents in hard-surface systems, although their primary contribution to the cleaning process may differ in the two cases.

### **3. FORMULATION**

Detergents are formulated to clean a defined set of soiled substrates under an expected range of washing conditions. Some detergents, the familiar bar or toilet soap, for example, consist essentially of only one component. There are few systems, however, in which a suitably formulated detergent consisting of several components does not outperform the best single-component system. Although detergents for hand dishwashing rarely contain builders, those currently used in the United States contain at least three surfactants, and they may contain up to six. Ingredients of laundering detergent formulations for fabrics may be divided into the following groups: surfactants, including soap and various others; the inorganic salts, acids, and bases, including builders, and other compounds that do not contribute to detergency but provide other functions, such as regulating density and assuring crispness of powdered formulations; organic additives that enhance detergency, foaming power, emulsifying power, or soil-suspending effect of the composition; and special-purpose additives, such as bleaching agents, fluorescent whitening agents, antimicrobial agents, blueing agents, or starch, which provide desirable performance functions but have no direct effect on soil removal.

Fabric detergent formulations for special applications, such as the various specific operations within the textile mill, are frequently much simpler. They tend to contain little if any builder or special-purpose additive. The indispensable ingredient in fabric detergency is the organic surfactant. Formulations for hard-surface detergency, such as those used in automatic machine dishwashing, are simpler than fabric-washing compositions. An organic surfactant is frequently not needed, and inorganic salts are the deterative ingredients.

### **4. SURFACTANTS**

The most important components of deterative systems are the surfactants. Although a detailed discussion of surfactants and surfactant systems is beyond the scope of this chapter, and the reader is referred elsewhere for such (4), a limited discussion of oleochemically derived surfactants is appropriate here.

The first deterative systems were essentially "pure" soaps, formed by mixing animal or vegetable fats with water and alkali from wood ashes. The earliest literary

reference to washing with soap is found in Sumerian clay tablets dating from about 2500 B.C. (5), and for the next 4500 years, soap remained the dominant deterative system. Tallow-based soaps provide excellent detergency in soft water but not in hard water. This is one reason why in the 1940s, they began to be replaced by synthetic detergents, i.e., combinations of synthetic surfactants, largely alkylbenzene sulfonates (ABS), and builders, originally pentasodium tripolyphosphate (STPP). Environmental factors have led to the replacement of ABS by linear alkylbenzene sulfonate (LAS), and the replacement of STPP by zeolite, or more complex builder/water-softening systems. However, despite occasional calls for a return to soap-based cleaning systems (6), soaps play a minor role in today's fabric washing detergents. Basically, soaps do not provide detergents that are competitive on a cost-performance basis with suitably formulated synthetic detergents.

Thus, over the last half-century, petrochemically derived surfactants, led by LAS, have largely replaced soap, which has been the norm for almost five millenia. Nevertheless, oleochemically based surfactants continue to play a vital role in detergent formulations. Soap itself is generally present as a minor component to control suds profile, reduce dye transfer, control powder properties, and act as a cosurfactant or cobuilder.

Although LASs are petrochemically based, the other major surfactants in use today, alcohol ethoxylates, alcohol ethoxysulfates, and primary alcohol sulfates, are derived from long-chain alcohols that can be either petrochemically or oleochemically sourced. There has, of late, been a substantial debate over the relative advantages of "natural" (oleochemical) vs. "synthetic" (petrochemical) surfactants, centering around reputed environmental advantages for the oleochemical materials. However, studies have shown that similar oleochemically and petrochemically based surfactants have similar biodegradability. Further, life cycle analysis studies, which consider all resources, energy, and waste associated with a product from production through disposal, have shown no substantial overall differences between the two (7, 8). This is hardly surprising because cost is a rough measure of the resource expended to produce a material. Similar materials that are cost competitive might be expected to require similar resource expenditure to produce.

Thus, in the end, it will be seen there is "no real difference between petrochemical- and oleochemical-based (alcohol) feedstocks for surfactants" (9). On this basis, the primary determinants of hydrophobe choice for alcohol-based feedstocks have been price and availability.

In addition to soaps, there are other groups of oleochemical surfactants having no petrochemical analogues that are assuming increasingly important roles in detergents, including methyl ester sulfonates, alkyl polyglycosides, and glucamides. Methyl ester sulfonates (MES), or fatty acid ester sulfonates (FAES), have been studied since the 1960s. However, until their recent adoption by a major Japanese detergent manufacturer, they have not seen high-volume usage. Used basically as an anionic replacement for LAS, FAES's claimed benefits include good detergency at low concentrations, low environmental load, and good supply of high-quality material (10).

In all of the surfactants discussed so far, except perhaps soap, the hydrophilic portion of the surfactant has been from petrochemical or inorganic sources. The alkyl polyglycosides (APG) and polyhydroxyamides are thus exceptional in offering a hydrophilic group based on sugar chemistry. Although APGs have been known for many years, they were not offered on a large scale until the 1980s. Despite many claimed advantages (11), APGs have not been strongly embraced by any major detergent manufacturer. An unattractive cost-performance profile has been a major factor in APG's limited success to date.

The polyhydroxyamides (PHA), or glucamides, have recently been adopted by a major detergent manufacturer in the United States and Europe (12, 13). To date they have appeared in dishwashing liquids and heavy-duty liquid detergents. In the case of U.S. heavy-duty liquids, inclusion of PHAs has been accompanied by a major reduction in LAS levels. Potential benefits include improved enzyme stability in fabric washing detergents and improved mildness of dishwashing detergents.

#### 4.1. Builders

Builders are substances that augment the deterative effects of surfactants (14). Most important is the ability to remove hardness ions from the wash liquor (i.e., soften the water) and thus to prevent them from interacting with the surfactant. Such interaction reduces deterative effectiveness. Hardness ions can also interact with the negative charges present on soil and fabric surfaces (formed, e.g., by ionization of  $-OH$  or  $-COOH$  groups), reducing electric repulsion between them, thus hindering the deterative process. Hardness ions are best removed by sequestration to form soluble chelates. Less desirable is the formation of insoluble precipitates that may deposit on fabrics and machines and can, over many wash-and-wear cycles, lead to incrustation on machine parts and harshening of fabric. A third mechanism for removing hardness ions from wash liquors is through ion exchange in which calcium in solution displaces sodium ions in the ion exchanger, thus effectively removing the hardness from solution. The ion exchanger, in general, is a solid. Unlike precipitated calcium carbonate, however, the particle size of the ion exchanger can be controlled, and the problem of the presence of insoluble particles in the wash liquor can be reduced.

In general, builders supply alkalinity to the wash liquor and thus function also as alkalis. In addition, they can exert a suspending (antiredeposition) effect and keep detached soil from depositing on the fabric; builder ions with multiple charges are especially effective in this area.

*Phosphates.* Pentasodium triphosphate, sodium tripolyphosphate, STPP,  $Na_5P_3O_{10}$ , is the most widely used and most effective builder in heavy-duty fabric washing compositions. It is a strong sequestrant for calcium and magnesium, with a  $pK_{Ca}$  of ca. 6, and provides excellent suspending action for soils. Because of its high sequestration power, it also finds extensive application in automatic-dishwashing detergents. Sodium tripolyphosphate forms stable hydrates and thus aids in the manufacture of crisp spray-dried laundry powders.

Tetrasodium pyrophosphate,  $\text{Na}_4\text{P}_2\text{O}_7$ , is another important primary builder and detergent. In sequestration, it is not as effective as sodium tripolyphosphate and its usage in heavy-duty laundry powders has declined in recent years. Functionally, tetrasodium pyrophosphate is both a builder for surfactants (i.e., water softener) and alkali.

Where hardness is present in excess of the sequestering capacity of sodium tripolyphosphate and pyrophosphate, both can function as precipitant builders.

Trisodium phosphate, trisodium orthophosphate,  $\text{Na}_3\text{PO}_4$ , is an important constituent of hard-surface cleaners including those for ceramic, metal, or painted surfaces. It may be used with soaps, surfactants, or other alkalis. It precipitates many heavy-metal ions but does not sequester to form soluble chelates. It is thus a precipitant builder and additionally an alkali.

Glassy phosphates (sodium polymetaphosphate, sodium hexametaphosphate) vary in composition, depending on the manufacturing process. They exert a powerful sequestering and suspending effect combined with a low-solution pH, about 6 or 7, and tend to hydrolyze or revert in aqueous solution and heat to pyrophosphates and orthophosphates.

Potassium phosphates, particularly tetrapotassium pyrophosphate,  $\text{K}_4\text{P}_2\text{O}_7$ , are considerably more soluble than their sodium analogs. They have been used as builders in liquid detergents.

In the early 1970s, a number of U.S. jurisdictions banned the use of phosphates as detergent builders. The trend has continued to the point today that phosphates are banned in a sizable proportion of the United States (about 45%). Consequently, several alternatives to phosphates have been introduced into heavy-duty U.S. laundry detergents. No entirely satisfactory single substitute for sodium tripolyphosphate has been found that is as cost effective. Sodium tripolyphosphate aids detergency not only via water softening (calcium/magnesium sequestration) but also via soil suspension, soil removal, and antiredeposition benefits, all of which are closely related mechanistically. Additionally, STPP provides excellent spray-dried powder properties. However, it has been found that use of a water softener such as 4A Zeolite, in combination with a mixed active system, buffer such as carbonate, and soil suspension antiredeposition agents such as NaCMC, poly(ethylene glycol), polacrylate, polyacrylate/maleate copolymers, plus other cobuilders such as citrate, can provide general cleaning at least as good as the old high P formulations. Indeed current products containing enzymes and effective low-temperature bleach are superior. It seems likely that all principal U.S. detergent manufacturers will remove phosphate from their products in the near future.

*Sodium Carbonate.* Sodium carbonate softens water by forming insoluble calcium carbonate with calcium ions in hard water. Carbonate can also reduce calcium levels by ion pairing, although the benefit to detergency is questionable. Buildup of calcium carbonate on machine and fabrics, which can occur with time, is undesirable. Sodium carbonate does not provide any suspending action. It does, however, provide alkalinity to the wash liquor and is an effective alkali.

*Silicates.* Sodium silicates have been used extensively as soap builders in laundering formulations since well before the advent of synthetic surfactants. Silicates

are more effective in removing magnesium than calcium hardness. Again, they function primarily as alkalis. In addition, they act as anticorrosive agents and prevent deterioration of washing machines, specifically metal pump parts. However, in recent years, many of these machine parts have been replaced by engineering plastics and the anticorrosion function has lost some of its importance. Alkaline silicates act as primary detergents in machine-dishwashing formulations. Commercial alkaline silicates are characterized by the ratio of  $\text{SiO}_2$  to  $\text{Na}_2\text{O}$  in the molecule. The silicates used in detergent formulations generally show a ratio  $>1$ , usually 2.0–2.4. The 1 : 1 compound, sodium metasilicate, is considered too corrosive to be widely used in consumer product formulations.

*Zeolites.* Certain zeolites have found application as builders in heavy-duty detergent formulations. The zeolite of choice is a so-called type A zeolite, of empirical composition  $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 4.5\text{H}_2\text{O}$  and particle size of the range of 10  $\mu\text{m}$ . This builder functions by ion exchange in which sodium ions released from the zeolite crystal are replaced by calcium ions in hard water, thus lowering the free hardness in the wash. Its pore size accommodates calcium ions but is not sufficiently large for the highly hydrated magnesium ions. Zeolite A, therefore, is not an effective builder for magnesium hardness. Like sodium carbonate building, the ion exchange process is appreciably slower than soluble chelate formation by strong sequestrants like sodium tripolyphosphate. Type A zeolite is used principally to replace sodium tripolyphosphate in areas where phosphates are limited by law, i.e., in the United States, Canada, and western Europe. However, it is not practical as the sole builder in a nonphosphate detergent formulation, because it does not contribute to alkalinity, soil suspension, or bind magnesium.

*Clays.* Clays, such as kaolin, the montmorillonites, and bentonites, have been recommended and used from time to time as ingredients of washing compositions and other formulations containing surface-active agents. Under certain favorable conditions, particularly in soft water of low dissolved solids content, clay suspensions can have a marked deterative effect on ordinary soiled fabrics. Bentonite also acts as a suspending agent. In addition, sodium bentonite has some water-softening effectiveness by virtue of its ability to sorb calcium ions. However, clays are considerably less effective than type A zeolite in water softening.

In one U.S. laundry powder, a montmorillonite clay serves as the main softening component. It is combined with a waxlike cationic granule (14–17). Both are absorbed or filtered onto the cloth during the wash and spin rinse. The clay absorbs onto the fabric in thin sheetlike layers, providing a lubricating effect. The cationic particles melt in the heat of the automatic dryer providing an antistatic benefit and augmenting the softening benefit of the clay. A softening-in-the-wash effect is thus achieved with minimal interference with detergent performance.

*Nitrilotriacetic Acid.* The trisodium salt of nitrilotriacetic acid,  $\text{N}(\text{CH}_2\text{COOH})_3$ , so-called NTA, is a powerful sequestrant builder, comparable with sodium tripolyphosphate. It is therefore noted here, even though it is an organic builder. NTA has been recommended and used as a phosphate replacement in areas where phosphate is banned. However, because of adverse laboratory reports of possible teratogenic effects, NTA was withdrawn in 1970 from consumer products at the suggestion of

the U.S. Surgeon General. Because it is a smaller molecule than sodium tripolyphosphate, NTA is theoretically a more effective sequestrant on a weight basis. It is, however, less effective than sodium tripolyphosphate as a suspending agent and is not as easily processed in spray-dried laundry powders.

*Alkalies.* Caustic soda (sodium hydroxide) is used largely in mechanical bottle washing, glass washing, and metal cleaning. Sodium carbonate, either anhydrous (soda ash) or in hydrated form, has been used as builder or filler in soaps, surfactants, and with inorganic constituents in cleaners for hard surfaces and fabrics. It forms insoluble calcium carbonate with calcium ions in hard water but does not provide any suspending action. Sodium bicarbonate,  $\text{NaHCO}_3$ , sodium sesquicarbonate,  $\text{NaHCO}_3 \cdot \text{Na}_2\text{CO}_3$ , sodium borate,  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ , and borax are used in place of soda ash when a lower pH is desired. In cases where high solubility is required, the potassium analogs are used.

The alkalies do not sequester heavy-metal ions and have little soil-suspending effect. They are effective in maintaining a high pH and saponify the acidic constituents of soil and thus promote cleaning. In the cleaning of ceramics, glass, and metal surfaces, however, the alkalies act as primary detergents even in the absence of surfactants in these systems.

*Neutral Soluble Salts.* Sodium sulfate and, to a considerably lesser extent, sodium chloride are the principal neutral soluble salts used in laundering compositions. They are often considered to be fillers although they perform an important standardizing function enabling the formulator to manufacture powders of a desired, controlled density. Sodium sulfate, in addition, lowers the critical micelle concentration of organic surfactants and thus the concentration at which effective washing can be achieved.

In wool-scouring systems for textile processing that contain nonionic surfactants, sodium chloride acts as a true builder, i.e., detergency promoter.

## 4.2. Organic Additives

Certain nonsurfactant organic additives improve cleaning performance and exhibit other desirable properties.

Such additives are usually present in low percentage and serve one or more of the following specific functions: reduced redeposition of soil from the detergent bath onto the substrate; increased whiteness or appearance of cleanliness; enhanced cleaning effect on specific types of solid and stains; promotion or inhibition of foaming power and stability; increased solubility or other modification of the physical form of the detergent composition; sequestering of heavy-metal ions, both in the concentrated detergent and in the diluted cleansing bath; and reduced injurious effects the detergent may have on the substrate or the washing machine, such as tarnishing of silverware or etching of glassware, corrosion of metals, or irritation of skin in toilet and cosmetic applications.

Antiredeposition agents contribute to the appearance of washed fabrics. Sodium carboxymethylcellulose, NaCMC, is the most widely used, and on cotton fabrics, the most effective. With the advent of synthetic fabrics, other cellulose derivatives,

e.g., methylcellulose, hydroxybutylcellulose, hydroxypropylcellulose, and mixed methyl and hydroxybutylcellulose ethers have been shown to be more effective than NaCMC (19).

Fluorescent whitening agents were first disclosed in 1940 in combination with detergents (20). They absorb ultraviolet radiation and subsequently emit some of the radiation energy in the blue part of the visible spectrum. As a result, they confer enhanced whiteness to the appearance of washed articles. Highly effective cotton-substantive fluorescent whitening agents were in widespread use at relatively high concentrations (ca. 0.5%) in the 1950s and 1960s. For synthetic fabrics such as polyester, it has proved to be more effective to prebrighten the fabric by incorporating the fluorescent whitening agent in the spin-melt during manufacture rather than depend on adsorption from the detergent bath. As a result, the usage of fluorescent whitening agents in formulated laundry products has decreased in recent years.

Bluing agents, which are dyes, provide another approach to maintaining fabric whiteness by a mechanism in which a yellow cast of washed fabrics is covered by the blue dye. As this approach reduces reflectance, it is less desirable than the use of fluorescent whitening agents that increase reflectance.

Proteolytic enzymes have been generally used in European detergent formulations. In the United States, they were used in the late 1960s and early 1970s, but their use declined until they were present in only a few detergents. There has been a resurgence of their use in the last decade. Proteolytic enzymes in particular are widely used in premium products. They degrade proteinaceous stains and aid the cleaning performance of other formulation ingredients (21–23). Amylases and lipases have been used in a few U.S. detergents, the former to remove starches and the latter fatty esters and triglycerides. Cellulases have appeared in a few laundry detergents around the world. As there are few, if any, cellulase-based soils present on home laundry, any laundering benefit from cellulase would be expected to come from action on cotton fabric. The nature and magnitude of such benefits is uncertain.

Bleaching agents, such as sodium perborate trihydrate,  $\text{NaBO}_3 \cdot 3\text{H}_2\text{O}$ , are commonly present in significant amounts in European laundry detergents. At high washing temperatures, sodium perborate effectively bleaches chemical stains such as wine, fruit juices, and so on. As European wash temperatures have declined, so has the efficacy of perborate alone. Bleach activators, primarily TAED (tetracetyl ethylenediamine), have been widely used in Europe to provide effective bleaching at these low temperatures. Because of the lower washing temperature in U.S. machines, sodium perborate is considerably less effective and its usage is restricted to some individual brands of laundry detergents (24, 25). In the United States, even TAED is ineffective due to still lower wash temperatures, shorter wash times, and lower product concentration. One U.S. detergent manufacturer has introduced detergents with the bleach activator sodium nonanoyloxybenzene sulfonate (26). This activator forms the surface-active species pernonanoic acid that does provide a bleach benefit under U.S. conditions. In automatic-dishwashing formulations, bleaching agents are needed to remove food stains from dishware and break down proteinaceous soil. Chlorine is the most cost-effective agent available for this purpose and is present in all U.S. products as chlorinated isocyanurate.



Foam regulators such as amine oxides, alkanolamides, and betaines are present in products where high foam value is functionally or esthetically desirable, mainly hand-dishwashing liquids and shampoos. In automatic-dishwashing products, on the other hand, copious foam volumes interfere with the efficiency of the mechanical rotors during operation. In this type of product, a foam depressant is often present.

Organic sequestering agents serve the same purpose as the sequestering phosphates, i.e., to remove interfering metal ions from the detergent bath. They would appear to also provide some benefits through ionic strength and soil suspending effects. They are used where the less expensive phosphates are, for one reason or another, not applicable. Nitrilotriacetic acid, EDTA, and a variety of organic phosphonate structures are commonly used in a wide range of detergent compositions. With the elimination of phosphate, sodium citrate and synthetic analogs such as sodium tartrate monosuccinate and sodium tartrate disuccinate (27) have found wide use, especially in heavy-duty liquids. They are used to some extent in detergent powders. In the latter case, they can also be used to modify powder properties, as can sodium polyacrylates. Certain hard-surface cleaning products such as sanitizing cleaners for hospital use are examples of products generally containing small amounts of organic sequestrants.

## 5. FACTORS INFLUENCING DETERGENCY

Detergency is mainly affected by the concentration and structure of surfactant, hardness and builders present, and the nature of the soil and substrate. Other important factors include wash temperature; length of time of washing process; mechanical action; relative amounts of soil, substrate, and bath, generally expressed as the bath ratio, i.e., the ratio of the bath weight to substrate weight; and rinse conditions.

### 5.1. Effect of Surfactant Concentration

A plot of soil removal versus surfactant concentration is generally sigmoid. It starts at the soil removal of water without surfactant, rises slowly, and then more steeply until a plateau is reached when detergency is little affected by increase in surfactant concentration. This plateau can be correlated with the critical micelle concentration (CMC) of the surfactant and is generally higher than the CMC. In general, detergency attains its maximum when the CMC of the surfactant is reached, taking into account surfactant adsorption on the soil and substrate. However, with certain surfactants, there is evidence that oily-soil detergency continues to increase above the CMC (28–31).

### 5.2. Surfactant Structure

The chemical structure of the surfactant is an important factor in deterative effectiveness. When relating deterative power to chemical constitution, within limited series, certain regularities can be observed, but few if any general principles apply to the

whole range of surfactants. Among the homologous fatty acid soaps and the straight-chain alkyl sulfates, optimum detergency under usual washing conditions occurs at a chain length of about 16 carbon atoms. Detergency among the ethoxylated nonionic surfactants varies in a regular manner with the length of the ethylene oxide chain as well as with the structure of the hydrophobic group. In general, optimum detergency occurs with 12–16 carbon atoms in the hydrophobe chain, and a hydrophile–lipophile balance (HLB) of about 12. For oily-soil detergency by nonionics, it is often found that the optimum corresponds with the phase inversion temperature of the system under consideration (32).

Within a series with a fixed hydrophilic head group, detergency increases with increasing carbon chain length, reaches a maximum, and then decreases. This behavior frequently reflects a balance between increased surface activity of the monomer and decreased monomer concentration with increased surface activity. Similar effects are seen in surfactants in biological systems.

The numerous studies of the effect of surfactant structure in detergency include the classical paper on the series of sulfated secondary straight-chain alcohols (33) and various papers on detergent-builder combinations (34); nonionic–anionic mixtures (35, 36); the polyethenoxy nonionic series (32, 37, 38); detergency of isomeric alkylbenzene sulfonates (39); practical home laundering (40); laundering and detergency effects in seawater (41); *o*- and *p*-alkylbenzene sulfonates with straight and branched chains (42); and studies on alkylbenzene sulfonates (43).

### 5.3. Water Hardness and Builders

The presence of heavy-metal ions, especially calcium and magnesium, has an effect on washing second only to that of the surfactant itself. Distilled water, used in a system where soil and substrate do not contain substantial calcium ions, is a surprisingly effective detergent, when the soils themselves contain fatty acids or sodium soaps. Detergency can be improved by the addition of surfactants in amounts smaller than might be expected. Conversely, the detergency of surfactants is generally decreased by the presence of hardness ions ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) and hard water alone is a poor soil remover. With traditional formulations containing anionic surfactants or soaps, cotton can be cleaned well by washing only if the calcium concentration is reduced to  $<0.01$  mM. In current synthetic detergent compositions, builders sequester calcium and magnesium ions and thus reduce interaction with surfactants, soils, and fabrics as described above.

The sodium soaps of fatty acid form calcium soaps of such low solubility that they act as their own builders. Initial soap additions precipitate the calcium ion, and the soap added thereafter functions in soft water. At high temperatures, the calcium soaps are relatively soluble compared with calcium tripolyphosphate. Thus, STPP can build (revert) soaps in a hot-water wash. However, at low temperatures, the relative affinity of STPP for calcium decreases so that STPP cannot build soaps in a cold-water wash.

Calcium ion enters the system not only in the form of water hardness but also in the form of calcium salts contained in the soil. Other heavy-metal ions such as

aluminum and ferric iron may also be present in the soil and must be removed by an appropriate builder to achieve good soil removal. Effective builders for cotton washing are those for which the calcium dissociation constant, expressed as  $pK_{Ca}$ , or  $-\log K_{Ca}$  is  $>4$  and preferably  $>7$  (44). Much of the work that led to elucidating the role of builders as calcium sequestering agents in detergency was done in connection with redeposition studies (45).

Legislatively mandated reductions in detergent phosphate concentrations have resulted in numerous attempts to compensate for the attendant cleaning losses. Problems caused by phosphate reduction can be ameliorated by changes in surfactant systems. Thus, calcium-sensitive surfactants such as LAS can be replaced by calcium-insensitive ones such as alcohol ethoxylates (AE) or alcohol ether sulfates (AES). Proper blends greatly reduce surfactant sensitivity to calcium ions (46, 47). Increasing the amount of surfactants partially compensates for phosphate reduction or elimination. Other calcium-lowering agents include NTA, citrate, zeolite, and carbonate. Citrate and NTA lower calcium levels in solution by sequestration, zeolite by ion exchange, and carbonate by ion pair formation and precipitation. To date, no entirely satisfactory single replacement for phosphate has been found. Indeed, some soil-cloth detergency data show that the 1978 nonphosphate products are less effective than the 1969 high-phosphate products (48). However, subsequent refinements in formulation technology have substantially improved nonphosphate product performance to equal that of their phosphate predecessors.

In the presynthetic surfactant era, soap was built (and still is) with alkaline salts such as soda ash, silicates, orthophosphates, and borates. These materials buffer the wash solution to a high pH and prevent soap protonation; thus, the soap remains effective. Another type of builder is the neutral inorganic salt such as sodium chloride and sodium sulfate. These materials may improve detergency by increasing the ionic strength and altering the CMC of anionic surfactants.

#### 5.4. Antiredeposition Agents

The redeposition effect is best illustrated by agitating a clean swatch of white cotton in a washing bath that has been used and is turbid with dispersed soil. Even though the bath may still be effective and capable of removing soil, the white swatch picks up some soil from the bath and darkens. Any agent that minimizes this redeposition improves net soil removal and decreases the soil accumulation through several laundering cycles. Redeposition is in many ways the inverse of soil removal and is influenced by the same factors. Thus, calcium ion promotes redeposition in much the same way that it inhibits soil removal. Surfactants themselves are effective antiredeposition agents. Builders reduce redeposition independently of their ability to sequester hardness ions.

Sodium carboxymethylcellulose, NaCMC, greatly reduces redeposition in cotton-washing systems based on synthetic surfactants. It is effective at remarkably low concentrations of ca. 1% of the standard washing compositions used at ca. 0.1–0.2% in the bath. Thus, ca. 0.001–0.002%, or 10–20 ppm, NaCMC is sufficient to significantly inhibit redeposition.

A very large number of hydrophilic polymers have been tested with regard to their power of inhibiting redeposition, and it is remarkable how few materials can match NaCMC. Some proteins, polyvinylpyrrolidinones, and vinyl alcohol polymers with rather specific molecular weight ranges are in the same class as NaCMC. Certain starch derivatives, cellulose sulfates, and cellulose ethers are also effective. Currently, both NaCMC and cellulose ethers are used in home laundry products specifically to control redeposition. The cellulose ethers are used to control oily-soil redeposition upon synthetic fibers, especially polyester. Using tracer methods, it was proved that NaCMC is adsorbed on the soil particles and also, under normal laundering conditions, on cotton fiber (49, 50). Adsorption on cotton appears to be more important in preventing soil redeposition. This absorption occurs to a significant extent only in the presence of metallic cations that are furnished as sodium ions from STPP and the filler salts present in the detergent. In the absence of cations, except for the  $\text{Na}^+$  present in the NaCMC itself, the adsorption is so slight as to be almost undetectable, and the antiredeposition effect is correspondingly lowered.

### 5.5. Liquid Soil

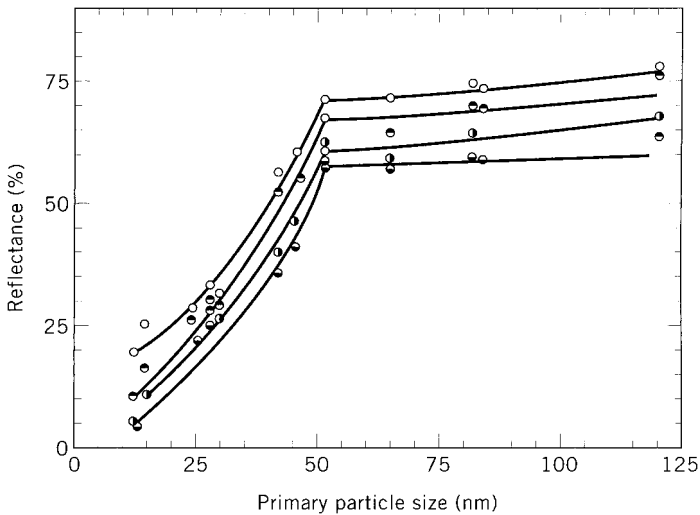
Liquid soils are frequently removed from fibers by a roll-up mechanism (50). In a study of wool grease removed from single fibers, it was found that the grease layer, originally continuous, rolled up in relatively large globules that detached themselves from the fiber. The detergent alters the contact angle at the fiber-grease-water interface. In pure water, the contact angle is  $<90^\circ$ , measured through the oil. Addition of detergent increases the angle to ca.  $180^\circ$ . The receding contact angle, i.e., that measured as the oil rolls up, is more important in detergency than is the advancing contact angle. Detergency mechanisms are similar on wool, viscose rayon, and cuprammonium rayon (52). The soil removal depends primarily on the nature of the oil. Those containing free fatty acids are removed more rapidly, followed by neutral glycerides and less polar mineral oils. Similarly, polar oils are more easily removed from wool than nonpolar oils (53). This difference correlates well with differences in contact angle.

Liquid soil can be removed by direct emulsification and solubilization as well as by roll-up. Oils that are more readily emulsified or solubilized are removed more rapidly and more completely.

### 5.6. Solid Soil Type and Size

Different solid soils differ greatly in ease of removal and redeposition behavior. These differences can be traced to particle size and soil-substrate bonding. The effect of particle size variation on detergency has been studied with soil removal and redeposition techniques.

In an early systematic investigation of this relationship, a series of carbon black samples was applied to chopped cotton fibers from an aqueous suspension with vigorous agitation (54, 55). The degree of soiling was estimated by filtering the fibers



**Figure 1.** Effect of primary particle size in a 1% carbon black dispersion on rate of change in reflectance. ○ = 5 min, ● = 20 min, ● = 180 min, ● = 360 min.

with suction to form a mat, drying, and measuring the reflectance of the mat. The degree of soiling was found to be directly related to the particle size of the carbon black. As shown in Figure 1, when the primary particles of the carbon black are  $<50$  nm, the soil deposition is severe, whereas at particle sizes  $>50$  nm, the soil deposition is much lighter and relatively independent of particle size. Electron microscope studies of the cotton fiber show that 50 nm is about the upper limit of the crevice width on the surface of cotton fibers. Furthermore, the soil deposition by this procedure using fine-particle carbon blacks is practically irremovable, and the fibers are to all intents and purposes dyed a very fast gray. On this basis and on the basis of other supporting evidence, it was concluded that soiling in this system takes place by microocclusion, i.e., that the particles of soil are so small that they can be trapped mechanically by the irregularities of the fiber surface, even though these irregularities are scarcely larger than the dimension of a polymeric molecule. This theory accounts well for the observed facts, but the facts could also be accounted for by simply assuming that van der Waals forces control the attachment of the carbon particles to the fiber. As these forces operate at the surface of the particle, there should be a critical particle size below which the surface forces become more important than the inertial and gravitational forces that are proportional to mass. Electron micrographs of soiled cotton show adherent soil particles in size ranges up to  $0.5\text{--}2.0\ \mu\text{m}$  clinging to apparently smooth surfaces (56–58). These and other supporting detergency data indicate that mechanical binding is not the basic mechanism of solid soil binding, even though the binding varies greatly with particle size.

As might be expected, large differences in the removability of solid particulate soil are due to differences in the chemical nature of the particle surface. Thus, iron

oxides, lampblacks, and clays, all of the same particle size, differ greatly in their redeposition behavior and the manner in which they are removed.

### 5.7. Fabrics and Fibrous Substrates

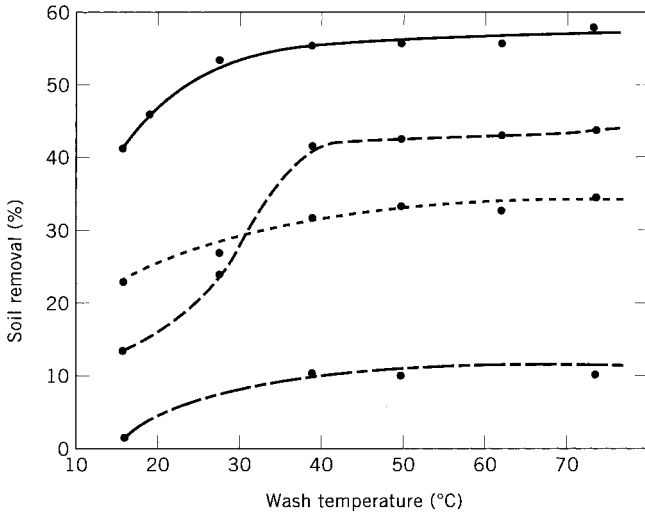
Certain fibers are easier to wash than others. The hardness of the fiber surface, which varies not only with the basic fiber but also with the surface finish, affects both soilability and soil removal. Generally, soft finishes pick up and retain soil more readily than hard finishes, since soft surfaces permit greater soil–surface contact. The hydrophobicity of the fiber surface also influences soilability and soil removal, and the more hydrophobic fibers show greater soil retention especially for hydrophobic soils such as oils. These effects are explained by the high-energy interface existing between hydrophobic fibers and water (59, 60). Many soil components lower the interfacial energy and therefore locate at the fiber–water interface.

Soils can also penetrate into the fiber. The interior of the cotton fiber, the lumen, is relatively hollow, and soils may collect there. Polyester fibers are solid, but if polyester is washed above its glass transition temperature  $T_g$ , it becomes relatively fluid. In this case, oils on the fiber surface can mix with the polyester itself. When soils penetrate into the fiber, they are nearly impossible to remove.

### 5.8. Temperature and Mechanical Action

Absorption of external thermal or mechanical energy by a deterative system influences the rate and extent of soil removal. Raising the temperature generally increases the cleaning rate and, therefore, the amount of soil removed during any fixed-time laundering cycle. The effect is only strong at two critical temperatures; one is the temperature at which the fatty soil in the system liquifies. As temperature increases through this region, the detergency increases markedly. Typical curves of detergency versus temperature are shown in Figure 2 (61). The second critical temperature is the boiling point. Boiling greatly increases detergency because of the localized mechanical action of steam bubbles forming, expanding, and breaking away at the solid–liquid interfaces. However, in the United States, boiling effects are never encountered in home washing machines. U.S. laundry wash temperatures have declined to an average wash temperature of about 35°C, and the average hot-water wash temperature is only 54°C. From 1970 to 1988, hot-water wash declined from 48% to 20% of washes (62). Temperature effects are also important with certain detergent additives. Thus, the efficacy of certain bleaches and enzymes is markedly reduced at the low wash temperatures now popular. These effects emphasize the problems inherent in the current trend to lower wash temperatures.

As with the case of energy input, detergency generally reaches a plateau after a certain wash time as would be expected from a kinetic analysis. In a practical system, each of its numerous components has a different rate constant; hence its rate behavior generally does not exhibit any simple pattern. Many attempts have been made to fit soil removal (63) rates in practical systems to the usual rate equations of



**Figure 2.** Effect of wash temperature on removal of — sebum, — lanolin, and ····· lard from cotton (0.25% built detergent) (58). - · - · - represents sebum removal with no detergent.

physical chemistry. The rate of soil removal in the Launder-Ometer could be reasonably well described by the equation of a first-order chemical reaction; i.e., the rate was proportional to the amount of removable soil remaining on the fabric (64, 65, 66). In a study of soil removal rates from artificially soiled fabrics in the Terg-O-Tometer, the percent soil removal increased linearly with the log of cumulative wash time.

In detailed studies with a number of different artificial test cloths, first-order kinetics were obtained for soil removal during the first 6–20 min of the cycle (67).

In the washing of fabrics, machine and timing must be carefully adjusted. Too large an energy input for too long a time can injure fabric severely and eventually tear it to shreds. The same effect is quite possible with purely mechanical agitation.

## 5.9. Foam

The relationship of foaming power to detergency has always been of interest, and foaming power has become associated in many consumers' minds with high detergency. However, foam has no direct relationship to detergency in ordinary fabric-washing systems, and does not improve cleaning in a laundry or home-washing machine. Indeed, excessive foam can inhibit agitation and reduce cleaning. Additionally, excess foam levels may concentrate certain surface-active cleaning agents in the foam, reducing their contact with the fabrics to be cleaned. In systems of low bath ratio, on the other hand, foam may play an important role. The individual foam laminae tend to imbibe and segregate particles of both liquid and solid soil that have been removed from the substrate. This prevents redeposition and enables the soil to be easily removed by scraping off or rinsing away the soil-laden foam. This effect is

very important in the on-location shampooing of rugs, and to a certain extent in the shampooing of human hair. An excellent series of photomicrographs show the imbibition of liquid-soil droplets by foam films during the washing of oiled glass plates (68). The soil tends to be carried by capillary convection to the low-pressure region in plateau borders where three individual laminae join to form the edge of a foam cell.

## 6. MECHANISMS

Even the simplest deterative system is surprisingly complex and heterogeneous. It can nevertheless be conceptually resolved into simpler systems that are amenable to theoretical treatment and understanding. These simpler systems are represented by models for substrate–solid soil and substrate–liquid soil. In practice, many soil systems include solid–liquid mixtures. However, removal of these systems can generally be analyzed in terms of the two simpler model systems. Although these two systems differ markedly in behavior and structure, and require separate treatment, there are certain overriding principles that apply to both.

The first principle is that soil systems can be regarded and treated as classical systems of colloid and surface chemistry. A cotton fiber bearing attached small particles of clay or carbon soil, immersed in an aqueous medium, is fully analogous to the sulfur sols or gold sols of classical colloid chemistry. Although the fiber is not of colloidal dimensions, the area over which any individual soil particle contacts the fiber is of colloidal dimensions. Furthermore, the ratio of the soil particle's area to the area of contact, and the ratio of the soil particle's mass to the area of contact put this system in the colloidal range. These ratios are the same as the ratios prevailing when one sulfur sol particle makes contact with another. In addition, in the substrate–soil system two different types of surfaces are interacting, whereas in the classical sol systems the particles are all of the same chemical nature and the interacting surfaces are therefore similar. The problem of mixed sols has, however, been explored in classical colloid chemistry to at least a limited extent, and it is fully analogous to the problem of the model soil–deterative system. A fiber or steel plate covered with oil and immersed in an aqueous solution is a good representative of the liquid–liquid–solid system of classical surface chemistry. The well-established concepts of interaction at the liquid–liquid interface and at the three-phase boundary line are fully applicable.

A second principle applying to these model systems is derived from their colloidal nature. With the usual thermodynamic parameters fixed, the systems come to a steady state in which they are either agglomerated or dispersed. No dynamic equilibrium exists between dispersed and agglomerated states. In the solid-soil systems, the particles (provided they are monodisperse, i.e., all of the same size and shape) either adhere to the substrate or separate from it. In the liquid-soil systems, the soil assumes a definite contact angle with the substrate, which may be anywhere from  $0^\circ$  (complete coverage of the substrate) to  $180^\circ$  (complete detachment). The governing thermodynamic parameters include pressure, temperature, concentration of dissolved



components, and electrical conditions. This concept is at variance with the idea of a dynamic detergency equilibrium, which was advanced as a working hypothesis by many of the early investigators. It is, however, much more in keeping with the established concepts of colloid chemistry. In the classical theory of lyophobic colloids, no consideration is given to a dynamic equilibrium existing between the peptized state and the agglomerated state. This all-or-nothing behavior has been studied experimentally in model deterative systems by observing, with the help of microscopic techniques, the behavior of single textile fibers in aqueous carbon black suspensions containing dissolved soap (69). In the absence of soap, the carbon particles deposit on the fiber. When sufficient soap is added, the carbon particles do not deposit on the fiber but remain in suspension. The quantity of soap necessary to maintain the carbon in suspension is proportional to the amount of carbon present in the system. There is a sharp demarcation between the state in which the carbon is suspended and the state in which the carbon is attached to the fiber.

In applying this concept, the factor of particle size must be continuously borne in mind. A heterodisperse system can reach a steady state wherein the smaller particles are agglomerated and the larger particles are dispersed, giving the apparent effect of an equilibrium. In ideal monodisperse systems under steady conditions, however, no such effects are noted.

Purely mechanical disturbances (which are not usually considered thermodynamic variables) may influence the state of aggregation of a colloidal system; for example, floc size in carbon and iron oxide suspensions varies with the degree of agitation being imposed on the system (70). When the agitation is stopped, the flocs revert to their steady-state size. To have any effect, the shear field must be high and the particles relatively large. With particles  $<0.5 \mu\text{m}$ , thermal agitation becomes more important than even vigorous mechanical agitation in determining the state of aggregation.

A final consideration in resolving practical deterative systems into their simpler components relates to soil removal versus redeposition. Superficially, it would appear that the redeposition phenomenon contradicts the all-or-nothing concept that the system must exist in either the agglomerated or the dispersed state. Keeping in mind both the composite nature and the kinetics of a practical system, it is readily shown that no such contradiction exists. The soil particle that redeposits is in a different state from what it was during its initial removal from the substrate. It may have changed its state by becoming detached from adherent oil or from a cluster of similar solid particles. During the time interval between detachment and redeposition, it may have altered its surface character by adsorption or desorption. In this time interval, the state of the substrate surface may also have changed by adsorption or desorption, or by the loss or gain of an oily layer. Additionally, there are probably varying substrate types present in the wash, with varying affinities for the soils present. For example, oily soil could be detached from a cotton fiber, but then deposited on a polyester fiber for which it would have a greater affinity. Thus, the initial group of agglomerated systems, which composes the soil-substrate-bath complex before soil removal, is different from the agglomerated system composed of substrate and redeposited soil.

## 7. SOLID-SOIL DETERGENCY

### 7.1. Adsorption

Many studies have been made of the adsorption of soaps and synthetic surfactants on fibers in an attempt to relate detergency behavior to adsorption effects. Relatively fewer studies have been made of the adsorption of surfactants by soils (71–74). Plots of the adsorption of sodium soaps by a series of carbon blacks and charcoals show that the fatty acid and the alkali are adsorbed independently, within limits, although the presence of excess alkali reduces the sorption of total fatty acids (75, 76). No straightforward relationship was noted between detergency and adsorption.

In a study of the adsorption of soap and several synthetic surfactants on a variety of textile fibers, it was found that cotton and nylon adsorbed less surfactant than wool under comparable conditions (77). Among the various surfactants, the cationic types were adsorbed to the greatest extent, whereas nonionic types were adsorbed least. The adsorption of nonionic surfactants decreased with increasing length of the polyoxyethylene chain. When soaps were adsorbed, the fatty acid and the alkali behaved more or less independently just as they did when adsorbed on carbon. The adsorption of sodium oleate by cotton has been shown independently to result in the deposition of acid soap (a composition intermediate between the free fatty acid and the sodium salt), if no heavy-metal ions are present in the system (78). In hard water, the adsorbate has large proportions of lime soap.

The adsorption behavior of commercial ABSs and several other anionic detergents on cotton, wool, and some of the synthetic fibers has been carefully plotted (79, 80). The equilibrium adsorption isotherms, with a few exceptions, do not show any startling abnormalities. A number of other studies have attempted to correlate adsorption with detergency (81–83). In general, no significant correlations were found for a number of possible reasons. On porous fibers such as cotton, adsorption may occur on fiber areas that are not beneficial to detergency. The presence of soils on the fiber surface may significantly alter the adsorption behavior. The presence of trace amounts of surface-active agents in the soils themselves could modify adsorption of the active detergent component. For instance, soap present in natural soils could significantly change nonionic adsorption behavior. Finally, and perhaps most importantly, equilibrium adsorption measurements may not realistically reflect adsorption taking place in a 10-min wash cycle.

Adsorption of bath components is a necessary and possibly the most important and fundamental detergency effect. Adsorption is the mechanism whereby the interfacial free energy values between the bath and the solid components (solid soil and substrate) of the system are lowered, thereby increasing the tendency of the bath to separate the solid components from one another. Furthermore, the solid components acquire electrical charges that tend to keep them separated, or acquire a layer of strongly solvated radicals that have the same effect. If it were possible to follow the adsorption effects in a detergative system, in all their complex ramifications and interactions, the molecular picture of soil removal would be greatly clarified.

## 7.2. Mass Transfer Near the Substrate Surface

Mechanical action has a great effect on soil removal, probably by influencing mass transfer, i.e., the diffusion of soluble material away from the immersed fibers (84, 85). Mechanical action tends to maintain a high concentration gradient near the fiber, and the resulting increased diffusion causes stronger diffusion currents to flow. These diffusion currents are presumably responsible for carrying away the soil particles that have already been detached or loosened from the fiber surface by physicochemical action. In a Terg-O-Tometer investigation of mass transfer, using only water-soluble substances, the transfer coefficient was found to be directly proportional to agitator speed and stroke angle, inversely proportional to the water-holding capacity of the cloth load and independent of bath volume (84, 85). The soil removal behavior is very similar to the mass transfer behavior, supporting the idea that diffusion currents are an important operating factor in soil removal, even though they may play no part in breaking the primary soil-substrate bond. Considering the wide variety of soils and substrates encountered in washing, as well as the wide variation in particle size and fabric geometry, mass transfer effects could play a principal or minor role in the overall wash process, depending on the specific system encountered.

## 7.3. Colloidal Stabilization

Surfactant adsorption reduces soil-substrate interactions and facilitates soil removal. For a better understanding of these interactions, a consideration of colloidal forces is required.

The model solid-soil detergent system is advantageously treated as a solagglomerate colloid system or, in more general terms, a lyophobic colloid. In the typical lyophobic colloid, consisting of a single disperse phase in an aqueous suspending medium, only one type of liquid-solid interface and one type of solid-solid interface is present. The simplest detergent system, however, has an added degree of complexity in the presence of two types of liquid-solid interface: soil-bath and substrate-bath. Also present are two effective types of solid-solid interface: soil-substrate and soil-soil. The soil-soil interface relates to flocculation or dispersion of soil particles remote from the substrate, and is not of primary concern in the present discussion. The soil-soil interface is, of course, important in practical detergency since soil aggregates can be regarded as large single particles.

There are two general theories of the stability of lyophobic colloids, or, more precisely, two general mechanisms controlling the dispersion and flocculation of these colloids. Both theories regard adsorption of dissolved species as a key process in stabilization. However, one theory is based on a consideration of ionic forces near the interface, whereas the other is based on steric forces. The two theories complement each other and are in no sense contradictory. In some systems, one mechanism may be predominant, and in others both mechanisms may operate simultaneously. The fundamental kinetic considerations common to both theories are based on Smoluchowski's classical theory of the coagulation of colloids.

As the particles in a colloidal dispersion diffuse, they collide with one another. In the simplest case, every collision between two particles results in the formation of one agglomerated particle, i.e., there is no energy barrier to agglomeration. Applying Smoluchowski's theory to this system, the half-life,  $t_{1/2}$ , i.e., the time for the number of particles to become halved, is expressed as follows, where  $\eta$  is the viscosity of the medium,  $k$  Boltzmann's constant,  $T$  temperature, and  $N_0$  is the initial number of particles:

$$t_{1/2} = \frac{3\eta}{4kTN_0}.$$

For dispersions at moderate concentration, ca.  $10^9$  particles per  $\text{cm}^3$ ,  $t_{1/2}$  has a value of the order of a few seconds. This expression assumes there is no barrier to collision and every collision is effective. For stable dispersions to exist, an energy barrier  $W$  is assumed that prevents collision. In this case, the expression for half-life becomes:

$$t_{1/2} = \frac{3\eta}{4kTN_0} \exp(kT/2W).$$

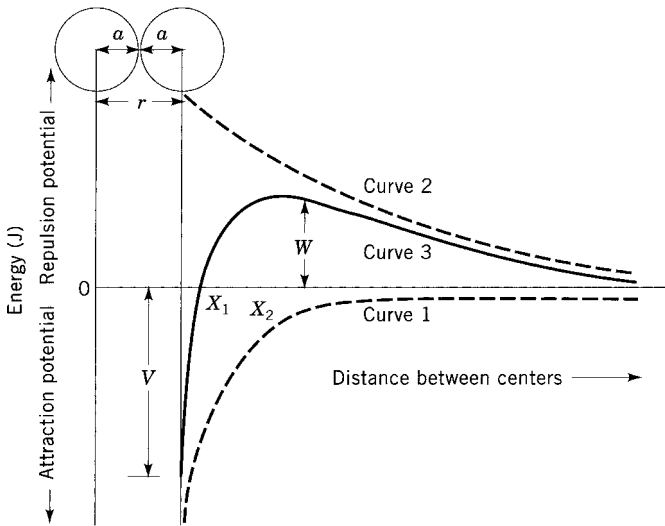
If  $W$  is  $15 - 20kT$ , the half-life is several days, and the dispersion is reasonably stable. The problem of colloidal stability thus becomes a problem of the energy barrier  $W$ , which lessens the unifying collisions.

*Ionic Stabilization.* The quantitative theory of colloid stability based on electrical barriers was developed independently in the USSR by Derjaguin and Landau (86, 87) and by Verwey and Overbeek in Holland (88). It is generally referred to as DLVO theory. There are two opposing forces between particles: a force of attraction that is responsible for agglomeration when the particles approach close enough, and a force of repulsion that prevents close approach. The attractive forces are the London dispersion forces or van der Waals forces. These have the general form shown below, where  $r$  is the distance between particles and  $n$  is between 3 and 7, depending on the geometry of the interacting surfaces:

$$F = K/r^n.$$

The repelling forces are caused by the electrical double layers that surround the particles in aqueous dispersions. The interaction of these two forces is conveniently shown in a plot of potential energy versus distance between particle centers, as in Figure 3. The ordinate in this plot can be either force or potential; for quantitative purposes, they are interconvertible through the definitive relationship that force is the negative gradient of potential. To separate two agglomerated particles ( $r = 2a$ ), it is necessary to overcome the energy barrier  $V + W$ , to bring them to distance  $X_2$  at which point repulsive forces predominate. Conversely, to cause agglomeration of two separated particles, it is necessary to overcome the barrier  $W$  at  $X_2$ .

According to DLVO theory, the shape of curve 2, which controls the shape of the effective curve 3, is determined by the thickness and charge density of the electrical



**Figure 3.** Attraction–repulsion potentials as a function of distance between particle centers. Curve 1 represents the attractive potential caused by van der Waals forces, curve 2 is the repulsive potential caused by double-layer forces, and curve 3 is the resultant force experienced by the two particles.

double layer that surrounds each particle. These factors, in turn, are determined by the nature of the ionic species adsorbed at the interface, the degree of adsorption, and the ionic strength of the surrounding medium. The quantitative aspects of the DLVO and the double-layer theory (i.e., the structure, formation, and interactions of electrical double layers at phase interfaces) are beyond the scope of this Chapter, and it is recommended that the reader consult the standard publications in this field. Qualitatively, high charge density increases the repulsion forces and thus shifts curve 3 upward, increasing the potential  $W$  and decreasing  $|V|$ . High ionic strength shrinks the double layer, lowering curve 3, lowering  $W$ , and tending to increase  $|V|$ .

Electrical potentials can, of course, be negative or positive. In most detergency systems, the charges acquired by both substrate and solid soil are negative. The adsorption of detergent anions increases the effective density of negative charge and tends to increase the double-layer repulsion potentials. The counterions, in this case metallic cations, exert the controlling effect on the ionic strength factor, with increasing valence greatly magnifying the effect. Thus, calcium ions have a much greater effect than sodium ions in shrinking the double layer and promoting agglomeration. Aluminum ions have an even greater effect than calcium ions. This is in accord with the well-known Schulze–Hardy rule that was proposed as an empirical finding many years before this quantitative DLVO theory was developed.

The electrokinetic effect is one of the few experimental methods for estimating double-layer potentials. If two electrodes are placed in a colloidal suspension, and a voltage is impressed across them, the particles move toward the electrode of opposite charge. For nonconducting solid spherical particles, the equation controlling

this motion is presented below, where  $u$  = velocity of particles;  $\eta$  = viscosity of medium;  $V$  = applied field, V/cm;  $R$  = factor for electrical relaxation;  $D$  = dielectric constant of medium;  $F$  = factor for size of spheres; and  $\zeta$  = zeta potential.

$$u = \frac{RFVD}{6\pi\eta} \zeta.$$

This equation is a reasonable model of electrokinetic behavior, although for theoretical studies many possible corrections must be considered. Correction must always be made for electrokinetic effects at the wall of the cell, since this wall also carries a double layer. There are corrections for the motion of solvated ions through the medium, surface and bulk conductivity of the particles, nonspherical shape of the particles, etc. The parameter zeta, determined by measuring the particle velocity and substituting in the above equation, is a measure of the potential at the so-called surface of shear, i.e., the surface dividing the moving particle and its adherent layer of solution from the stationary bulk of the solution. This surface of shear lies at an indeterminate distance from the true particle surface. Thus, the measured zeta potential can be related only semiquantitatively to the curves of Figure 3.

There have been many attempts to correlate detergency and deflocculation with measurements of zeta potential (89); such measurements have been made on both fibers and soil particles using various model detergents as the media. Aside from the expected result that high zeta potentials tend to correlate with deflocculation, few direct relationships of theoretical significance have become evident. In an extensive and productive study, it was pointed out that DLVO theory relates the height of the maximum in the potential curve ( $W$  in curve 3, Figure 3) to the square of the zeta potential rather than to its first power (90, 91). From the DLVO theory the surface potential  $\psi$  was calculated, which is necessary to give a barrier of height (in joules)  $W = 15kT$  for soil particles of various size in electrolytes of various concentrations;  $\zeta$  was measured experimentally. When the ratio  $\zeta^2/\psi^2$  was greater than unity, good stability was attained. In calculating soil-substrate interaction, good results were obtained using the ratio of  $[(\zeta \text{ substrate}) (\zeta \text{ soil})]/[\psi \text{ substrate}(\psi \text{ soil})]$ . Application of DLVO theory to detergency has also been made very successfully when the shape factors and factors involving the structure of the double layer were taken into consideration (92-94).

The strong adverse influence of calcium ions on the stability of lyophobic suspensions is predicted by DLVO theory, and has been demonstrated with many types of simple soils. That calcium ions have an overwhelming effect on the redeposition of carbon soil onto cotton tends to support the idea that DLVO theory is a principal key in explaining detergents action. The redeposition of carbon onto cotton has been correlated quantitatively with the calcium ion content of the system, both in the presence and absence of surfactant (95). The adverse effect of calcium ions on wet soil removal in practical washing has also been well established (96). The effect of calcium in detergency cannot be explained solely, however, by its shrinking of

the double layer. Calcium is very strongly adsorbed onto cotton through the carboxyl and hydroxyl groups present on the cellulose chains. This adsorption is so strong that cotton rinsed in even slightly hard water transfers a harmful amount of calcium to the next wash liquor. Calcium present in soil particles can act as a bonding agent at the soil-substrate interface and inhibit soil removal by this secondary mechanism. In one of the most thorough studies of the above effects, it was also demonstrated that calcium promotes the deposition of oil onto cotton (97). The sorption of calcium during washing is complicated by its apparent ability to coadsorb with surfactants and complex phosphate builders, further obscuring the mechanism by which it exerts its adverse effect (98, 99).

Despite its successes and its great usefulness as a guide, the limitations of the DLVO theory in explaining detergency are most evident when considering the nature of the soil-substrate bond. The DLVO theory in its simplest form postulates van der Waals' bonding as the primary agglomerating mechanism. Even revised theories of double-layer interaction, which do not assign such an important role to van der Waals' forces, do not encompass agglomerating mechanisms such as polyvalent cation bridges and hydrogen bonding (100, 101). There is ample evidence that these latter mechanisms prevail in some important detergency systems.

*Steric Stabilization.* Double-layer repulsion cannot satisfactorily explain stabilization of lyophobic suspensions by nonionic surfactants nor nonionic detergency in solutions of high salt content. Not only nonionic surfactants but even anionic ones can overcome the agglomerating effects of salt. To explain these phenomena, steric interaction between the surfactant molecules adsorbed at the solid-solution interface must be considered. The adsorbed molecules are oriented with the hydrocarbon tails adherent to the essentially hydrophobic solid surface and the water-soluble heads sticking into the solution. This adsorption and orientation is governed by the same factors governing micelle formation, i.e., the amphipathic nature of the surfactant molecule and solution forces. The head groups at both nonionic and anionic surfactants are heavily hydrated. As a result the lyophilic particle is surrounded by an inner layer of hydrophobic surfactant tails and an outer layer of hydrated surfactant heads. In Figure 3, it can be seen that the attractive potential does not greatly increase until the particles are relatively close to each other.

The bulk of the hydrated head groups prevents the particles from approaching near enough to each other for the attractive forces to cause agglomeration. In the case of polyoxyethylene nonionic surfactants, the head group is quite large, extending for several nanometers into solution; the head group is associated with a large amount of water of hydration caused by ether-water interaction. The bulk of a hydrated anionic head group is not so great; however, the anionic surfactant retains some ionic stabilization even in salt solution. Agglomeration is achieved only if the particles overcome the energies involved in steric compression of the head groups and desolvation of the head groups. Conversely, if two particles, adherent over a small area of contact, are immersed in a surfactant solution, oriented adsorption on the noncontacting areas, followed by hydration, presumably breaks the adhesive bond and forces the particles apart. This picture would suggest some degree of correlation between the wetting power of a surfactant for a given material and its

stabilizing power for sols of the same material. Such a correlation has indeed been found, using paraffin wax with both nonionic and anionic surfactants (102, 103).

## 8. OILY-SOIL DETERGENCY

### 8.1. Roll-Up

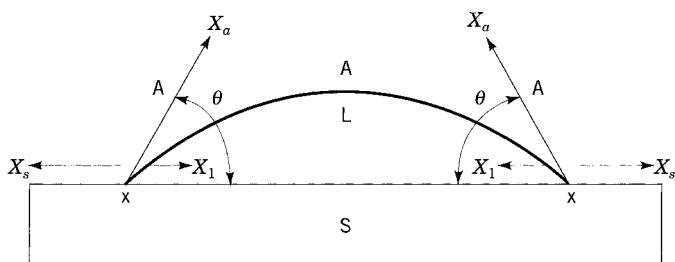
The principal means by which oily soil is removed is probably roll-up. The applicable theory is simply the theory of wetting. In briefest outline, a droplet of oily soil attached to the substrate forms at equilibrium a definite contact angle at the oil–solid–air boundary line. This contact angle (Figure 4) is the result of the interaction of interfacial forces in the three phase boundaries of the system. These interfacial forces, expressed in  $\text{mN/m}$  ( $= \text{dyn/cm}$ ), or interfacial free energy values expressed in  $\text{mJ/m}^2$  ( $\text{erg/cm}^2\text{s}$ ) are conveniently designated  $\gamma_{LA}$ ,  $\gamma_{SA}$ , and  $\gamma_{SL}$ , the subscripts relate to the liquid–air, solid–air, and solid–liquid interfaces, respectively. The equilibrium contact angle at the boundary line  $\theta_{SLA}$  may be regarded as a thermodynamic quantity since it is functionally related to the free energy values through the Young–Dupre equation:

$$\gamma_{SA} = \gamma_{SL} + \gamma_{LA} \cos \theta_{SLA}.$$

When the solid substrate is placed in the bath, the air is displaced by the bath, B, and the SA interface is replaced by an SB interface. Similarly, an LB interface replaces the LA interface. The equilibrium free energy values of these new interfaces are not established immediately but gradually through mass transfer (if there is any mutual solubility between L and B; it is assumed that B does not dissolve S) and through adsorption of dissolved components. When these processes have gone to completion the new relationship is

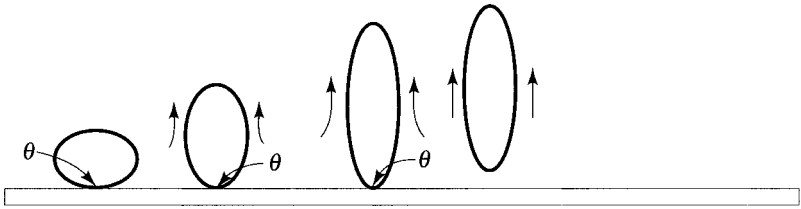
$$\gamma_{SB} = \gamma_{SL} + \gamma_{LB} \cos \theta_{SLB}.$$

In general,  $\gamma_{SB}$  and  $\gamma_{LB}$  are lower than  $\gamma_{SA}$  and  $\gamma_{LA}$ . If B contains a surface-active agent, they tend to become exceptionally low and  $\theta_{SLB}$  is therefore much greater



**Figure 4.** Contact angle at the oil–solid–air boundary line in the rollback process of oily solid detergency.  $X_s$ ,  $X_1$ , and  $X_a$  are force vectors at the surface and tangential to the droplet.





**Figure 5.** With  $\theta$  remaining constant at  $>90^\circ$ , oil droplet can be removed completely by hydraulic currents (arrows).

than  $\theta_{SLA}$ . Because  $\theta_{SLB}$  is greater than  $90^\circ$ , even though less than  $180^\circ$ , hydraulic action is capable of removing the oil droplet from the soil and surface.

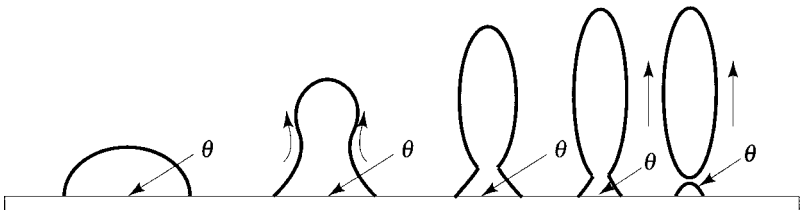
Rearrangement of the above equation gives

$$\cos \theta = \frac{\gamma_{SB} - \gamma_{SL}}{\gamma_{LB}}$$

For  $\theta > 90^\circ$ , there must be  $\cos \theta < 0$ . As the interfacial tension terms are positive, for  $\cos \theta < 0$ , it follows that  $\gamma_{SL} > \gamma_{SB}$ . In other words, for effective roll-up, i.e.,  $\theta > 90^\circ$ , the interfacial tension between the solid and bath must be less than that between the solid and liquid. As shown in Figure 5, the area of contact can be reduced to zero while maintaining the contact angle and its equilibrium value, and avoiding any necking down and division of the droplet. If  $\theta_{SLB}$  is less than  $90^\circ$ , it is impossible to separate the oil completely from the surface by hydraulic action alone. As the droplet is withdrawn and the area of contact is reduced, a neck is formed directly above the contact region. If the contact angle is maintained at equilibrium, the drop must divide at this neck, leaving a small quantity of oil adherent to the substrate. This effect is shown in Figure 6.

The value of  $\theta_{SLB}$  can be estimated on purely theoretical grounds from estimates of the adsorption of surfactant which, in turn, can be estimated from the Gibbs adsorption equation relating adsorption to surface tension lowering.

Even when the equilibrium value of  $\theta_{SLB}$  approaches  $180^\circ$ , it is quite difficult in practice to displace all the oil from the substrate by the bath for the following reasons: The contact angle of the oil, as it is rolling back, is a receding contact angle that is considerably smaller than the equilibrium angle. Any surface roughness



**Figure 6.** With  $\theta$  remaining constant at  $<90^\circ$ , droplet cannot be removed completely by hydraulic currents (arrows). A small droplet is left attached to the substrate.

further increases the hysteresis. Thus, an actual angle of  $180^\circ$  may not be achieved even though the theoretical equilibrium angle has this maximum value. Solid surfaces are notoriously inhomogeneous. Even a small surface area exhibits roughness or spots of high interfacial energy where the moving boundary tends to stick. The hydraulic currents then tear away the bulk of the droplet, leaving some oil at the rough or sticky spot.

In this model, the surface is oil free under steady-state conditions only when  $\theta_{\text{SLB}}$  is  $180^\circ$ . At any lower value, free-floating oil droplets redeposit and become attached to the substrate, and at equilibrium (on the all-or-nothing basis) all the oil is attached. When  $\theta_{\text{SLB}}$  is high, however, even though it is not  $180^\circ$ , agitation keeps a large proportion of oil separated from the substrate and moving around in the bath. Thus, if the substrate is removed from the agitated bath and rinsed, it is freed of most of its soil burden.

This model for oily-soil removal does not apply to systems where the oil soil becomes emulsified. Emulsified oil droplets are characterized by a surface layer that acts either as a physical barrier or as a potential energy barrier against coalescence, or against coming into intimate contact with the substrate. Such droplets must overcome this potential barrier to redeposit on the substrate, whereas the free-floating unemulsified oil droplets of the roll-up model redeposit on contact without surmounting any barrier, energetic or physical. The mathematical model for emulsification can be found in standard treatises on emulsification. It resembles the model for solid-soil dispersion outlined above, although there are many important differences of detail.

## 8.2. Solubilization

The role of micellar solubilization (as the term is used in the physical chemistry of surfactants) in oily-soil removal has been debated for many years. The amount of oily soil that could be present in a normal wash load could not all be removed and held in micellar solution by anionic surfactants. On the other hand, nonionic surfactants could do so, because of their greater solubilizing ability. High solubilizing power is definitely linked with good detergency (104). Thus, a very direct relationship between the solubilizing power of a surfactant for the test dyestuff Orange OT and its ability to remove polar solid from steel surfaces was established (105). In a practical detergency range lying between the surfactant concentration that gives 90% soil removal and a surfactant concentration twice that high, the relationship is expressed by the equation below, where  $D$  is the detergency value,  $S$  is the solubilization value, and  $K_1$  and  $K_2$  are constants:

$$D = K_1 S + K_2.$$

Furthermore, in a series of polyoxyethylene nonylphenol nonionic surfactants, the value of  $K_1$  varied linearly with the HLB number of the surfactant. The value of  $K_2$  varied linearly with the log of the interfacial tension measured at the surfactant concentration that gives 90% soil removal. Carrying the correlations still further, it was

found that from the detergency equation of a single surfactant with three different polar soils,  $K_1$  was a function of the soil's dipole moment and  $K_2$  a function of the soil's surface tension (105).

Detailed thermodynamic and mechanistic analyses of solubilization and related mechanisms are given in (28) and (106–108). These works show that under proper circumstances, solubilization can make a significant contribution to oily-soil removal.

### 8.3. Phase Changes at the Soil-Bath Interface

Closely related to solubilization is a phenomenon that involves polar organic soils and surfactant solutions. If a complete phase diagram is plotted for a ternary system containing sodium dodecyl sulfate (or glycerol oleate) and water, several important and unusual features are noted. A large area represents a liquid phase consisting of a microemulsion, where the dispersed particles are so small that the system is isotropic, like the familiar soluble oils. Also, over another large area, a liquid crystalline phase is formed, containing all three components. This liquid crystalline phase flows like a liquid, at least in one direction. Flow perpendicular to the oriented planes is accomplished by folding the planes cylindrically, but the physical flow is still of the purely viscous type, with no yield point evident. These two phases, particularly the liquid–crystal phase, play an important part in detergency (109). Furthermore, liquid–crystal formation lowers interfacial tension (110). Although this phenomenon was demonstrated in tertiary oil recovery, the principles could also apply to oily-soil detergency.

When the polar organic component is a solid at ordinary temperatures, the addition of detergent and water markedly lowers the melting point; more specifically, as the temperature is raised, a point is reached where surfactant and water penetrate the solid. Thus, the ternary liquid–crystal phase might form spontaneously at room temperature by mixing the components, or, more precisely, an aqueous detergent solution can literally melt and liquify a relatively large proportion of solid polar fatty matter. What actually happens when these two phases are placed together at a low temperature and slowly warmed is a slow interaction. At a definite critical temperature, however, penetration of the detergent solution into the polar material starts to take place rapidly and the mass soon becomes fluid (28). This interaction is especially favorable in the case of nonionic surfactants that are relatively soluble in polar organic compounds and are the active ingredients of choice when forming microemulsions. In addition, above certain temperatures they display multilayer adsorption isotherms (111–116).

These phenomena are most rapid and easiest to observe in fairly concentrated aqueous detergent solutions, that is, minimally 2–5% detergent solutions. In a practical qualitative way, this is a familiar effect, and there are many examples of the extraordinary solvency and cleaning power of concentrated detergent solutions, for example, in the case of fabric pretreatment with neat heavy-duty liquid detergents. Penetration can also be demonstrated at low detergent concentrations. As observed microscopically, the penetration occurs in a characteristic manner with the

formation of a sheathlike structure, termed myelin; they are filled with isotropic liquid but have a liquid crystalline birefringent skin.

In a deterative system containing a dilute surfactant solution and a substrate bearing a solid polar soil, the first effect is adsorption of surfactant at the soil–bath interface. This adsorption is equivalent to the formation of a thin layer of relatively concentrated surfactant solution at the interface, which is continuously renewable and can penetrate the soil phase. Osmotic flow of water and the extrusion of myelin forms follows the penetration, with ultimate formation of an equilibrium phase. This equilibrium phase may be microemulsion rather than liquid crystalline, but in any event it is fluid and flushable from the substrate surface. This phase change effect explains the deterative behavior of sucrose fatty esters in admixture with alkylarenesulfonates (117).

## 9. MEASUREMENT OF DETERGENCY

The measurement of detergency can be approached from two different points of view. The theoretical approach is concerned with the relative quantity of soil bound to the substrate before and after washing. In this case, measurement is a necessary analytical procedure in the study of the detergency mechanism. The second approach emphasizes the development of reproducible laboratory methods that predict the results of practical cleaning operations. In the development of new household-cleaning compositions, for example, the formulator must know whether his products outperform others under actual use conditions. Realism, or accuracy as it is usually termed, is a prime requisite of any detergency test. It means good correlation between laboratory evaluation and the results of field testing. The practical field evaluation is usually made on the basis of specifications, sometimes implied rather than explicitly expressed, that determine whether or not the cleaning results were satisfactory. In removing spinning lubricants from wool yarns, for example, the most satisfactory cleaning procedure is that which removes most soil from the substrate. In certain metal-cleaning operations, however, the satisfactory outcome is a piece of metal free of solid soil but carrying an even and easily perceivable layer of rust-preventive oil. In judging the cleanness of white fabrics, more interest is usually shown in fabric whiteness rather than in its actual soil content. Thus, most detergency evaluations in which white fabric is the substrate specify cleanness in terms of fabric whiteness or reflectance.

With these limitations in mind, the measurement of detergency in the laboratory requires the following components: A means for measuring or estimating the amount of soil on the substrate or the degree of cleanness both before and after washing; satisfactory substrates and soiling compositions; a means for applying soil to substrate in a realistic manner; and a realistic and reproducible cleaning device. These fundamental requirements apply regardless of the particular type of substrate that is being cleaned. More attention in this area has been centered on textile fabrics than on other substrates, but the various substrates and even human skin can be considered from the same point of view.

## 10. FABRIC DETERGENCY

### 10.1. Laundering

Reflectance is the most commonly used measurement for the whiteness of fabrics, although the transmittance of light by fabric specimens can also be used. The most commonly used instrument for reflectance measurement is the Gardner colorimeter, although the Zeiss Elrepho is also used. For general detergency, the grayness of the fabric is measured. Color effects can also be measured, and fabric yellowing is especially important. It is masked by fluorescent whitening agents (FWA). Special filters are available to eliminate this effect, and whitening caused by soil removal can be distinguished from that of FWA deposition.

As would be expected, no single artificial soil or combination of artificial soils can adequately model natural soils in the household laundry. Natural soils vary widely within a single household, between households, and between regions. In addition, natural soiling includes effects of aging and repetitive soiling that are difficult to simulate artificially. Consequently, there is no single set of test conditions that can consistently rate a group of detergents in a completely realistic manner. Limited correlations are usually obtained by suitably adjusting both the laboratory procedure and the practical full-scale procedure.

These problems can be dealt with by using artificial test cloths impregnated with various approximations of natural soils such as vacuum cleaner dust, dirt from air conditioner filters, clays, carbon black, fatty acids dirty motor oil, and artificial sebum, either alone or in combination (48, 118–123). The soils are applied by spraying, immersion, or padding. If the soils are carefully applied, reproducible results can be obtained. Soil test cloths can be of great help in detergency studies, when used with an understanding of their limitations.

The device most widely used for laboratory fabric detergency measurements is the Terg-O-Tometer (U.S. Testing Co.), a miniature agitator washer. Just as it is difficult to model natural soils, it is difficult to model a full-scale washing machine. The Terg-O-Tometer consists of four or six small agitator washers in 2-L beakers. Water and detergent are placed into the beakers, and the temperature controlled by a water bath. Standard-soil cloths are added and washed for 5–15 min. The speed as well as the angle of oscillation of the agitators is adjustable. The soil cloths are then rinsed, usually in the Terg-O-Tometer, dried, and the reflectances read. The performance of various detergent mixtures can be compared on the basis of the final reflectance  $R_f$  of the washed soil cloths. However, it is generally more useful to express the cleaning as percentage detergency, % $D$  where  $R_i$  is the initial reflectance (before the wash) of the soil cloths and  $R_0$  the reflectance of the unsoiled fabric used to prepare the soil cloths, as shown in the following equation:

$$\%D = \frac{R_f - R_i}{R_0 - R_i} \times 100.$$

Redeposition of soil can be estimated simultaneously with net soil removal (detergency) by including a white swatch with the soiled swatches. It also can be

estimated separately by adding a measured amount of soil to a fresh wash bath, then treating the white swatch in this standard-soil bath. The former method is, superficially at least, more realistic. The latter method, however, is frequently of greater value in development work because both the quantity and composition of the soil are known and can be controlled.

Grayness of a fabric swatch is not directly proportional to its content of black pigment (or artificial soil). A basic formula relating reflectance to the pigment content or concentration can be applied to the evaluation of detergency test swatches (64, 125–127). In simple form, an adaptation of the Kubelka-Munk equation, it states that the quantity  $(1 - R)^2/2R$  (where  $R$  is the fraction of light reflected from the sample) is a linear function of the soil content of the sample.

In some cases, it may be impossible, or undesirable, to measure the amount of soil by reflectance. Soil can also be determined by extraction and weighing the cloths, or weighing the washed cloths.

A number of excellent studies have used a variety of radiolabeled soils to investigate the removal of small amounts of colorless soils such as oils (128–130). By proper use of different radiolabels (such as  $^3\text{H}$  and  $^{14}\text{C}$ ), the preferential removal of various components in a soil mixture can be followed. In these cases in particular, detergency can also be calculated from measurements of the amount of radioactivity that is removed from the fabric and is found in the wash liquor.

The above tests all measure the ability of a washing system to remove soil from a fabric in a single wash. However, the detergent or washing machine is often judged by the way the linen appears after several soil-wash cycles. After a series of soilings and washings, the linen acquires an off-white gray or yellowish shade caused by soil accumulation and chemical changes in the soil. Although soil accumulation tests are more tedious than soil removal tests, they give the most realistic results. A laboratory-scale soil accumulation test using vacuum cleaner dirt and small swatches of fabric has been described (131).

## 10.2. Textile Mill Operations

Detergency is important in textile finishing because small quantities of foreign matter on the goods can interfere seriously with dyeing and other finishing treatments. Furthermore, the goods are expected to be uniformly and thoroughly clean when sold. Many detergency tests in this area are of the semipractical type, i.e., test swatches are analyzed for soil content. This analysis generally consists of gravimetric determination of the soil content either directly from the fabric weight or by extraction.

## 11. HARD-SURFACE DETERGENCY

Despite the variety of hard-surface objects that are purposefully cleaned at regular intervals, detergency has been studied quantitatively in relatively few cases only. The small-scale user normally judges washing results as satisfactory or unsatisfac-

tory. If satisfactory results are obtained with the amount of detergent and the degree of mechanical action employed, the user is not interested in minor qualitative differences. In those areas where specifications are important and where differences among detergents or mechanical washing equipment are readily perceivable, quantitative methods for measuring detergency have been developed.

In specific cases of metal cleaning where small amounts of residual soil must be detected and are difficult to measure by conventional means, radiotracer methods have been employed (132). Interest in these techniques has been stimulated by the development of methods for decontaminating hard surfaces subjected to atomic fallout (133).

Quantitative measurements have been obtained for ceramics and glass, metals, and organic surfaces such as painted and plastic tile.

### 11.1. Glassware and Dishwashing

Dishes are washed either by hand or in an automatic dishwashing machine. Hand-dishwashing detergents are generally high-foaming compositions containing organic surfactants as the main ingredient. The consumer judges efficiency not only by the cleanness of plates but also by foam persisting throughout the operation. Evaluation of hand-dishwashing products by manufacturers simulates this procedure. The number of plates that can be washed clean, judged visually without or with a color or fluorescence indicator, and the number of plates necessary to kill the foam in the dishpan is taken as the measure of detergency efficiency (134, 135).

More objective laboratory methods employ a mechanical device such as a Terg-O-Tometer (136). Food soils are applied to microscope slides or glass tape rather than to actual plates. The soils are tagged with fluorescent materials or with dark pigment to facilitate measurement of residual soil. Reflectance or transmittance may also be read directly (137).

The foam stability of hand-dishwashing compositions can also be measured more directly and more quantitatively using mechanical means to whip up a foam and adding increments of food soil to a predetermined no-foam end point (138).

The detergents in automatic-dishwashing compositions are largely inorganic, but evaluation of residual soil is essentially the same as in hand-dishwashing tests. Low foam avoids inhibition of the free movement of the rotor. Clarity of glassware is a particularly prized performance feature of home dishwashers and special photometric methods have been reported for measuring freedom from water spots or filming (haze) (139). More frequently, however, the presence of spots and filming is assessed visually in a box in which light is beamed at the interior of inverted glasses in an otherwise black environment.

In restaurant operations, sterilization of dishes is an additional requirement. Sterilization is determined by the usual swabbing and culturing methods or by employing bacteria tagged with radioactive phosphorus and counting residual radioactivity on the washed dishes (134).

## 11.2. Metal Cleaning

The purpose of cleaning steel is to remove dirt and leave the article in a state in which it can be delivered for use without further finishing. The surface must therefore be covered with a tenacious corrosion-resistant coating as it emerges from the cleaning bath. Many emulsion cleaners remove lubricants and other unwanted dirt while depositing an anticorrosive coating on the metal. The primary test for efficacy in this situation is a corrosive test of the cleaned article.

A more thorough cleaning prepares the surface for further finishing, e.g., electroplating or painting, or application of an oxide finish. The basic objective in this case is to ensure maximum adhesion of the finish by freeing the surface of all foreign matter (140). Frequently, the cleaning compositions are straight organic solvents, hydrocarbons or chlorinated hydrocarbons, but in many cases aqueous detergent solutions are used. Regardless of the composition of the cleaning product, detergency test methods involve soiled test specimen, a standard method or device for performing the cleaning operation, and a quantitative method for estimating the extent of soil removal. The test specimens are mostly metal panels, or coupons, 10–300 cm<sup>2</sup> in area. They are carefully cleaned, usually by abrasive action, and then soiled. Soils may vary widely but often are based on lubricating oil or asphalt. A dark pigment or fluorescent marker is added if the cleaning result is to be evaluated visually or by photometry. A radioactive tracer, compatible or identical with the soil, may also be added for assessment of residual soil. The cleaning device is often a miniature version of large-scale cleaning equipment that provides soaking of the test article and agitation by hydraulic action or boiling.

When a quantitative estimate of residual soil is not called for and the suitability of a metal surface for further finishing needs to be assessed, the water-break test is used. The term water-break refers to the behavior of a water film on a smooth greasy surface. When the film becomes sufficiently thin by drainage, it suddenly breaks into islands or droplets between which the surface appears dry. On the other hand, when a film drains from a clean water-wettable, nongreasy surface, it becomes progressively thinner and finally disappears by evaporation without ever breaking into droplets. Such a surface is said to be free from water-break.

In a similar procedure, the atomizer test, which depends on the behavior of an advancing rather than a receding contact angle, a fine mist of water is applied to the metal surface and the spreading of water is observed. On a clean surface, water spreads to a uniform film. With oleic acid as the test soil, the atomizer test can detect the presence of 10<sup>-5</sup> mg of soil per cm<sup>2</sup>, less than a monomolecular layer (141). For steel that is to be electroplated, the copper dip test is often employed. Steel is dipped into a cupric salt solution and the evenness of the resulting metallic copper deposit is noted.

Correlation of all aspects of the test method with the practical system of interest is always important. The test used for dairy cleaning is an excellent example (142). Milk is used to tag the soil with radioactive <sup>45</sup>Ca by an exchange with radioactive CaCl<sub>2</sub>. This treatment is applied to stainless steel planchets by suspending the planchets in milk under actual pasteurizing conditions.



### 11.3. Organic Surfaces

Tests for detergency on organic surfaces such as painted walls and plastic tile generally include a rubbing or sponging step corresponding to the manner in which such surfaces are cleaned in practice. The method adopted by the Chemical Specialties Manufacturers' Association includes scrubbing a painted glass surface soiled with a marking pencil in a Gardner Straight Line Washability Machine and assessing deterative efficacy visually on a seven-point scale (143). In a similar method, a preconditioned linoleum tile is coated with an oily pigment soil and scrubbed in a Gardner machine; the deterative efficacy is measured by reflectance change (144).

## 12. DETERGENT MANUFACTURE

### 12.1. Liquid Products

The manufacture of liquid detergent products is generally a straightforward process requiring batch equipment with provisions for metered addition of individual ingredients, agitation, and if needed, heating and cooling. Capital cost can vary depending on the degree of automation.

### 12.2. Spray-Dried Products

The manufacture of powdered product is more complicated. High-pressure spray-drying of an aqueous slurry has replaced the earlier process in which a solidified cake of the product had to be broken up mechanically. Spray-drying equipment requires a relatively high capital outlay. The resulting product, however, is characterized by several desirable features: a high concentration of surfactant can be accommodated in the finished product; the product consists of hollow beads that dissolve readily in the washing solution; powders are considerably less dusty than those produced by the earlier process; powders generally do not lump and most compositions can be packaged in cartons without special liners; and bulk densities are obtainable within the general range of 0.25–0.65 g/L.

The first stage in preparing spray-dried products involves producing a slurry of liquid and solid ingredients. Two processes are available, a batch process or a continuous process. The batch process involves preparing a slurry in a crutcher, which is a mixing vessel with heavy-duty agitation and provision for heating. Solid and liquid components are combined to form a homogeneous slurry. It is normal with a batch process that two crutchers are employed; while one crutcher batch is discharged to the spray-drying tower, another batch is prepared in the second crutcher. The automated batch process is preferred when there are reaction, hydration, and crystallization processes involved.

The continuous process can offer shorter residence times and requires a high degree of automation (145). In addition, continuous slurry preparation can permit

high solids concentrations and hence reduce the evaporative load in the spray-drying tower. The resulting energy savings can be significant in large installations.

Acids such as fatty acids and alkylbenzene sulfonic acids are neutralized with NaOH during slurry preparation to form soap and sodium alkylbenzene sulfonate, respectively.

After preparation, the slurry is transported to an aging vessel. During residence time of 20–30 min, the neutralization process, hydration of sodium tripolyphosphate and structural changes in the slurry are completed to provide a homogeneous composition. By means of a high-pressure pump, the slurry is conveyed to the spray-drying tower under a pressure of ca. 10 MPa (100 atm). A representative spray-drying tower layout is shown in Figure 7.

The slurry, at 80–100°C, is forced through nozzles of 2.5–3.5 mm diameter arranged on a nozzle ring. In the tower, the slurry encounters hot air that has entered the tower at 250–350°C. Airflow is normally countercurrent. Air circulation is provided by two blowers. A third blower provides suction that carries fines into the collecting cyclones. From there the fines may be reintroduced into the upper part of the tower where they are contacted by wet slurry descending in the tower. Alternatively, the fines may be added to subsequent crutcher batches. Upon exit, the powder is conditioned during passage via belt conveyer and an airlift to the packaging machinery.

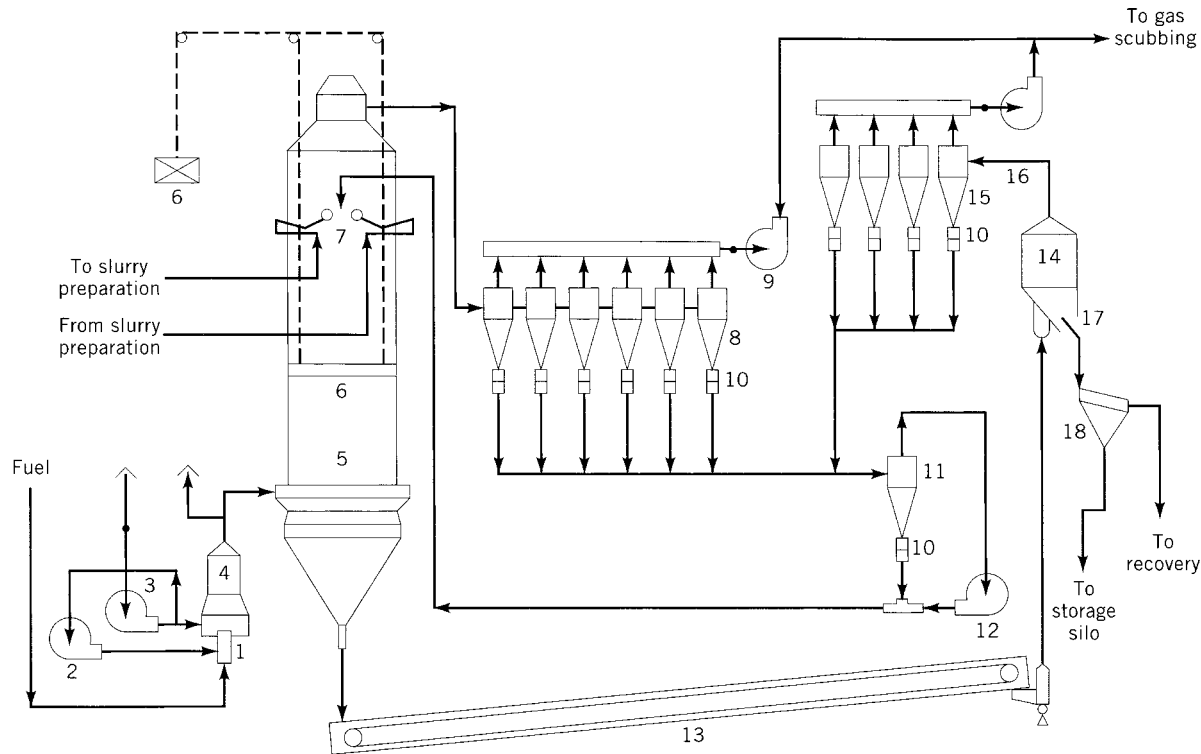
The capacity of spray-drying towers is influenced by the formulation of the powder being produced. For large spray-drying towers, throughputs of up to 30 tons per hour can be expected.

Because of stringent air pollution rules, the exit gases are wet-scrubbed in brine since high NaCl concentrations reduce foam formation. NaOH scrubs out SO<sub>2</sub> from sulfur-containing fuel. The scrubbing solution, saturated with detergent fines, is recycled to the water tank for slurry preparation. As shown in Figure 7, the tower is also provided with a cleaning ring that is moved along the tower walls.

Although spray-drying accommodates relatively high content of surfactants, certain types, such as the alkanolamides and some nonionic surfactants are best added to the product after spray-drying. Postaddition not only protects the surfactant from the heat of the tower but also prevents the formation of aerosols in the exit gas. Aerosols are more difficult to trap in the scrubbing system than solid fines. They are formed by unsulfonated matter from the manufacture of LAS and nonionic surfactants with short ethylene oxide chains (146).

### **12.3. Dry-Blended Product**

In addition to lower capital outlay, dry-blending requires considerably less processing energy. Final product density, which is usually near unity, depends on the density of the starting materials and the nature of equipment used to blend these materials. Modern mixing and blending equipment, if properly controlled, can give product density and particle sizes comparable to spray-dried products.



**Figure 7.** Plant for spray-drying of detergents and soaps. (1) Burner, (2) air combustion, (3) cold air fan, (4) hot air generator, (5) spray-drying tower, (6) cleaning ring, (7) nozzles circuit, (8) tower suction cyclones group, (9) tower suction fan, (10) double-flap dischargers, (11) recovery powder cyclone, (12) recovery powder fan, (13) belt conveyor, (14) air lift, (15) air lift suction cyclones, (16) air lift suction fan, (17) double-flap dischargers, (18) vibrating sieve.

## 12.4. Agglomerated Products

The process of agglomeration is intermediate between spray-drying and dry-blending. Process water concentrations are between 35% and 40% in a crutcher slurry and essentially zero in dry blending. In agglomeration, a liquid is sprayed onto a continuously agitated powder. Equipment designs include stationary mixers, rotating mixers with spray nozzles, and rotating blenders with a liquid dispersion bar, either twin shell or continuous zigzag (147). Automatic dish-washing detergents are mostly manufactured by agglomeration (148). Liquid components of the formulation, for example, silicate, detergent actives, and water, can be used as the agglomerating liquid. Other examples of agglomeration processes include the hydration of sodium tripolyphosphate, coloring (speckling) of detergent powders, and the agglomeration of spray tower fines.

## 13. ANALYSIS

The literature on analytical methods is voluminous and not easily summarized (149–156). Often the greatest expertise in the analysis of complex detergent mixtures resides with in-house personnel in individual companies who may regard their methods as proprietary information.

An unknown commercial detergent may contain some combination of anionic, nonionic, cationic, and possibly amphoteric surfactants, inorganic builders and fillers as well as some minor additives. In general, the analytical scheme includes separation of nonsurfactant and inorganic components from the total mixture, classification of the surfactants, separation of individual surfactants, and quantitative determination (157).

## 14. HEALTH AND SAFETY FACTORS

As a class, surfactants and detergent products are among the most widely used chemical compositions. Almost everyone is exposed to these products on a daily basis in situations that range from ingestion of food-grade emulsifiers to intimate contact of skin and eyes with personal-care and laundry products. Safety is therefore a matter of great importance (158, 159). Ranges of surfactant LD<sub>50</sub> values are shown in Table 2.

**TABLE 2. Rat Oral LD<sub>50</sub> Values of Surfactant (158).**

Type of Compounds	Oral LD <sub>50</sub> (mg/kg)
Alkylbenzene sulfonates	700–2,480
Alcohol ethoxylates	1,600 to greater than 25,000
Sulfated alcohol ethoxylates	7,000 to greater than 50,000
Alcohol sulfates	5,000–15,000

Under conditions of normal use, detergent products are not hazardous to users. Nonetheless, surfactants possess some toxicity, and they are mild irritants. Particularly under conditions of misuse, such as accidental ingestion or spillage, they can produce irritation and discomfort in the form of nausea and vomiting, as well as irritation to skin and eyes. The long-term effects, however, are minimal (160).

In the 1980s and 1990s, governmental concern with the safety of chemicals has led to a number of legislative acts that regulate the manufacture and sale of chemicals including detergents. Even before this, the Food and Drug Act was passed in 1938. Food-grade emulsifiers fall under its provisions, as well as products containing antimicrobial agents such as deodorant soap bars.

Like other chemicals, new substances introduced into detergent products are regulated by the TSCA of 1977. Since its purpose is to prevent chemicals with long-term deleterious effects from entering the marketplace, the emphasis in testing is on long-term effects such as carcinogenicity, mutagenicity, and teratogenicity.

The manufacture of surfactants and of detergent products is regulated by the Occupational Safety and Health Administration (OSHA). Dust concentration in detergent plants as well as factory noise levels are the primary areas of relevance, since the individual components in these products are essentially nonhazardous. Of more immediate concern to the detergent industry is the Federal Hazardous Substances Act (FHSA) of 1960 and the Consumer Products Safety Act (CPSA) of 1972. The FHSA defines specific labeling requirements, such as "Danger" for extremely flammable, corrosive, or highly toxic substances, and "Warning" or "Caution" for less hazardous materials.

To assess the degree of hazard, animal acute oral toxicity data are generally relied upon even though such data have limitations in their applicability for predicting possible human effects in the case of a specific exposure. Ranges of detergent LD<sub>50</sub> values ratings are given in Table 3.

**TABLE 3. Detergent Animal Acute Oral LD<sub>50</sub> versus Probable Lethal Dose for a Human Adult (161)**

Animal Acute Oral LD <sub>50</sub> (g/kg)	Toxicity Rating	Probable Lethal Oral Dose for 70 kg Person (g) <sup>a,b</sup>
<0.005	6 super toxic	taste <0.35
0.005–0.050	5 extremely toxic	0.35–4.9
0.05–0.50	4 very toxic	4.9–28.3
0.5–5.0	3 moderately toxic	28.3–45.3
5.0–15.0	2 slightly toxic	453–1000
>15	1 practically nontoxic	>1000

<sup>a</sup>0.35 g = 7 drops; 4.9 g = 1 tsp; 28.3 g = 1 oz.

<sup>b</sup>To convert grams to pounds, multiply by 0.0022. 1 lb is approximately 1 pt.

**TABLE 4. Acute Oral LD<sub>50</sub> Ranges for Detergent Products (162).**

Detergent Type	Albino Rat, Oral LD <sub>50</sub> (g/kg)
Heavy-duty, laundry	
Granular	2-7
Liquid	2-9
Hand-dishwashing, liquid	5-20
Automatic dishwashing	2-7
Floor and wall cleaner	
Crystalline	4-6
Liquid	8->16
Rug cleaner	7-9
Laundry presoak with enzyme, granular	3-11
Fabric softener	>10
Toilet bar, soap, or synthetic base	7-20
Shampoo	
Plain	>10
Medicated	3-10

The range of values for several representative categories of detergent products is given in Table 4.

Because of emesis, it is unlikely that appreciable quantities of most common soaps and detergents could be ingested accidentally.

Methods of testing for eye and skin irritation potential have been reviewed (163). The official FHSA procedure for evaluating ocular irritation potential of detergent products is a modified Draize rabbit eye test (164). Some controversy surrounds this method at present, and a search for a procedure less injurious to test animals is in progress. In general, the order of irritation is cationic > anionic > nonionic (165).

Skin irritation potential is assessed by patch tests. More serious than simple irritation is the potential of a product to cause sensitization, i.e., to cause a subject to become allergic to even very small amounts of the product.

Several test methods have been devised for sensitization. In the Magnusson-Kligman procedure, guinea pigs are injected just below the skin with a slightly irritating dose of the test substance with and without an antigen. After some days, a patch test is taken on the injection site to stimulate the skin to react. After several weeks, another patch test is taken, this time on an untreated site. A positive reaction is an indication of the sensitizing potential of the test substance (166).

Photosensitization, the potential of a product to cause sensitization in the presence of sunlight, is similarly evaluated by taking a patch test on guinea pigs before and after exposure to ultraviolet radiation (167).

Controversy over test methodology, and concern with the welfare of test animals, has been highly publicized in the last decade, and various states have proposed legislation to ban animal tests. Significant effort has been devoted to developing non-animal alternative tests and predictive methods. Progress has been made, but no entirely satisfactory substitute has been found to date (168).

## 15. ENVIRONMENTAL CONSIDERATIONS

The introduction of surfactant products into the environment, after use by consumers or as part of waste disposed during manufacture, is regulated by the Clean Water Act, the Clean Air Act, and the Resource Conservation and Recovery Act. In this respect, surfactants are subject to the same regulations as chemicals in general. There are, however, two areas of specific relevance to surfactants and detergent products, i.e., biodegradability and eutrophication.

As early as 1947, incidents of foam blankets on bodies of water were reported. This was at a time when detergents containing synthetic surfactants were in the process of displacing soap as the principal heavy-duty laundry product. It was suspected that they were the cause of foam formation since they were less readily degraded by the microorganisms present in water. In contrast to soap with a straight-chain hydrophobe, ABS, the predominant surfactant at the time, contained a highly branched hydrophobe, e.g., a propylene tetramer alkyl group. The U.S. surfactant industry, represented by The Soap and Detergent Association (SDA), took the lead in the investigation of the problem, sponsoring a large number of field studies in the development of test methods and analytical procedures for measuring the presence of surfactants in the environment. For anionic surfactants like ABS, the Methylene Blue Active Substance (MBAS) test, which depends on the formation of a chloroform-soluble complex of the anionic surfactant with cationic methylene blue, provides the first indication of the extent to which a surfactant has been degraded (169). Nonionic surfactants are assayed by complex formation with cobalt thiocyanate (170).

Among the methods for simulating the fate of the surfactant in the environment, the river die-away test (171) and the shake-flask method (172) have proved acceptable for fast screening and routine use. The semicontinuous activated sludge (SCAS) method is more time-consuming but is more accurate and reproducible (173). Determination of biological oxygen demand (BOD) also provides useful data on biodegradation (174, 175).

The extensive investigations led to the conclusion that a branched hydrophobe impedes the rate and extent of degradation of surfactants by microorganisms. The most immediately apparent remedy, therefore, was to replace the propylene tetramer in ABS with a straight hydrocarbon chain giving straight-chain ABS, so-called linear alkanesulfonate (LAS). At the same time, commercialization of the Ziegler process for the oligomerization of ethylene provided another route to straight-chain hydrophobes that could easily be converted to detergent alcohols and straight-chain nonionic surfactants. By 1965, the U.S. detergent industry had completed a voluntary switch from hard to soft surfactants at a cost that has been estimated at ca.  $\$150 \times 10^6$ . In addition to ABS, other surfactants based on propylene oligomers, such as alkylphenol derivatives, have largely disappeared from U.S. consumer laundry products.

Even though the biodegradability problem has been solved for all practical purposes, the subject continues to receive considerable attention. The biodegradation of LAS has been studied intensively, and several mechanistic pathways have been

identified such as  $\beta$  and  $\omega$  oxidation as well as reductive and oxidative desulfonation (176). Investigation of the biodegradation of LAS, alcohol ethoxylates, and alkylphenol ethoxylates in the laboratory and under sewage plant operating conditions showed that LAS and straight-chain alcohol ethoxylates and their sulfates degrade to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  (177, 178).

### 15.1. Eutrophication

This term, which denotes excessive nutrition or overfertilization, has been applied to the contribution excessive amounts of phosphorus may make to the growth of algae under certain conditions. Phosphorus in water supply originates from runoff of agricultural fertilizers, human excrement, and sodium tripolyphosphate present in detergent formulations. It has been estimated that 25–30% of the phosphorus in wastewater comes from laundry detergents, and that detergents contribute about 3% of the phosphorus annually entering U.S. surface waters (179). Excessive algal growth in stagnant bodies of water contributes to oxygen depletion, which causes starvation of marine life and eventually leads to the death of lakes through silting. Phosphorus can be removed from the effluent of sewage treatment facilities by treatment with materials such as alum. Expansion of sewage treatment has been the method of choice in some areas of the world, notably Sweden to assure acceptably low phosphorus content in environmental waters (180). In the United States, and later in Western Europe, detergent phosphates were singled out in the early 1960s as the cause of eutrophication, and their removal from consumer laundry formulations was proposed as a feasible approach to improvement of environmental water quality. Many states and a number of local jurisdictions have banned detergent products containing phosphate.

The efforts of the detergent industry toward solution of its part of the eutrophication problem are, at this point, less complete than its response to the biodegradability problem. Soda ash,  $\text{Na}_2\text{CO}_3$ , sodium silicate, and, to a lesser extent, sodium citrate formed the basis of the early formulations marketed in the areas where phosphates were banned. Technically, these substances are considerably less effective than sodium tripolyphosphate. As a precipitant builder, soda ash can lead to undesirable deposits of calcium carbonate on textiles and on washing machines.

At the same time, the industry embarked on an intensive search for phosphate substitutes. Of a very large number of experimental organic builders, a few substances reached commercialization or near-commercialization, including trisodium nitrilotriacetate (NTA), trisodium carboxymethoxysuccinate (CMOS) (181) and trisodium carboxymethyltartronate (182). As discussed above, sodium citrate ether carboxylates have achieved widespread use as phosphate substitutes. Polymeric builders (polyelectrolytes) proved to be effective calcium sequestrants, but failed to satisfy the criterion of acceptable biodegradability. Interestingly, some monomeric polycarboxylates proved to be even more powerful calcium sequestrants than sodium tripolyphosphate but were not sufficiently biodegradable (183).

Trisodium nitrilotriacetate, a sequestrant of effectiveness comparable to sodium tripolyphosphate, reached commercialization in the late 1960s. However, because



some laboratory findings suggested potential teratogenicity, it was withdrawn from the market in 1970 at the request of the U.S. Surgeon General. It has continued to be used in Canada and elsewhere. On the basis of many studies supporting the safety of NTA, the Environmental Protection Agency dropped its opposition to NTA and this builder, in combination with type-A zeolite, briefly appeared in some nonphosphate consumer laundry products. However, continuing opposition to NTA on the state level resulted in its withdrawal from detergents and its reappearance seems unlikely at this time.

Laboratory assessment of the eutrophication potential of an experimental substance is less clear-cut than that of biodegradation. A frequently used method is the algal assay procedure in which a variety of algal cultures is grown in open shake flasks and the effect of test material on their growth is determined (179). In a second procedure, the MAAP test, one observes the behavior of a microcosm, consisting of bacteria, algae, zooplankton, sediment, and water taken from an oligotrophic lake. The diversity index, an indication of the number of algal species present, is a measure of the nutritive index of the system. A reduction in the diversity index is taken as an indication of the eutrophic potential of the test substance (184).

## REFERENCES

1. C. B. Brown, *Research* (London), **1**(10), 46 (Oct. 1947).
2. W. C. Powe and W. L. Marple, *J. Amer. Oil Chem. Soc.*, **37**, 136 (1960).
3. E. Kissa, in W. G. Cutler and E. Kissa, eds., *Detergency: Theory and Technology*, Marcel Dekker Inc., New York, 1977, Ch. 1.
4. A. Cahn and J. L. Lynn, Jr., in J. I. Kroschwitz, ed., *Encyclopedia of Chemical Technology*, vol. 22, 3rd ed., John Wiley & Sons, Inc., New York, 1983, pp. 332–432.
5. L. Spitz, *Soap Technology for the 1990s*, Am. Oil Chem. Soc., Champaign, Illinois, 1990, p. 2.
6. W. R. Noble, R. G. Bistline, Jr., and W. M. Linfield, *Soap Cosmet. Chem. Spec.*, **38**, 62 (1972).
7. W. E. Bishop, C. C. Kuta, and C. A. Pittinger, *Life Cycle Analysis and Its Relevance to the Detergent Industry*, paper presented Sept. 14, 1992, New Horizons' 92 CSMA/AOCS Detergent Industry Conference, Bolton Landing, New York.
8. C. Fusler, in A. Cahn, ed., *Proceedings of the 3rd World Conference on Detergents: Global Perspectives*, AOCS Press, Champaign, Illinois, 1994, pp. 58–63.
9. W. J. B. Voge, in Ref. 8, p. 123.
10. T. Satsuki, in Ref. 8, pp. 135–140.
11. A. D. Urfer et al., in A. R. Baldwin, ed., *Proceedings of the Second World Conference on Detergents: Looking Toward the 90s*, Am. Oil Chem. Soc., Chicago, Illinois, 1987, pp. 268–271.
12. H. Andree and B. Middelhaue, in A. Cahn, ed., *Proceedings of the 3rd World Conference on Detergents: Global Perspectives*, AOCS Press, Champaign, Illinois, 1994, pp. 95–98.

13. D. S. Connor, J. J. Scheibel, and R. G. Severson (to Procter & Gamble Co.), U.S. Patent 5,194,639, March 16, 1993.
14. W. W. Morgenthaler, *Inform*, **2**, 6 (1991).
15. J. P. Nirschl and R. A. Gloss (to Procter & Gamble Co.), U.S. Patent 3,862,058, January 21, 1975.
16. R. J. Baskerville, Jr., and F. G. Schiro (to Procter & Gamble Co.), U.S. Patent 3,936,537, February 3, 1976.
17. T. D. Storm and J. P. Nirschl (to Procter & Gamble Co.), U.S. Patent 4,062,647, December 13, 1977.
18. S. R. McDonald (to Procter & Gamble Co.), U.S. Patent 4,141,841, February 27, 1979.
19. G. K. Greminger, Jr., and A. S. Teot, *J. Amer. Oil Chem. Soc.*, **55**, 122 (1978).
20. A. E. Siegrist, *J. Amer. Oil Chem. Soc.*, **55**, 114 (1978).
21. P. N. Christensen, P. Holm, and B. Sønder, *J. Amer. Oil Chem. Soc.*, **55**, 109 (1978).
22. C. A. Starace, *J. Amer. Oil Chem. Soc.*, **60**, 1025 (1983).
23. G. Jensen, *Tenside Surf. Det.*, **27**, 30 (1990).
24. A. Gillis, *J. Amer. Oil Chem. Soc.*, **66**, 734 (1989).
25. A. P. James, *Chemistry of Peroxygen Bleaching*, paper presented at 83rd American Oil Chemists' Society, Toronto, Canada, May 1992, Interox America Reprints, Houston, Texas, 1992.
26. S. Y. Chung and G. L. Spadini (to Procter & Gamble Co.), U.S. Patent 4,412,934, November 1, 1983.
27. R. D. Bush, D. S. Connor, S. W. Heinzman, and L. N. Mackey (to Procter & Gamble Co.), U.S. Patent 4,663,071, May 5, 1987.
28. M. E. Ginn and J. C. Harris, *J. Amer. Oil Chem. Soc.*, **38**, 605 (1961).
29. J. A. Shaeiwitz et al., *J. Colloid Interface Sci.*, **84**, 47 (1981).
30. K. W. Dillan, E. D. Goddard, and D. A. McKenzie, *J. Amer. Oil Chem. Soc.*, **56**, 59 (1979).
31. K. W. Dillan, E. D. Goddard, and D. A. McKenzie, *J. Amer. Oil Chem. Soc.*, **57**, 230 (1980).
32. H. L. Benson and K. H. Raney, *Shell Technical Bulletin, SC: 1182-90*, Shell Chemical Co., Houston, Texas, 1990.
33. E. E. Dreger et al., *Ind. Eng. Chem.*, **36**, 610 (1944).
34. H. Stupel, *Synthetische Wasch-und Reinigungsmittel*, Konradin-Verlag, Stuttgart, Germany, 1954.
35. G. E. Barker, *J. Amer. Oil Chem. Soc.*, **28**, 317 (1951).
36. H. R. Suter and M. G. Kramer, *Soap Sanit. Chem.*, **27**(8), 33 (1951).
37. R. L. Mayhew and R. C. Hyatt, *J. Amer. Oil Chem. Soc.*, **29**, 357 (1952).
38. J. V. Karabinos, G. E. Bartels, and G. E. Kapella, *J. Amer. Oil Chem. Soc.*, **31**, 419 (1954).
39. F. N. Baumgartner, *Ind. Eng. Chem.*, **46**, 1349 (1954).
40. M. S. Furry and E. M. O'Brien, *Am. Dyestuff Rep.*, **41**, 861 (1952).
41. R. Bernstein and H. Sosson, *Soap Sanit. Chem.*, **30**(1), 46 (1954).
42. F. W. Gray, J. F. Gerecht, and I. J. Krems, *J. Org. Chem.*, **20**, 511 (1955).
43. H. A. Ludeman, J. A. Balog, and J. C. Sherrill, *J. Amer. Oil Chem. Soc.*, **35**, 5 (1958).

44. T. G. Jones, in K. Durham, ed., *Surface Activity and Detergency*, Macmillan and Co., Ltd., London, 1961.
45. T. G. Jones and J. P. Parke, *Proceedings of the 4th International Congress on Surface Activity*, 3rd ed., University of Mainz Press, Germany, 1960, p. 178.
46. K. L. Stellner and J. F. Scamehorn, *J. Amer. Oil Chem. Soc.*, **63**, 566 (1986).
47. K. L. Stellner and J. F. Scamehorn, *Langmuir*, **5**, 79 (1989).
48. B. J. Rutkowski, in W. G. Cutler and R. C. Davis, eds., *Surfactant Science Series*, vol. 5, part 3, Marcel Dekker, Inc., New York, 1981, p. 1013.
49. S. Stawitz, W. Klaus, and P. Hoepfner, *Fette Seifen Anstrichm.*, **60**, 94 (1958).
50. J. W. Hensley and C. G. Inks, *Textile Res. J.*, **29**, 505 (June 1959).
51. N. K. Adam, *J. Soc. Dyers Colour*, **53**, 121 (1937).
52. W. Kling, E. Langer, and I. Haussner, *Melliand Textilber.*, **25**, 198 (1945).
53. J. C. Stewart and C. S. Whewell, *Textile Res. J.*, **30**, 903 and 912 (1960).
54. J. Compton and W. J. Hart, *Textile Res. J.*, **23**(3), 158 and 418 (1953).
55. J. Compton and W. J. Hart, *Textile Res. J.*, **24**(6), 263 (1954).
56. W. Kling and H. Mahl, *Melliand Textilber.*, **35**, 640 (1954).
57. T. H. Shuttleworth and T. G. Jones, *International Congress on Surface Activity*, vol. 4, 2nd ed., Academic Press, New York, 1957, pp. 52–59.
58. V. W. Tripp et al., *Textile Res. J.*, **28** (1958).
59. J. Berch and H. Peper, *Textile Res. J.*, **33**, 137 (1963).
60. J. Berch, H. Peper, and G. L. Drake, Jr., *Textile Res. J.*, **34**, 29 (1964).
61. W. C. Powe, *J. Amer. Oil Chem. Soc.*, **40**, 290 (1963).
62. J. L. Lynn, Jr., *Detergents in Depth '89*, Soap and Detergent Association, New York, 1989, p. 11.
63. J. C. Dickinson and R. C. Palmer, *J. Textile Inst.*, **42**, T510 (1951).
64. T. H. Vaughn, A. Vittone, Jr., and L. R. Bacon, *Ind. Eng. Chem.*, **33**, 1012 (1941).
65. W. P. Utermohlen, Jr., and E. L. Wallace, *Textile Res. J.*, **17**, 676 (1947).
66. L. Loeb, P. B. Sanford, and S. D. Cochran, *J. Amer. Oil Chem. Soc.*, **41**, 120 (1964).
67. H. Schott, *J. Amer. Oil Chem. Soc.*, **52**, 225 (1974).
68. D. G. Stevenson, *J. Soc. Dyers Colour*, **68**, 57 (1952).
69. I. J. Gruntfest and E. M. Young, *J. Amer. Oil Chem. Soc.*, **26**, 236 (1949).
70. I. Reich and R. D. Vold, *J. Phys. Chem.*, **63**, 1497 (1959).
71. J. C. Harris, *Textile Res. J.*, **18**, 669 (1948).
72. J. C. Harris, *Soap Chem. Spec.*, **34**(11), 40 (1958).
73. J. C. Harris, *Soap Chem. Spec.*, **34**(12), 59 (1958).
74. J. C. Harris, *Soap Chem. Spec.*, **35**(2), 57 (1959).
75. A. S. Weatherburn, G. R. F. Rose, and C. H. Bayley, *Can. J. Res.*, **27F**, 179 (1949).
76. A. S. Weatherburn, G. R. F. Rose, and C. H. Bayley, *Can. J. Res.*, **27**, 426 (1949).
77. A. S. Weatherburn and C. H. Bayley, *Textile Res. J.*, **22**, 797 (1952).
78. K. W. Gardiner and L. B. Smith, *J. Amer. Oil Chem. Soc.*, **26**, 194 (1949).
79. L. H. Flett, L. F. Hoyt, and J. Walter, *Am. Dyestuff Rep.*, **41**, 139 (1952).
80. W. A. Fessler, *Am. Soc. Test. Mater. Spec. Tech. Publ.*, **115**, 9 (1951).

81. A. L. Meader, Jr., and B. A. Fries, *Ind. Eng. Chem.*, **44**, 1636 (1952).
82. K. McLaren, *J. Soc. Dyers Colour*, **66**, 521 (1950).
83. J. M. Lambert, *Ind. Eng. Chem.*, **42**, 1394 (1950).
84. J. Tuzson and B. A. Short, *Textile Res. J.*, **30**, 983 (1960).
85. B. A. Short, in Ref. 48, pp. 237–238.
86. B. Derjaguin, *Discuss. Faraday Soc.*, **18**, 85 (1954).
87. B. Derjaguin and L. Landau, *Acta Physicochim. USSR*, **14**, 633 (1941).
88. E. J. W. Verwey and J. T. G. Overbeek, *Theory of the Stability of Lyophobic Colloids*, Elsevier Publishing Co., Amsterdam, 1948.
89. J. C. Harris, *Textile Res. J.*, **28**, 912 (Nov. 1958).
90. K. Durham, *J. Appl. Chem.*, **6**, 153 (1956).
91. K. Durham, in Ref. 57, p. 60.
92. H. Lange, *Kolloid-Z.*, **154**, 103 (1957).
93. H. Lange, *Kolloid-Z.*, **156**, 108 (1958).
94. H. Lange, *Am. Dyest. Rep.*, **50**(12), 25 (1961).
95. J. Ross, P. T. Vitale, and A. M. Schwartz, *J. Amer. Oil Chem. Soc.*, **32**, 200 (1955).
96. P. T. Vitale, *J. Amer. Oil Chem. Soc.*, **31**, 341 (1954).
97. A. S. Porter, in Ref. 54, p. 103.
98. W. J. Schwarz, A. R. Martin, and R. C. Davis, *Textile Res. J.*, **32**, 1 (1962).
99. B. J. Rutkowski and A. R. Martin, *Textile Res. J.*, **31**, 892 (1961).
100. A. Bierman, *J. Colloid Sci.*, **10**, 231 (1955).
101. O. F. Devereux and P. L. De Bruyn, *Interaction of Plane Parallel Double Layers*, MIT Press, Cambridge, Massachusetts, 1963.
102. H. Lange, *J. Phys. Chem.*, **64**, 538 (May 1960).
103. H. Lange, *Kolloid-Z.*, **169**, 124 (1960).
104. G. S. Hattiangdi, W. W. Walton, and J. I. Hoffman, *J. Res. Natl. Bur. Stand.*, **42**, 361 (1949).
105. A. M. Mankowich, *J. Amer. Oil Chem. Soc.*, **39**, 206 (1962).
106. R. Mallikarjun and D. B. Dadyburjor, *J. Colloid Int. Sci.*, **84**, 73 (1981).
107. H. L. Rosano and M. Clausse, *Microemulsion Systems*, Marcel Dekker, New York, 1987.
108. R. Zana, *Surfactant Solution*, Marcel Dekker, New York, 1977.
109. A. S. C. Lawrence, *Nature*, **183**, 1491 (1959).
110. E. I. Franses and co-workers, *J. Phys. Chem.*, **874**, 1547 (1980).
111. J. M. Corkill, J. F. Goodman, and J. R. Tate, *Trans. Faraday Soc.*, **62**, 979 (1966).
112. N. A. Klimenko and A. M. Koganovskii, *Kolloidn. Zh.*, **36**, 135 (1974).
113. N. A. Klimenko, A. A. Tryasorokova, and A. A. Permilovskaya, *Kolloidn. Zh.*, **36**, 624 (1974).
114. N. A. Klimenko, A. A. Permilovskaya, and A. M. Koganovskii, *Kolloidn. Zh.*, **36**, 728 (1974).
115. N. A. Klimenko, A. A. Permilovskaya, and A. M. Koganovskii, *Kolloidn. Zh.*, **37**, 870 (1975).
116. N. A. Klimenko and co-workers. *Kolloidn. Zh.*, **37**, 873 (1975).

117. L. I. Osipow and F. D. Snell, *J. Amer. Oil Chem. Soc.*, **38**, 184 (1961).
118. F. L. Diehl and J. B. Crowe, *J. Amer. Oil Chem. Soc.*, **31**, 404 (1954).
119. E. Goette, *Melliand Textilber.*, **34**, 534 (1954).
120. C. Z. Draves and O. L. Sherburne, *Am. Dyestuff Rep.*, **9**, P771 (1950).
121. H. L. Sanders and J. L. Lambert, *J. Amer. Oil Chem. Soc.*, **27**, 153 (1950).
122. R. E. Wagg, in Ref. 54, pp. 35–42.
123. A. R. Martin and R. C. Davis, *Soap Chem. Spec.*, **36**(4), 49 (1960).
124. A. R. Martin and R. C. Davis, *Soap Chem. Spec.*, **36**(5), 73 (1960).
125. O. C. Bacon and J. E. Smith, *Ind. Eng. Chem.*, **40**, 2361 (1948).
126. J. C. Harris, M. R. Sullivan, and L. E. Weeks, *Ind. Eng. Chem.*, **46**, 1942 (1954).
127. I. Reich, F. D. Snell, and L. I. Osipow, *Ind. Eng. Chem.*, **45**, 137 (1953).
128. B. E. Gordon and E. L. Bustin, *J. Amer. Oil Chem. Soc.*, **45**, 754 (1968).
129. B. E. Gordon and W. T. Shebs, *J. Amer. Oil Chem. Soc.*, **46**, 537 (1969).
130. J. C. Illman and co-workers, *J. Amer. Oil Chem. Soc.*, **47**, 379 (1970).
131. A. M. Schwartz and J. Berch, *Soap Chem. Spec.*, **39**(5), 78 (1963).
132. E. H. Armbruster and G. M. Ridenour, *Soap Chem. Spec.*, **31**(7), 47 (1955).
133. G. Segura and co-workers, *Chem. Ind. (London)*, 1270 (1953).
134. W. G. Mizuno, F. T. Lanners, and J. L. Wilson, *J. Amer. Oil Chem. Soc.*, **32**, 437 (1955).
135. A. Segesser and H. Stupel, *Seifen Oele Fette Wachse*, **80**, 686 (1954).
136. G. R. Edwards and H. Stüpel, paper presented at the 4th International Congress on Surface-Active Agents, Brussels, Sept. 1964, Technical Bulletin SC:365–80, Shell Chemical Co., Houston, Texas, 1980.
137. L. K. Bradacs and W. Schetelig, *J. Amer. Oil Chem. Soc.*, **41**, 161 (1964).
138. L. E. Weeks, J. C. Harris, and E. L. Brown, *J. Amer. Oil Chem. Soc.*, **31**, 254 (1954).
139. A. L. Kimmel, H. M. Gadbury, and D. O. Darby, *Soap Chem. Spec.*, **37**, 51 (1961).
140. J. C. Harris, W. Stericker, and S. Spring, *ASTM Bull.*, **204**, 31 (1955).
141. E. A. Saubestre in M. J. Schick, ed., *Surfactant Science*, vol. 1, Marcel Dekker, New York, 1967, ch. 19.
142. F. H. Firsching and H. E. Everson, *J. Amer. Oil Chem. Soc.*, **34**, 547 (1957).
143. CSMA Designation DCC-04, Chemical Specialties Manufacturers' Association, Washington, D.C., July 1973.
144. R. R. Adler, R. B. Albin, and B. M. Finger, *J. Amer. Oil Chem. Soc.*, **46**, 520 (1969).
145. A. Davidsohn, *J. Amer. Oil Chem. Soc.*, **55**, 134 (1978).
146. J. Kretschmann, H. Latka, and H. Reuter, *Waschmittelchemie*, Henkel & Cie, Heidelberg, 1976, pp. 235–252.
147. S. A. Kuti, *J. Amer. Oil Chem. Soc.*, **55**, 141 (1978).
148. R. J. Fuchs, J. M. Polkowski, and P. P. Carfagno, *Chem. Times Trends*, 37 (1977).
149. F. G. Longman, *The Analysis of Detergents and Detergent Products*, John Wiley & Sons, New York, 1975.
150. M. J. Rosen and H. Goldsmith, *Systematic Analysis of Surface-Active Agents*, 2nd ed., Wiley-Interscience, New York, 1972.

151. J. Kross, in M. J. Schick, ed., *Surfactant Science Series*, vol. 8, Marcel Dekker, New York, 1975.
152. J. G. Karabinos, G. E. Kapella, and G. E. Bartels, *Soap Chem. Spec.*, **6**, 30 and 41 (1954).
153. K. Burger, *Z. Anal. Chem.*, **196**, 22 (1963).
154. L. E. Weeks, M. E. Ginn, and G. E. Baker, *Proc. Chem. Specialties Mfrs. Assoc.*, 150 (1957).
155. *Standard Methods for the Examination of Water and Wastewater*, 14th ed., American Public Health Association, New York, 1975.
156. R. A. Llenado, *Household Personal Products Industry*, **18**, 69, 71, 73 (1981).
157. H. G. Nadeau and S. Siggia, in Ref. 141, pp. 422–431.
158. *Human Safety and Environmental Aspects of Major Surfactants*, NTIS document PB 301193, Soap and Detergent Association, Arthur D. Little, Inc., Cambridge, Massachusetts, 1977.
159. Ref. 142, supplement, NTIS document PB 1182452, 1981.
160. A. R. Temple, *Detergents: In Depth '78*, Soap and Detergent Association, New York, 1978, pp. 36–41.
161. *Cleaning Products and Their Accidental Ingestion*, Scientific and Technical Report No. 5R, Soap and Detergent Association, New York, 1980, p. 9.
162. Ref. 161, p. 10.
163. F. N. Marzulli and H. I. Maibach, *Advances in Modern Toxicology*, vol. 4, John Wiley & Sons, New York, 1977.
164. *Food, Drug, and Cosmetic Law Reports*, 233, 440, and 476.
165. Ref. 163, p. 440.
166. A. H. Gilbert, *J. Amer. Oil Chem. Soc.*, **58**, 66A (1981).
167. Ref. 163, Chapt. 14.
168. H. North-Root, Ref. 62, p. 32.
169. J. Longwell and W. D. Maniece, *Analyst*, **80**, 685 (1955).
170. S. L. Boyer and co-workers, *Environ. Sci. Technol.*, **11**, 1167 (1977).
171. P. J. Weaver, *Soap Chem. Spec.*, **41**, 45 (1965).
172. Subcommittee on Biodegradation Test Methods, *J. Amer. Oil Chem. Soc.*, **42**, 989 (1965).
173. Ref. 172, p. 990.
174. C. A. Vath, *Soap Chem. Spec.*, **40**(2), 56 and 182 (1964).
175. C. A. Vath, *Soap Chem. Spec.*, **40**(3), 55 and 108 (1964).
176. R. D. Swisher, *Surfactant Science Series*, Vol. 3, Marcel Dekker, Inc., New York, 1970.
177. R. J. Larson and G. E. Wentler, *Soap Cosmet. Chem. Spec.*, **58**(5), 33 (1982).
178. R. L. Huddleston and A. M. Nielson, *Soap Cosmet. Chem. Spec.*, **55**, 34, 36, 37, 44 (1979).
179. *C and E News*, **70**, 36 (1992).
180. H. O. Bouveng, *J. Amer. Oil Chem. Soc.*, **55**, 178 (1978).
181. V. Lamberti and M. Konart (to Lever Brothers Co.), U.S. Patent 3,635,830, January 18, 1972.
182. M. M. Crutchfield, *J. Amer. Oil Chem. Soc.*, **55**, 58 (1978).

183. H. C. Kemper et al., *Tenside*, **12**, 47 (1975).  
 184. D. Mitchell and J. C. Buzzell, Jr., *J. Sanit. Eng.*, (8333), 453–465 (1971).

## GENERAL REFERENCES

- A Handbook of Industry Terms*, Soap and Detergent Assoc., New York, 1981.
- Adamson, A. W., *Physical Chemistry of Surfaces*, 4th ed., John Wiley & Sons, New York, 1982.
- Baldwin, A. R., ed., *Proceedings of the Second World Conference on Detergents: Looking Toward the 90's*, Am. Oil Chem. Soc., Chicago, 1987.
- Balsam, M., and E. Sagarin, eds., *Cosmetics, Science and Technology*, vols. 1–3, 2nd ed., Wiley-Interscience, New York, 1972, 1974.
- Becher, P., *Emulsions: Theory and Practice*, 2nd ed., Reinhold Publishing Corp., New York, 1977.
- Bluestein, B. R., and C. L. Hilton, eds., *Amphoteric Surfactants*, *Surfactant Science Series*, vol. 12, Marcel Dekker, Inc., New York, 1982.
- Cahn, A., ed., *Proceedings of the 3rd World Conference on Detergents: Global Perspectives*, AOCS Press, Champaign, Illinois, 1994.
- Cahn, A., and J. L. Lynn, Jr., in *Encyclopedia of Chemical Technology*, vol. 22, 3rd ed., 1983, pp. 332–432.
- Carroll, B. J., in E. Matijevic, ed., *Surface and Colloid Science*, vol. 9, John Wiley & Sons, New York, 1976.
- Cross, J., ed., *Anionic Surfactants*, *Chemical Analysis, Surfactant Science Series*, vol. 8, Marcel Dekker, New York, 1977.
- Cross, J., ed., *Nonionic Surfactants: Chemical Analysis, Surfactant Science Series*, vol. 19, Marcel Dekker, New York, 1987.
- Cross, J., and E. J. Singer, *Cationic Surfactants: Analytical and Biological Evaluation, Surfactant Science Series*, vol. 53, Marcel Dekker, New York, 1994.
- CSMA Detergents Division, *Test Methods Compendium*, 2nd ed., CSMA, Inc., Washington, D.C., 1985.
- Cutler, W., and R. C. Davis, eds., *Detergency: Theory and Test Methods, Surfactant Science Series*, vol. 5, Marcel Dekker, New York, 1972.
- Cutler, W. G., and E. Kissa, eds., *Detergency: Theory and Technology, Surfactant Science Series*, vol. 20, Marcel Dekker, New York, 1987.
- Detergents: In Depth, '89*, Soap and Detergent Assoc., New York, 1989.
- Falbe, J., ed., *Surfactants in Consumer Products: Theory, Technology, and Application*, Springer-Verlag, New York, 1987.
- Friberg, S. E., and B. Lindman, *Organized Solutions: Surfactants in Science and Technology, Surfactant Science Series*, vol. 44, Marcel Dekker, New York, 1992.
- Garrett, P. R., *Defoaming: Theory and Industrial Applications, Surfactant Science Series*, vol. 45, Marcel Dekker, New York, 1993.
- Gloxhuber, C., and K. Kunstler, eds., *Anionic Surfactants: Biochemistry, Toxicology, Dermatology, Surfactant Science Series*, vol. 43, Marcel Dekker, New York, 1992.

- Hiemenz, P., *Principles of Colloid and Surface Chemistry*, Marcel Dekker, New York, 1977.
- Huthig, A., *Waschmittelchemie*, Henkel & Cie, Heidelberg, 1978.
- Jungerman, E., ed., *Cationic Surfactants, Surfactant Science Series*, vol. 4, Marcel Dekker, New York, 1970.
- Kitahara, A., and W. Watanabe, eds., *Electrical Phenomena at Interfaces: Fundamentals, Measurements, and Applications, Surfactant Science Series*, vol. 15, Marcel Dekker, New York, 1984.
- Linfield, W. M., ed., *Anionic Surfactants: Surfactant Science Series*, vol. 7, Marcel Dekker, New York, 1976.
- Lucassen-Reynders, E. H., ed., *Anionic Surfactants: Physical Chemistry of Surfactant Actions, Surfactant Science Series*, vol. 11, Marcel Dekker, Basel, 1981.
- McCutcheon's Emulsifiers & Detergents*, North Am. & International Ed., Glen Rock, New Jersey, 1994.
- McCutcheon's Emulsifiers & Functional Materials, Detergents*, North Am. & International Ed., Glen Rock, New Jersey, 1994.
- Miller, C. A., and P. Neogi, eds., *Interfacial Phenomena: Equilibrium and Dynamic Effects, Surfactant Science Series*, vol. 17, Marcel Dekker, New York, 1985.
- Milwidsky, B. M., and D. M. Gabriel, *Detergent Analysis: A Handbook for Cost-Effective Quality Control*, Micelle Pr., Cranford, New Jersey, 1989.
- Mittal, K. L., ed., *Colloidal Dispersions and Micellar Behavior*, ACS Symposium Series 9, Am. Chem. Soc., Washington, D.C., 1975.
- Mittal, K. L., ed. *Micellization, Solubilization, and Microemulsions*, vols. 1 and 2, Plenum Press, New York, 1977.
- Mittal, K. L., ed., *Solution Chemistry of Surfactants*, vols. 1 and 2, Plenum Press, New York, 1979.
- Mittal, K. L., and B. Lindman, eds., *Surfactants in Solution*, vols. 1–3, Plenum Press, New York, 1984; Vols. 4–6, 1987; Vols. 7–10, 1990.
- Mittal, K. L., and D. O. Shah, *Surfactants in Solution*, vol. 11, Plenum Press, New York, 1992.
- Myers, D., *Surfactant Science & Technology*, VCH, New York, 1988.
- Piirma, I., ed., *Polymeric Surfactants, Surfactant Science Series*, vol. 42, Marcel Dekker, New York, 1992.
- Richmond, J. M., ed., *Cationic Surfactants: Organic Chemistry, Surfactant Science Series*, vol. 34, Marcel Dekker, New York, 1990.
- Rosen, M. J., ed., *Structure/Performance Relationships in Surfactants*, Am. Chem. Soc., Washington, D.C., 1984.
- Rosen, M. J., *Surfactants and Interfacial Phenomena*, 2nd ed., Wiley-Interscience, New York, 1989.
- Rubingh, D. N., and P. M. Holland, eds., *Cationic Surfactants: Physical Chemistry, Surfactant Science Series*, vol. 37, Marcel Dekker, New York, 1991.
- Schick, M. J., ed., *Nonionic Surfactants, Surfactant Science Series*, vol. 1, Marcel Dekker, New York, 1966.
- Schick, M. J., ed., *Nonionic Surfactants: Physical Chemistry, Surfactant Science Series*, vol. 23, Marcel Dekker, New York, 1987.



- Schmitt, T. M., ed., *Analysis of Surfactants, Surfactant Science Series*, vol. 40, Marcel Dekker, New York, 1992.
- Schwartz, A. M., in E. Matijevic, ed., *Surface and Colloid Science*, vol. 5, John Wiley & Sons, New York, 1972.
- Schwartz, A. M., and J. W. Perry, *Surface Active Agents: Their Chemistry and Technology*, Krieger, Huntington, New York, 1978.
- Schwartz, A. M., J. W. Perry, and J. Berch, *Surface Active Agents and Detergents*, vol. 2, Interscience Publishers, New York, 1958.
- Shaw, D. J., *Introduction to Colloid and Surface Chemistry*, 3rd ed., Butterworth, London, 1981.
- Shinoda, K., ed., *Solvent Properties of Surfactant Solutions, Surfactant Science Series*, Vol 2, Marcel Dekker, New York, 1967.
- Swisher, R. D., ed., *Surfactant Biodegradation, Surfactant Science Series*, vol. 3, Marcel Dekker, New York, 1970.
- Swisher, R. D., ed., *Surfactant Biodegradation, Surfactant Science Series*, vol. 18, 2nd ed., Marcel Dekker, New York, 1987.
- Tadros, T. F., *Surfactants*, Academic Press, London, 1984.
- Zana, R., ed., *Surfactant Solutions: New Methods of Investigation, Surfactant Science Series*, vol. 22, Marcel Dekker, New York, 1986.

# 5

## Glycerine

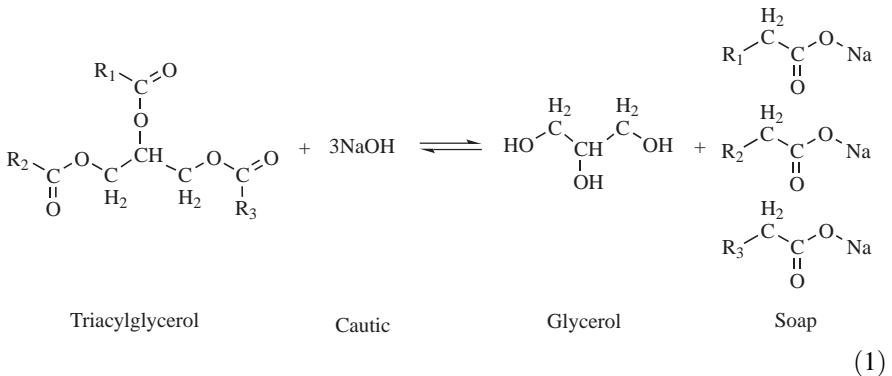
Keith Schroeder  
CC Engineering, Ltd.

### 1. INTRODUCTION

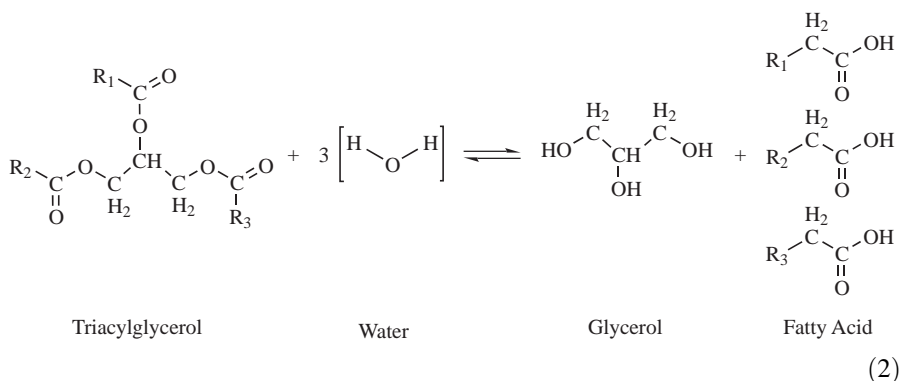
#### 1.1. Chemistry

Glycerine (sometimes called “glycerin”) is the name of the commercial product consisting of glycerol and a small amount of water. Glycerol is actually trihydric alcohol  $C_2H_5(OH)_3$ , which is more accurately named 1,2,3-propanetriol. Its chemical structure is shown in the formulas given below.

Much of the naturally produced (as opposed to synthetic) glycerine is a coproduct of saponification of fats using caustic soda. The reaction is (1)



The resulting coproduct stream, called spent lye, typically contains 10–25% glycerol (depending on the production process) as well as contaminants such as salt (NaCl), water, and various organics usually known as matter organic nonglycerol (MONG). Probably the more abundant source of glycerol is the hydrolysis (splitting) of fats and oils, which produces fatty acid and glycerol. This reaction is (1)



The concentration of glycerol in the resulting sweetwater is 10–18% and gets its name from the characteristic sweet taste imparted by the glycerine (2). Glycerin is also recovered as the coproduct of methyl esters (from fats and oils) and fatty alkanolamides; the amount produced from these sources has historically been relatively small but has increased significantly due to worldwide construction of fatty alcohol plants and, more recently, methyl ester biodiesel plants. There are some other interesting sources of glycerol, which will be discussed below. This Chapter concentrates on the recovery of glycerol from soap lyes (water and glycerol from soap making) and sweetwaters, as they represent the majority of the recovery applications. Synthetic glycerine will not be covered, as it is not derived from fats and oils.

## 1.2. History of Glycerine Processing

Glycerine was first identified in 1770 by Scheele, who produced it by heating olive oil and litharge. In 1784, he observed that the same substance could be produced from other vegetable oils and animal fats, such as lard and butter. He called this new substance “the sweet principle of fats” due to glycerine’s characteristic sweet taste (3). In 1811, Chevreul, while studying Scheele’s Sweet, as it had come to be called, coined the modern name glycerine from the Greek word *glyceros*, meaning “sweet.” After closely studying glycerol, he was awarded the first patent relating to its manufacture in 1823. Chevreul also distinguished himself by doing some important early research work on fats and soaps. By 1836, Pelouze had determined the formula for glycerol, and finally Berthlot and Luce published the structural formula in 1883 (4).

Nitroglycerine was discovered in 1847 by Sobrero. This compound is dangerously unstable, which limited its potential for commercial applications. In 1863,

Alfred Nobel demonstrated nitroglycerine's explosive capabilities, and in 1866 he invented dynamite. He followed that discovery by the invention of blasting gelatin, a mixture of nitroglycerine and nitrocellulose, in 1875. Nobel's commercial success and humanitarian efforts are well known, but equally important was the vital role his inventions played in advancing the Industrial Revolution. The demand for his explosive products helped to form a large and growing demand for glycerine.

The history of glycerine is closely related to the history of soap making because one of the earliest commercial sources of glycerine was recovery from soap lyes, and soap lyes continue to be a common feed stock for glycerine recovery today. In the early 1870's, the first U.S. patent "for the recovery of glycerine from soap lyes by distillation" was issued. The process was further developed by Runcorn in 1883. In the decades following, the soap industry began recovering glycerine from the "waste streams" of their soap-making operations on a relatively large scale, thus making glycerine a readily available commodity.

A prominent source of glycerine is from the sweetwaters of fat splitting, which initially came from the manufacture of stearine for candle making (3). The famous Twitchell process for fat splitting was developed at the turn of the century. Twitchell developed a process for splitting fat using a catalyst and dilute sulfuric acid that produced an acceptable product (5). This was followed by high-pressure autoclave splitting, which relied on high-pressure steam for hydrolysis of fat and produced a superior product. Today's modern fat-splitting plants, using stainless steel columns with counter current flow of fatty acid and sweetwater, are the latest development in the splitting process. The high-quality sweetwaters obtained allow efficient refining into the high-purity grades of glycerine used today.

### 1.3. Economics

The consumption of glycerine has remained strong over the years, and trends indicate that this is not likely to end soon. Although glycerine is a mature product with a multitude of uses that continue to increase, the Soap and Detergent Association is trying to develop new uses for the product. In 2003, the market price of natural USP glycerine in the United States was at \$0.55–0.75/lb., but has been as low as \$0.25/lb. and as high as \$1.00/lb. throughout the 1990's. The U.S. production of glycerine is approximately 600 million pounds (270 million kg) per year and grows 2–5% annually. Imports add about 10% more supply, while only a small amount is exported. The world glycerine production is well over one billion pounds (450 million kg) per year (6). Many new fatty acid splitting facilities have come online, especially in Asia, where palm and coconut plantations are plentiful. China continues to add capacity as more products come into demand.

Synthetic glycerine is produced in the United States by a single supplier. There are only three producers worldwide. The strong supply of natural glycerine and the rising cost of feedstock (propylene) are likely to pressure producers to revise their production. Also, some epichlorohydrin, an intermediate product, can be diverted into other products of potentially high profitability. Synthetic glycerine may contain

small quantities of chlorinated hydrocarbons, which has caused some concern in the health care applications.

## 2. PROCESSING PRINCIPALS AND DETAILS

### 2.1. Sources of Glycerine

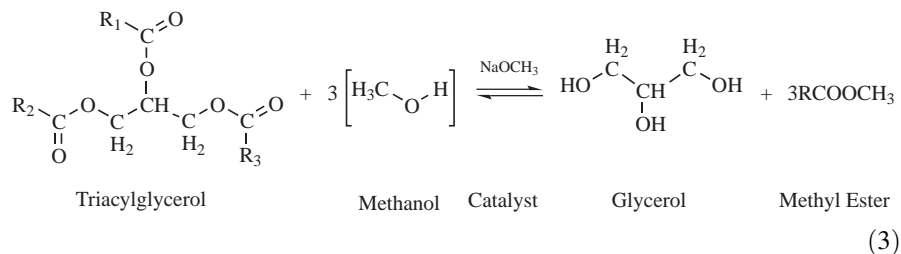
Natural glycerine is essentially a coproduct of certain processes performed on animal or vegetable fats and oils:

1. Fat splitting under high pressure and temperature, in the presence of water, to obtain fatty acids and sweetwater glycerine. The fat-splitting process provides the majority of glycerine in the United States. Processing of splitter "sweet-water" requires less intensive processing and lower equipment costs because salt content is not high and common stainless steels can be used as the material of construction.
2. Saponification of fats with sodium hydroxide (caustic soda, NaOH), yielding spent lyes containing glycerol, water, sodium chloride (NaCl), and other impurities; depending on the process for saponification and soap washing, the concentration of the glycerol varies greatly. The presence of high salt levels requires higher cost metallurgies as is discussed later in this chapter.

The chemical reactions for these processes were given earlier in formulas 1 and 2.

3. Transesterification of fats, typically with methanol, using sodium methoxide as a catalyst, to produce methyl esters and glycerol (7).

The chemical reaction for this process is as follows:



Although the initial chemical reactions for the above processes yield high theoretical concentrations of glycerol, subsequent washing steps dilute the glycerol to typical values:

Fat Splitting	10–18%
Saponification, kettle	10–15%
Saponification, continuous	20–25%
Transesterification	25–30%

Synthetic glycerine is produced (Dow process) by chlorinating propylene to allyl chloride, converting this to dichlorohydrin, which is then converted to glycerine

through the addition of small amounts of dilute sodium hydroxide and sodium carbonate (8).

## 2.2. Pretreatment Techniques

The purpose for treatment of spent soap lyes or sweetwater is to prepare the feedstock for glycerine recovery by removing impurities that cause operating problems in subsequent processing steps, resulting in a poor-quality product. In the handling of dilute glycerol feedstocks, care must be taken to prevent fermentation, which can cause serious loss of glycerine due to the formation of trimethylene glycol (TMG), (1,3-propyleneglycol), gases, and acids during decomposition. Fermentation can occur in glycerol solutions of 25% by wt. held at temperatures of 125°F or less. Glycerol solutions above 25% prevent bacterial growth as does the presence of salt or caustic. Furthermore, these gases produced by the bacteria may cause problems in evaporation of the lyes, and any TMG can be difficult to separate from the glycerine by distillation due to their similar vapor-pressure characteristics. Excess impurities in soap lye crudes can act as nucleation sites for crystal growth in the evaporator, and aid the growth of larger, more easily separated salt crystals.

Spent soap lyes, as they are drawn from the continuous saponification area or soap kettles, consist primarily of glycerine, sodium chloride, and water as well as small quantities of sodium hydroxide, sodium sulfate, sodium carbonate, soap, and fatty acids, and also some albuminous and oleaginous matter. To remove these impurities, the soap lye is generally treated in batches.

First, a cooling and settling step is used to separate the soaps and fatty acids. The fatty acids are less soluble at lower temperatures and rise to the surface, where they can be removed by skimming. Other heavy materials will settle to the bottom and remain in the tank until it is cleaned periodically. This skimming of the fatty acid is best done in the soap plant before the lyes are transferred to the treatment plant (Figure 1).

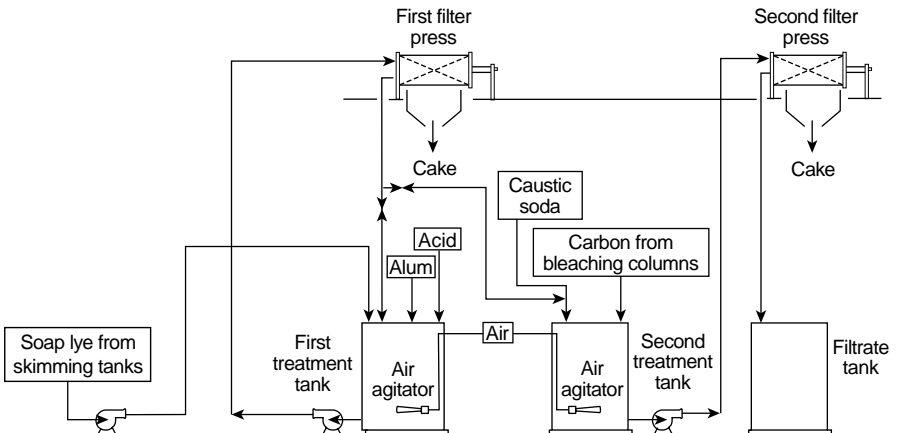
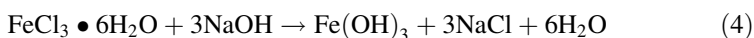


Figure 1. Batch soap lye treatment flowsheet. Courtesy of Crown Iron Works Co. (9).

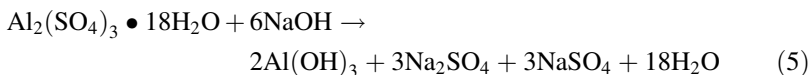
Second, the skimmed and settled lye is transferred to the first treatment tank. It is good practice to heat the lye to 50–60°C during the transfer pumping or in the treatment tank; the treatment process is most efficient at this temperature. In the first tank, the lye is acidulated to pH 4.6–5.0 with sulfuric or hydrochloric acid. Hydrochloric acid is recommended to avoid the formation of sulfates. These sulfates can build up in the recovered salt, which is returned to the soap-making area where sulfates are not desirable. This acidulation step liberates fatty acids, which may be skimmed, as most are insoluble in water.

Third, a coagulant may be added to cause the residual fatty acids to form insoluble soaps, which coagulate. The pH is adjusted to 4.5. At the same time, compressed air is bubbled into the tank and is used for mixing and to ensure oxidation. The coagulant is usually either aluminum sulfate or ferric chloride. If ferric chloride is used, the air agitation is particularly needed to oxidize the iron to its ferric state (10). Although ferric chloride is more difficult to handle than aluminum sulfate, it is strongly corrosive to iron, and is more expensive, but is usually preferred because the salts formed using aluminum sulfate introduce additional sulfates. The precipitates of either aluminum hydroxide or ferric hydroxide will adsorb other impurities such as fatty acids or proteinaceous matter. The chemical reactions for these two precipitants neutralizing the excess sodium hydroxide are described below.

It takes 1 kg of ferric chloride to neutralize approximately 0.44 kg of sodium hydroxide, according to the following formula:



Exactly 0.36 kg of sodium hydroxide is required to neutralize 1 kg of aluminum sulfate according to the following reaction:



The composition of the first treatment can be checked by filtering a sample in the laboratory and then adding a small amount of dilute ferric chloride or aluminum sulfate to the filtrate. Any precipitate indicates that the reaction has not been taken to completion (11–13). The contents of the first treatment tank are then filtered, typically in an open plate and frame filter. The filter cake is discharged periodically for disposal. Filter aid can be added, if required.

Finally, the filtrate from the first press flows to the second treatment tank, where sodium hydroxide is used to adjust the pH to approximately 9.0. Compressed air is again used to agitate and oxidize the batch, before filtration through a second press. A smaller amount of precipitate is produced in this step, and therefore the filter can be sized for less area. In addition, spent bleaching carbon from the glycerine distillation plant can be added at this point, with the goal of recovering some residual glycerine into the treated lye.

Treatment can also be carried out using a continuous or semicontinuous operation. In each case, accurate, in-line pH measurement is needed to control the addition of the treatment chemicals. Filtration can be accomplished on a semicontinuous basis using plate and frame filters by sizing the treatment tanks to allow surge capacity for filter cleanout intervals, or a continuous vacuum drum filter can be used. These processes lend themselves to the large capacity plants.

Treatment of sweetwaters is less involved than the treatment of soap lyes as described above. The purpose of treating sweetwaters is to remove any unreacted fats and to prevent bacteriological growth in the glycerine. Typically, the sweetwater is settled for several hours to allow any free fats and fatty acids to rise to the surface, where they are skimmed off the top. Then, using air agitation, an excess of lime is added to neutralize the fatty acids still present. Excess lime is calculated as 0.23% of calcium oxide, and the batch must be adjusted appropriately to obtain this excess (1). The sweetwater is then filtered. Sodium carbonate is sometimes used to remove the calcium excess as carbonate. Some processes treat using barium chloride and sulfuric acid, which is a more effective and expensive process. Also, it has been shown that addition of a small amount of polyelectrolyte, such as used in water treatment, will significantly improve the quality of the crude glycerine (10).

An alternative method for treating sweetwater, implemented in many facilities, is to settle the sweetwater for approximately 24 h at 80–90°C and a pH of 4–5. Phosphoric acid is sometimes used to help break any emulsion, but this is not always required. The fats and fatty acids are decanted from the top of the sweetwater and returned to the splitter feed for recycle.

The settled sweetwater is then sent to the evaporators for concentration. This alternative method requires two tanks, one for settling and one for collecting the sweetwater. The tanks are alternated every 24 h to run continuously.

## 2.3. Evaporation

**2.3.1. Overview** Following treatment, the soap lyes or sweetwaters are concentrated to 80–88% glycerol through an evaporation process. This is an energy-intensive process and, depending on the plants capacity and energy costs, can have either a single-, double-, or even triple-effect evaporator. The primary design consideration is the amount of water to be evaporated. With soap lyes, the secondary consideration is the method for handling the salt that precipitates as the water is evaporated.

The amount of water to be evaporated is calculated from the amount of liquor (soap lye or sweetwater) and its glycerol concentration. Table 1 shows the

**TABLE 1. Example of Concentrations in Soap Lye and Crude Glycerine.**

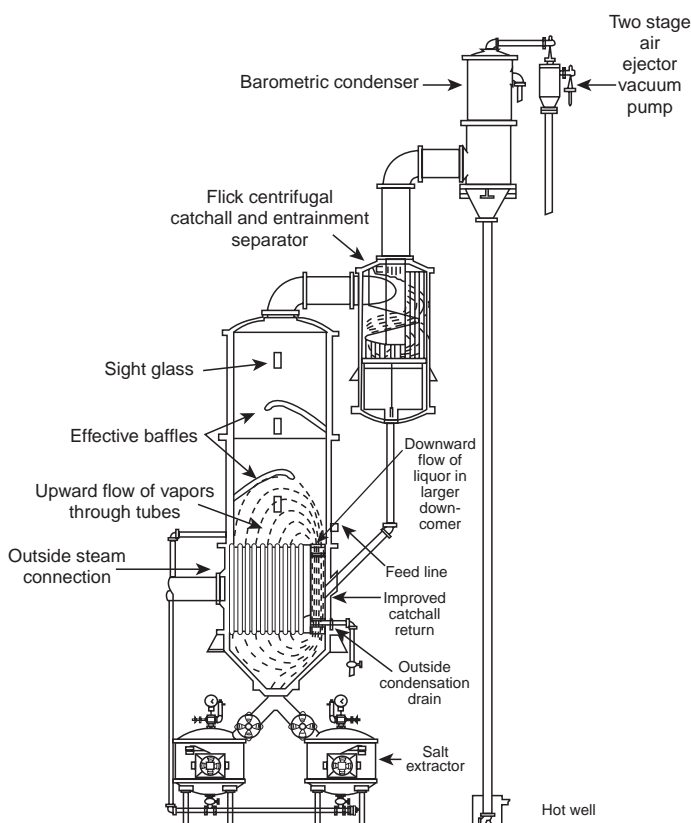
Substance	Percent	Weight (kg)	Percent	Weight (kg)	Change
Glycerol	10	100	80	100	None
Salt	11	110	9	14	96 kg Salt
Water	79	790	11	16	774 kg Water



concentrations that occur, starting with a dilute soap lye and concentrating to 80% crude glycerine. The table does not include losses that occur.

Two general types of evaporators are used, and their names refer to the type of circulation used to transfer heat to the liquor for evaporating the water. Natural circulation evaporators rely on a thermosiphon to circulate liquors while forced circulation units use a pump to achieve the required circulation. The heating tubes may be inside or outside the evaporator body, but most designs, especially the older calandria style evaporators, use internal tubes for heating (Figure 2).

Forced circulation heating is used for most new evaporation plants for several reasons. Primarily, pumping the liquor allows the selection of a higher flowrate and velocity than can be achieved by natural convection, which improves heat transfer, thus reducing the surface area required for heating and evaporation of water. Higher velocities in the tubes also reduce fouling and scaling of the tube surfaces. One drawback to forced circulation is the increase in energy consumption from pump motors. In addition, if the velocity is too high, there can be problems with crystallization and crystal growth when processing soap lye.



**Figure 2.** Calandria-style single-effect evaporator with flick separator. Courtesy of Crown Iron Works Co. (9).

Evaporation can be either single effect or multiple effect, depending on the number of evaporation stages used. Single-effect evaporation offers the simplest process and minimum equipment investment. In a single-effect evaporator, a batch of dilute glycerine can be evaporated to a half crude state with a concentration of 40–50% glycerol and stored. When sufficient quantities of half crude are available, it is processed to full crude (80% concentration). The steam economy for a single-effect evaporator is 0.8–1.0 kg of water evaporated for each kilogram of steam used. Single-effect evaporators, operating as a semicontinuous unit, as described above, have been used with some frequency in the past, but are now primarily used for small plants because of the economy and convenience of operation inherent in continuous multieffect plants.

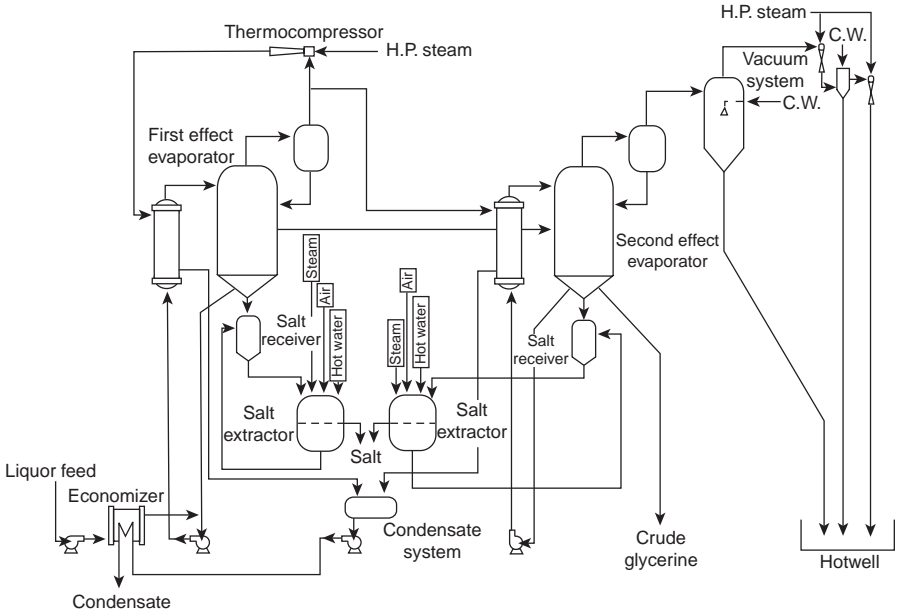
Multiple-effect evaporation is accomplished by joining two or more evaporators in series using the heat in the waste vapor from one effect to heat the subsequent effect. Live steam is added in the first effect only. The difference in the working pressure in each effect (each effect working at a lower pressure than the previous one) allows the use of lower pressure heating steam in the following effects. This pressure difference also allows transfer of liquor from one effect to the other.

As a rough guideline, it can be assumed that approximately 0.8 kg of water can be evaporated per kilogram of steam for each evaporator effect. Thus, one effect will evaporate 0.8 kg of water per kilogram of steam, two effects will evaporate 1.6 kg, and three effects will evaporate 2.4 kg (14).

Multiple-effect evaporators are most often used in glycerine recovery plants of medium to large size, with the typical plant having a two-effect evaporator. The user must calculate and compare the savings in steam use for operating additional effects against the additional equipment and maintenance costs as well as space and operational complexity for additional effects.

Additional economy in steam consumption can be obtained through implementation of thermal vapor recompression (TVR). Water vapor from the evaporator is captured and recompressed in a thermocompressor, using high-pressure motive steam, so the vapor can be condensed in the first effect's heat exchanger. The pressure after the thermocompressor is between that of the water vapor and motive steam. Only a portion of the vapor is recompressed in the thermocompressor, with the remaining vapor used to heat the subsequent effect. The thermocompressor can be used on a single-effect evaporator or on the first effect of multieffect units. This equipment favors low  $\Delta T$  in the heat exchanger to minimize the compression ratio. The thermocompression effect will add additional economy equivalent to at least half of an additional evaporation effect at a modest extra cost, typically just a fraction of the cost of an additional effect. Recovered condensate can be economized by using it to preheat incoming liquor (15).

The operation of a two-effect, continuous evaporator is shown in Figure 3. Treated lye or sweetwater is fed continuously and regeneratively heated with condensates coming from the heaters. Water is evaporated in the first evaporation chamber, which is kept at a pressure slightly above atmospheric pressure (4–6 psig), using heat provided by the thermocompressor. The thermocompressor takes part of the vapor from the first-effect heater and recompresses it to increase its temperature



**Figure 3.** Double-effect evaporator with thermocompressor flowsheet. Courtesy of Crown Iron Works Co. (9).

and pressure. The remaining vapors go to heat the second effect. In the first evaporator, about 60% of the water is evaporated (In a three-effect evaporator, the first effect evaporates about 45–50% of the water, with the remaining two effects roughly splitting the amount of water evaporated).

The liquor from the first effect is then fed by gravity and by pressure difference to the second effect, which is under a slight vacuum (115 mm Hg absolute). There, it is concentrated to 80% for soap lyes or up to 88% for sweetwater crude (containing no salt) and then pumped to crude glycerine storage.

As soap lyes are concentrated, the concentration increases until the dissolved salts begin to come out of solution and form crystals. These crystals grow and settle in the bottom of the evaporator, where they are removed as described below. Some salt crystals will drop out in the first effect, but most of the salt will drop in the second effect. The proportions are determined by several factors, including the operation of the evaporators, the characteristics of the soap lye, and the concentrations reached in each effect. The solubility of sodium chloride in glycerol is relatively unaffected by temperature, however.

**2.3.2. Entrainment Separation** Some kind of entrainment separation is necessary to reduce losses of glycerine entrained in the vapor stream from each evaporator. Control of the vapor velocity by proper evaporator and duct sizing will minimize the amount of entrainment. An entrainment separator should be installed at the outlet of each evaporator body, and the glycerol recovered should be returned

to its evaporator. The separator is usually of the cyclonic type, which can be equipped with internal impingement baffles (Flick type). Mesh- or baffle-type separators are possible, but care must be taken to prevent any buildup on the internal components, which would cause excessive pressure drop. Figure 2 shows a Flick separator installed.

As the glycerol concentration increases during evaporation of soap lyes, salt crystals begin to form as the liquor saturates. These salt crystals, as they grow, will tend to sink to the cone in the bottom of the evaporator. Proper treatment and filtration of the soap lye is important to ensure proper crystal growth in this stage because impurities in the soap lye can hamper the growth of crystals. There are several methods to recover the salt from the evaporators, and all of the methods address the need to wash as much glycerol from the salt as practical. Washing is done with water and sometimes lyes, with the purpose of recovering glycerol that is removed with the salt. The wash liquor is subsequently returned to the treatment plant. Obviously, any water that is added must be evaporated, so an economic balance must be achieved between glycerol loss and water to be evaporated.

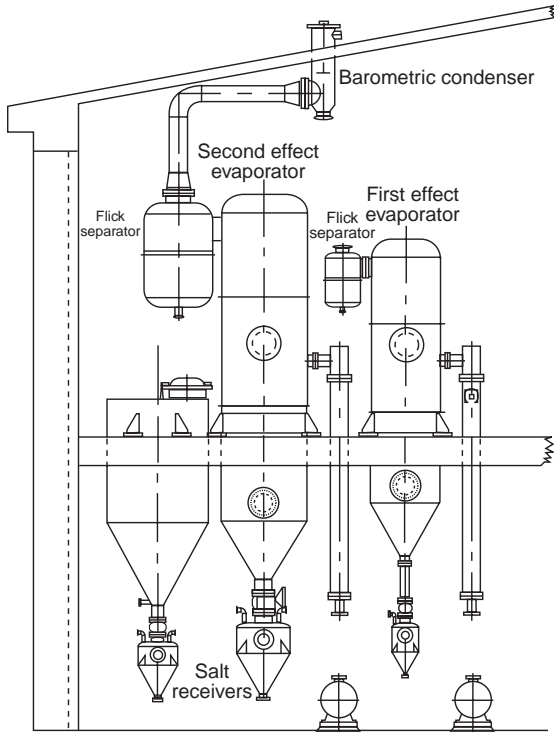
**2.3.3. Salt Removal** All salt removal systems start with the salt crystals (and some glycerol) being removed from the evaporator, through an open valve, and collected in a salt receiver or directly into a salt extractor, depending on the configuration of the plant. If a salt receiver is used, the salt level is allowed to build up to a certain level, determined by observation through a sight glass or by time interval. Then the valve above the receiver is closed, the one below it is opened, and the salt slurry is blown to a salt extractor using compressed air or steam. Salt extractors are usually provided in pairs and used alternatively. The valves are then returned to their original positions for recovering salt. If salt receivers are not used, the salt is dropped directly into the salt extractor, in a similar manner as described above. Salt receivers are placed below each evaporator and under a salt settling tank (Figure 4).

The salt extractor is fitted with a screen to catch the salt and allow the liquor to pass out of the extractor by being returned to the salt receiver from which it originated. Several batches of salt from the receivers are accumulated in a salt extractor before the salt is removed. The salt is then washed to remove glycerol that is contained with the salt. The wash fluid can be soap lye for the first wash and water for subsequent washes. The wash water is returned to the treatment plant.

Some plants remove the salt manually by shoveling it out through a door in the salt extractor. Before this salt is removed, it is dried in the extractor by steaming. Removal of dried salt can be a labor-intensive practice. The salt is usually returned to the soap plant, where it is dissolved into brine to be used again in the soap-making operation.

Other plants will dissolve the salt in the salt extractor. There, after the final wash, water is introduced into the extractor, the salt is dissolved, and the brine is pumped back to the soap plant.

Centrifugal separation can be used to extract and wash the salt obtained in the evaporation process. The centrifuge used for this process can be either a continuous

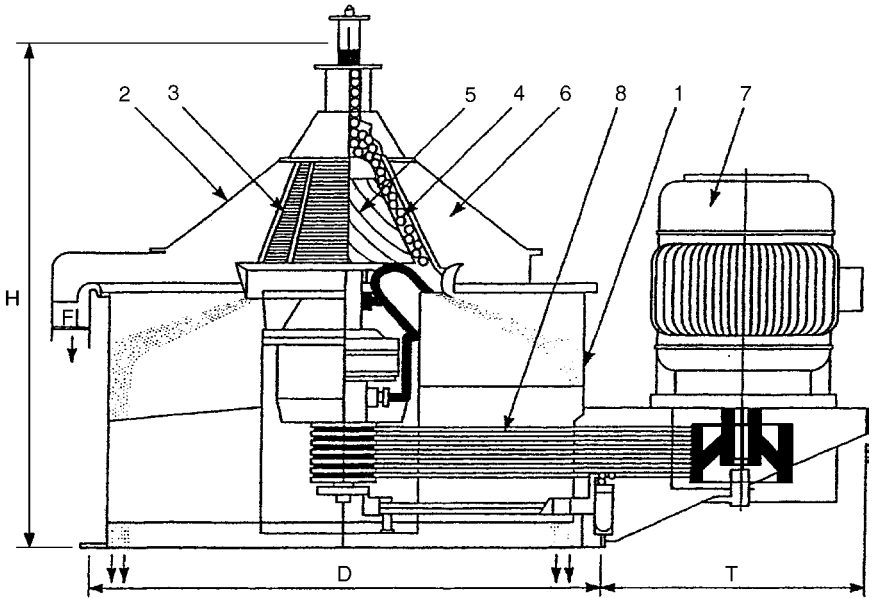


**Figure 4.** Double-effect evaporation with forced circulation and external heating. Courtesy of Crown Iron Works Co. (9).

or a batch operation model. The continuous model typically offers higher capacity and efficiency, but at a higher cost. The salt slurry is fed to the centrifuge in a similar fashion as described above for feeding a salt extractor, but there is typically a slurry tank ahead of the centrifuge.

A batch centrifuge has a basket with a fine screen (or cloth bag), which catches the salt while the liquor is drained back to the evaporation plant. The centrifuge is fed with salt slurry from the evaporators and is spun until the basket is full of salt. Then the salt is washed with treated lye or water to remove glycerol contained with the salt, and then the salt is removed. The salt can be removed by mechanical means or dissolved for reuse in the saponification plant. In the case of the cloth-bag type centrifuge, the entire bag of salt is lifted out of the basket, and a new bag is installed. The salt is then dissolved from the bag in a separate tank, freeing up the centrifuge for salt extraction. Batch centrifuges require a relatively high amount of operator attention.

The continuous centrifuge is fed with salt slurry from the evaporators. Figure 5 shows the main components and operating principles for this type of unit. The salt slurry from the evaporators is continuously fed from the top inlet where it falls between the helicoidal blades of the extractor and the conical basket. The basket



**Figure 5.** Continuous centrifuge operation diagram. 1, Frame; 2, discharge channel; 3, conical basket; 4, screen; 5, helicoidal blades; 6, reduction gear unit; 7, electric motor. Courtesy of Guinard Centrifugation. (16).

has a specially designed, slotted screen installed over it to contain the salt crystals. The slurry experiences a centrifugal load of 300–500 g, due to the spinning of the basket. The liquid passes through the screen and is collected in the discharge channel. The extractor rotates at a positive differential speed relative to the screen, which pushes the salt that has settled toward the bottom of the screen where it is discharged out the bottom of the machine's frame. The salt can be continuously washed while it is on the screen. Some machines have more than one washing zone, allowing the use of different wash liquor concentrations for more efficient washing. The efficiency of salt removal depends on crystal size. For best results, the crystals should be at least 100  $\mu\text{g}$  in size (17).

**2.3.4. Fat Settling and Saponification** For sweetwaters that have been pre-treated by settling alone, the concentrated crude glycerine will require an additional step before the refining step. In this step, any excess fat that has been forced out of the solution during evaporation requires decanting and saponification.

In the crude glycerine settling step, the crude is fed to one of two (or more) tanks. The crude glycerine is maintained at 80–90°C and the fat and fatty acids are allowed to rise to the top of the crude glycerine for a 24-h period. As with the sweetwater settling step, alternate tanks are used in parallel. One tank is for collecting the concentrated crude glycerine and one tank is used for settling the crude glycerine. After settling, the crude glycerine is drawn off to another set of tanks for saponification.

The saponification step involves reacting any fats and fatty acids remaining in the crude concentrated sweetwater with sodium hydroxide. The resulting sodium soap will be removed with the foots, or pitch, that make up the still bottoms.

A sample of crude glycerine is analyzed for saponification (SAP) value using the AOCS Official Method Cd 3-25 analytical test (18). The SAP value is then converted to a sodium hydroxide basis. This number represents the pounds (or kg) of pure sodium hydroxide that must be added to 1000 pounds (or kg) of the crude glycerine. Typically, an excess of sodium hydroxide is added based on the batch size. This excess is usually in the range of 0.1–0.25%. This excess must be minimized because excess sodium hydroxide will generate polyglycerols in the distillation step. Empirical evaluations have shown approximately seven pounds (or kg) of glycerine are lost as polyglycerols for every pound (or kg) of excess sodium hydroxide present in the crude glycerine.

The saponification reaction is carried out at a temperature of 90°C with agitation. This process is typically accomplished on a batch basis using alternate tanks to simultaneously carry out the reaction in one tank and feed the distillation system with reacted crude glycerine from a second tank. The system can be run on a continuous basis with this two (or more) tank system.

**2.3.5. Vacuum System** The vacuum system for the evaporation plant plays a dual role by condensing the water evaporated and providing the required vacuum of approximately 50 mm Hg absolute pressure. The vapor-condensing function and initial vacuum device is either a barometric condenser, using water spray and a barometric leg discharging into a hotwell, or a water-cooled surface condenser. Despite a somewhat higher equipment cost, the surface condenser system offers several significant advantages over a barometric condenser: (1) Any glycerine in the vapor that is condensed is recovered and is not lost into the hotwell water, and, (2) The surface condenser is cooled with clean water, typically circulated from a cooling tower. In a barometric system, the spray water becomes contaminated, requiring a separate cooling tower system from the “clean” cooling water used elsewhere in the plant. The “dirty” cooling tower water must be periodically cleaned or replaced to prevent excess buildup and fouling in the cooling system.

Following the condensing section, and depending on the utility situation at the plant site, either a liquid ring vacuum pump or a two-stage steam jet with an inter-condenser is used. A booster ejector is not required to maintain the vacuum level required for evaporation.

## 2.4. Refining of Glycerin

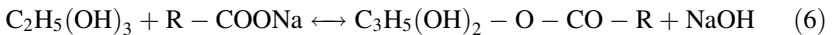
**2.4.1. Distillation** Distillation of glycerine is accomplished through distillation with steam, under high vacuum, and at elevated temperatures. The vapor pressure of glycerine at atmospheric pressure is 760 mm Hg at 290°C, and because glycerol will begin to polymerize at approximately 200°C, distillation must occur at low pressure. When distilling with steam, the partial pressure of the glycerol is reduced,

while maintaining the same total pressure, as related by the following well-known equation:

$$\frac{\text{Weight of glycerine vapor}}{\text{Weight of water vapor}} = \frac{\text{Partial pressure of glycerine vapor}}{\text{Partial Pressure of water vapor}} \times \frac{\text{MW of glycerine}}{\text{MW of water}}$$

Glycerine distillation plants typically operate at 5–6 mm Hg absolute pressure and at about 165°C. Certain undesirable chemical reactions can occur in crude glycerol at distillation temperatures:

1. Formation of nitrogen compounds from proteinaceous matter present in the crude glycerine (not removed in the treatment process) by thermal breakdown; these, along with volatile decomposition products, form impurities in the refined glycerin; therefore, it is important to limit the time the glycerol is at high temperature as well as the maximum temperature it is exposed to.
2. Formation of volatile glycerol esters by reaction with soaps (low molecular weights) by the following equilibrium reaction:



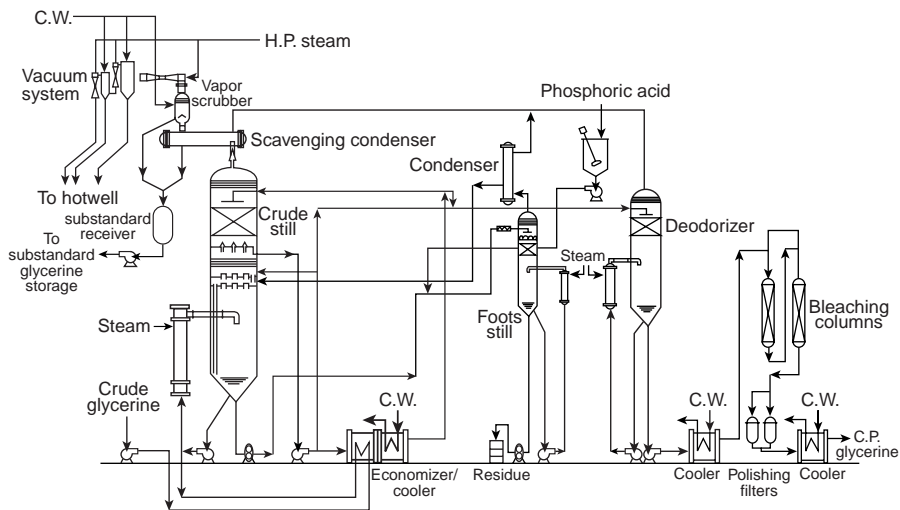
The formation of ester is reduced in the presence of alkali.

3. Formation of polyglycerols, which occur in the presence of NaOH; thus, it can be seen that it is important to control the alkalinity of the crude glycerol to an optimum level.
4. Formation of acrolein ( $\text{CH}_2 = \text{CHCHO}$ ), which is an odor constituent that is difficult to eliminate.

The amount of total stripping steam for distillation is about 20% of the amount of glycerol processed. This amount is greater with poorer quality feedstocks. However, not all of this steam is injected, as the water in the incoming crude glycerine (80%) flashes to steam and provides a significant portion of the stripping steam requirement. It may be undesirable to produce crude glycerine at a higher concentration in the evaporation stage, as additional steam would be needed.

**2.4.2. Residue Recovery and Disposal** After the glycerine has been distilled from the crude glycerine, a residue remains that is continuously removed from the still. The residue contains some glycerol, polymerized glycerol, aldehyde resins, organic products of decomposition, and salt, which must not be allowed to become too concentrated as buildup of residue will cause quality and capacity problems in the plant (19). If the residue is allowed to concentrate in the still, it displaces volume for incoming crude glycerol, thus reducing the still's capacity. At least two methods of removing the residue are used:





**Figure 6.** Flowsheet of Wurster & Sanger glycerine refining plant. Courtesy of Crown Iron Works Co. (9).

1. A residue receiver vessel placed below the still accumulates residue, which is periodically discharged to the treated lye tank for reprocessing.
2. Residue is removed continuously from the still and is redistilled to recover the remaining glycerine; the concentrated residue from the bottom of the foots still is discharged to a drum for disposal, typically to land fill as solid waste.

The details of glycerine refining via the Crown Iron Works Co. process, which is representative of the continuous distillation process used by a large portion of suppliers for soap lye crude glycerine, are detailed here (Figure 6).

The crude glycerine is preheated regeneratively by hot distilled glycerine. The liquor then enters the still heater where it is further heated to about 165°C and circulated by means of a circulation pump. The circulated liquor is partially vaporized through the aid of vacuum (6 mm Hg) and sparging steam in the flashing chamber. The vapor rises through an entrainment separating section and then enters the condensing section. Here, the glycerine vapors are condensed in a layer of packing, wetted by recirculated, cooled, distilled glycerine. The remaining vapors enter the scavenging condenser where remaining traces of glycerine are condensed and recovered as 80–90% substandard glycerine, which is sent to intermediate storage. The substandard glycerine is re-refined after sufficient quantities have been collected for a 2- to 3-day run every month. Typically, the substandard glycerine is processed into a lower grade of glycerine, such as high gravity or dynamite grade.

The residue in the bottom of the still is continuously discharged from the crude still to the foots still. The residue should be kept rich in glycerol (>25%) to improve handling and distillation characteristics. A small amount (0.5–1%) of

phosphoric acid is added to keep the residue soft by lowering the pH to retard the formation of polyglycerols. Here, it is reheated by recirculation through an external heater to about 175°C and partially vaporized, under vacuum and with about 25% stripping steam. Most of the vapors are condensed in the foot still's condenser, and the condensate is returned to the crude still. Any remaining glycerine is recovered where the vapors pass through the scavenging condenser. The residue at the bottom of the foot's still is discharged to a drum for disposal.

The distilled glycerine from the crude still is re-evaporated in the deodorizer at about 130–140°C, again in the presence of high vacuum and stripping steam and with external heating, to ensure optimal removal of odoriferous materials and residual moisture. The vapor passes through a packed section where the incoming feed condenses the vaporized glycerine. Proper reflux rates are needed to ensure removal of close boiling impurities. Residual volatiles are condensed in the scavenging condenser.

An alternate method for distilling glycerine, especially sodium-hydroxide-treated sweetwater crude glycerine, has been implemented in many facilities and differs from the above system in many features (Figure 7). Most notably is the use of a fractional (or partial) condenser for the glycerine product followed by a final condenser to capture a “yellow” glycerine stream. A wiped film evaporator is used for recovering glycerine from the foot's, or pitch, stream.

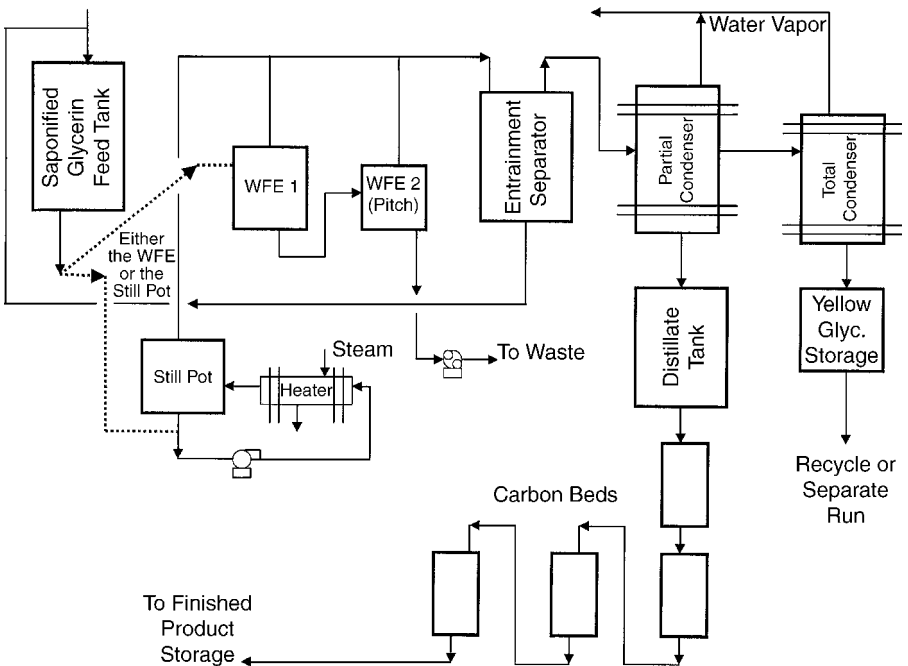


Figure 7. Hydrolyzer crude refining system process flow diagram. Courtesy of CC Engineering, Ltd. (20).

The still for this system is a simple flash still with a forced circulation reboiler. The saponified, crude glycerine stream is fed into the recirculation pump on the suction side. The recirculation rate is in the range of 40–50 times of the crude glycerine feed rate. The top of the still vessel is equipped with a pad style entrainment separator to eliminate carryover.

The still bottoms are drawn off and sent to a second distillation step to remove as much of the remaining glycerine as possible. This system can be a small still, similar to a foot's still, or a wiped film evaporator. The foot's still bottoms, or pitch, from this step is comprised mostly of sodium soaps, polyglycerols, salts, and minor quantities of organic compounds formed in the treatment and distillation steps.

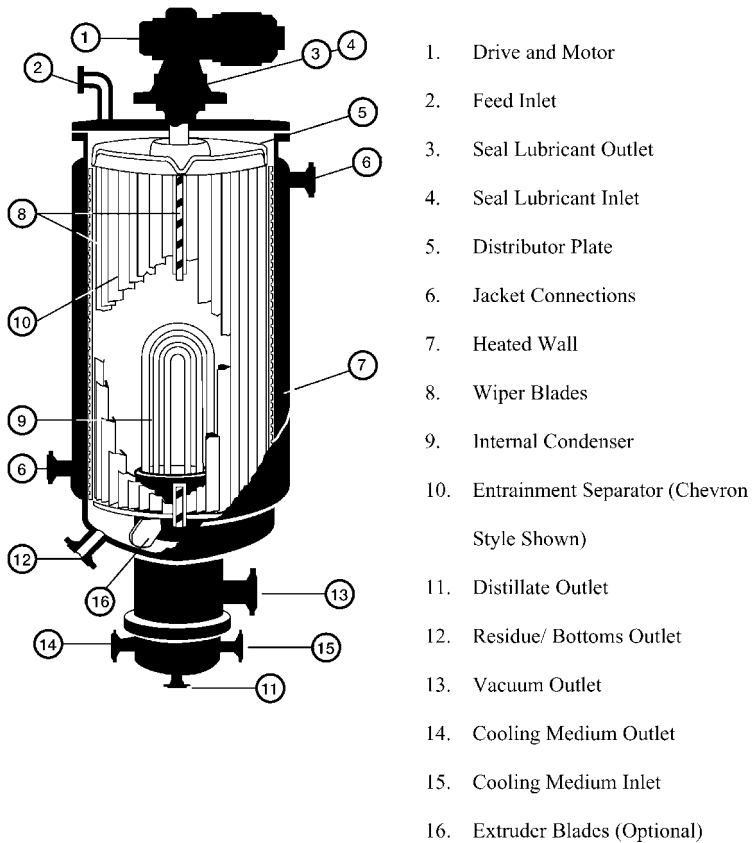
The glycerine and water vapors leave the still and enter the partial condenser. This condenser is a vertical, shell and tube heat exchanger that condenses the glycerine on the shell side. The tube side of the heat exchanger is configured to allow water to boil in the tubes. The tube side outlet is equipped with a valve to control the pressure of the steam leaving, thereby controlling the temperature at which the water boils and glycerine vapors are condensed. Typical condensing temperatures are 115–135°C. A balance must be reached with the temperature of condensation so that odor and color impurities are at a minimum, yet the glycerine yield is economically sufficient.

The remaining glycerine and water vapor leaves the partial condenser and enters the full condenser. This condenser is a shell and tube type condenser that condenses the remaining glycerine and a small amount of the water. This glycerine stream is a low-gravity glycerine and is very yellow. This stream is collected and can be run as a separate recycle batch as mentioned above. The full condenser uses cooling tower water on the tube side. Water vapor leaves the full condenser shell side and goes through the vacuum system where it is condensed.

**2.4.3. Wiped Film Evaporator (WFE)** An alternate method of distilling glycerine is by using a wiped film evaporator system (Figure 8). Pfaudler Corp. describes the process as follows.

Feed material enters the inlet [2] and flows onto a distributor plate [5], which is part of the rotor assembly. The initiation of feed to the WFE occurs at the same time that the drive and motor [1] are started. As the rotor unit turns, centrifugal force spreads the feed from the distributor plate onto the heated wall of the WFE. Volatile components, such as glycerine, are rapidly evaporated. Slotted wiper blades [8] connected to the rotor evenly distribute the feed material into a uniform, agitated, thin film and continuously move material down the heated wall, including highly viscous materials.

The vaporized glycerine stream passes through the entrainment separator [10] and condenses on the internal U-tube condenser [9]. Droplets of material entrained with the vapor stream impinge on the entrainment separator and flow back to the heated wall through centrifugal force of the rotating assembly. Distillate flows out the distillate outlet [11] and noncondensables flow out through the vacuum outlet [13].



**Figure 8.** Wiped film evaporator with internal condenser components. Courtesy of Pfaudler Corporation. (21).

The bottoms, or residue, continues to move down the heated wall into the bottoms collector and out through the residue/bottoms outlet [12]. Optional extruder blades [16] mounted on the bottom of the rotor assembly act to push highly viscous material out through the residue/bottoms outlet.

The heating temperature is chosen based on the vapor pressure of glycerine. The WFE is typically under a high vacuum (3–5 mm Hg). Noncondensables and water vapor are removed by the vacuum system.

One option used in many facilities is to use a WFE that is not equipped with an internal condenser. The vapor stream is then taken from the WFE to the partial condenser inlet for fractional condensation previously discussed.

The advantages of this type of continuous process over batch or continuous distillation in a “still pot” include low residence time (1–2 minutes) and a small difference between the vapor temperature and the liquid film temperature. The temperature of the glycerine can be tightly controlled, thereby reducing the formation of odor, color, and other impurities. This system does not require stripping

steam, which reduces the capital and operating costs of the vacuum system and overall energy consumption.

Wiped film evaporators are very effective for processing sweetwater crudes. Yields of glycerine are very good and the quality of finished product is equal to glycerine from other distillation systems discussed previously. Salt crude's can be distilled in these systems, but special modifications are required to handle the salt that precipitates out during distillation.

**2.4.4. Vacuum System** The vacuum system for the distillation plant is typically a single system for the still, deodorizer, and foots still (if supplied). To achieve a vacuum of 2–10 mm Hg required in the distillation equipment, the vacuum system is usually designed for 1–8 mm Hg absolute, to allow for equipment pressure differentials, ducting losses, leaks, etc. Most systems have a surface condenser or a barometric condenser, followed by a booster jet or multiple-stage vacuum pump. The cooling and condensing water going to the hotwell is typically of good quality, as virtually all condensables are removed by the condensing sections.

**2.4.5. Carbon Adsorption** Distilled glycerine requires one final processing step prior to final storage and shipment. The final step is processing through activated carbon columns. The carbon adsorption process step removes any final traces of odor and color from the distilled glycerine and enhances stability to provide a glycerine product that will not degrade in the final storage tanks (22).

Typical activated carbon usage is in the range of 0.5–1.0%. This can vary depending on the amount of odor and color impurities in the distilled glycerine.

The typical carbon column configuration (Figure 9) consists of three carbon columns in series with a standby carbon column filled with fresh carbon. The column

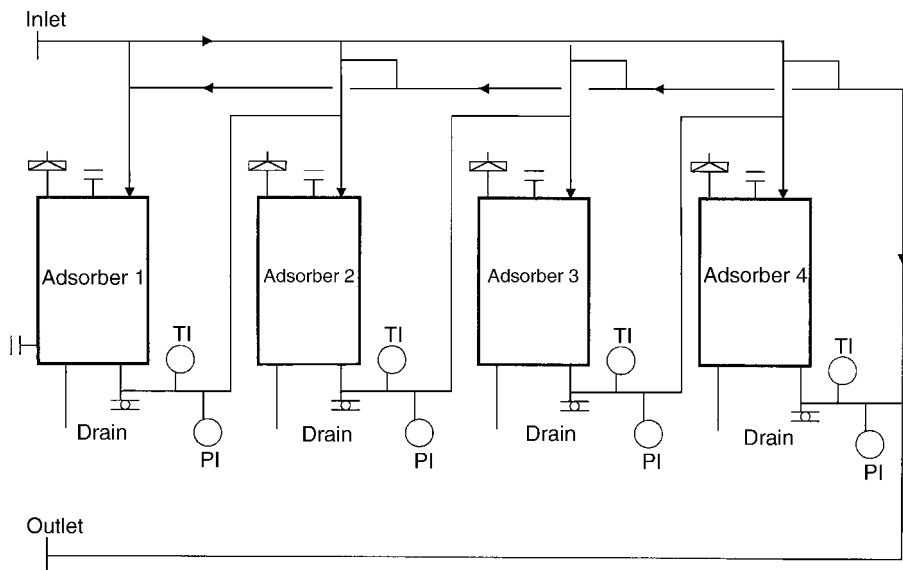


Figure 9. Carbon adsorption process flow diagram. Courtesy of CC Engineering, Ltd. (20).

is filled with dry, activated carbon using any of several methods, including bag dumping, supersacks, or pneumatic conveying. The column is then filled with water or with heated (75–85°C) final glycerine product to saturate the carbon and displace air from the carbon granules. The filled column needs to settle while vented to the atmosphere. This ensures the carbon bed is thoroughly saturated, all air has been expelled, and channeling of glycerine through the carbon bed is prevented. Channeling in the carbon bed leads to increased carbon usage and poor-quality glycerine that does not meet required specifications.

Heated (75–85°C) glycerine is pumped to the carbon column system from the distilled glycerine storage tank. It is critical to the adsorption process to avoid “bumping” the carbon beds with a sudden surge of glycerine because this disrupts the beds and can cause odor and color quality degradation in the finished glycerine. The “bumping” of the carbon bed can sometimes occur with a centrifugal pump. A positive displacement pump, preferably equipped with a variable frequency drive, is recommended to feed the glycerine at a constant flow rate.

A filter is used after the carbon columns to remove any carbon fines from the glycerine. The filter is typically a bag type using polypropylene or PTFE cloth bags with a pore size of 5–10 micron. The bleached and deodorized glycerine product is then sent to final storage for shipment.

## 2.5. Storage and Stability

Dilute and crude glycerine contain certain amounts of suspended material (precipitates, salt, etc.) that tend to settle out during storage. Therefore, to avoid introducing these materials into the process when liquor is drawn, it is recommended to have discharge nozzles be approximately six inches above the bottom of the tank. It is necessary to empty and clean the storage tank(s) periodically.

Dilute solutions of glycerol (<50%) are subject to fermentation, which reduce yields and introduce breakdown products that degrade the glycerol. Holding the glycerol above 70°C or higher concentrations will alleviate this problem. Concentrated glycerine is difficult to pump at lower temperatures because of its high viscosity. It is recommended that glycerine be pumped at 40–50°C; lower temperatures make pumping difficult and higher temperatures can affect the color. If heating coils or steam tracing are used, it is important to use low-pressure steam so as not to overheat the glycerol and cause breakdown of products.

Stainless steel vessels or stainless-steel-lined vessels are recommended to prevent the formation of color complexes, especially if moisture or residual fatty acids are present in a carbon steel tank. Since glycerine is hygroscopic, care should be taken to exclude moisture from the refined glycerine storage tank. Glycerine subjected to heat should not be stored in vessels containing copper or tin, as copper or iron salts will catalyze oxidation of glycerine under those conditions (23).

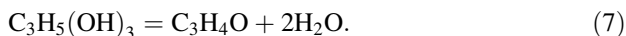
## 2.6. Odor and Color

As noted, color and odor problems can be mostly avoided by using a high-quality raw material, treating and storing crude glycerol properly, and avoiding high

temperatures for an extended time period. Impurities in crude glycerine, especially matter organic nonglycerol (MONG), affect the quality and quantity of distilled glycerine. If the MONG content is high (3–5%), odor, taste, and color reversion problems may exist in the final product. Tremethylene glycol, which is present in MONG, can affect the color of glycerine and lead to problems in storage.

A properly designed deodorizer or “stripper” vessel, located after the distillation column and operating at high vacuum and with open or “sparging” steam, will remove close boiling impurities and most odor bodies. Remaining color bodies are removed by adsorption with activated carbon.

Acrolein may be formed in the presence of iron, iron salts, acid, or neutral salts:



Acrolein odor is distinctive and is detectable to the human nose in small quantities (ppm).

It has been found that glycerine left inside processing vessels, especially carbon columns, can degrade and develop hard-to-remove odors. Therefore, process equipment should be cleaned completely after shutdown, and carbon columns blown down with air and washed with water. Also, fresh carbon can sometimes contain odor-causing impurities. The carbon can be washed with distilled glycerine that can be subsequently redistilled or sold as a lower grade.

### 3. PROCESSING PLANTS

#### 3.1. Layout Considerations

Plant layout considerations for a glycerine recovery plant are similar to those for other processing plants: adequate space must be allowed for equipment installation, operation, and maintenance, as well as operator safety. As some of the operation requires material handling, consideration must be given to these specific requirements. These operations include batch feeding of treatment chemicals (most of which are hazardous), disposal of filter cake, and charging of the bleaching columns with carbon. It generally makes sense to arrange the equipment in order of process flow, as piping and other connections can be made much more conveniently. In addition, if process tanks are located to allow gravity feeds, some pumps can be eliminated. Locating equipment on several levels also helps in reducing the overall footprint, or floor space requirements.

All areas should be equipped with wash-down equipment, and all levels must have adequate floor drains with the floor surfaces properly pitched. Care must also be exercised when selecting flooring materials, as glycerine is slippery; this is a real concern, because spills and leaks inevitably occur. Provisions must be made in the plant for efficient cleaning procedures for the equipment, piping, and building.

## 3.2. Material and Construction

**3.2.1. Overview** Careful selection of the materials of construction is important to ensure adequate life of equipment in the glycerine processing plant and to ensure a high-quality finished product. This is especially true when recovering glycerine from soap lyes, where the presence of sodium chloride, in crystal and solution forms, greatly increases the corrosion potential. The reader is encouraged to consult textbooks and manufacturer's data sheets regarding corrosion for the compounds and materials in the glycerine plant.

**3.2.2. Pretreatment Plant** When treating sweetwater glycerine, ductile iron (DI) can generally be used for pumps, although lined DI and stainless steel pumps can be used with an expectation of longer life.

More care must be used when selecting material for treating spent lye. Treatment vessels can be either lined carbon steel or stainless steel. Fiberglass-reinforced polyester (FRP) is a good choice for both tanks and piping in the treatment plant, but care must be taken to ensure that the temperature range of the FRP material is observed and that the pipelines are correctly supported. Also, steam blowing of pipes should not be allowed as the high temperature can damage the FRP piping.

The open plate and frame filter presses used typically have cast iron frames with polypropylene plates that give excellent life. Filter clothes are usually constructed of synthetic materials such as polypropylene felt, rather than natural fibers such as cotton. Plastic tools must be used to clean cake from the clothes to avoid damage.

A plate and frame heat exchanger is sometimes used to preheat incoming soap lye, as treatment is more effective with warm lye. The plates can be of 316L stainless steel, or even titanium for effective corrosion resistance.

**3.2.3. Evaporation Plant** Material selection for the evaporation plant is the greatest challenge in the glycerine recovery plant, again, especially if there is salt present. For evaporating splitter sweetwater, 304L stainless steel may be used. The corrosive nature of the brine-glycerol solution makes carbon steel and most grades of stainless steel a poor choice for evaporators handling soap lye. The heavy-walled, cast-iron callandria that have been used extensively for soap lye evaporation in the past have long lives, however, economic considerations discourage new evaporators of that style from being built. The material of choice for the past 25 years for welded plate evaporators (and related components handling salt or brine) is copper-nickel alloy. The two principal alloys to consider are 9010 (90% copper, 10% nickel), which has the best corrosion resistance to the glycerol-brine solution, and 7030, which has a higher cost (24).

An interesting class of materials that should be considered for evaporator service are the duplex stainless steel alloys. These high-performance stainless steel alloys have a "duplex" structure. The alloy contains both the austenitic and ferritic phases



in the grain structure at the same time. These materials have shown high resistance to stress corrosion cracking in chloride-bearing environments and have the characteristics of high corrosion and erosion resistance. (A description of the corrosion protection mechanism is outside the scope of this chapter; the reader is referred to corrosion handbooks and manufacturers data sheets for more information.) Two alloys that show promise for this application are Sandvik's SAF 2205 and SAF 2507 (20, 25). Other, more expensive alloys, such as Ferralium 225, Hastelloy, and Monel, have been used in various applications.

Pumps for the evaporation plant must stand up under the hot glycerol-brine solution and are also generally of the centrifugal design. Cast stainless steels (CF8M alloy) are often used for this application, but a superior material for pumps is a chromium-nickel-copper-molybdenum stainless steel known as CD-4M, which should be considered when choosing the pump material. The extra cost is usually not significant.

Seals for pumps are generally mechanical. If the fluid contains salt crystals, which are abrasive, it is recommended that water be used to flush the seal. Both seal faces on the pump side should be of a hard material; using carbon for one of the seal faces has not proven to be successful. The reader is again urged to contact seal manufacturers for their recommendation.

**3.2.4. Refining Plant** Materials for the refining plant can be selected based on the impurity content of the glycerol being handled. The initial distillation vessel or residue still, if it is handling crude glycerine with salt in it, should be a high grade of stainless steel. The L grades generally exhibit slightly better corrosion resistance than their base versions. The 316 and 316L stainless steels are recommended for stills handling soap lye crude glycerine, while 304 and 304L stainless steel is adequate for sweetwater stills, vapor scrubbers, condensers, deodorizers, and bleaching equipment where salt is not present. The 316L is especially recommended for foot stills where salt is present but water is not.

In some cases, more corrosion-resistant materials have been used; the Ittner refining stills in use at some Colgate-Palmolive plants were fabricated from pure nickel. The life of such equipment is exceptionally long (26).

Pumps for handling glycerine can be centrifugal as long as the temperature and viscosity of the product is satisfactory for the pump being used. For lower temperatures and pumping residue, a positive displacement pump with a pressure relief loop (internal) should be used.

### 3.3. Instruments and Controls

Control techniques for glycerine plants have not changed much over the years, as it is necessary to control pH, levels, temperatures, and vacuums in the various portions of the plant. Most operations can be accomplished manually, and traditionally, manual operation of these plants have been the norm. However, the development

and refinement of digital single-loop controllers and associated sensing equipment have increased the ease and accuracy of control. Reliable inline pH monitoring has made treatment plants easier to operate.

A caution must be given, however, to select instrumentation that can handle the sometimes severe conditions in the glycerine recovery plant. The crude glycerine in the evaporation and crude refining sections contains both dissolved and suspended salt, while the residue in the refining plant contains salt as well as other impurities that make the viscosity of the residue high. Vane-type flow meters have been used successfully for measurement of difficult flows such as residue from the crude still. Rotameters often will be plugged by suspended solids and are recommended only for flows that are “clean” and will maintain a constant viscosity over time. Mass flow meters (Coriolis effect), such as those manufactured by Micromotion, have proven successful in many areas of the treatment and refining plants. These flow meters have no moving parts and can handle a wide range of viscosities and densities.

Materials of construction for wetted portions of the instrumentation must be selected using similar considerations to those used for the equipment itself. In addition, plastic diaphragm seals are available to cover and protect the pressure-sensing faces of immersed level transmitters. This allows the use of stainless steel in applications where, if unprotected, the corrosion rate would be unacceptably high on the thin face of the instrument. A diaphragm seal is recommended for pressure instruments in the presence of suspended or entrained solids.

### 3.4. Piping Considerations

In addition to the few suggestions on material selection given earlier, other issues need to be considered when designing or evaluating the piping for a glycerine recovery plant. As in any piping system, the piping must be properly supported, be given correct slopes for proper flow and self drainage, accommodate thermal expansion, and be designed with adequate bypasses for equipment maintenance and proper operational control. In addition, consideration should be given for sampling points, drains, and for blowout-wash-down connections. Fluid velocities should be maintained within standard design limits. The above information can be obtained in reference books dealing with industrial piping design. Another area of concern may be the possible problem of electrolysis as a result of dissimilar materials used together in the piping system.

## 4. PROPERTIES OF GLYCEINE

Glycerine has a unique set of physical properties that allow glycerine to be used in a variety of industries. Some of these properties are summarized in the following tables:

**TABLE 2. Physical Properties of Glycerine.**

Molecular Weight	92.09
Boiling Point	290°C (760 mm Hg)
Melting Point	18.17°C
Freeze Point (eutectic)	(66.7% glycerol solution) -46.5°C
Specific Heat	0.5795 cal/gm°C (26°C)
Refractive Index	(N <sub>d</sub> <sup>20</sup> ) 1.47399
Flash Point	(99% glycerol) 177°C
Fire Point	(99% glycerol) 204°C
Autoignition Point	(on platinum) 523°C (on glass) 429°C
Heat of Combustion	397.0 Kcal per gram
Surface Tension	63.4 dynes cm (20°C) 58.6 dynes cm (90°C) 51.9 dynes cm (150°C)
Coefficient of Thermal Expansion	0.0006115 (15-25°C Temp. Interval) 0.000610 (20-25°C Temp. Interval)
Thermal Conductivity	0.000691 cal cm deg/sec (0°C)
Heat of Formation	159.8 Kcal/mol (25°C)
Heat of Fusion	47.5 cal/gram
Heat of Vaporization	21,060 cal/mol (55°C) 19,300 cal/mol (105°C) 18,610 cal/mol (175°C)

**TABLE 3. Vapor Pressure of Pure Glycerine.**

mmHg	1	5	10	20	40	60	100	200	400	760
°C	125.5	153.8	167.2	182.2	198.0	208.0	220.1	240.0	263.0	290.0

**TABLE 4. Viscosity of Glycerine-Centipoise.**

°C	80	90	100	110	120	130	140	150	158	167
cp	32.18	21.2	14.60	10.48	7.797	5.986	4.726	3.823	3.282	2.806

## 5. QUALITY AND TESTING

### 5.1. Glycerine Grades

Several grades of glycerine have been established or are in common use in North America. U.S. Pharmacopoeia (USP) or chemically pure (CP) glycerine standards have been established for the highest grades of glycerine, water (white in color), and with glycerol contents of not less than 95%. It conforms to standards given in *U.S. Pharmacopoeia*. It is used by the food and pharmaceutical industries because of the high purity. Both natural and synthetic glycerine meet these specifications (Table 5).

**TABLE 5. Refined Glycerine Specifications.**

Characteristic	99.7% USP	99.5% USP	99.0% USP
Specific Gravity, 25/25°C	1.26092	1.26073	1.25945
Color	25 hazen maximum, APHA 10 maximum	25 hazen maximum, APHA 10 maximum	25 hazen maximum, APHA 10 maximum
Ash	0.01% maximum	0.01% maximum	0.01% maximum
Chlorides	10 ppm maximum	10 ppm maximum	10 ppm maximum
Sulfate	20 ppm maximum	20 ppm maximum	20 ppm maximum
Arsenic	1.5 ppm maximum	1.5 ppm maximum	1.5 ppm maximum
Heavy metals	5 ppm maximum	5 ppm maximum	5 ppm maximum
Readily carbonizable	—	Not darker than matching fluid H	Not darker than matching fluid H
Chlorinated Compounds	30 ppm maximum	30 ppm maximum	30 ppm maximum
Acrolein, glucose and ammonia compounds	Not yellow, no ammonia odor	Not yellow, no ammonia odor	Not yellow, no ammonia odor
Fatty acids and esters (FA & E)	1.0 milliequivalent/100 gm MAX.	1.0 milliequivalent/100 gm MAX.	1.0 milliequivalent/100 gm MAX.
PH	Neutral	Neutral	Neutral

High-gravity glycerine is a commercial grade of glycerine, near white in color, and with a high glycerol content (>98.7% with specific gravity minimum of 1.2583 at 25/25°C). It conforms to Federal specification O-G-491C and to ASTM D1257. Dynamite glycerine meets all high-gravity specifications, except that a darker color is allowed.

Hydrolyzer (88%) and soap lye (80%) crude's are crude, unrefined grades of glycerine offered for sale to glycerine refiners. Hydrolyzer crude is concentrated sweetwater from fat splitting, while soap lye crude comes from soap making and contains some salt.

For salt crude glycerine, the typical concentration for sale is 80%. Adjustments in price can be made for concentrations above or below 80%, however, these adjustments are negotiated by buyers and sellers. The standard for ash content in salt crude is 10% maximum. The standard for water is 10% maximum. Limits on minor constituents include a 0.5% limit for trimethylene glycol (TMG), 2.5% limit for MONG, and a limit of 2 ppm for arsenic.

Hydrolyzer (sweetwater) crude glycerine is traded at a standard 88% glycerol. Limits on minor constituents include ash at 1% maximum, MONG at 1.5% maximum, and TMG at 0.5% maximum. The limit on arsenic is 2 ppm maximum.

## 5.2. Test Methods

The American Oil Chemists' Society (AOCS) has published several methods for sampling and testing of glycerine (18). Included are procedures for sampling crude glycerine and test methods for glycerol, apparent specific gravity at 25/25°C,

moisture, and color (Table 6). Tests are usually conducted to analyze the amount of ash, alkalinity, salt, and organic residue. In addition, the refractive index of glycerine can be used to estimate its concentration (13, 25). Color is determined using the APHA scale. APHA color is determined using AOCS Method Ea 9-65, which measures “color by comparison with artificial empirical standards” (23).

## 6. PROCESSING LOSSES

Losses are a natural part of any processing plant. The control and reduction of the losses will, in part, determine the economic effectiveness of the plant's operation. All areas of the glycerine recovery plant can produce losses. In storage, fermentation can cause serious losses of glycerine due to the formation of trimethylene glycol, gases, and acids during decomposition.

In the evaporation plant especially, glycerine can be carried out with the vapor during evaporation and lost in the vacuum system's condensing of water. The amount of carryover can be minimized in several ways:

1. Entrainment separators, or “catchalls”, and mist eliminator pads can be installed where the vapor is removed from the vessel by the vacuum system; the recovered product can be returned to the process at an appropriate point.
2. Control the vapor velocity and maintain an adequate vapor space at the top of the vessel; it becomes important to control the liquid level to maintain the required vapor space.
3. Use a surface condenser to recover any additional condensable material that escapes into the vacuum system.

The treatment process should be done carefully to control any tendency to foam. Other sources of glycerine losses include the following:

1. Glycerine is carried out with salt coming from soap lyes; proper washing of the salt is required to minimize this loss.
2. The filter cake and skimming from the treatment plant contain glycerine.
3. Spent bleaching carbon contains glycerine of the highest concentration produced in the plant. It should be allowed to drain and then the columns should be blown down with air or nitrogen to extract as much free glycerine as possible. Then, the spent carbon can be added to the first treatment tank, where additional glycerine will be washed from the carbon. The carbon is separated in the filter press and disposed of with the rest of the filter cake.
4. Residue removed from the distillation process contains a certain percentage of glycerine; much of the glycerine can be recovered from this residue by a separate distillation step in a wiped film evaporator, or foots still.
5. Glycerine leaks into heat exchangers.

6. There is spillage from all sources; careful plant design and operation are needed to minimize spills from overflowing tanks, equipment malfunctions, and other operational errors.
7. Opening of equipment for maintenance can result in loss of product. Where possible, the liquid should be blown into the previous or next process vessel or drained back into a bucket for recovery. Any product that is recovered that is not badly contaminated can be returned to a separate storage tank upstream in the process. If a “substandard” or “off-spec” glycerine tank is provided in the refining section, it can be equipped with an easily removable cover.

## 7. WASTE MANAGEMENT

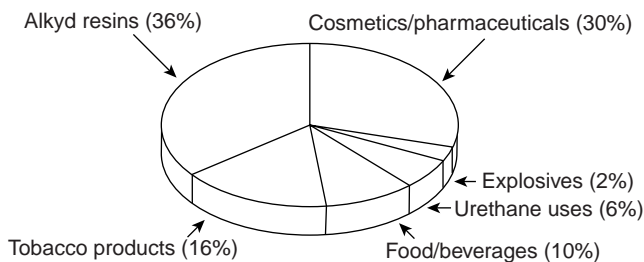
The three primary waste streams from a glycerine recovery plant are skimming and filter cake from the treatment plant, contaminants in the vacuum system condensing water, and residue (foots) from the glycerine refining plant. Filter cake discharge is typically sent to a solid landfill. The concentrated residue from a foots still, when allowed to cool, will typically solidify and must be disposed of as required by the local environmental authority.

Contaminants that get into the vacuum system’s condensing water supply are a dual problem. There is glycerol and other condensables that are carried over from the vapor stream from the evaporation plant. First, the glycerol represents a loss of product. Second, these contaminants will foul the condensing water supply, which is typically recirculated through a cooling tower. A certain amount of the condensing water is generally bled away and replaced with makeup water to maintain an acceptable water-quality level. An important alternative here is the inclusion of a closed-loop system that incorporates a surface condenser cooled with clean water recirculated from a cooling tower. The surface condenser, usually a horizontal shell and tube heat exchanger, replaces the barometric condenser in the vacuum system and, while using clean cooling water, allows any condensables to be recovered.

## 8. USES, APPLICATIONS, AND ECONOMICS

The number of uses of glycerine is truly phenomenal (Figure 10). Depending on the publication surveyed, up to 1700 uses have been identified. Its wide range of applications is, in part, related to a few of its key properties:

1. Glycerine is a natural product, nontoxic, and generally recognized as safe (GRAS) for human consumption.
2. It is an excellent humectant, emulsifier, and plasticizer.
3. It is compatible with a wide variety of materials and mixes well.
4. It possesses antioxidant properties.



**Figure 10.** Glycerine uses.

Below is a list of some of the more prominent or interesting uses for glycerine (4, 23).

- Adhesives, used for plasticizing and penetrating properties.
- Agriculture, used in sprays, dips, and washes.
- Antifreeze: properties include low freezing point and excellent compatibility.
- Cleaners and polishes, used in a wide range for the home and automotive markets.
- Corrosion prevention, used in gums and resins for surface-coating metals.

Cosmetics, acting as a bodying agent, emollient, humectant, lubricant, and solvent; present in skin creams and lotions, shampoos and hair conditioners, soaps, and detergents.

Dental creams, up to 50% of typical dental creams, used as a humectant, to ensure good dispersion and to serve as a vehicle for dyes and flavorings.

Explosives, a large amount is consumed in the manufacture of nitroglycerine-based explosives.

Food and beverages, used in a wide variety of applications; serves as a solvent, carrier, emulsifier, conditioner, freeze preventer, and coating; used in wine, liqueurs, chewing gum base, confectioneries, and chewy bars; kosher glycerine is used in kosher foods.

Leather, used in tanning and finishing processes.

Metal processing, widely used for pickling, quenching, stripping, electroplating, galvanizing, and soldering.

Paper, acts as a humectant, plasticizer, softening agent, and barrier against grease and solvents; used for greaseproof and glassine papers.

Pharmaceuticals, used in salves and dressings, antibiotic preparations, capsules, and suppositories; used as a vasodilator for angina pectoris (nitroglycerine).

Photography, properties include wetting and plasticizing.

Resins, includes ester gums, phthalic acid and malic acid resins, polyurethanes, and epoxies.

Textiles, facilitates printing and dyeing, lubricating and snagproofing; used for treatments for antistatic, antishrink, anticrease, waterproofing, and flameproofing.

Tobacco, used as a humectant, softening agent, and flavor enhancer.

## 9. FUTURE CONSIDERATIONS

### 9.1. Technology

Future technologies that appear most likely to impact the production of natural glycerine center around recovery of glycerine from nontraditional sources. As demand for biodiesel fuel from vegetable oils grows, large amounts of high-quality glycerine will become available. Other technologies that show promise for use in glycerine recovery include ultrafiltration and reverse osmosis. As processes become more effective and efficient and as economic conditions change, some of these processes may come into more common use.

An interesting source of glycerol is through recovery from ethanol fermentation still bottoms by chromatographic separation and ion exchange process. In a process proposed by IWT/US Filter, raw stillage is first clarified by filtration through a membrane, concentrated (by evaporation), and then fed to the ADSEP chromatography process where the glycerol is separated. The glycerol is then further purified by ion exchange, followed by traditional concentration and distillation (27).

### 9.2. Future Economics

The future economics of glycerine worldwide will be sure to change as fundamental changes on the supply side occur. Animal fat is a significant raw material for glycerine production, both for fat splitting and saponification. Recent health trends emphasizing lower fat content in meats has caused animal producers to develop leaner meats, reducing the supply of animal fats. Other uses of fat add additional price pressures. However, the soap and detergent industry is expected to increase its use of glycerine as nonionic surfactants.

Additional glycerine production is being added from several growing and significant sources. The Asian fatty alcohol and fatty acid industry is expected to add an additional 30,000–60,000 tons to the world market in the next few years. There is a similar amount expected to arrive in the market, primarily from the European biodiesel (methyl ester production from vegetable oil) industry. Other developing sources of glycerine, such as glycerine recovery from ethanol stillage, may add further stocks to the world supply.

The purification of glycerine from these sources is obtained using the traditional methods outlined in this chapter. However, the economics of production of the crude glycerol will determine how these new sources affect the world market.

## REFERENCES

1. K. P. Radhakrishnan, in L. Spitz, ed., *Soap Technology for the 1990's—Glycerine Processing from Spent Lye and Sweet Water*, AOCS, Champaign, Illinois, 1990, p. 128.
2. W. I. Rowell, *Fat Splitting and Glycerine Recovery*, AOCS, Champaign, Illinois, 1987.



3. L. L. Lamborn, *Modern Soaps, Candles, and Glycerin*, Van Nostrand Co., New York, 1906, p. 542.
4. A. A. Neuman, *Glycerol*, CRC Press, Cleveland, Ohio, 1968.
5. A. S. Langmuir, *J. Soc. Chem. Ind.*, **36**, 180 (1917).
6. J. Hoffman, *Chem. Market. Reporter*, **243**, 5 (1993).
7. B. Freedman, *Methyl Esters—Manufacture and Utilization*, USDA.
8. A. A. Neuman, *The History of Glycerol*, CRC Press, Cleveland, Ohio, 1968, ch. 1.
9. Crown Iron Works Co.
10. E. Woollatt, *The Manufacture of Soaps, Othe Detergents and Glycerine*, Elliss Horwood Ltd., Chichester, United Kingdom, 1985.
11. O. H. Wurster, *Oil Soap*, **13**, 246–253 (1936).
12. O. H. Wurster, *Oil Soap*, **13**, 283–286 (1936).
13. C. S. Miner and N. N. Dalton, eds., *Glycerol*, Reinhold Publishing Co., New York, 1953, ch. 6.
14. Bulletin. SW-200R, Swenson Evaporators.
15. P. E. Minton, *Handbook of Evaporation Technology*, Noyes Publications, Park Ridge, New Jersey, 1986.
16. Guinard Centrifugation.
17. *Centrifuge Type C*, brochure, Guinard Centrifugation.
18. American Oil and Chemists Society.
19. J. W. Lawrie, *Glycerol and the Glycrols*, Chemical Catalog Co., New York, 1928.
20. CC Engineering, Ltd.
21. Pfaudler Corp.
22. *Glycerine Purification*, brochure, CC Engineering.
23. *Glycerine: An Overview*, Soap and Detergent Assoc., New York, 1990.
24. *APV Evaporator Handbook*, 3rd ed., APV Crepaco, Inc.
25. L. F. Hoyt, *Oil Soap*, **10**, 43–47 (1933).
26. M. H. Ittner (to Colgate Palmolive), U. S. Patent 2,164,274, June 27, 1939.
27. B. D. Burris, *Recovery of Glycerol from Still Bottoms*, Illinois Water Treatment, 1987.

# 6

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## *Vegetable Oils as Biodiesel*

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### 1. INTRODUCTION

Renewable fuels are increasingly being used to displace fuels from non-renewable sources, though the goals of using these bio-based fuels are often complex. In Austria, where much of the pioneering commercial development occurred, biodiesel development was driven by the need to: (1) increase security of energy supply for the transportation sector by having a renewable source at hand, (2) have an environmentally friendly fuel available for the diesel combustion engine, (3) reduce health and security risks, and (4) provide the customer with a reliable fuel at a reasonable cost-benefit ratio (1).

Generally, renewable fuels are produced to reduce greenhouse gas emissions, improve combustion of fuels, and to extend supplies of fossil fuels, although their production may also be used to subsidize the production of agricultural commodities and improve the balance of trade for countries that produce little fossil fuel.

Biodiesel is a renewable fuel that is usually narrowly defined as esters of lower alcohol and fatty acids, where the fatty acids are derived from vegetable oil, animal fat, or tallow (2). In spite of the restrictive nature of this definition, it is possible to include a wide range of molecules derived from living organisms in fuels for compression ignition engines (3). In light of the diverse potential for using biocomponents for fuels, a section of this chapter will be dedicated to specific molecules

derived from fatty acids and their derivatives that are used as biodiesel fuel and fuel components. Pyrolysis oils, derived by heating biomass in the absence of oxygen, are derived from a broad range of molecules including carbohydrates, proteins, phenolic compounds, and lipids. Their formation and use is beyond the scope of this discussion.

Fatty acid derivatives are used in diesel fuel primarily and include a narrow range of current and proposed applications (1). Commercial fuels are predominantly methyl esters of fatty acids or blends of fatty acid methyl esters with conventional diesel fuels from fossil sources. Although other esters have been studied as fuels, the relative cost benefits and ease of preparing and using methyl esters have allowed these sources to dominate current commercial production (1). Other options are being explored, including conversion of vegetable oils to *n*-alkanes to improve combustion (4–6) and developing diesel technology that combusts triacylglycerol oils directly (7–9). These latter technologies bear discussion but are not part of the major global commercialization effort that is currently under way.

## 2. BIODIESEL QUALITY

Diesel fuels are complex mixtures of hydrocarbons defined by physical and chemical properties. Petroleum diesel fuels are based on molecules with 9 to 20 carbon atoms and a boiling range between 170°C and 350°C (10). These fuels are produced by sequential chemical treatments and refining of heavy petroleum oils followed by distillation. In general, specifications for fuels are inclusive so as not to exclude compositions with similar operational characteristics. However, environmental concerns regarding toxic emissions from diesel engines have led to legislation that has forced manufacturers to modify diesel fuel chemistry (11).

Fatty acid methyl esters and other esters of fatty acids and a lower alcohol can be added at a low ratio to most diesel fuels without substantially changing fuel characteristics. It has been reported that in blends containing 30 percent biodiesel, low-temperature flow properties are not greatly affected (12), but at higher blend levels, the properties of the methyl ester may affect the properties of the fuel. With few exceptions, pure biodiesel does not meet minimum low-temperature requirements and may exceed manufacturers' maximum viscosity for diesel fuels.

### 2.1. Physical Parameters

Official methods of physical analysis used to characterize conventional diesel are applicable and meaningful when applied to biodiesel and provide useful information. Biodiesel chemistry leads to a number of physical characteristics that are unique when compared with diesel fuels. Most biodiesel preparations have higher viscosity, density, initial boiling point, final boiling point, cold-filter plugging point, and flash point than conventional diesel fuels. Virtually all of these characteristics are due to the high average molecular weight of the component esters of biodiesel.

Boiling point and flash point, for example, are related to vapor pressure. Fatty acid saturation does not appear to impact surface tension significantly.

Physical properties may affect fuel characteristics and combustion in a number of ways. Diesel fuel must be efficiently atomized to effect combustion. Surface tension and viscosity of diesel fuel are properties that determine atomization characteristics (19). Allan and Watts (19) compared the atomization characteristics of 15 biodiesel fuels with number two diesel. They found that molecular weight was the main property affecting atomization. As an example, the mean oil droplet diameter of coconut oil methyl esters, which are composed of 12 and 14 carbon chains, was not significantly different from diesel fuel, whereas methyl esters, which had predominantly 18 carbon chains, produced droplets with mean diameters 20–30 percent larger than diesel fuel. Rapeseed oil, with a mean carbon chain length of 20 carbons, increased droplet size by 40 percent over that of diesel fuel. The larger size of atomized droplets of biodiesel may account for some of the differences in emissions observed from diesel fuels. Vapor pressure and, consequently, the flash point of all diesel fuels is largely determined by the molecular species with the lowest molecular weight present in the fuel. As most of the diesel supply infrastructure was built for a fuel with a high flash point, it is advantageous that properly made biodiesel has an elevated flash point (2, 14–18).

## 2.2. Chemical Parameters

Official methods of chemical analysis of conventional diesel are often not adequate to characterize biodiesel. Tests for the levels of sulfur and aromatic components in biodiesel are useful but usually reveal that the concentrations of compounds containing these atoms or functional groups are very low. Analysis of biodiesel chemistry can reveal characteristics conferred by the source of the oil, the method of manufacture, and duration of storage (20, 21). For example, free and bound glycerol is measured to ascertain if biodiesel has been completely formed during synthesis. Fatty acid content, residual soaps, iodine value, peroxide value, and fatty acid composition all may reflect the quality of biodiesel (Table 1) but are unimportant and inapplicable in conventional diesel fuel quality determination.

Significant differences in fuel standards also exist among different countries (2, 14–18; Table 1). In the European Union (EU), member countries have adopted a standard requiring an iodine value of less than 115 (15, 16), 120 (14), or 125 (18). This iodine value reflects the upper extreme iodine value of canola (low erucic acid rapeseed) oil. The American Society for Testing Measures (ASTM) and Italian National Standards Body (UNI) standards do not include iodine value (2, 17) and thus allow higher iodine value oils such as soy and sunflower.

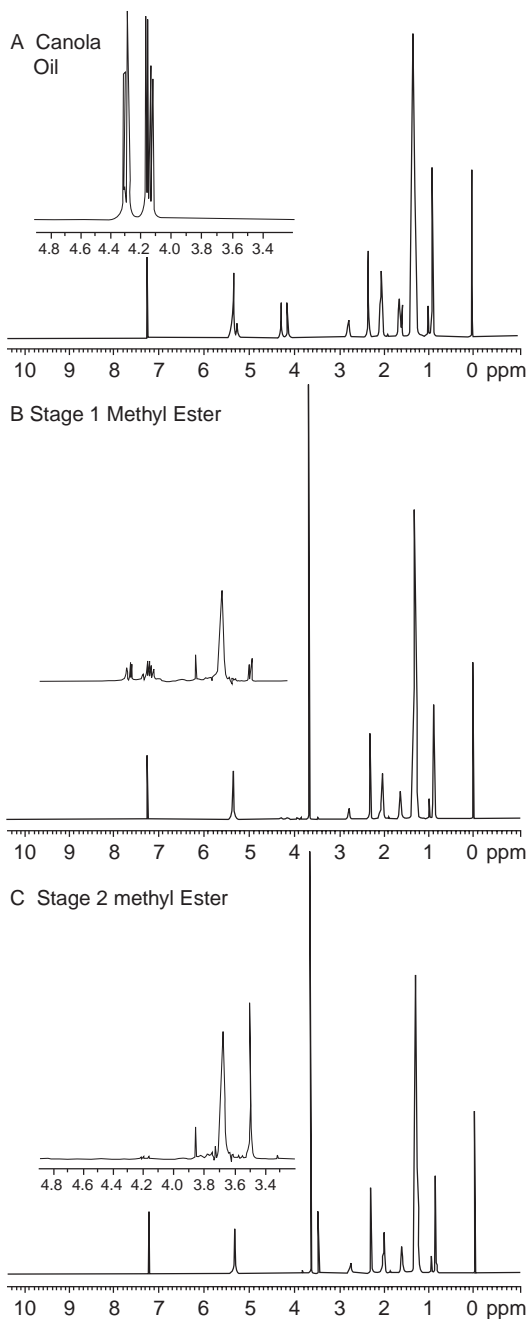
Chemical analyses of biodiesel may be accomplished through standard methods as recommended by ASTM and other organizations, but these measures do not determine all aspects of biodiesel chemistry (20, 21). Knothe (21) extensively described methods of measuring *trans*-esterification progress and assuring biodiesel quality, and suggested that no single test could assure quality. Online methods, such as NIR (near infrared spectroscopy), could be used to rapidly screen products to

**TABLE 1. International Standards for Biodiesel.**

Standard/ Specification	Test Value	Unit	AUSTRIA ON C1191 (14)*	FRANCE Journal Officiel (15)*	GERMANY DIN E 51606 (16)*	ITALY UNI 10635 (17)*	SWEDEN SS 15 54 36 (18)*	USA D-6751-02 (2)*
Date			1 July 1997	14 Sep 1997	Sep 1997	21 April 1997	27 Nov 1996	10 Jan 2002
Application			FAME**	VOME**	FAME**	VOME**	VOME**	FAME**
Density	15°C	g/cm <sup>3</sup>	0.85–0.89	0.87–0.90	0.875–0.90	0.86–0.90	0.87–0.90	—
Viscosity	40°C	mm <sup>2</sup> /s	3.5–5.0	3.5–5.0	3.5–5.0	3.5–5.0	3.5–5.0	1.9-6.0
Distillation	I.B.P	°C	—	—	—	>300	—	—
Distillation	95%	°C	—	<360	—	<360	—	—
Flashpoint		°C	>100	>100	>110	>100	>100	>130
CFPP		°C	<0/–15	—	<0/–10/–20	<0/–15	<–5	—
Pourpoint	summer	°C	—	<10	—	—	—	—
Total Sulfur		% mass	<0.02	—	<0.01	<0.01	<0.001	—
CCR	100%	% mass	<0.05	—	<0.05	—	—	<0.050
CCR	10%	% mass	—	<0.3	—	<0.5	—	—
Sulfate ash		% mass	<0.02	—	<0.03	—	—	<0.02
(Oxide) Ash		% mass	—	—	—	<0.01	<0.01	—
Water content		mg/kg	—	<200	<300	<700	<300	<500
Impurities total		mg/kg	—	—	<20	—	<20	—
Cetane No.		—	>49	>49	>49	—	>48	>47
Neutral No.		mgKOH/g	<0.8	<0.5	<0.5	<0.5	<0.6	<0.80
Methanol cont.		% mass	<0.20	<0.1	<0.3	<0.2	<0.2	—
Ester content		% mass	—	>96.5	—	>98	>98	—
Monoacylglycerol		% mass	—	<0.8	<0.8	<0.8	<0.8	—
Diacylglycerol		% mass	—	<0.2	<0.4	<0.2	<0.1	—
Triacylglycerol		% mass	—	<0.2	<0.4	<0.1	<0.1	—
Free glycerol		% mass	<0.02	<0.02	<0.02	<0.05	<0.02	<0.020
Total glycerol		% mass	<0.24	<0.25	<0.25	—	—	<0.240
Iodine No.		—	<120	<115	<115	—	<125	—
Phosphorous		mg/kg	<20	<10	<10	<10	<10	<10
Alkaline met.	NA/K	mg/kg	—	<5/5	<5	—	<10/10	—

\*Reference No.

\*\* Abbreviations: FAME: Fatty Acid Methyl Ester, VOME: Vegetable Oil Methyl Ester, CFPP: Cold Filter Plugging Point, CCR: Conradson Carbon Residue.



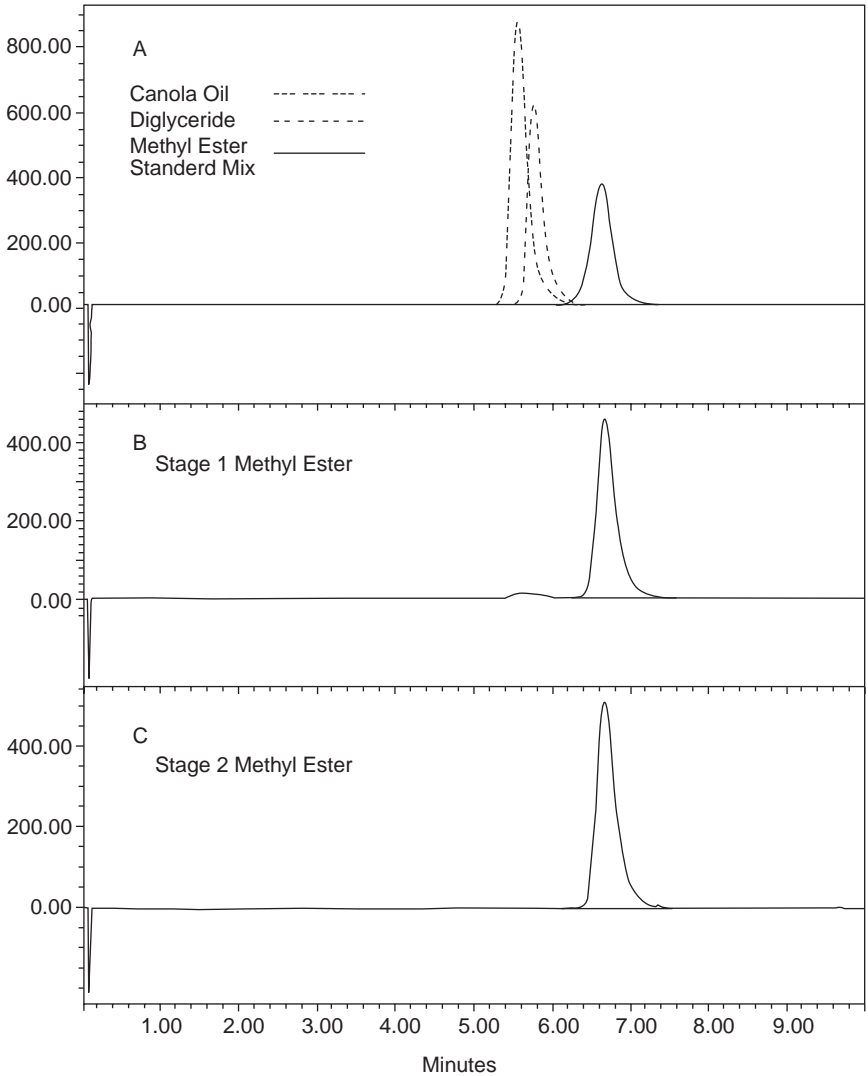
**Figure 1.** Proton NMR (400 MHz) spectra: a) spectrum of bulk refined canola oil in deuterated chloroform ( $\text{CDCl}_3$ ) with the expansion of the region containing protons from glycerine (3.4–4.8 ppm); b) spectrum of single stage reaction of canola oil with methanol and KOH in  $\text{CDCl}_3$  with the expansion of the region containing protons from glycerine (3.4–4.8 ppm); c) spectrum of two stage reaction of canola oil with methanol and KOH in  $\text{CDCl}_3$  with the expansion of the region containing protons from glycerine (3.4–4.8 ppm).

identify those materials that warrant further analyses. Samples that failed NIR scrutiny would be subject to more stringent testing methods, such as gas chromatography (GC), to determine the exact problem with the fuel. Mittlebach (20) suggested that GC methods provided most of the needed information for determining biodiesel quality.

In many specifications, there is redundancy in biodiesel testing. The ASTM specification is unique because it does not limit methanol concentration, but the specified flashpoint is only possible if the methanol content is very low (2). Other specifications define both methanol content and flash point. Some biodiesel standards are also redundant in the measurement of mono-, di-, and triacylglycerols as separate analyses from the determination of free and bound glycerol (15–18). The quantitation of individual acylglycerols is achieved by liquid or gas chromatography and may require sample preparation and a significant period for analysis. Free and bound glycerol can be determined by relatively simple colorimetric iodometry (20, 21). In the future, rapid spectrophotometric methods will likely lead to online analyses, as predicted by Knothe (21). Online methods will be based on techniques that have sufficient signal-to-noise ratio to determine the presence of low levels of contaminants while accurately measuring target components. Figure 1a shows the 400 MHz NMR spectrum of canola oil, whereas Figure 1b and 1c show the first and second stage of a two-stage *trans*-esterification, respectively. The inset to all figures shows the expansion of the spectrum from 3.5 ppm to 4.5 ppm. The loss of the proton signals between 4.1 and 4.4 and their replacement by a singlet at 3.7 ppm is indicative of the replacement of glycerol with methanol. The NMR method allows direct quantitation of glycerol and methanol without derivatization. New NMR technology based on microcells could allow development of a cost-effective instrument for rapid and precise determination of glycerol (22). Although NMR technology remains costly, HPLC methods involving gel permeation chromatography (GPC) are also able to separate acylglycerides. Figure 2a shows the separation of four standards using a gel permeation column where larger molecules elute first followed by smaller molecules. Therefore, the elution order is triacylglycerols, diacylglycerols, monoacylglycerols, and, finally, methyl esters, which coelute with fatty acids. In Figure 2b, the residual acylglycerols in the first stage of a biodiesel synthesis reaction are clearly visible. These components are not detected in the second stage of the reaction. The largest problem with both NMR and GPC is low sensitivity. The liquid chromatography method relies on a light scattering detector, which does not provide a linear response and may only be used for qualitative analysis.

### 2.3. Toxicological Parameters

Methyl and ethyl esters of fatty acids have been used in consumer products for a long time and are generally recognized as safe in specific applications (23, 24). However, the new use of biodiesel as a fuel has led to tests to determine its potential toxicity in new applications.



**Figure 2.** Gel permeation chromatograms: a) chromatogram of four standards of triglyceride (triolein), diglyceride (diolein), and methyl ester standards as detected by evaporative scattering light detection; b) chromatogram of single stage reaction of canola oil with methanol and KOH; c) chromatogram of a two stage reaction of canola oil with methanol and KOH.

Acute oral toxicity was determined in fasted male and female albino rats to be greater than 5000 mg/kg body weight for both methyl and ethyl esters of canola oil (25). Dermal toxicity was tested on albino rabbits. Applying levels of up to 2000 mg/kg body weight was found to have no observable effect for systemic toxicity (25). The treatment produced only slight and temporary erythema (redness) and edema (swelling).



Acute aquatic toxicity was determined on both *Daphnia magna* and rainbow trout (25). The *Daphnia magna* became trapped in oil sheen and the concentration that produced 50% death was determined to be 99 ppm (compared with table salt at 3.7 ppm and diesel at 1.4 ppm).

Rainbow trout survived 48 hours of treatment with 100 ppm and 300 ppm canola methyl and ethyl esters, but were in poor condition (25). Biodiesel exhibits acute toxicity in aquatic systems but its rapid degradation and low overall toxicity make it greatly preferred to diesel fuel in environmentally sensitive areas.

### 3. BIODIESEL AND DIESEL EMISSIONS

#### 3.1. Local Emissions

The U.S. National Institute for Occupational Safety and Health (NIOSH) has identified diesel exhaust as a “potential occupational carcinogen” (26). Particulate matter in diesel exhaust has been identified as a potential risk factor. Biodiesel emissions are generally found to be more benign than diesel fuel, but it cannot be said that all components of the emissions are reduced compared with conventional diesel fuels. The results can be especially confusing as diesel fuels can differ greatly depending on the source of crude oil and the refining process that was used to produce the fuels (10). Simply stated, biodiesel emissions compare favorably with emissions from most diesel fuels, but the fuels used for comparisons have varied among tests. In addition, as fuel specifications are changed, often in response to legislation, combustion characteristics of “conventional” diesel fuels are also changing. Older data obtained using fuels with higher sulfur and aromatic components and lower cetane numbers may no longer represent current industrial practice.

Furthermore, biodiesel is variable depending on the source of the fuel and its age (27). For example, Monyem and Van Gerpen (27) observed decreased carbon monoxide and hydrocarbon emissions from oxidized neat biodiesel when compared with unoxidized biodiesel. In addition, cetane number is inversely proportional to iodine value (28) and proportional to biodiesel age (29).

Hydrocarbon, carbon monoxide, and particulate matter are all reduced in both direct and indirect inject diesel engines fueled with biodiesel when compared with diesel, whereas nitrogen oxide emissions are increased (30–33).

In addition to the measurements on regulated emissions mentioned above, unregulated emissions and, in particular, aldehyde and polyaromatic hydrocarbon emissions, have been studied extensively. When compared with engines operating on conventional diesel, formaldehyde and acrolein were found to increase by 40% in engines operating on biodiesel fuel while polyaromatic hydrocarbons greatly decreased (33). Although mutagenicity was not attributed to specific compounds, diesel fuel exhaust was found to have greater total mutagenic potential in modified Ames testing when compared with biodiesel fuel exhaust (33).

The combined emissions improvements obtainable with 100% biodiesel provide an advantage in enclosed areas where the use of this fuel mitigates the potentially

toxic diesel fumes. This toxicity of biodiesel exhaust was thoroughly tested by the Lovelace Respiratory Research Institute (34). The authors concluded that “no pronounced toxicity resulted from the exposure of rats to biodiesel exhaust emissions at any concentration.” Histological analysis revealed small changes in the lungs of female rats treated with the highest level of exhaust indicating an adverse exposure to high-level exhaust. The study also noted some mutagenic activity in both the particulate and semivolatile fractions. The authors of the study assert that “the no-adverse-effect-level for this study of inhaled biodiesel exhaust emission was the intermediate exposure level” (34).

Blends of biodiesel with conventional diesel fuel have also produced substantial improvements in combustion products similar to those reported for pure biodiesel. Several research groups (35–37) have investigated the potential for construction of a fuel sensor that may be able to detect biodiesel (and ethanol) content in fuel and adjust the engine during operation to minimize  $\text{NO}_x$  emissions. Although they have met with some technical difficulties, such a detector is likely practical. With improvements obtainable through combinations of biodiesel, low-sulfur diesel, and emission controls including oxidation catalysts and adaptive fuel systems, it may be possible to meet stringent new standards.

### 3.2. Global Emissions

The impacts of biodiesel on global greenhouse gas emissions have been extensively and repeatedly studied. The exercise of Life Cycle Assessment (LCA) is a form of accounting that is used to determine the net impact of a process or series of processes. In an early LCA assessment of biodiesel production, Krahl et al. (38) reported a positive output of energy from the cultivation of winter canola grown under European conditions. Other assessments have followed and generally reveal a ratio of one unit of fossil energy used in the production of two to four units of renewable energy embodied in the biodiesel fuel (38–41). The major energy consumption in biodiesel production revealed by LCA includes field tillage, synthetic fertilizer (N, P, K, and S) production, oilseed processing to recover oil, and methanol used in *trans*-esterification (38–41). Substantial improvements in LCA are possible. For example, herbicide-resistant crops may be grown using minimum tillage technology to save substantially on fuel consumption (42). Nitrogen-fixing organisms like soybean have an enhanced positive LCA mainly because of their minimal need for nitrogen fertilizer.

It may be assumed that LCA is not static and that numerous improvements may be made to improve the overall assessment. In Canada, substantial improvements to the ratio of yield to energy input have been attained through the adoption of low-tillage systems made possible by the introduction of herbicide-tolerant crops (42). Oilseed crops have been developed using hybrid seed technology that displays yield improvement over nonhybrids (43). Agronomic practices are continuously improving the efficiency of fertilizer use and decreasing field emissions.

Improvements in the LCA of biodiesel may be realized by using ethanol in biodiesel production. The LCA of ethanol is positive (39, 40) but not as positive as

biodiesel (38–41). As ethanol comprises a greater portion of the biodiesel fuel compared with methanol, it may be seen to displace a portion of the higher LCA fuel. It is important to note that substitution of methanol with ethanol in biodiesel production yields more fuel. Therefore, the impact of substituting ethanol for methanol is a decrease in total greenhouse gas emissions due to the larger potential volume of fuel produced, but the LCA assessment on a fuel weight basis is not greatly affected.

Life Cycle Assessment of canola and other oilseeds could be substantially improved by increasing seed oil content while decreasing overall plant phosphorous and nitrogen content. Canola meal is particularly high in phosphorous in the form of phytate (inositol hexaphosphate) (44). Phytate is indigestible by nonruminants and can impair digestion by making mineral nutrients, particularly zinc, unavailable. It is likely that a reduction in phosphorous in canola meal or a conversion of the phytate phosphorous to other forms would have a net positive impact on LCA as it would be easier to use the meal as feed. To date, corn varieties released with low phytate characteristics have had reduced yields when compared with higher phytate counterparts (45). A similar reduction in canola phytate level with consequent energy savings may also be possible through conventional breeding or through genetic engineering. Although it is not desirable to lower the protein content of canola meal, it is possible to reduce the total meal production with respect to oil. This has been accomplished with the development of yellow-seeded canola varieties that have significantly higher oil content and a higher ratio of oil to protein

**TABLE 2. Biodiesel Production Capabilities, North America 2002.\***

Name	Location	Capacity	Feedstock
Griffin Industries	Butler, Kentucky	6.8 million L/year	Waste Oil & Grease and animal fats
Ocean Air	Lakeland, FL	38 million L/year	Waste Oil & Grease
Pacific Biodiesel	Kahului, Hawaii	567,000 L/year	Waste Oil & Grease
Stepan Co.	Joliet, IL	UNK	Soy Oil
Ag Environmental	Lenexa, Kansas	22.7 million L/year	Soybeans
Imperial Western Products	Coachella, CA	46 million L/year	Waste oil and grease/and soy
West Central Co-operative	Ralston, Iowa	45 million L/year	Soybeans
Columbus Foods	Chicago, Illinois	5.7 million L/year	
Biodiesel Industries	Las Vegas, Nevada	18 million L/year	Waste oil and grease
American Bio-Fuels/ Green Star Products	Adelanto, CA	10 million L/year	Soybeans
Montana Biodiesel	Missoula, Montana	1 million L/year	
Proctor and Gamble Co.	Cincinnati, OH	Unknown	
Government of Saskatchewan	Saskatoon Saskatchewan	8 million L/year	Refined Canola Oil

\*Survey performed by telephone October 2002 by the senior author.

**TABLE 3. Biodiesel Production in Selected Countries (Approximate Use in 2000).**

Region	Biodiesel
Annual Biodiesel Production (millions of liters)	
North America	
USA	22
Canada	<1
Europe	
Austria	22
Belgium	90
France	275
Germany	230
Italy	90
Sweden	11

than conventional dark seed coat varieties (46). These varieties also have higher average meal protein contents than the brown seed coat counterparts. Owing to its high molecular weight, rapeseed (high erucic acid rapeseed) oil produces less glycerol and requires less methanol for *trans*-esterification than canola oil. The best combinations of plant traits would improve LCA of canola biodiesel well beyond current practice.

Soybean, corn and sunflower oil content can also be manipulated by plant breeding (47, 48). Historic attempts to increase oil content of soy have lowered protein production (47). If the domestic demand for soybean biodiesel increases in North America, diversion of plant photosynthate from meal to oil production may prove advantageous and limit market distortions.

### 3.3. Global Biodiesel Production

The Austrian Biofuels Institute currently maintains an ongoing database on the global biodiesel industry (49). Their current statistics reveal that most biodiesel is obtained from canola (low erucic acid rapeseed; 84%) and sunflower (13%). European biodiesel production dominated world production in 2000 and Europeans have chosen canola oil as the mainstay for manufacturing biodiesel. However, recent facility construction (50) and legislative action (51) in North America may lead to rapid increases in biodiesel production (Table 2). The State of Minnesota has passed legislation that mandates the inclusion of 2% biodiesel in all diesel fuel sold in the state (51). European and American biodiesel production is rapidly increasing. In 2000, U.S. production was just 22 ML (Table 3).

## 4. RESOURCES FOR BIODIESEL PRODUCTION

Currently, most biodiesel is synthesized from higher quality vegetable oils, including canola, sunflower, and soy. More recently, used frying oil and tallow have

increasingly been used as sources of oil for biodiesel production (Table 2) (1). Modern biodiesel facilities are usually designed with capability to cleanly process multiple-feed stocks (MFS) to accept lower cost oils. The capability of dealing with lower cost feed stocks is also important in recovering esters and acids that are often entrained in glycerol during processing. Modern MFS biodiesel plants can convert almost 100% of fatty acids and esters to usable biodiesel (1).

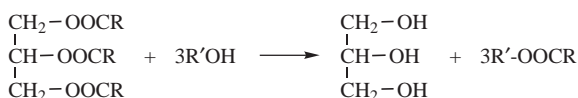
New sources of oil are continually being suggested for biodiesel production. Rubber seed (*Hevea brasiliensis*) produces unsaturated oil that has little value and is often not recovered but could be effectively used to produce biodiesel (52). *Jatropha curcus*, a bushy tree that grows quickly and produces a fruit with a high-oil seed, has been touted as an energy plant for tropical climates (53).

Research has also been conducted into the development of unique high-glucosinolate producing *Brassica sp.* hybrids (54). These species are suggested as industrial crops because the meal they produce is inedible and would not enter feed markets, but the glucosinolates in the meal provide the potential for use of the meal or meal extracts as pesticides. The tuber, yellow nut-sedge (*Cyperus esculentus* L.), is also a rich source of oil, and blends of this oil with diesel fuels have been recommended (55).

Northern regions with short growing seasons have limited total production potential. Typical yields of canola and flaxseed in Canada, for example, average 1300 kg/ha (56), whereas in Europe, yields of these crops are often double this amount (57); the EU average yield for canola was 2700 kg/ha in 1998. The primary limitation in growing more productive crops is the cold winter conditions that prevent the introduction of winter varieties. Enormous potential exists for increasing the production of oilseed in northern climates (e.g., Europe, North America, and Northern Asia) through the introduction of higher yielding species.

## 5. PRODUCTION TECHNOLOGY

Biodiesel is produced from vegetable- and animal-based oils by esterification or *trans*-esterification with a lower alcohol (3). Most vegetable oils and animal fats are predominantly triacylglycerols (TAGs). Although TAGs may be incorporated in diesel fuels without chemical modification, these compounds increase fuel viscosity, are poorly combusted, and tend to prematurely foul upper cylinder engine parts. Therefore, chemical processes of converting fats and oils to alkyl esters of monohydric alcohols are now in common use to produce a fuel with lower viscosity that may be used as a direct replacement for diesel fuel. The core synthetic process of most biodiesel production technologies is *trans*-esterification (3). In this process, animal fat or vegetable oil consisting mostly of acylglycerols is reacted with a catalyst and alcohol. The reaction that ensues consumes the acylglycerols and liberates glycerol and alkyl esters of lower alcohols. Reaction 1 shows the *trans*-esterification of a triacylglycerol with three moles of alcohol releasing one mole of glycerol and three moles of ester. Ma and Hanna (3) reviewed biodiesel production, and this article is suggested as an additional reference.

**Reaction 1**


where

R = long chain alkyl group (Example:  $(\text{CH}_2)_7(\text{CH})_2(\text{CH}_2)_7\text{CH}_3$ )

R' = short chain alkyl group (Example:  $\text{CH}_3$ )

**5.1. Oil**

Oil quality and composition are important determining factors in optimizing esterification strategy. Oils may contain numerous components that will affect the efficiency of esterification. Common oil contaminants include fatty acids, partial acylglycerols, phospholipids, unsaponifiables, and water (20, 21).

Fatty acids react with alkaline catalysts to form catalytically inactive soaps (3). The chemical reaction consumes one mole of fatty acid per mole of alkaline catalyst. Although fatty acid composition of the starting material varies, the content determined by titration reflects the amount of catalyst that would be consumed in a chemical reaction. By calculation, it may be determined that one gram of fatty acid (expressed as oleic acid) will react with about 0.2 g of anhydrous potassium hydroxide or 0.14 g of anhydrous sodium hydroxide. Often, additional catalyst must be added to esterify a vegetable oil containing higher levels of fatty acids (3). Conversely, acid catalysts are not inactivated by fatty acids (3). In a unique reaction, fatty acids produced during biodiesel manufacture are actually used as a catalyst in their own esterification (see below).

Partial acylglycerols, including mono- and diacylglycerols (DAGs), often occur in high concentrations in the same reaction mixtures as fatty acids (58). These materials can be thought of as interim products of the biodiesel manufacturing process and do not normally present significant problems with reactions. However, monoacylglycerols (MAGs) can increase the viscosity and melting point of oils (59). Furthermore, lower concentrations of alcohol would be required to esterify oils that are enriched in partial acylglycerols. One mole of glycerol mono-oleate (356 g/mole) may react with one mole of methanol to produce one mole of methyl ester (296 g/mole) and one mole of glycerol. In this reaction, the mass ratio of alcohol to MAG is lower than that produced in a reaction with a TAG. The yield of glycerol is higher than that produced in reaction with the TAG. As emulsifiers, MAGs may also serve to accelerate the formation of esters.

Phospholipids are often removed in refining steps prior to biodiesel synthesis processes (3). Refining steps for phospholipid extraction often include the industrial-standard degumming with acids or water that form oil-insoluble materials, which are readily removed by centrifugation. These methods have the disadvantage of removing a portion of the oil, which is lost to further reaction. Other methods include removing the gums while the oil is dissolved in a hexane solution (60).

This process may mitigate some neutral oil losses. It is predictable from chemistry that the impact of the presence of phospholipid during *trans*-esterification is mostly a contamination of the glycerol phase with compounds that include glycerol phosphate, inositol, and basic compounds (choline, serine) derived from the phospholipid. The authors are not aware of studies describing the presence of these products in the glycerol derived from *trans*-esterification of unrefined oils. In our experience, oils with high levels of phospholipid (50–300 ppm) are readily *trans*-esterified using alkaline catalysts. The remaining oil is depleted in phosphorous by the reaction, and the resulting methyl esters contain less than 10 ppm phosphorous after refining (unpublished results). The impact of this process on glycerol production is not known.

Unaponifiable materials are rarely considered as significant components in biodiesel, though they may constitute several percent of the fuel weight (61). Unaponifiables are primarily alcohols and hydrocarbons that do not affect the overall *trans*-esterification reaction. Unaponifiable components do not require alcohol for esterification and decrease the total alcohol used in *trans*-esterification to a very small extent. The presence of unaponifiable matter in biodiesel raises questions regarding the fate of these materials during combustion. In most oils, the unaponifiable fraction is composed mainly of sterols that are higher in molecular weight than other fuel constituents. To the authors' knowledge, little is known of the fate of sterols during combustion in a diesel combustion cycle (62). However, research has been conducted on the effects of unaponifiables on cetane number. The slight changes that were observed were less than the ASTM repeatability and reproducibility specifications.

Biological oils are often contaminated with water in emulsion (3). Although some water is tolerable in the synthesis of esters, its presence is generally not preferred. Water can act with alkali catalysts to saponify esters that form soaps. Water can also change the equilibrium of esterification with acidic catalysts favoring the formation of free fatty acids over that of esters. Water has the potential to react with 16 times its weight with TAG or ester, assuming the ester or TAG is of oleic acid. As will be described later, this reaction can be mitigated even if water is present.

Oil composition specifically affects the esterification strategy and also the potential uses of the ester. Esters rich in highly saturated fats and high-melting *trans*-fatty acids, for example, may need to be esterified or *trans*-esterified at elevated temperatures. (3). The product esters of these saturated compounds will typically have elevated melting points (59). Such esters are often not well suited for use in fuels that may be exposed to lower temperature (62). With dilution in fuel, low-melting esters or solvent esters of saturated fatty acids may be included in fuels suitable for use at low temperatures (62). Unsaturated fats have lower freezing points and are often preferred in lower temperature applications (63). Polyunsaturated fats are prone to oxidation and polymerization. The changes induced by oxidation may alter the quality of biodiesel deriving from these fats (64). Polymerization can increase the fuel viscosity and alter combustion characteristics (27, 64). In addition to the increase in viscosity with oil oxidation, in an accelerated aging test, acid value

**TABLE 4. Impact of Oil Type on Alcohol Required for *Trans*-Esterification.**

Oil Type	Oil <i>moles/kg</i>	Methanol <i>kg/kg Oil</i>
Palm oil	1.17	0.112
Canola oil* (LEAR)	1.13	0.108
Rapeseed oil (HEAR)	1.04	0.100

\*Moles/kilogram is similar for canola, soy, corn, sunflower, and flax.

and specific gravity also increased (64). The antioxidants  $\alpha$ -tocopherol and tert-butylhydroquinone (TBHQ) were partially effective in mitigating effects of accelerated aging (64).

The ratio of alcohol to oil in ester is determined by the molar weights of both the alcohol and the oil. Lower molecular weight fatty acids comprise a smaller portion of an ester product than higher molecular weight substances (Table 4). For common fatty acids, changes in chain length have the greatest impact on ester weight, whereas changes in saturation have little impact. Biodiesel made with oils rich in palmitic acid will require more alcohol than biodiesel made with oils rich in oleic or higher alcohols. Erucic acid, found in rapeseed oil, requires 10% less alcohol in the synthesis of its esters than oleic acid.

## 5.2. Alcohol

Many choices of alcohol are possible, including methanol, ethanol, isopropanol, n-propanol, butanol (all isomers), and pentanol (all isomers) (65). In general, improved conversion is achieved by adding extra alcohol (3). Higher molecular weight alcohols have lower polarity. Phase separations become more difficult with higher alcohols (66). Mixing higher alcohols with methanol has been used to improve reaction completion and phase separation (66). Adding glycerol and water can have the same effect (67). Small amounts of water are tolerable only if a glycerol phase forms (68). Otherwise, soaps form slowly in a reversible reaction. In reactions where water is present, the key to avoiding saponification is to minimize reaction time and have water only in the glycerol phase. The amount of alcohol contributing to the weight of the ester increases with the molecular weight of the alcohol. Table 5 indicates the contribution of various alcohols that may be used in esterification of triolein. Approximately 5% weight contribution increase in the proportion of alcohol to ester is contributed with each additional carbon atom.

## 5.3. Catalyst

**5.3.1. Alkali** In base catalysis of fatty acids, little or no esterification occurs and saponification is essentially irreversible. Therefore, it is necessary to optimize con-

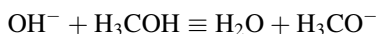


**TABLE 5. Alcohol Molecular Weights and Percent Weight of Three Moles of Alcohol to One Mole of Pure Triolein.**

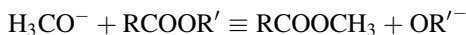
Alcohol	Molecular Weight (g/mole)	Ratio (3 mole alcohol/ 1 mole triolein; w/w)
Methanol	32	11
Ethanol	46	15
Propanol	60	20
Butanol	74	25
Pentanol	88	30

ditions so that hydrolysis (saponification) is minimized. Water present in the oil, alcohol, or catalyst may result in saponification (3). As the reaction proceeds and sufficient glycerol is formed to separate from solution, water will dissolve preferentially in the glycerol phase (67). The separation of the glycerol phase can prevent substantial lipid hydrolysis even when substantial amounts of water are present (67, 68). In alkali catalyzed reactions, the catalyst is presumed to be metal alkylate of the alcohol. For example, potassium hydroxide reacts with methanol to reversibly form potassium methoxide and water. The water of reaction is not sufficient to produce significant quantities of fatty acids through hydrolysis (3).

Reaction 2:



Reaction 3:



**5.3.1.1. NaOH vs KOH** Sodium hydroxide has a lower molecular weight than KOH, and thus it also has more moles per gram (Table 6). The potassium ion is larger and forms slightly more soluble salts, including more oil-soluble ethoxide. On a mole basis, KOH is a more efficient catalyst than NaOH. Weight for weight both catalysts are equivalent in reaction rate. However, water and fatty acids are known to inactivate both catalysts with one mole of fatty acids reacting with one

**TABLE 6. Comparison of Alkaline Catalysts Required for Efficient *Trans*-Esterification.**

Catalyst	Purity	Mol. Wt.	Moles Base (100 g/oil)
0.25% KOCH <sub>3</sub>	0.95	70	0.0034
0.25% NaOCH <sub>3</sub>	0.95	54	0.0044
1.0% KOH	0.9	56	0.0161
1.0% NaOH	0.9	40	0.0225

mole of each catalyst. In this reaction, it requires significantly more fatty acid to inactivate sodium hydroxide than potassium hydroxide.

**5.3.1.2.  $\text{NaOCH}_3$  vs  $\text{NaOH}$**  Where oils and alcohols contain little water, it is possible to use alkoxide catalysts to produce alkyl esters from vegetable oils. In anhydrous systems, as little as 0.1% to 0.2% alkoxide by weight of oil can efficiently catalyze *trans*-esterification, but higher concentrations are normally reported (3). Alkoxides also have the advantage of minimizing the base content of the glycerol product. For example, a *trans*-esterification process using 0.25% potassium methoxide would require approximately 0.034 moles of methoxide per kg of oil, whereas 1% potassium hydroxide would require 0.16 moles per kg of oil (Table 6). The relatively small level of ions simplifies the requirements of glycerol refining. An additional benefit of alkoxides is the avoidance of water. Typically commercial hydroxides contain small amounts of water and form additional water in reaction with alcohol to form the alkoxide. By definition, commercial methoxides are anhydrous. Water produced by or released from catalyst in biodiesel manufacture will eventually be recovered with methanol evaporation. If the water content of the alcohol is substantial, a rectification distillation column and a zeolite-drying column are required to distill dry alcohol for further esterification (69). Methoxide catalysts can be used to avoid adding water to the reaction and to prevent water accumulation in the alcohol. Alcohol recovered from alkoxide-catalyzed reactions may be dried using a zeolite-packed column.

**5.3.2. Acid** Esters are readily synthesized from fatty acids and alcohols in the presence of acid catalysts. In general, a large excess of alkylating reagent is required to drive the equilibrium. Preferred acids include sulfuric acid and hydrogen chloride, although *p*-toluenesulfonic acid is also used (3). Acid-catalyzed esterification of fatty acids is relatively rapid and is usually performed at the reflux temperature of the alcohol. Small amounts of water are known to reverse the reaction, and thus methods that require large additions of alcohol are often used (3, 70). However, methods that significantly lower water content or provide a second phase that contains the water can lead to esterification with smaller amounts of alcohol (67).

As a result of the need for acid-resistant alloys and other equipment required for acid esterification, the process is typically more capital intensive than base *trans*-esterification. The higher capital costs associated with the use of acidic catalysts are usually offset by the ability of the process to accept lower cost feedstocks (1). Acidic catalysts may be used to recover soap byproducts of alkali-catalyst based *trans*-esterification processes (3, 71). In these processes, acid is used to convert soap to free fatty acids and then to esters (see below).

Many oil products are available that cannot be esterified directly using alkali catalysts as they have sufficient fatty acid content to neutralize an alkali catalyst, yet have high ester contents. An acid esterification step may be employed in advance of base catalyzed esterification. These pre-esterification methods need only provide sufficient acid and alcohol to esterify the fatty acids present (3, 68, 71), although

some methods use large molar excesses of alcohol (72). Acid-catalyzed *trans*-esterification of acylglycerols with alcohol is a relatively slow reaction when compared with acid-catalyzed esterification. *Trans*-esterification and release of glycerol is avoided by maintaining abbreviated reaction times. In essence, the bulk of the acylglycerol oil is not reacted and, therefore, inert during the pre-esterification reaction. These pre-esterification methods will be discussed in detail below.

**5.3.3. Exotic** Ideally, catalysts are long lasting, hasten reactions, lower energy inputs, and simplify product refining. Alkali catalysts, used as the mainstay of many biodiesel production processes, are entrained in glycerol released by the process and are lost for further catalysis. Alkali catalysts are neutralized by fatty acids forming soaps with limited alkalinity that do not act as catalysts. This latter property limits the utility of basic catalysts to applications with low acid value oils. Liquid acid catalysts may be reacted with oils with high acid numbers but, like basic catalysts, these materials tend to be soluble in the glycerol phase.

Most innovative approaches to catalysis involve new heterogeneous (solid) catalysts that can be repeatedly used to manufacture biodiesel, although a few novel approaches describe using homogeneous catalysts. For example, Demmering (72) was able to use oleic acid as a catalyst for biodiesel synthesis with reaction conditions of 240°C for 3 hours. Georghiu (73) was able to generate esters using an organotitanate catalyst at just 0.15% of the reaction mixture using a reaction time of 2.5 hours at 220°C. Acidic resins are also capable of forming esters but may also produce ethers as a coproduct. Lundquist (74) describes esterification using acidic resins at 110°C. The reaction produced a significant amount of dimethyl ether in spite of adjusting reaction conditions to minimize ether formation. Resin-based acid catalysts may also be used in pre-esterification of acidic oils. Jeromin et al. (75) achieved a reduction of acid value from 10 to less than 1. The elevated temperature, high autogenic pressure, and exotic catalysts often combine to detract from the economics of these novel catalysts. Improved catalysts are anticipated.

Ultimately, enzymatic esterification holds great promise in biodiesel production. Foglia et al. (76) and Haas (77) have worked to develop enzymatic methods for production of alkyl esters. Foglia et al. (76) found that 10% lipase by weight of TAG stirred at 200 rpm for 5 hours at 45°C was sufficient for high conversion of oils to alkyl esters. As a result of the current high cost and low activity of enzymes, the reaction described was limited in scale. Wu et al. (78) have been able to improve and regenerate lipase activity by washing the catalyst with alcohols with three or more carbon atoms. However, the enzymatic reactions and their improvements as described did not lead to a process that could economically yield commercial quantities of fuel. Enzymes will not be components of commercial biodiesel synthesis until significant improvements have been developed.

## 5.4. Ester Production Processes and Strategies

An abundance of intellectual property has been developed to improve production of fatty acid esters. The goals of most inventions are to increase the yield of product,

use lower quality materials, ease refining requirements, or minimize inputs such as energy, catalyst, or alcohol. Simple principles of chemistry would seem to limit the efficiency of all processes (i.e., esterification is reversible) but combinations of principles have been used to design processes and reactions that achieve almost 100% efficient conversion of materials. Basic catalyzed reactions often appear to go beyond theoretical yields; but this is caused by the separation of glycerol into a second phase during the reaction. This phenomenon prevents feedback inhibition of glycerol on ester synthesis. Soaps do not accumulate to high levels in the reaction as they dissolve in water, lower alcohol, and glycerol. Removal of soaps in a glycerol phase can greatly assist in the refining required for biodiesel production.

Some total processes define the extraction and refining of the oilseed within their invention. Stidman et al. (79) describes a process for extracting oils from soybean and converting those oils to methyl esters. The process includes standard steps for degumming, caustic refining, and bleaching followed by esterification. The process finishes with glycerin removal and a washing step.

In one process, the inventor has reported that sugars from oilseed meals are readily fermented to form ethanol (80). With soybeans, the concentration of fermentable sugars can constitute 12% of the meal weight. These sugars could readily be fermented to produce enough ethanol for esterification of oil from the seed. However, it is improbable that seeds with higher oil contents would produce sufficient ethanol from fermentation to esterify the oils present.

**5.4.1. Unique Reaction Sequence** Advantageous methods have been developed to improve esterification processes. In an early development, Glossop (81) reported the removal of fatty acids from high acid oils by washing with methanol or another solvent that was not miscible with oil in a countercurrent extractor. After washing, the oil phase had low free fatty acids and was suitable for base *trans*-esterification. The methanol phase was treated with acid to esterify the fatty acids.

It is often found to be more difficult to produce esters of ethanol and higher alcohols than methanol. Dreger (66) overcame this inefficiency in forming esters by using two-stage *trans*-esterification and two alcohols, one of which is methanol. The reaction produced a mixed ester, but the yield was significantly improved.

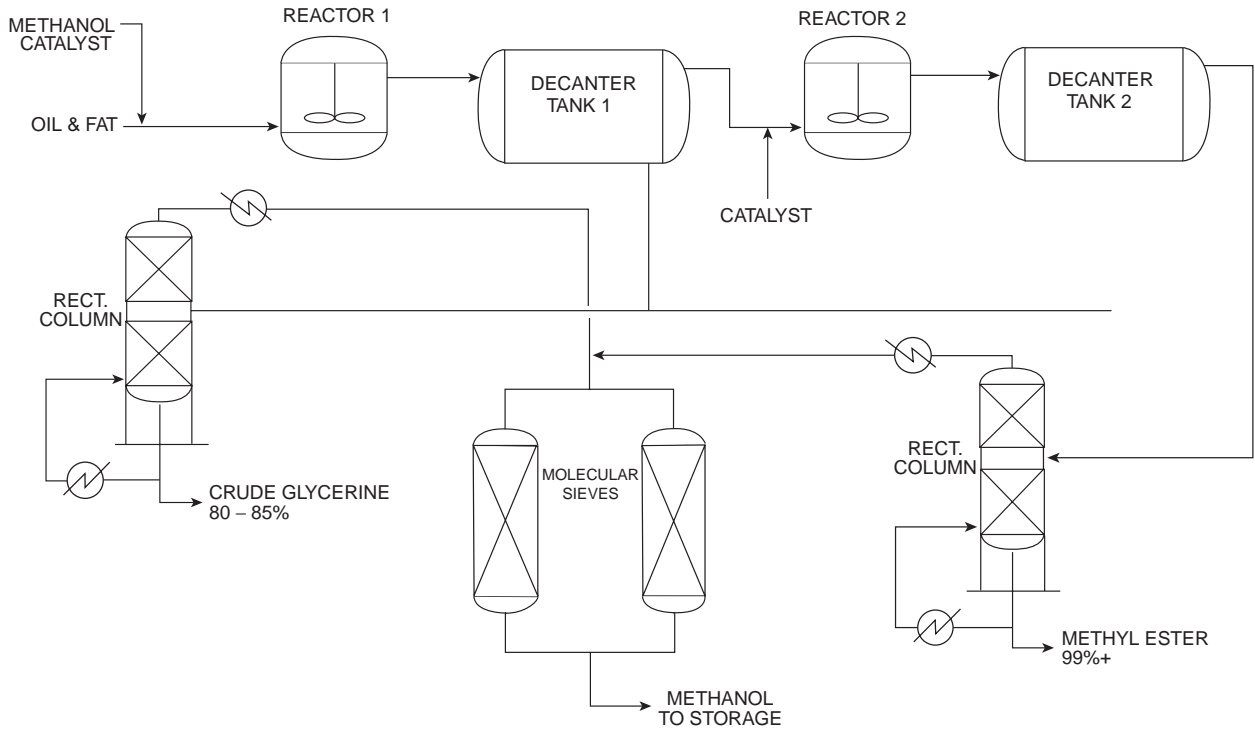
**5.4.2. Unique Reaction Conditions** Billenstein et al. (82) reported 498-g tallow plus 27 g of 30% sodium methylate were combined in a reactor and successfully reacted with alcohol vapor at  $>210^{\circ}\text{C}$ .

Klok and Verveer (83) were able to achieve extensive esterification with the minimum addition of alcohol. According to their description, 40-kg soy oil and 6.2-kg methanol were heated to  $65^{\circ}\text{C}$  and 0.95-kg 30% sodium methoxide was added. The mixture was stirred for 1.5 h then settled and the glycerol separated. Subsequently, a further 0.2 kg of 30% sodium methoxide was added to the ester layer. The reaction mixture was heated and methanol recovered from the methyl ester layer by distillation.

Soapstock is a novel low-cost material that is rich in fats. Soapstock is generated during alkali refining of vegetable oils, where oils are mixed with sodium or potassium hydroxide solutions (84). This mixture is centrifuged to yield a low acidity, light-refined oil phase and a heavy phase composed of soap, neutral lipid, and water. Haas et al. (85) developed a process for converting these lipids into esters. In the process, the soap solution is treated with additional alkali to complete the saponification, and then it is dried. The dried solids may be esterified by the addition of inorganic acid catalysts and alcohol. Reaney (86) also approached the conversion of soapstock to esters. In the patented technology, soapstock was treated with acid and an alcohol, where the alcohol was selected from among alcohols that remained insoluble in salt water. The process efficiently generates separate phases of water and oils without evaporation. Fatty acids and alcohol in the mixed upper layer are condensed to esters through treatment with heat. The resulting product, including mixed esters and glycerides, may be *trans*-esterified using base catalysis to remove glycerol. Preferred alcohols for conducting this reaction include n-propanol, i-propanol, n-butanol, and isopentanol alcohol. Mixtures of these alcohols, called fusel oils, may be obtained as byproducts from the production of ethanol by fermentation.

The early stages of most biodiesel reactions proceed slowly, but the slow reaction is readily overcome when the reaction is induced to form a single phase. Boocock (87) described methods of accelerating methyl ester synthesis by adding ether cosolvents. In one example, 100 g of soy oil was reacted with 28 mL of methanol in the presence of 35 mL of tetrahydrofuran and 1 g of sodium hydroxide. The reaction proceeded rapidly and separated in 20 min. After the reaction was complete, the solvents were flashed off the methyl ester phase and a small amount of glycerol separated. The ester yield was 90.1%.

Stern et al. (71) recovered free fatty acids from alkaline esterification. Fatty acids are converted to soaps in *trans*-esterification. When the alkaline glycerol layer forms, the soaps partition into the glycerol layer leaving a high-quality methyl ester. The alkaline glycerol is then neutralized with acid and the soaps are converted to fatty acid in the process. The glycerol and fatty acids are mutually insoluble and separate. When separated from the glycerol, the fatty acids are recovered as a concentrate. Stern et al. (71) recommend a process of mixing the fatty acids with glycerol and heating to more than 200°C. The acidity of the mixture is sufficient to allow the formation of esters from the glycerol and fatty acid mixture. The esters may be added to future batches for alkaline *trans*-esterification. This method increases yield of the reaction while allowing some tolerance for fatty acid content in the oil and fatty acid production during processing. Stern et al. (71) described production of methyl esters by *trans*-esterification with 95% ethanol and recovery of the lost fatty acids by reaction with glycerol. Stern et al. (71) also proved that a high yield is possible with alkali-based *trans*-esterification even using 7% acidity palm oil. In this reaction, the level of base required for the reaction was increased from 0.4% to 1.2% by weight. The method may require an additional amount of alkaline catalyst to overcome acids encountered in the reaction. The reaction requires equipment for handling acidic materials but has the advantage that the total



McDonald Patent 6,262,285 Flow  
 Figure X  
 Courtesy of Crown Iron Works, Co.

**Figure 3.** Biodiesel production schematic as taught in US Patent 6,262,285. Flow diagram shows the vessels and devices included in the invention required to mix, react, heat, separate glycerine, recover solvents, and dry solvents for reuse.

material reacting under acidic conditions is less than 10% of the total reaction volume.

McDonald (69) reported a total system for ester synthesis that described a problem that is seldom alluded to in other published methods (Figure 3). Alcohol recycled in biodiesel synthesis commonly accumulates water from catalysts, leaks, and fresh alcohol. If the water content is low, a column containing molecular sieves may remove it, but with higher amounts of water present, a process for drying the alcohol is required. The method described by McDonald (69) also avoids a water wash step for fatty acid methyl esters altogether by decanting the glycerol in continuous decanters and recovering the methanol from the methyl ester directly.

Lepper and Friesenhagen (67) were able to esterify oils rich in free fatty acids by adding alcohol, glycerol (or other polyhydric alcohol), and *p*-toluenesulfonic acid (or other acid catalyst). The unique contribution of this technology is the use of a polyhydric alcohol to form a second phase that does not occur if the reaction does not release glycerol. Fatty acids react quickly to form esters, and the water formed by the reaction is largely partitioned into the glycerol phase. The reaction products include a phase of alkyl esters of lower alcohols and glycerol mixed with a phase of glycerol, catalyst, and methanol. The alkyl ester phase is readily *trans*-esterified using alkaline catalysts to form fatty acid esters suitable for use as biodiesel. The process relies on inexpensive means to refine or reuse the polyol to remain efficient. Lepper and Friesenhagen (67) reported that the polyol was effectively recycled nine times (67).

Wimmer (88) described the need in base catalysis to compensate for the consumption of base by fatty acids. In their patent, they claim the need for 0.025 moles of base catalyst for each 100 g of fatty acid ester. Fatty acids are overcome by the addition of more catalyst, on an equivalent molar amount, to the fatty acids in the free state. Clearly, this method can be used with some fatty acids being present in the oil; but it is not adaptable to oils with elevated fatty acid contents.

All biodiesel plants risk emission of the alcohol used in *trans*-esterification reactions, evaporation, and other processes. Emissions are typically restricted by government regulations and sophisticated recovery processes are required to meet these regulations. Traditionally, methanol can be recovered from plant emissions by condensation, absorption into organic solvents (e.g., triethylene glycol), or adsorption onto activated carbon. In a unique process, Granberg and Schafermeyer (89) have designed an agitated column that uses a chemical reaction to recover methanol entrained in inert gases used in a biodiesel process. Their reaction intimately mixes the inert gas with a catalyst and a "fatty source." The reaction that ensues converts the vapor to nonvolatile alkyl esters and essentially recovers most of the waste alcohol vapor from plant exhaust. In this process, Granberg and Schafermeyer (89) indicated a reduction of alcohol from 16,000 ppm to less than 80 ppm.

Oils with high acid values may be esterified with synthetic heterogeneous catalysts. Jeromin et al. (75) successfully reduced the free fatty acid content of coconut oil in reaction columns containing Lewatit SPC <sup>®</sup>118 BG ion exchange resin (Sybron Chemicals Inc., Birmingham, NJ) and a related product. They claimed methods for pre-esterification of crude fat or oil of vegetable or animal origin in

an admixture with methanol, in a mole ratio of methanol to free fatty acid content of the fat or oil of 10:1 to 50:1 over a fixed bed, heterogeneous catalyst. Although this method holds the promise of directly *trans*-esterifying the resultant mix of esters and alcohol, the authors proceed to evaporate the alcohol to produce oils with low acid numbers.

Kawahara and Ono (90) reviewed methods of pre-esterifying oils with high acid numbers and found that these methods often produced poor quality esters. They attributed the low quality to impurities that were readily extracted by washing the esters with alcohol immediately after pre-esterification.

## 5.5. Summary of Biodiesel Production Technology

Clearly there are numerous technologies for biodiesel production and improvements to increase efficiency and lower costs. Evaluating these processes and comparing the costs of biodiesel production processes can and should be accomplished based on objective criteria such as comprehensive empirical data and comparative analysis of approaches. Many improvements to biodiesel technology may be considered as components that may be added or inserted into existing processes. For the purpose of this discussion, we consider the impact of these improvements on the core process of *trans*-esterification and alcohol recycling described by McDonald (69). The two-stage process described in this patent includes a complete engineering diagram with information on pumps, mixers, and solvent recovery systems. With appropriate insertion of processing data and engineering information, complete economic and operation models may be devised to emulate the process and determine potential advantages of new processes versus old.

As most biodiesel glycerol separations can occur quickly, the McDonald patent reports replacement of typical centrifuge technology with less capital-intensive decanters. Discharging from the decanter will be the lightest, therein the least contaminated, methyl ester from the top discharge and inversely, the heaviest component, glycerol, from the bottom of the decanter. As the methyl ester and methanol mixture from the top of the decanter is free of glycerol, the wash step commonly found in standard processing can be eliminated. The elimination of the wash water step improves energy efficiency, yield, and alcohol recovery.

The many methods of pre-esterification would complement the described process by allowing the use of acylglycerols with higher fatty acid content. The benefits of such methods may then be determined independent of a total process.

Cosolvents such as tetrahydrofuran would increase the need for energy used in evaporation of solvents and may increase the need for vapor compression to condense a mixed phase. The ether solvents described by Boocock (91) would add to the total energy consumed in biodiesel production and would detract from the efficiency of the McDonald design. However, the advantage of using cosolvents in pre-esterification, described later by Boocock (91), could be manifold if lower cost high free fatty acid materials are used. Pre-esterification without the need for evaporation may eventually prove the most efficient method of processing oils with high levels of free fatty acids.



McDonald's last step is then to recover the methanol using vacuum and rectifying the methanol stream using molecular sieves. Inclusion of the molecular sieves significantly lowers energy costs when compared with traditional distillation, as steam is only used during the regeneration of the sieves and steam usage in the rectification column is greatly reduced because a lower operating temperature is required as a result of the use of vacuum. The ingenious vapor recovery system developed by Granberg and Schafermeyer (89) could reduce the cost of condensing alcohol and could be incorporated into most biodiesel manufacturing plants, but it would not provide specific advantages in the recovery of ether solvents. If this technology were incorporated in the McDonald design, this could replace significant portions of the alcohol vapor condensation equipment and energy inputs.

It is possible that the need for water removal from methanol is not necessary if the entire system is maintained in a dry state and dry catalysts are used. For example, sodium methoxide brings less water to the reaction than the comparable hydroxide, and commercial methanol can be obtained in an essentially dry state. In the case where dry oil is available for processing, it may be advantageous to produce biodiesel using the dry ingredients and monitor water levels in recovered methanol.

Many glycol liquids form a two-phase mixture with biodiesel, and these compounds can be contacted with biodiesel to remove polar contaminants (92). The process has many advantages in refining biodiesel but glycols tend to have high boiling points and, thus, are difficult to recycle. The use of glycols as absorptive media described by Bam et al. (92) might consume significant amounts of energy and capital costs in the distillation to recover the liquid glycol products.

Single-stage reactions are normally inefficient, as they require the addition of large amounts of alcohol to drive the reaction to completion. In a single-stage reaction, it is normal to add 5–10 times the amount of alcohol required for a stoichiometric reaction. *Trans*-esterification in a two-stage reaction is advantageous when it allows the reduction of alcohol used for *trans*-esterification to 2–4 times stoichiometric requirements. A single-stage reaction using methanol and a typical vegetable oil with a molecular weight of 885 g/mole (glycerol trioleate) uses between 483 g and 976 g of extra methanol. In a two-stage reaction, the extra methanol is reduced to 108–325 g. In addition, as methyl esters are soluble in both glycerol and cosolvents, a two-stage reaction has lower losses of these materials. In any case, the methyl esters and fatty acids dissolved in alcohol are not lost and they may be recovered.

## 6. UTILIZATION TECHNOLOGY

### 6.1. Lubrication

At current oil crop production levels and fuel consumption rates, biodiesel is unlikely to replace more than a very small portion of total diesel fuel consumed globally (1). However, biodiesel has many potential niche markets where its low toxicity and improved emissions can provide value that outweighs the added costs of using this fuel. Toxicity and biodegradability tests have determined that biodiesel is a

preferred fuel for environmentally sensitive areas where fuel spillage poses an undue risk. This advantage is especially important in inland waterways and national parks. Fuels comprising 20% to 100% biodiesel also have special applications where the oxygen content of the fuel significantly reduces a broad spectrum of potentially toxic emissions. Applications include the use of ultra-low-sulfur-fuel blends with biodiesel and oxidation catalysts in mines to reduce the production of particulates and carbon monoxide. Additional technology is required to reduce NO<sub>x</sub> emissions that accompany the combustion of this fuel.

In addition to fulfilling market demand, governments (1, 51) have also enabled the development of new markets by mandating specific levels of biodiesel in fuels. Primarily, these mandated fuel markets develop demand for domestic agriculture, reduce reliance on foreign fuel sources, and improve the balance of trade. An unexpected improvement in overall fuel economy can be realized with the addition of low levels of biodiesel to certain fuels.

Concern for the environment has resulted in moves to significantly reduce the noxious components in emissions when fuel oils are burned. Attempts are being made to minimize sulfur dioxide emissions and, as a consequence, a strategy to minimize the sulfur content of fuel oils has been implemented. Although typical diesel fuel oils have, in the past, contained 1% or more of sulfur (expressed as elemental sulfur) by weight, environmental legislation in the United States has required that sulfur content of diesel fuel be less than 0.05% (11). These levels will be reduced to 15 ppm or less to protect new exhaust catalyst after-treatment devices. In Europe, various jurisdictions have moved to lower sulfur content. In Sweden, for example, taxation of higher sulfur, lower cetane fuels is elevated to reflect to their respective environmental cost (93).

The reduction in the sulfur content of diesel fuel is correlated with lubricity problems. It is generally accepted that the reduction in sulfur is also accompanied by a reduction in polar oxygen and nitrogen-containing compounds as well as polycyclic aromatic compounds (10). As these compounds are responsible for fuel boundary lubricating ability, their loss in severe refining results in low-sulfur fuels with reduced lubricity. Thus, low-sulfur content is not necessarily indicative of a lubricity problem, but it has become the measure of the degree of refinement of the fuel. In comparison with less refined diesel fuels, ultra-low-sulfur diesel fuels have been found to induce an increase in sliding adhesive wear and fretting wear of pump components including rollers, cam plates, couplings, lever joints, and shaft drive journal bearings. Reducing the level of one or more of the diesel fuel components that contributes to lubricity reduces the ability of the fuel oil to lubricate the injection system of the engine. The engine's upper cylinder liner and top piston rings also suffer from reduced fuel lubricity. When diesel fuels are compared using lubricity bench tests, including the high-frequency reciprocating rig (HFRR) or the Munson roller on cylinder lubricity evaluator (M-ROCLE), small additions of biodiesel have been found to reduce wear areas and friction coefficients for unadditized low-sulfur fuels (94–96). This additional lubricity translates into significantly reduced (10–50%) engine wear, as measured by accumulation of wear metals in crankcase oil, and improvements (2–13%) in fuel economy in field tests (94–101).

The consequences of lowered lubricity include fuel injection pump failures relatively early in the life of an engine. Fuel injection system failures due to low-lubricity fuel occur more frequently in high-pressure rotary distributor pumps, although inline pumps and injectors also experience reduced life. Poor lubricity problems in diesel fuel oils are likely to be exacerbated by the future engine developments aimed at further reducing emissions, which are predicted to have more exacting lubricity requirements than present engines. For example, the advent of high-pressure unit injectors is anticipated to increase fuel oil lubricity requirements. Poor lubricity can lead to wear problems in the engine upper cylinder areas and in other mechanical devices, such as valve guides dependent for lubrication on the natural lubricity of fuel oil. Diesel fuels exhibiting higher coefficients of friction in lubricity bench tests can be expected to increase engine upper piston ring drag on cylinder walls, thus increasing fuel consumption as well as engine wear.

Hertz et al. (95–101) have reported a lengthy series of diesel engine vehicle field studies with biodiesel lubricity additives applied at rates from 30% (soy methyl ester) to 0.5% (canola methyl ester). Unadditized as well as additized commercial summer and winter seasonal low-sulfur diesel fuels were referenced. Engine wear was inferred from ICP spectrometry and ferrographic analysis of the used engine oil wear particles as well as examination of oil filter debris. Both indirect and direct injection engines were compared, with and without exhaust gas recirculation and turbo charging. In these studies, it was found that biodiesel additization to low-lubricity diesel fuels resulted in significant iron wear reductions, typically in the 10% to 50% range. The benefit depended on the engine design, the lubricity of the reference diesel fuel, the additization rate, and the efficacy of the additive. A typical 0.5% canola methyl ester treatment in commercial diesel fuels resulted in 50% to 57% ICP iron wear reductions in a VW TDI engine (100). In this study, 0.5% canola methyl ester additization increased field fuel economy from 2% at moderate ambient temperatures to 13% under arctic winter conditions.

Numerous lubricity additives for fuel oils have been described (101). Caprotti et al. (102) disclosed an additive that is comprised of an ester of a carboxylic acid and an alcohol, wherein the acid has from 2 to 50 carbon atoms and the alcohol has one or more carbon atoms, e.g., glycerol monooleate. Although general mixtures are contemplated, no specific mixtures of esters were described.

Furey, in U.S. Patent No. 3,273,981 (103), disclosed a lubricity additive as being a mixture of A + B, wherein A is a polybasic acid or a polybasic acid ester made by reacting the acid with C<sub>1</sub>–C<sub>5</sub> monohydric alcohols; and B is a partial ester of a polyhydric alcohol and a fatty acid, for example, glyceryl monooleate, sorbitan monooleate, or pentaerythritol monooleate. The mixture finds application in jet fuels.

Beimesch and Zehler (104) described the uses of two esters with different viscosity in diesel fuel to reduce smoke emissions and increase fuel lubricity. In one preferred embodiment of that invention, methyl octadecenoate, a major component of biodiesel, was included in the formula. Similarly, Dilworth (105) also described a fuel composition comprising middle distillate fuel oil and two additional lubricating components; those components being (1) an ester of an unsaturated

monocarboxylic acid and a polyhydric alcohol and (2) an ester of a polyunsaturated monocarboxylic acid and a polyhydric alcohol having at least three hydroxyl groups.

The approach of using a two-component lubricity additive was pioneered by Fainman (106). He described an additive and a liquid hydrocarbon fuel composition consisting essentially of a fuel and a mixture of two straight-chain carboxylic acid esters, one having a low molecular weight and the other having a higher molecular weight.

In U.S. Patent 5,713,965, Foglia et al. (76) describe the synthesis of alkyl esters from animal fats, vegetable oils, rendered fats, and restaurant grease. The resultant alkyl esters are reported to be useful as additives to automotive fuels and lubricants. The addition of alkyl esters of fatty acids derived from vegetable oleaginous seeds were recommended at rates between 100 ppm to 10,000 ppm to enhance the lubricity of motor fuels in U.S. Patent 5,599,358, (107). Similarly, a fuel composition was disclosed by Stoldt and Harshida (108) comprising low-sulfur diesel fuel and esters from the *trans*-esterification of at least one animal fat or vegetable oil triacylglycerol.

Lang et al. (109) investigated the lubricity of methyl, ethyl, 2-propyl and 1 butyl esters of canola, linseed, rapeseed, and sunflower oils. A statistical test of the results revealed effects of oil used and alcohol on the resultant lubricity measured. Canola and rapeseed esters were superior to linseed esters in reducing wear scar formation followed by the esters of sunflower. Esters of isopropyl alcohol had the greatest impact on reducing the coefficient of friction. Munson and Hertz (95, 96) surveyed the lubricity of a series of vegetable-based esters at 1% treatment rates in hydrotreated low-sulfur No.1 diesel fuel. The reported M-ROCLE lubricity numbers, based on the ratio of wear area stress to coefficient of friction, were found superior for canola methyl ester, linseed ethyl ester, and rapeseed methyl ester. The lubricity number response for canola-based esters was found to be a semilogarithmic function of treatment rates. Soy and sunflower methyl esters performed poorly as a result of their respective higher coefficients of friction and larger wear areas.

## 6.2. Low Temperature Behavior

In temperate climates, diesel fuel must remain fluid at temperatures below the minimum expected temperature for the season. Through much of North America, winter diesel fuels have low temperature flow points below  $-30^{\circ}\text{C}$ . Diesel fuels with low pour points have lower viscosity and often lack lubricity (110). These fuels typically provide little lubricity. For example, Nouredini (111) reports that biodiesel fuels that are simple esters of various vegetable oils have poor flow characteristics below a temperature of  $-2^{\circ}\text{C}$ . To overcome this difficulty, a solvent consisting of mixed ethers of glycerol is added to the biodiesel. The resultant fluid has a low temperature cloud point below  $-32^{\circ}\text{F}$  ( $-36^{\circ}\text{C}$ ). The pour point of this fluid is still above that necessary to effectively add to many winter diesels, as it may be necessary to pour the fuel component at temperatures as low as  $-45^{\circ}\text{C}$ .

Soybean ester fuel blends may also be produced that have improved low temperature performance (62). Thirty percent blends of soybean methyl esters with kerosene began crystallization at  $-14.7^{\circ}\text{C}$ , whereas the 2-butyl ester began crystallization at  $-30.2^{\circ}\text{C}$ . These solutions do not have sufficient low temperature performance for inclusion in many low temperature applications.

Vegetable oils have also been considered in applications as fuels and attempts to maintain solutions of triacylglycerol oils at low temperatures have also been attempted. Blends of vegetable oil and lower alcohols have suitable viscosity for use in fuels, but they separate into two phases as temperature is lowered (62). Higher molecular weight alcohols can be used to lower the temperature of phase separation. In a soy oil number 2 diesel blend with ethanol, phase separation was deferred to  $-16^{\circ}\text{C}$  whereas the cold filter plugging point was lowered to  $-24^{\circ}\text{C}$ .

**6.2.1. Hydrotreated Vegetable Oils n-Alkane Fuels** Tall oil, animal fats, yellow grease, and vegetable oils can be converted to combustible fuels suitable for diesel engines by hydrotreating, i.e., treating with hydrogen over catalysts at elevated temperatures (5, 6). The impact of hydrotreating was complete decarboxylation and hydrogenation of all alkene and hydroxyl functionality. Decarboxylation occurring during the reaction shortens the chain length of fatty acids by one and hydrogenation assures that the converted products are mostly odd chain length n-alkanes. Glycerol undergoing this conversion is primarily converted to propane. Water and carbon dioxide are the primary byproducts and they embody none of the energy present in the starting material. The n-alkane products have the desirable characteristic of high cetane values, which allows their use as fuel combustion improvers. However, these materials have the undesirable trait of high melting point, which limits their use in fuels suited for low-temperature applications.

The test results show that n-paraffin (1) linearly raises the cetane of unadditized diesel without a top-end limit; (2) can linearly raise the cetane of "nonresponsive" diesel blends; (3) is synergistic with traditional cetane improvers; (4) has a low sulfur content; and (5) increases endproduct volume added (4, 5). When n-alkanes and cetane enhancers were added to a diesel fuel with a cetane number of 32 to raise the cetane number to 43, a 10% reduction in carbon dioxide emissions was achieved.

**6.2.2. Triacylglycerols** Triacylglycerols (TAGs) may be combusted directly in diesel engines, and technology exists to efficiently use these resources without *trans*-esterification (8). Diesel engines may be modified to operate directly on vegetable oil, but low temperature performance can be difficult. This is overcome by heating the fuel and insulating fuel lines (112). As viscosity is the main difficulty in operating on TAG oils, treatments to lower viscosity have been attempted. Heating TAG oil has been partially successful in allowing combustion of the fuel without engine modifications, but engine failure has occurred when fueling with heated TAG (113). Fuel viscosity can be reduced by blending soybean oil with other fuels or by forming emulsions (7). This approach still may result in inadequate engine performance due mostly to blocked injectors.

Certain short-chain triacylglycerols (SCT) produced by *Cuphea viscosissima* (113) could be used as sources of low viscosity triacylglycerols. There is no current abundant and commercial short-chain triacylglycerol source available but in the future new plant domestication, genetic engineering, or breeding efforts may lead to SCT biodiesel fuels.

Most biodiesel synthesis processes produce a fuel that has a slight contamination with acylglycerols, and this fact is recognized in most current biodiesel standards (Table 1). In spite of their low concentrations, these acylglycerols may contribute disproportionately to fuel lubricity.

## 7. COPRODUCT USE

During methyl ester production, glycerol is produced at approximately 10% of the weight of most fats and oils, with exceptions being fats with higher average molecular weights, which produce less glycerol, and fats with lower molecular weights, which produce more glycerol. As the source for most biodiesel produced to date has been canola, sunflower, and soy oils, the glycerol production is approximately 10% of biodiesel production. An increasing percentage of the world's glycerol supply is coming from the production of biodiesel. As biodiesel production increases, new markets must be found to deal with the rising production levels of the byproduct glycerol.

In addition to traditional uses, which are reviewed elsewhere, glycerol may be used in animal feed, reaction solvents, and chemical synthesis (113–115). Glycerol containing strong alkali may be used in the synthesis of conjugated linoleic acid (114). It is expected that substantial new demand will be generated for glycerol in the production of diacylglycerols that are being promoted for their beneficial dietary effects. Glycerol can also be converted through chemical and biological processes to polyglycerol ethers, glycerol acetates, isopropylidene glycerol, glycerol ethers, and propylene glycol (115). As the value of glycerol falls, the opportunities to use glycerol will necessarily expand.

Salt is a coproduct of biodiesel production. Usually, salts are formed in the step of glycerol use or during glycerol distillation. The salts formed are dependent on the catalyst used in ester formation and the acid used to neutralize the glycerol. Sodium- or potassium-based catalysts can be reacted with phosphoric, hydrochloric, sulfuric, carbonic, or other acids to produce the respective phosphates, chlorides, sulfates, carbonates, or other salts. Preferred technologies include the use of potassium hydroxide and phosphoric acid to generate a final product of potassium phosphate, which is suitable for fertilizer. Sodium salts are more difficult for land disposal, but they may be suited to production of specific commercial products. Sodium chloride is the resultant salt in processes developed by Lurgi and Crown Iron Works. Although chloride ions are corrosive on equipment, advanced materials available from these technology suppliers allow cost-effective recovery of glycerol and brines.

## 8. THE FUTURE

If current trends continue, biodiesel will grow to become the largest market for triglyceride oil, expanding in size beyond current markets for food, feed, and industrial products. Research will be required to improve biodiesel production technology at all levels. With innovation, the footprint of agriculture land required for biodiesel production will be minimized. Energy inputs for biodiesel production will also be minimized, whereas protein and glycerol coproducts of biodiesel production will be improved to allow total use. In spite of the diversion of food crops to energy production, it is anticipated that technology development will enable agriculture production to meet future needs.

The steps taken to the future will be small at first. Over the next several years, feedstocks, including yellow grease, soybean, corn, and sunflower oil, will be the foundation of profitable biodiesel production. However, in the long term, the demand for oil will drive the development of new biodiesel sources. Canola produces a desirable ratio of oil to meal; but it cannot fix nitrogen, whereas soy will inevitably be found to produce too much protein and too little oil to be considered over the long term for global biodiesel production. Breeding existing crops and introducing new species will produce new biodiesel crops for our future.

## REFERENCES

1. W. Korbitz, "New trends in developing biodiesel worldwide," in *Asia Biofuels: Evaluating & Exploiting the Commercial Uses of Ethanol, Fuel Alcohol & Biodiesel*, Singapore, 2002.
2. Standard specification for biodiesel fuel (B100) blend stock for distillate fuels, ASTM Standards D-6751, .
3. F. Ma and M. A. Hanna, *Bioresource Technol.*, **70**, 1–15 (1999).
4. M. Stumborg, A. Wong, and E. Hogan, *Bioresource Technol.*, **56**, 13–18 (1996).
5. D. W. Soveran, M. Sulatisky, K. Ha, W. Robinson, and M. Stumborg "The effect on diesel engine emissions with high cetane additives from biomass oils," in *Proc. American Chemical Society (Division of fuel chemistry) Meeting*, San Francisco, CA, April 5–10, 1992.
6. J. Monnier, G. Tourigny, D. W. Soveran, Douglas, A. Wong, E. N. Hogan, and M. Stumborg (to Natural Resources Canada), U.S. Patent 5,705,722, January 6, 1998.
7. J. W. Goodrum, in J. S Cundiff et al., eds., *Liquid Fuels and Industrial Products from Renewable Resources Proceedings of the Third Liquid Fuel Conference*, The American Society of Agricultural Engineers, Nashville, Tennessee, 1996, pp. 128–135.
8. Conversion of Diesel Engines. (2004). Available: [www.elsbett.com](http://www.elsbett.com).
9. S. Bari, T. H. Lim, and C. W. Yu, *Renewable Energy*, **27**, 339–351 (2002).
10. D. P. Wei and H. A. Spikes, in W. J. Bartz et al., eds., *Fuels International: Advances in Fuels and Automotive Energy*, vol. 1, no. 1, Leaf Coppin Publishing, Ltd., United Kingdom, 2000, pp. 45–62.

11. Control of air pollution from new motor vehicles, Code of Federal Regulations 40 CFR parts 69.80 and 69.86.
12. Y. Ali, M. A. Hanna, and S. L. Cuppert, *JAOCS*, **72**, 1557–1564 (1995).
13. R. O. Dunn and M. O. Bagby, *JAOCS*, **72**, 895–904 (1995).
14. Österreichisches Normungsinstitut, Kraftstoffe-Dieselmotoren: Fettesäuremethylester, Anforderungen, NORM C-1191 (1997).
15. Association Française de Normalisation, *Journal Officiel*, No. 214, p. 13374 (1997).
16. Deutsches Institut Für Normung e.V., Flüssige Kraftstoffe; Dieselmotoren aus Fettesäuremethylester (FAME); Mindestanforderungen. DIN E 51606 September (1997).
17. Ente Nazionale Italiano di Unificazione, UNI 10635 Esteri metilici di oli vegetali; Caratteristiche chimico-fisiche. 21. April (1997).
18. Standardiseringskommissionen i Sverige, SS 15 54 36: Motorbränslen – Vegetabiliska fettesäuremethylester – Krav och provningsmetoder. 27. 11 (1996).
19. C. A. W. Allen and K. C. Watts, *Trans. Amer. Soc. Agric. Eng.*, **43**, 207–211 (2000).
20. M. Mittelbach, *Bioresource Technol.*, **56**, 7–11 (1996).
21. G. Knothe, *Trans. Amer. Soc. Agric. Eng.*, **44**, 193–200 (2001).
22. D. M. Freeman and S. A. Swedberg (to Agilent Technologies, Inc.), U.S. Patent 6,194,900, February 27, 2001.
23. Federal government of the USA, Methyl and ethyl esters of fatty acids produced from edible fats and oils, Code of Federal Regulations 21 CFR 172.225, pp. 40–41.
24. Federal government of the USA, Synthetic flavoring substances and adjuvants, Code of Federal Regulations 21 CFR 172.515, pp. 56–63.
25. D. L. Reece, X. Zhang, and C. L. Peterson, in J. S. Cundiff et al., ed., *Liquid Fuels and Industrial Products from Renewable Resources Proceedings of the Third Liquid Fuel Conference*, The American Society of Agricultural Engineers, Nashville, Tennessee, 1996, pp. 166–176.
26. NIOSH Carcinogen list. (2002). Available: <http://www.cdc.gov/niosh/npotocca.html>.
27. A. Monyem and J. H. Van Gerpen, *Biomass and Bioenergy*, **20**, 317–325 (2001).
28. G. Knothe, M. O. Bagby, and T. W. Ryan III, in J. S. Cundiff et al., ed., *Liquid Fuels and Industrial Products from Renewable Resources Proceedings of the Third Liquid Fuel Conference*, The American Society of Agricultural Engineers, Nashville, Tennessee, 1996, pp. 54–58.
29. J. C. Thompson, C. L. Peterson, D. L. Reece, and S. M. Beck, in J. S. Cundiff et al., ed., *Liquid Fuels and Industrial Products from Renewable Resources Proceedings of the Third Liquid Fuel Conference*, The American Society of Agricultural Engineers, Nashville, Tennessee, 1996, pp. 104–113.
30. A. R. Womac, R. J. Stange, J. A. Crouch, and C. Easterly, in J. S. Cundiff et al., ed., *Liquid Fuels and Industrial Products from Renewable Resources Proceedings of the Third Liquid Fuel Conference*, The American Society of Agricultural Engineers, Nashville, Tennessee, 1996, pp. 177–185.
31. J. Krahl, J. Bunger, O. Schroder, A. Munack, and G. Knothe, *J. Amer. Oil Chem. Soc.*, **79**, 717–724 (2002).
32. W. G. Wang, D. W. Lyons, N. N. Clark, and N. Gautam, *Environ. Sci. Technol.*, **34**, 933–939 (2000).



33. J. Krahl, A. Munack, M. Bahadir, L. Schumacher, and N. Elser, in J. S. Cundiff et al., ed., *Liquid Fuels and Industrial Products from Renewable Resources Proceedings of the Third Liquid Fuel Conference*, The American Society of Agricultural Engineers, Nashville, Tennessee, 1996, pp. 136–148.
34. Lovelace Respiratory Research Institute, Tier 2 testing of biodiesel exhaust emissions. Available: <http://www.nbb.org/resources/reportsdatabase/reports/gen/gen-278.pdf> (2000).
35. M. E. Tat and J. H. Van Gerpen, ASAE Paper No. 01-6052, ASAE, St. Joseph, Michigan, 2001.
36. A. Munack, J. Krahl, and H. Speckmann, ASAE Paper No. 02-6081, ASAE, Chicago Illinois, 2002.
37. G. Schmitz, R. Bartz, U. Hilger, and M. Siedentop, SAE Paper No. 900231, SAE, Warrendale, Pennsylvania, 1990.
38. J. Krahl, J. Bunger, H. E. Jeberien, K. Prieger, C. Schutt, A. Munack, and M. Bahadir, in J. S. Cundiff et al., ed., *Liquid Fuels and Industrial Products from Renewable Resources Proceedings of the Third Liquid Fuel Conference*, The American Society of Agricultural Engineers, Nashville, Tennessee, 1996, pp. 149–165.
39. T. Beer, T. Grant, G. Morgan, J. Lapszewicz, P. Anyon, J. Edwards, P. Nelson, H. Watson, and D. Williams, “Comparison of Transport Fuels,” Report EV45A/2/F3C, CSIRO Atmospheric Research, 2001.
40. T. Beer, T. Grant, D. Williams, and H. Watson, Fuel-cycle greenhouse gas emissions from alternative fuels in Australian heavy vehicles, Atmospheric Environment (in press).
41. K. Scharmer, G. Golbs, and I. Muschalek, “Pflanzenolkraftstoffe und ihre Umweltauswirkungen, Argumente und Zahlen zur Umweltbilanz,” in Bericht für die UFOP, Bonn, Germany, p. 35. (1993)
42. An Agronomic and Economic Assessment of GMO Canola. (2004). Available: [http://www.canola-council.org/production/gmo\\_main.html](http://www.canola-council.org/production/gmo_main.html).
43. Variety of Grain Crops. (2002). Available: [www.agr.gov.sk.ca/DOCS/crops/var2002.pdf](http://www.agr.gov.sk.ca/DOCS/crops/var2002.pdf).
44. B. Uppstrom, “Seed Chemistry,” in D. Kimber and D. I. McGregor, eds., *Brassica Oilseeds Production and Utilization*, 1996, pp. 217–242.
45. M. A. Schmitt, Environmental Ramifications and Production Implications of Growing Low Phytate Corn. Available: [www.manure.coafes.umn.edu/research/corn.html](http://www.manure.coafes.umn.edu/research/corn.html) (2004).
46. G. C. Buzza, “Plant Breeding” in D. Kimber and D. I. McGregor, eds., *Brassica Oilseeds Production and Utilization*, 1996, pp. 153–157.
47. R. F. Wilson, “Seed Metabolism,” in J. R. Wilcox, ed., *Soybeans: Improvement, Production and Uses*, 2nd ed., No. 16, American Society of Agronomy Inc., 1987, pp. 643–686.
48. J. R. Lofgren, U.S. Patent 6,229,079, 2001.
49. Biodiesel Courier. (2004). Available: [www.biodiesel.at](http://www.biodiesel.at).
50. Current and Potential Biodiesel Production. (2004). Available: <http://www.biodiesel.org/buyingbiodiesel/guide/ProducersMap-existingandpotential.pdf>.
51. Biodiesel content mandate State of Minnesota, S.F No. 1495, 3rd Engrossment; 82nd Legislative Session (2001–2002).
52. O. E. Ikwuagwu, I. C. Ononogbu, and O. U. Njoku, *Industrial Crops and Products*, **12**, 57–62 (2000).

53. N. Foidl, G. Foidl, M. Sanchez, M. Mittelbach, and S. Hackel, *Bioresource Technol.*, **58**, 77–82 (1996).
54. A. P. Brown, J. Brown, and J. Davis, in *New Horizons for an Old Crop Proceedings of the 10th International Rapeseed Conference*, Canberra, Australia. (1999). Available: ([www.regional.org.au/au/gcirc/4/281.htm](http://www.regional.org.au/au/gcirc/4/281.htm)).
55. H. Y. Zhang, M. A. Hanna, Y. Ali, and L. Nan, *Industrial Crops and Products*, **5**, 177–181 (1996).
56. Canadian Grains Industry Statistical Handbook 01, Canadian Grain Council, Winnipeg, Canada, 2001.
57. Economic Research Service: United States Department of Agriculture, Briefing Room; European Union available at: <http://www.ers.usda.gov/briefing/EuropeanUnion/data/rapeseed.xls> (2004).
58. J. Giacometti, A. Miloevi, and C. Milin, *J. Chromatogr. A*, **976**, 47–54 (2002).
59. C. A. W. Allen, K. C. Watts, R. G. Ackman, and M. J. Pegg, *Fuel*, **78**, 1319–1326 (1999).
60. L. Lin, K. C. Rhee, and S. S. Koseoglu, in “Advances in Oils and Fats, Anti-oxidants, and Oilseed By-Products,” vol. 2, in *Proceedings of the World Conference on Oilseed and Edible Oils Processing*, S. S. Koseoglu, K. C. Rhee, and R. F. Wilson, eds., AOCS Press, Champaign, Illinois, 1996, pp. 76–82.
61. J. Van Gerpen, in J. S. Cundiff et al., eds., *Liquid Fuels and Industrial Products from Renewable Resources Proceedings of the Third Liquid Fuel Conference*, The American Society of Agricultural Engineers, Nashville, Tennessee, 1996, pp. 197–206.
62. R. O. Dunn, *JAOCS*, **79**, 709–715 (2002).
63. M. E. González Gómez, R. Howard-Hildige, J. J. Leahy, and B. Rice, *Fuel*, **81**, 33–39 (2002).
64. R. O. Dunn, *JAOCS*, **79**, 915–920 (2002).
65. X. Lang, A. K. Dalai, N. N. Bakshi, M. J. Reaney, and P. B. Hertz, *Bioresource Technol.*, **80**, 53–62 (2001).
66. E. E Dreger (to Colgate-Palmolive-Peet Company), U.S. Patent 2,383,596, August 28, 1945.
67. H. Lepper and L. Friesenhagen (to Henkel Kommanditgesellschaft auf Aktien), U.S. Patent 4,608,202, August 26, 1986.
68. M. Canakci and J. Van Gerpen, *Trans. ASAE*, **44**, 1429–1436 (2001).
69. W. M. McDonald (to Crown Iron Works Company), U.S. Patent, 6,262,285 B1, July 17, 2001.
70. G. I. Keim (to Colgate-Palmolive-Peet Company), U.S. Patent 2,383,601, August 28, 1945.
71. R. Stern, G. Hillion, and M. N. Eisa (to Institut Francais du Petrole), U.S. Patent 6,013,817, January 11, 2000.
72. G. Demmering, C. Pelzer, and L. Friesenhagen (to Henkel Kommanditgesellschaft auf Aktien), U.S. Patent 5,455,370, October 2, 1995.
73. M. Gheorghiu (to M. Gheorghiu), U.S. Patent 5,532,392, July 2, 1996.
74. E. G. Lundquist (to Rohm and Haas Company), U.S. Patent 5,426,199, June 20, 1995.
75. L. Jeromin, E. Peukert, and G. Wollmann (to Henkel Kommanditgesellschaft auf Aktien), U.S. Patent 4,698,186, October 6, 1987.

76. T. A. Foglia, L. A. Nelson, and W. N. Marmer (to The United States of America as represented by the Secretary of Agriculture), U.S. Patent 5,713,965, February 3, 1998.
77. M. J. Haas (to The United States of America, as represented by the Secretary of Agriculture, Washington D.C.), U.S. Patent 5,697,986, December 16, 1997.
78. W. T. Wu and J. W. Chen (to W.T. Wu), U.S. Patent, June 4, 2002.
79. W. D. Stidham, D. W. Seaman, and M. F. Danzer (to West Central Cooperative), U.S. Patent 6,127,560, October 3, 2000.
80. K. W. Anderson (to Henkel Corporation), U.S. Patent 5,710,030, January 20, 1998.
81. G. A. Glossop (to Colgate-Palmolive-Peet Company), U.S. Patent 2,383,599, August 28, 1945.
82. S. Billenstein, B. Kukla, and H. Stuhler (to Hoechst Aktiengesellschaft), U.S. Patent 4,668,439, May 26, 1987.
83. R. Klok and H. H. Vermeer (to Van den Bergh Foods Co., Division of Conopco, Inc.), U.S. Patent 5,116,546, May 26, 1992.
84. D. Anderson, in Y. H. Hui, ed., *Baile's Industrial Oils and Fats Products*, 5th ed., John Wiley and Sons, Inc. New York, p. 22 (1996).
85. M. J. Haas, S. Bloomer, and K. Scott (to The United States of America as represented by the Secretary of Agriculture), U.S. Patent 6,399,800, June 4, 2002.
86. M. J. T. Reaney (KRU Feed Energy Company, Des Moines, Iowa), U.S. Patent 6,399,802, June 4, 2002.
87. D. G. B. Boocock (to D.G.B. Boocock), Canadian Patent Application 2,316,131, February 18, 2001.
88. T. Wimmer (to Vogel and Noot Industrieranlagenbau Gellschaft m.b.H., Graz, Austria), U.S. Patent 5,399,731, March 21, 1995.
89. E. P. Granberg and R. G. Schafermeyer (to Procter & Gamble Company), U.S. Patent 5,844,111, December 1, 1998.
90. Y. Kawahara and T. Ono (to Kao Soap., Ltd., Tokyo, Japan), U.S. Patent 4,164,506, August 14, 1979.
91. D. G. B. Boocock (to D.G.B. Boocock), Canadian Patent Application 2,381,394, February 22, 2001.
92. B. Bam, D. C. Drown, R. Korus, D. S. Hoffman, T. G. Johnson, and J. M. Washam (to Idaho Research Foundation), U.S. Patent 5,424,467, June 13, 1995.
93. Directive 98/70/EC of the European Parliament and of the Council of 13 October 1998 relating to the quality of petrol and diesel fuels and amending Council Directive 93/12/EEC, *Official Journal*, L 350, 58–68 (1998). [europa.eu.int/comm/taxation\\_customs/french/publications/info\\_doc/taxation/306-98\\_en.pdf](http://europa.eu.int/comm/taxation_customs/french/publications/info_doc/taxation/306-98_en.pdf).
94. J. H. Van Gerpen, S. Soyulu, and D. Y. Z. Chang, Evaluation of the lubricity of Soybean Oil-based Additives in Diesel Fuel (1998). Available: [http://www.nbb.org/resources/reportsdatabase/reports/gen/19980201\\_gen-070.pdf](http://www.nbb.org/resources/reportsdatabase/reports/gen/19980201_gen-070.pdf).
95. J. W. Munson and P. B. Hertz, "Seasonal Diesel Fuel and Fuel Additive Lubricity Survey Using the 'Munson ROCLE' Bench Test," SAE Paper 1999-01- 3588, Toronto, Ontario, Canada, (1999).
96. J. W. Munson and P. B. Hertz, "Gasoline and Diesel Fuel: Performance Additives," SAE Publication SP-147 (1999).

97. P. B. Hertz, "Biodiesel Engine Wear and Highway Performance," Proceedings of CANCAM '95, Victoria, British Columbia, Canada (1995).
98. P. B. Hertz, Summer 95 Engine Wear Investigations Using Canola Methyl Esters and No. 2 Diesel Fuels. (1996). Available: <http://www.nbb.org/resources/reportsdatabase/reports/gen/gen-234b.pdf>.
99. P. B. Hertz, Winter Engine Wear Comparisons with a Canola Biodiesel Fuel Blend (1995). Available: <http://www.nbb.org/resources/reportsdatabase/reports/gen/gen-233.pdf>.
100. P. B. Hertz, Extending Diesel Engine Life And Fuel Economy With Canola Based Fuel Additives (2001). Available: [http://www.scdc.sk.ca/html/rese\\_fs.html](http://www.scdc.sk.ca/html/rese_fs.html).
101. P. B. Hertz, Bio-diesel Engine Wear Tests Using Canola Fuel Additives (1997). Available: [http://www.scdc.sk.ca/html/rese\\_fs.html](http://www.scdc.sk.ca/html/rese_fs.html).
102. R. Caprotti, C. H. Bovington, and C. J. D. MacRae (to Exxon Chemical Patents, Inc.), World Patent Organization 94/17160, April 8, 1994.
103. M. J. Furey (to Esso Research and Engineering Co.), U.S. Patent 3,273,981, September 20, 1966.
104. G. J. Beimesch and E. R. Zehler (to Henkel Corporation), U.S. Patent 6,080,212, June 27, 2000.
105. B. Dilworth (to Exxon Chemical Patents, Inc.), U.S. Patent 5,882,364, March 16, 1999.
106. M. Z. Fainman (to M. J. Fainman), U.S. Patent 4,920,691, May 1, 1990.
107. F. Giavazzi and F. Panarello (to Euron S.p.A.), U.S. Patent 5,599,358, February 4, 1997.
108. S. H. Stoldt and D. Harshida (The Lubrizole Corp.), U.S. Patent 5,730,029, March 24, 1998.
109. X. Lang, A. K. Dalai, M. J. Reaney, and P. B. Hertz, *Tribotest J.*, **8**, 2 (2001).
110. A World-wide 1997- Winter Diesel Fuel Quality Survey, Paramins Fuel Additives, Exxon Chemical Limited, Abingdon, Oxfordshire, United kingdom (1997).
111. H. Nouredini (to The Board of Regents of the University of Nebraska), U.S. Patent 6,174,501 January 2001.
112. K. Pramanik, *Renewable Energy*, **28**, 239–248 (2003).
113. D. P. Geller, J. W. Goodrum, and S. J. Knapp, *Industrial Crops and Products*, **9**, 85–91 (1999).
114. A. Schroder and K-H Sudekum, "Glycerol as a By-product of Biodiesel in Diets for Ruminants," in Proceedings of the 10th Rapeseed Congress, Canberra, Australia (1999). Available: <http://www.regional.org.au/au/gcirc/1/241.htm>.
115. M. J. T. Reaney (to Her Majesty the Queen in right of Canada, as represented by the Minister of Agriculture), U.S. Patent 6,409,649, June 25, 2002.

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## *Vegetable Oils as Lubricants, Hydraulic Fluids, and Inks*

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### **1. INTRODUCTION**

The major components in lubricants are the basestock (usually 80–100%) and additives. The additives are used to enhance the most important properties for each specific application. Most of the basestocks originate from petroleum, including many synthetic esters and poly-alpha-olefins. Less than 2% of the basestocks are the product of oleochemical and related industries, the primary area of their application has been as hydraulic fluids. This is consumed at approximately 5 million metric tons/year in the U.S. market, which has the highest demand for biodegradable lubricants (1). A major application area is industrial hydraulic fluids that represent an 838 million liter (222 million gallon) market in the United States, with potential use in waterways, farms, and forests. Other significant niche market areas are cutting and drive chain oils, two-stroke engine oils, chainsaw bar oil, wire rope oil, marine oils and outboard engine lubricants, oil for water and underground pumps, rail flange lubricants, agricultural equipment lubricants, metal cutting oils, tractor oils, dedusting, and several others.

Environmental concerns over the use of petroleum-based products in various industries, such as forestry, farming, mining, boating, and others, has led to increased interest in the use of environmentally friendly fluids. The beneficial aspects of vegetable oils as lubricants are mainly their biodegradability and non-toxicity, which are not exhibited by conventional mineral base oils (2, 3). Their volatility is low because of the high molecular weight of the triglyceride structure and the oils have a narrow range of viscosity change with temperature. The ester linkages deliver inherent lubricity on metallic surfaces because of their adhesive property. Further, vegetable oils have superior solubilizing power for contaminants and additive molecules compared with mineral base fluids.

Certain performance limitations of vegetable oil basestocks are poor oxidative stability due to bis-allylic protons in the fatty acyl chain, deposit forming tendency, low-temperature solidification, and low hydrolytic stability. Oxidation results in increased acidity, corrosion, viscosity, and volatility of the lubricant. On the other hand, parameters like lubricity, antiwear protection, load carrying capacity, rust prevention, foaming, demulsibility, etc., are mostly additive dependent. Antioxidant additives (4) provide limited improvement of oxidative stability; therefore, other approaches are required to improve the above characteristics.

Low-temperature testing shows that vegetable oils solidify at  $-20^{\circ}\text{C}$  upon long-term exposure, a problem in some parts of the world. Chemical modifications are therefore necessary to suppress or eliminate triacylglycerol crystallization. The inherently narrow viscosity range limits use in various viscosity grades, especially at lower viscosities. The polar nature of triacylglycerols contributes to air entrainment and problems of foaming, compressibility, and bulk modulus.

The performance limitations of vegetable oil basestocks can be overcome by genetic modification, chemical modification, processing changes, and improved additive technology. Soybean oil is the largest and cheapest vegetable oil in the U.S. market; others include corn, canola, safflower, sunflower, and their various genetically modified forms. Soybean oil could have a distinct advantage over other oils if it can be modified to improve stability (oxidative and low temperature), a major step for commercialization as a base fluid.

Current research efforts are directed toward improving the thermal and low-temperature stability of vegetable oils by chemical modification, blending with functional fluids and additive response studies.

## 2. VEGETABLE OIL STRUCTURE AND COMPOSITION

Physical and chemical properties of vegetable oils are determined by the fatty acid (FA) profile. Table 1 shows typical FA contents of some vegetable oils that are being investigated as potential basestocks for industrial applications.

High unsaturation in the molecule increases the rate of oxidation resulting in polymerization and an increase in viscosity (5). On the other hand, high saturation increases the melting point of the oil (6). Therefore, suitable adjustment between low-temperature properties and oxidative stability must be made when selecting a vegetable oil basestock for a particular industrial application. Table 2 presents some of the chemical properties associated with  $\text{C}=\text{C}$  unsaturation in the molecule.

**TABLE 1. Fatty Acid Composition (%) of Vegetable Oils Used in Studies of Lubricant Basestocks (Gas Chromatography Analysis, AACC Method 58-18, 1993).**

Vegetable Oil	16:0	18:0	18:1	18:2	18:3
Safflower oil	6.4	2.5	17.9	73.2	—
High-oleic safflower oil	4.6	2.2	77.5	13.2	—
High-linoleic safflower oil	6.7	2.6	14.6	75.2	—
Sunflower oil	6.1	5.3	21.4	66.4	—
High-oleic sunflower oil	3.5	4.4	80.3	10.4	—
Soybean oil	6.0	5.2	21.2	65.7	0.5
High-oleic soybean oil	6.2	3.0	83.6	3.7	1.7
Corn oil	10.6	2.0	26.7	59.8	0.9
Cottonseed oil	18.0	2.0	41.0	38.0	1.0

16:0 palmitic; 18:0 stearic; 18:1 oleic; 18:2 linoleic; 18:3 linoleic.

**TABLE 2. Typical Analytical Data of Vegetable Oils Used in Studies of Lubricant Basestocks.**

Vegetable Oil	IV <sup>1</sup>	FFA <sup>2</sup>	PV <sup>3</sup>
Cottonseed oil	109.1	0.33	34.7
Corn oil	119.9	0.20	26.6
Canola (rapeseed)	99.1	0.22	22.4
Safflower oil	135.2	0.21	32.0
High-oleic safflower oil	83.6	0.31	27.8
High-linoleic safflower oil	121.2	0.00	32.2
Sunflower oil	124.8	0.30	39.1
High-oleic sunflower oil	80.8	0.10	23.9
Soybean oil	117.0	0.22	27.4

<sup>1</sup>Iodine value, AOCS method, Cd 1-25, 1993;

<sup>2</sup>Free fatty acid, AOCS method, Cd 5a-40, 1993;

<sup>3</sup>Peroxide value, AOCS method, Cd 8-53, 1993.

### 3. OXIDATIVE STABILITY

Oxidation is the single most important reaction of oils resulting in increased acidity, corrosion, viscosity, and volatility when used as lubricant base oils for engine oils. The triacylglycerol structure forms the backbone of most available vegetable oils, and these are associated with different FA chains. It is therefore a complex association of different FA molecules attached to a single triglycerol structure that constitute a vegetable oil matrix. The presence of unsaturation in the triacylglycerol molecule, due to C=C from oleic, linoleic, and linolenic acid moieties, functions as the active sites for various oxidation reactions. Saturated FAs have relatively high oxidation stability (5), which decreases with increasing unsaturation in the molecule.

Oxidation of hydrocarbons usually takes place through a radical initiated chain mechanism (7) involving: initiation ( $\text{RH} \rightarrow \text{R}^\bullet$ ,  $\text{R}^\bullet + \text{O}_2 \rightarrow \text{RO}_2^\bullet$ ); propagation

( $\text{RO}_2^\bullet + \text{RH} \rightarrow \text{RO}_2\text{H} + \text{R}^\bullet$ ,  $\text{R}^\bullet + \text{O}_2 \rightarrow \text{RO}_2^\bullet$ ); branching ( $\text{RO}_2\text{H} \rightarrow \text{RO}^\bullet + \bullet\text{OH}$ ,  $\text{RO}^\bullet + \text{RH} + \text{O}_2 \rightarrow \text{ROH} + \text{RO}_2^\bullet$ ,  $\bullet\text{OH} + \text{RH} + \text{O}_2 \rightarrow \text{H}_2\text{O} + \text{RO}_2^\bullet$ ); chain stopping inhibition ( $\text{In} + \text{H} + \text{RO}_2^\bullet \rightarrow \text{In}^\bullet + \text{RO}_2\text{H}$ ); peroxide decomposition ( $\text{RO}_2\text{H} \rightarrow \text{inert products}$ ). The free radicals generated during the initiation stage react with  $\text{O}_2$  to form peroxy free radicals and hydroperoxides (8). During this period,  $\text{O}_2$  is consumed in a zero-order process (9), apparently leading to intermediates that are not too well characterized, prior to the formation of peroxides (8). The latter undergoes further reaction to form alcohols, ketones, aldehydes, and carboxylic acids (10), leading to rancidity and toxicity (11), thereby accelerating the oil degradation process (12, 13). These compounds have molecular weights that are similar to vegetable oils and therefore remain in solution. As the oxidation proceeds, the oxygenated compounds polymerize to form viscous material that, at a particular point, becomes oil insoluble leading to oil thickening and deposits.

The extent of oxidation and formation of oxidation products are further complicated by the amount of unsaturation, structural differences in the various triacylglycerol molecules, and presence of antioxidants. All these factors, together or individually, can change the specific compounds formed and the rates of their formation (14). In addition to unsaturation in the molecule, oxidative degradation and kinetics of oxidation are also influenced by methylene chain length, bis-allylic methylene groups, etc. The cumulative effect of various structural parameters in the triacylglycerol molecule makes oxidation a highly complex process and no simple kinetic model alone would hold good for such systems.

Several bench top oxidation tests are available primarily as screening tools for oxidative stability of vegetable oils. Evaluation of oxidation is complex and a fully acceptable protocol is yet to emerge. Estimation of peroxide content (peroxide value, PV) can be used as an index of oxidation if the peroxides formed are stable and do not decompose after formation, which most often is not the case. The activation energy for the formation of peroxide is 146–272 kJ/mol (15) and that of decomposition of lipid peroxide is 84–184.5 kJ/mol, suggesting peroxides are less stable than lipids (16). Two other methods for measuring oxidative stability are the active oxygen method (AOM) (17) and the Rancimat method (18, 19). In the former method, test oil is heated to 100°C and the oxidation is followed by measuring the PV of heated sample at regular time interval until PV = 100 meq/kg is reached, which gives the AOM endpoint. A large amount of sample, numerous analysis, and critical control of airflow is required. With samples that form unstable peroxides, PV = 100 meq/kg may never be reached and such measurements have no meaning. In the AOM method, consumption of  $\text{O}_2$  may also be a measure for induction period. The Rancimat method is based on the fact that the volatile acids formed during oxidation (20, 21) can be used as automated endpoint detection. Gordon and Murshi (22) have shown good correlation of Rancimat method at 100°C with oil stability as measured by peroxide development during storage at 20°C. In another study, Jebe et al. (23) have pointed out the advantages of Rancimat method at higher temperature. In the Sylvester test (24), the sample is heated to 100°C in a closed vessel and the pressure decrease due to  $\text{O}_2$  consumption is monitored.



Oxidograph (24) is an automated version of this method and induction period is determined from the sudden decrease in the  $O_2$  pressure.

Chemiluminescence can be used to follow vegetable oil oxidation. The oxidative status of oil can also be obtained by integrating the light curve during the chemiluminescence reaction (25). The method is highly sensitive for the measurement of lipid oxidation. Matthäus et al. (26) have described a linear correlation ( $R^2 = 0.99$ ) between iodimetric peroxide determination (27) and chemiluminescence method. Another official method to measure induction period is oil stability index (OSI) (28). The OSI values generally correspond well with AOM values if PV is 100 meq/kg or greater (29). The method is automated and much easier as compared with AOM. However, lengthy experimental time, large errors associated with small changes in  $O_2$ /air-flow rate (30), and inability to differentiate between small changes in vegetable oil matrix are its main disadvantages.

The thin film micro-oxidation test is often the method of choice for studying vegetable oils because it is simple and reproducible. The test is especially effective when thermally induced, volatility is low, and insoluble deposit formation through polymerization is to be considered rather than rates of inhibitor depletion.

The procedure of micro-oxidation, analogous to that of heated block micro-oxidation, involves polishing the coupons and heating block, weighing the empty coupons, applying 40 mm<sup>3</sup> (~36 mg) of sample in a form of homogeneous film, placing the coupons on the thermally equilibrated aluminum slab, covering with the bottomless impinger, and supplying 0.1 MPa dry air flow at 20 cm<sup>3</sup>/min (31). After a given duration, the sample pan is removed and placed on a clean, cold metal surface for shock-cooling. Then the pan is weighed and the oxidized sample washed off with tetrahydrofuran (THF). Weighing before washing with THF permits determination of volatile loss (or gain due to oxidation), whereas weighing after the washing determines possible formation of solids or corrosion of the surface metal. Gel permeation chromatography (GPC) is employed to investigate oxypolymerization kinetics (Figure 1).

Figure 2 presents the results of evaporation at two different temperatures (175°C and 200°C) for the oils. The temperature is high enough to result in substantial evaporative loss of the oil as well as cause quantifiable polymerization resulting in oil-insoluble deposits. Upon epoxidation of SBO, percent evaporation is low at 175°C, and increased at higher temperature (200°C).

The plausible reason for such behavior could be the oxidative cleavage of the FA chain structure into smaller fragments. The thermal opening of the epoxy linkage could also result in unstable structure and consequently to C—C chain scission. Sunflower oil shows high evaporation on increasing the percent monounsaturated both at 175°C and 200°C. The percent increase of evaporation was substantial at higher temperature with high oleic content. Conversely, increase in polyunsaturation results in low evaporation of vegetable oil. Similar trends were observed in safflower, high oleic, and high linoleic safflower oil. High oleic oils, evaluated at 175°C and 200°C, exhibit the highest evaporative loss. On increasing the polyunsaturated FA content (High linoleic safflower oil), the percent evaporation decreased at 175°C and remained comparable with safflower oil at 200°C.

Sample analysis in micro oxidation

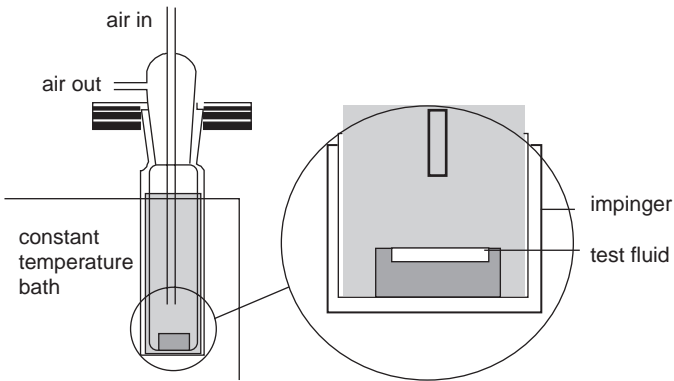
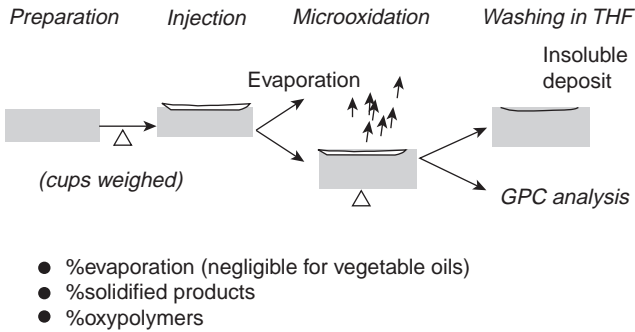


Figure 1. Sample analysis in micro-oxidation.

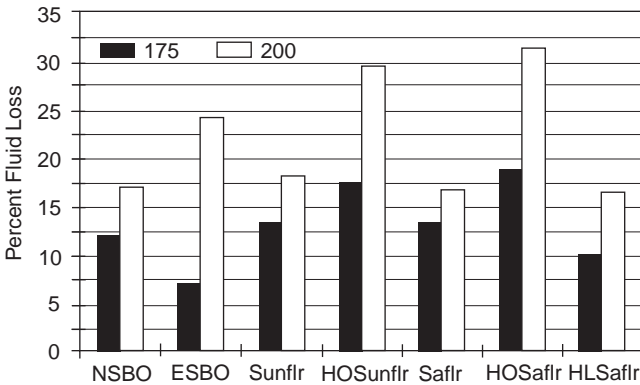
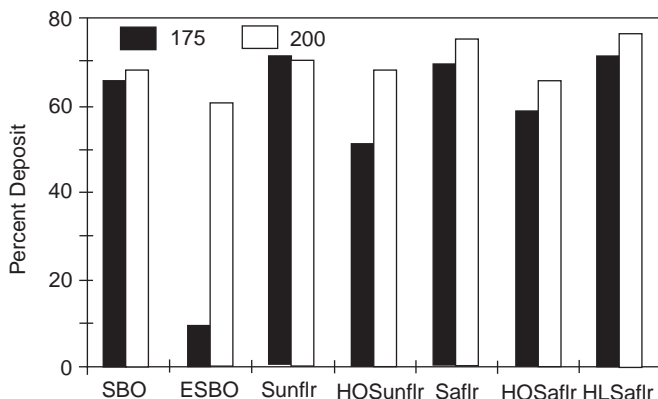


Figure 2. Percent fluid loss of vegetable oils during thin film micro-oxidation.



**Figure 3.** Percent insoluble deposit of vegetable oils during thin film micro-oxidation.

In Figure 3, epoxidized soybean oil showed substantial decrease in the formation of insoluble deposit at lower temperature. The selective removal of unsaturation through epoxy group has resulted in significantly reducing the tendency to oxidative degradation. The presence of polyunsaturation in the FA is the primary reason of low oxidative stability, as di-vinyl-CH<sub>2</sub> protons are highly susceptible to free radical attack leading to substitution with O<sub>2</sub> molecule and consequent formation of polymeric oxy-polar compounds. These compounds are the precursors of oil-insoluble deposits often encountered with high-temperature oxidation of vegetable oils.

Thermal analysis, such as pressurized differential scanning calorimetry (DSC), is another popular approach for rapid measurement of the oxidative stability of vegetable oils (32, 33). The procedure is fast, requires only a small quantity of sample, and is extremely reproducible. Nominally, 1.5 mg of sample is placed in a hermetically sealed aluminum pan with a pinhole lid for interaction of the sample with the reactant gas (oxygen). The sample amount has significant impact on the shape and reproducibility of DSC exotherm. An optimum weight in the range 1.0–1.5 mg is found to yield consistent results. A film thickness of less than 1 mm is required to ensure proper oil-O<sub>2</sub> interaction and eliminate any discrepancy in the result due to oxygen diffusion limitations (32, 34). The module is first temperature calibrated using the melting point of indium metal (156.6°C) at 10°C/min heating rate and later at 1°C/min, 5°C/min, 15°C/min, and 20°C/min to be used in the study. For kinetic studies, the system is equilibrated at 35°C and heated using the above heating rates. Oxygen gas (dry, 99% pure, obtained commercially) is pressurized in the module at a constant pressure of 3450 kPa and maintained throughout the length of the experiment. These conditions maintain maximum contact with the sample and eliminate any limitation due to oxygen diffusion in the oil medium. Extreme care should be taken to maintain the pressure of the reactant gas (O<sub>2</sub>) constant.

The inverse of peak height temperature corresponding to maximum oxidation from the exotherm is plotted against the log of heating rate (*b*). Using the linear

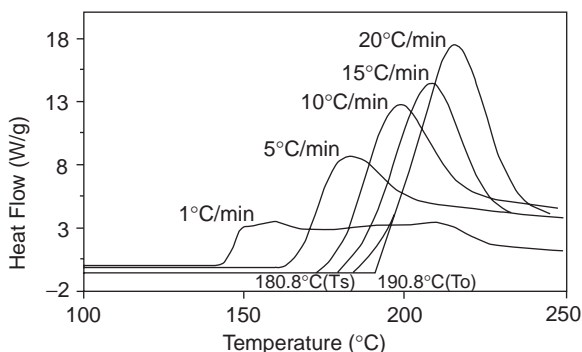


Figure 4. PDSC exothermic plots for high oleic sunflower oil at different heating rates.

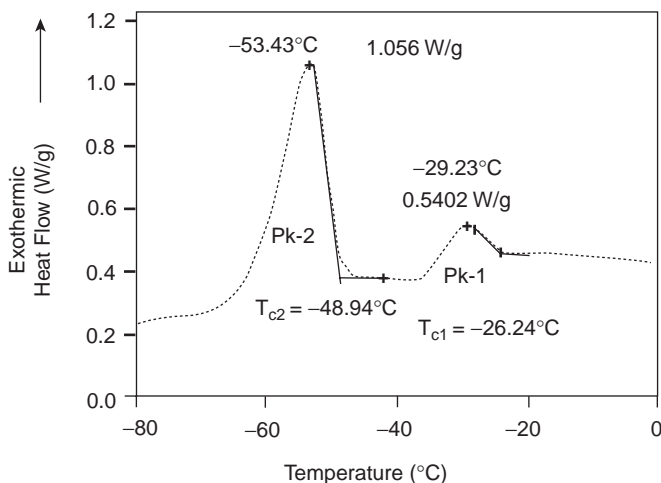
regression method and subsequent data computation, various kinetic parameters are obtained. Figure 4 presents the calorimetric curves (in scanning mode) of a typical vegetable oil at different heating rates. The figure shows the start temperature ( $T_s$ ) at 180.84°C and onset temperature ( $T_o$ ) representing temperature of rapid oxidation at 190.8°C (only at 20°C/min) for high oleic sunflower oil.  $T_o$  is defined as the temperature when rapid increase in the rate of oxidation is observed in the system. This temperature is obtained from extrapolating the tangent drawn on the steepest slope of reaction exotherm. A high  $T_o$  would suggest a high oxidative stability of the vegetable oil matrix. The oxidation start temperature ( $T_s$ ) is the temperature during which primary oxidation products begin to form in the vegetable oil matrix (33). It is also when loss of small molecular fragments due to evaporation are observed. Lower  $T_s$  and  $T_o$  indicates a thermally unstable matrix.

Table 3 shows that the activation energy ( $E_a$ ) of vegetable oil oxidation is affected by the degree and extent of polyunsaturation in the vegetable oils. High

**TABLE 3. Thermal, Kinetic, and Wax Appearance Data of the Vegetable Oil Basestocks Calculated from Differential Scanning Calorimetry Results;  $\beta = 10^\circ\text{C}/\text{minute}$ ; Cooling Range =  $+50^\circ\text{C}$  to  $-100^\circ\text{C}$ . ( $K = Z \cdot e^{-E_a/RT}$ ;  $R = \text{Rate Constant}$ )**

Vegetable Oil	$E_a$ (kJ/mol)	$T$ (K)	$k$ ( $\text{min}^{-1}$ )	$t_{1/2}$ (min)	$T_s$ (°C)	$T_o$ (°C)	$T_{c1}$ (°C)	$T_{c2}$ (°C)
Cottonseed oil	63.3	477.4	0.37	1.87	139.5	149.9	-10.7	—
Corn oil	77.8	464.3	0.43	1.61	162.6	177.0	-25.3	-43.4
Canola	88.5	454.0	0.51	1.36	119.8	143.0	—	—
Safflower oil	75.3	455.6	0.44	1.57	147.4	166.4	-24.2	-43.7
HOSaflr oil	88.7	471.8	0.48	1.44	172.0	177.8	-26.2	-48.9
HLSaflr oil	73.5	456.9	0.42	1.65	152.4	166.3	-21.3	39.8
Sunflower oil	63.8	446.2	0.38	1.82	129.8	145.4	-14.9	-36.7
HOSunflr oil	86.6	471.6	0.47	1.47	169.6	177.0	-21.5	-45.8
Soybean oil	79.7	464.5	0.44	1.57	161.3	178.2	-13.2	-35.5

HOSaflr = high oleic safflower; HLSaflr = high linoleic safflower; HOSunflr = high oleic sunflower oil; DSC data reported are the average values for three independent experiments.



**Figure 5.** Cooling curve of high oleic safflower oil.

linoleic content decreases the  $E_a$  for oxidation, and high oleic content increases it. The  $E_a$  for most of the oils studied varied in the range of 63–89 kJ/mol, which resulted in subsequent variation in the  $Z$  (Arrhenius constant),  $k$  (rate constant), and  $t_{1/2}$  values.

Figure 5 presents a modulated DSC trace of high oleic safflower oil (HOSaflr) obtained on cooling the sample from  $+50^\circ\text{C}$  to  $-100^\circ\text{C}$  at the rate of  $10^\circ\text{C}/\text{minute}$ . As a result of the presence of various triacylglycerol polymorphic forms ( $\alpha$  and  $\beta$ ), which can influence the crystal packing and the energy involved in the low-temperature crystallization process, vegetable oils do not crystallize over a narrow temperature range (35). Instead, crystallization of vegetable oil is a slow continuous process as initially microcrystalline structures are produced, which on further cooling, transform to macrocrystalline forms that rapidly lead to an overall solid-like consistency.

Unsaturation in the FA chain is inversely related to the liquidity of the oil or its freezing point, and is directly related to its solubility and chemical reactivity (thermal and oxidative). Oils with high saturation, in general, show high oxidative stability but poor low-temperature fluidity. Saturated FA have no  $\text{C}=\text{C}$  bonds to distort the geometry of the chain. Therefore, during the cooling process they pack very effectively into crystalline forms and, wax appears at a higher temperature than for unsaturated fatty acids of the same chain length.

During the initial cooling process,  $T_{c1}$  is the temperature at which microcrystals start forming through stacking of the “bent tuning fork” conformation. On further cooling, the solvation power of the vegetable oil matrix is lowered and the oil transforms from an increasingly viscous fluid to a solid material and is preceded by an onset temperature of freezing ( $T_{c2}$ ). Rationalization of the wax appearance temperature in a system like vegetable oil, consisting of a complex mixture of various

fatty acids in the triacylglycerol molecule, cannot be based on the content of oleic acid alone. The relative amount of other fatty acid components (which may function as solvents to stabilize the crystals formed during the cooling process) may also have an effect on crystallization.

Thermal analysis methods, such as pressurized differential scanning calorimetry (PDSC), are popular for the determination of oxidative stabilities of vegetable oils (33, 36, 37).

In conclusion, the complexity of vegetable oil oxidation is primarily due to the involvement of different structural parameters in the fatty acid chain. Different structural parameters participate in the reaction at different stages of oxidation. It is therefore not recommended to measure the extent of oxidative degradation in terms of a single parameter, i.e., polyunsaturation. Allylic, bis-allylic protons,  $\alpha$ -CH<sub>2</sub> to C=O group, chain length of the saturated methylene groups, etc., have significant effect on the oxidation process at different stages. The <sup>1</sup>H- and <sup>13</sup>C NMR-derived structural information can be used to explain most of the thermal and kinetic behavior of unmodified and genetically modified vegetable oils (38).

A critical analysis of the data in Tables 3 and 4 indicates that a high percentage of saturated CH<sub>2</sub> carbons and low percentage of bis-allylic CH<sub>2</sub> carbons translates to high E<sub>a</sub> of the vegetable base fluid. An increase in the methylene carbons of the FA chains, without being interrupted by -C=C- bonds, increases the thermal and oxidative stability. Protons on bis-allylic methylene groups (Table 5) are highly susceptible to rapid radical attack and initiate oxidative degradation of the triacylglycerol molecule.

Similarly, an increase in percentage olefin protons in the FA chain decreases the E<sub>a</sub> for oxidation. Conjugated olefin structures offer easy removal of H• due to better resonance stabilization.

**TABLE 4. Structural Parameters (Expressed as % of Carbon Atoms) of Vegetable Oil Basestocks Derived from Quantitative <sup>13</sup>C NMR Data.**

Vegetable Oil	Olefin	Saturated CH <sub>2</sub>	Bis-allylic CH <sub>2</sub>
Cottonseed oil	8.1	38.3	8.0
Corn oil	9.6	36.3	7.8
Safflower oil	9.1	32.4	9.4
HOSaflr oil	9.7	43.1	5.6
HLSaflr oil	9.5	32.4	10.2
Sunflower oil	9.0	34.9	9.7
HOSunflr oil	9.9	41.9	6.1
Soybean oil	9.1	36.6	10.8

HOSaflr = high oleic safflower; HLSaflr = high linoleic safflower; HOSunflr = high oleic sunflower oil; NMR data reported are the average values of three independent experiments. The repeatability of the data is  $\pm 0.2$ .

**TABLE 5. Structural Parameters (Expressed as %) of Vegetable Oil Basestocks Derived from Quantitative  $^1\text{H}$  NMR Data.**

Vegetable Oil	Olefin Proton	Bis-Allylic $\text{CH}_2$ Proton	$\text{CH}_2$ on Saturated Carbons
Cottonseed oil	8.8	2.83	54.87
Corn oil	10.0	3.4	51.4
Safflower oil	11.2	4.3	48.4
HOSaflr oil	7.2	0.8	57.03
HLSaflr oil	11.0	4.4	48.5
Sunflower oil	10.2	3.9	51.1
HOSunflr oil	6.9	0.6	57.8
Soybean oil	10.3	4.1	51.7

HOSaflr = high oleic safflower; HLSaflr = high linoleic safflower; HOSunflr = high oleic sunflower oil; NMR data reported are the average values of three independent experiments. The repeatability of the data is  $\pm 0.2$ .

#### 4. LOW-TEMPERATURE PROPERTIES

Vegetable oils, when subjected to low-temperature environment, undergo solidification through crystallization and, therefore, are a major hurdle for use in industrial applications. The relatively poor low-temperature flow properties of vegetable oils derive from the appearance of waxy crystals that rapidly agglomerate resulting in the solidification of the oil. Vegetable oil is a complex molecular system and, therefore, the transition from liquid to solid state does not occur at a particular temperature, but over a wide temperature range involving several polymorphic forms ( $\alpha$ ,  $\beta'$ ,  $\beta$ ) (39–41) contributing to the wax appearance and crystallization process. This deposition of waxy materials from oil results in a rapid viscosity increase leading to poor pumpability, lubrication, and rheological behavior.

Low-temperature behavior of triacylglycerols relates first of all to their crystallization kinetics. Major crystalline forms have been established for saturated triacylglycerols (42). Hagemann (43) has listed over a hundred reports on melting temperatures of mainly monoacid triacylglycerols. However, crystalline forms of unsaturated triacylglycerols have been established only for triacylglycerols with symmetrical distribution of monounsaturated fatty acids (44). Thus, investigations of crystallization of unsaturated mixed acid triacylglycerols are mostly empirical (45), and solidification of such triacylglycerols is too complex to be studied using traditional techniques, such as X-ray diffraction. Nonetheless, it has been firmly established (42, 43) that presence of *cis*-unsaturation, lower molecular weights, and diverse chemical structures of triacylglycerols favor lower temperatures of solidification.

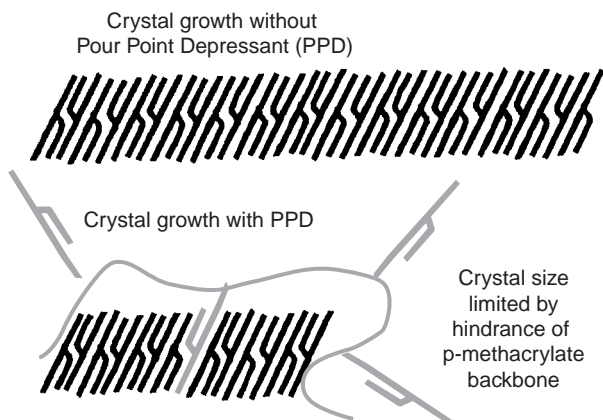
Crystallization kinetics generally is very sensitive to temperature fluctuations and related factors, such as cooling rate or thermal history. As can be expected from nucleation theory and crystallization thermodynamics, presence of contaminants,

foreign bodies, or other nucleation centers and even shaking may affect crystallization. As solidification thermodynamics of VO is exceedingly complex, only indirect semiquantitative data are available from techniques such as cooling the liquid and measuring its viscosity increase, precipitation, or loss of fluidity.

In the industry, one major characteristic of the low-temperature properties of lubricating fluids is pour point (PP). ASTM technique D-97 is used to determine PP by placing a test tube containing 50 mL of the sample into a metal cylinder, which is submerged into cooling media, and measuring the temperature at the top of the sample until it stops pouring (46). The temperature of the cooling media is kept constant below the sample temperature. When the sample temperature reaches the specified range (e.g., three of the ranges are  $+9^{\circ}\text{C}$  to  $-6^{\circ}\text{C}$ ,  $-6^{\circ}\text{C}$  to  $-24^{\circ}\text{C}$ , and  $-24^{\circ}\text{C}$  to  $-42^{\circ}\text{C}$ ), the temperature of cooling media is also reduced to the specified value ( $-18^{\circ}\text{C}$ ,  $-33^{\circ}\text{C}$ , and  $-51^{\circ}\text{C}$ , respectively). Statistically, the test shows satisfactory accuracy; as described in a precision statement (46), the difference between two test results from independent laboratories exceeds  $6^{\circ}\text{C}$  in only one case of 20, and repeatability is  $2.87^{\circ}\text{C}$  at 95% confidence.

Pour point depressants (PPD) have been created to suppress formation of large crystals during solidification, although the mechanism of PPD action on triacylglycerol crystallization remains undisclosed (47). One type of commercial PPD contains a polymethacrylate backbone with branching, which allows inclusion of the PPD molecule into the growing crystal (Figure 6). However, VO containing PPD have still shown unsatisfactory performance when exposed to low temperatures for durations significantly longer than those specified in the D-97 test (48).

Table 6 tabulates the pour point values for selected vegetable oils and synthetic fluids. It must be noted that some of the fluids may actually still pour after being held for significant durations at slightly lower temperatures than their determined PP. A good example is castor oil, which pours after more than 24 hours when stored at  $-25^{\circ}\text{C}$ , although its PP has been determined to be  $-24^{\circ}\text{C}$  in triplicate runs.



**Figure 6.** Crystal growth with and without PPD.



**TABLE 6. Pour Points of Test Fluids.**

Fluid	Pour Point (°C)
Canola oil	-18
Soybean oil	-9
High oleic sunflower oil	-12
Meadowfoam oil	+3
Castor oil	-24
Diisodecyl adipate	-60
PAO 2 <sup>a</sup>	-72

<sup>a</sup>Poly alpha olefin (PAO) with viscosity of 2 cSt at 100°C (hydrogenated dimmers/trimers of  $\alpha$ -decene).

There may be several arguments to explain this observation. First, the D-97 calls for determining the PP at either -24°C or -27°C, i.e., the actual loss of fluidity may occur at the temperature which is closer to -27°C than -24°C, but it still qualifies as a PP of -24°C, rather than -27°C.

Second, the D-97 requires lowering the bath temperature from -33°C to -51°C when the sample thermometer reaches -24°C. This produces pronounced changes in the cooling rate and temperature profile within the sample, which may have implications on the solidification kinetics. Despite the complexities of interpreting the data, it can be observed that increasing molecular weight of fatty acids and full saturation contribute to the increase in PP, whereas *cis*-unsaturation favors the decrease. Castor oil demonstrates PP notably lower than those of soybean, high-oleic sunflower, and canola oils, suggesting that interaction between the hydroxy groups (hydrogen bonding) of ricinoleic acid interferes with the crystal growth.

The effect of pour point depressant on pour points of vegetable oils (Table 7) shows that an amount of 0.4% by weight of PPD significantly reduces rates of solidification. Increased amount of PPD may still slow down solidification, however, any further depression ceases quite rapidly.

Differential scanning calorimetry (DSC) is a relatively simple and reproducible technique capable of providing a direct measurement of the  $\Delta H$  (change in enthalpy) for a system undergoing physical and chemical changes during heating or cooling (49-51). A number of studies have been carried out on the cooling behavior of

**TABLE 7. Effect of Pour Point Depressant (PPD<sup>a</sup>) on Pour Points (°C) of Vegetable Oils.**

Vegetable Oil	0	0.4	1	2
	(Amount of PPD <sup>a</sup> [% {w/w}])	(Amount of PPD <sup>a</sup> [% {w/w}])	(Amount of PPD <sup>a</sup> [% {w/w}])	(Amount of PPD <sup>a</sup> [% {w/w}])
Soybean oil	-9	-18	-18	-18
High oleic sunflower oil	-12	-21	-24	-24
Canola oil	-18	-30	-33	-33

<sup>a</sup>Poly(alkyl methacrylate) copolymer of -8,000 amu, canola oil carrier 1:1.

different wax-bearing crude and mineral base oils using DSC (52–57). Reports are also available on the application of DSC for identification of various stable polymorphs in pure single-acid triacylglycerol structure by heating and cooling experiments (58–63) and references therein. However, the situation in an unmodified or genetically modified vegetable oil is far more complex. This is primarily due to the presence of different fatty acid moieties, their chain length, abundance, structural difference, and configuration in the triacylglycerol that makes vegetable oils significantly different from other pure molecules. All the above factors individually and collectively are capable of influencing the physical properties of vegetable oils.

Overall, DSC is an excellent method to measure the wax appearance and crystallization temperatures of vegetable oils. Due to the complexity of the vegetable oil composition with respect to their FA distribution, the situation is not as simple as pure triacylglycerol molecules. Moreover, there is significant influence of the nature, relative abundance, and orientation of C=C bonds on the wax appearance temperatures. Further, presence of other saturated short-chain-length FAs in vegetable oil structure is found to affect the crystallization process. Statistical analysis of  $^1\text{H}$  NMR-derived vegetable oil structure support the influence of several predictor variables associated with FA unsaturation on the crystallization process.

Distance and dihedral angle calculations on triacylglycerol structures using energy minimization molecular modeling approach were able to explain the relative crystallization tendency of vegetable oils in the bent “fork” conformation (61).

## 5. VISCOSITIES, POUR POINTS, AND OXIDATIVE DEGRADATION TENDENCIES OF MAJOR LUBRICANT BASESTOCKS

Soybean oil (SBO) and high oleic (90%) sunflower oil (HOSO) were chosen for evaluation as examples of vegetable oils (62). Polyalphaolephin and adipate represented widely used synthetic biodegradable lubricating basestocks. The mineral oil was a typical “non-biodegradable” basestock mostly used for formulations of automotive lubricants. Except for natural antioxidants, the above fluids did not have any additives.

Kinematic viscosities of the fluids were measured according to ASTM D-445, 1991b (63). For Oxidative Stability testing, micro-oxidation experiments were carried out using the Pennsylvania State University Micro-Oxidation Kit (64). ASTM D-97 was used for pour points and cold storage tests (65).

Initially, kinematic viscosities at 40°C and pour points of the fluids were determined (results shown in Table 8). It appears that low-temperature properties of vegetable oils are much more inferior to those of synthetic basestocks or even mineral oil. Cold storage properties of vegetable oils do not appreciably respond to the pour point depressants (PPD), as opposed to mineral oils. This is consistent with earlier observations (35). Oxidative stabilities of the oils are compared in Table 9.

The data also compare oxypolymerization and volatility tendencies of the fluids. Temperature of 150°C and durations of 30–60 minutes chosen for testing were high

**TABLE 8. Viscosities and Pour Points of Major Lubricating Basestocks.**

Fluid	Viscosity, cST at 40°C	Pour Points, °C	Storage at -25°C, days (No additives)	Storage at -25°C, days (With 1% PPD <sup>a</sup> )
Soybean oil	31.5	-9	0	0
90% Oleic Sunflower oil	40.3	-12	0	0
Di i-C13 adipate	27.2	-54	7+	7+
PAO 4 <sup>b</sup>	17.4	-63	7+	7+
Mineral oil	71.2	-21	0	7+

<sup>a</sup>Poly alkyl methacrylate copolymer of ~8000 amu, canola oil carrier 1:1.

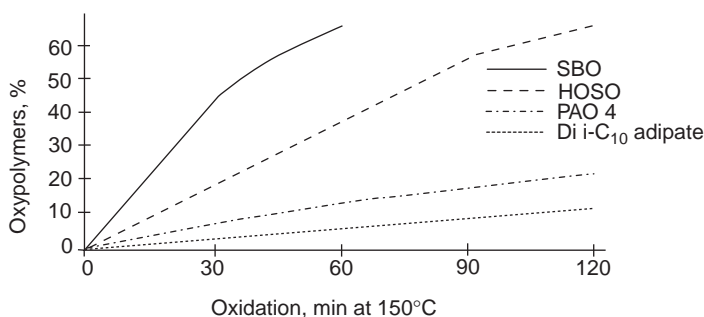
<sup>b</sup>Poly-alpha olefin (kinematic viscosity 4 mm<sup>2</sup>/s).

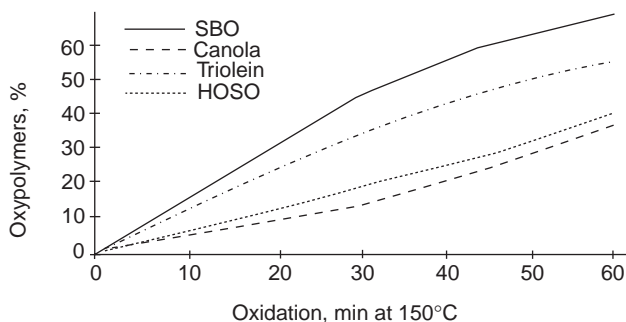
**TABLE 9. Oxidative Degradation Tendencies.**

Fluid	Micro-oxidation, 30 min at 150°C, % (Oxypolymers)	Micro-oxidation, 30 min at 150°C, % (Evaporation)
Soybean oil	48	2
90% Oleic Sunflower oil	13	0
Di i-C13 adipate	3	5
PAO 4 <sup>a</sup>	6	45
Mineral oil	5	5

<sup>a</sup>Polyalphaolefin with viscosity of ~4 cSt at 100°C (hydrogenated dimmers/trimers of  $\alpha$ -decene).

enough to cause a quantifiable polymerization in unsaturation-free basestocks, yet not too severe to result in oxidative gelation of vegetable oils. Therefore, the side processes, such as oxidative cleavage and formation of solids, were not substantial. It appears from the data that vegetable oils oxypolymerize considerably faster than unsaturation-free fluids. This is further highlighted in a kinetic chart for oxypolymerization, as shown in Figure 7.

**Figure 7.** Oxidative degradation and oxypolymerization tendencies of various oils.



**Figure 8.** Oxypolymerization tendencies using thin film micro-oxidation test.

Although HOSO containing only 5% of linoleic acid shows higher resistance to oxypolymerization than SBO, its oxidative stability is far less than those of PAO or adipate. Oxypolymerization proceeds much faster and slows down only when side processes, especially formation of solids, become more pronounced. It has been established that methylene interrupted polyunsaturation is the key factor causing low oxidative stability of vegetable oils (66, 67). This is also evident comparing micro-oxidation data for different vegetable oils (Figure 8).

Chemical modification is necessary to improve these performance limitations with the focus of eliminating bis-allylic hydrogen functionalities in methylene interrupted polyunsaturation and optimal extent of structural alteration for improved low-temperature performance.

## 6. EFFECT OF DILUENT AND ADDITIVES ON LOW-TEMPERATURE PROPERTIES

Studies show that diluents have a significant role in lowering the pour point of the base fluid (35). However, high dilution does not necessarily translate to proportionate depression of pour point, and no synergism exists between diluents and PPD molecules.

During the cooling process, the response to diluents and PPD molecules is dependent on the vegetable oils FA composition and its geometry to a certain extent. Pour point determinations (65) of safflower, high oleic safflower, and high linoleic safflower in the presence of diluent and additive molecules are presented in Table 10 (68).

The addition of a synthetic ester diluent to safflower and high linoleic safflower oils showed a larger decrease in the pour point compared with the high oleic oil. Due to the presence of multiple unsaturations in safflower and high linoleic safflower oil, the triacylglycerol molecules encounter significant steric-hindrance from the “zigzag” nature of the FA chain during the cooling process. The presence of diluent molecules in the system enhances this effect by lowering the viscosity

**TABLE 10. Response of Diluents and Additives on Genetically Modified Vegetable Oil.**

Fluid	Pour Point in °C <sup>b</sup>		
	Neat	+Diluent <sup>a</sup>	Diluent + PPD <sup>a</sup>
Safflower oil	-21	-39	-45
High oleic safflower oil	-21	-27	-36
High linoleic safflower oil	-21	-39	-48

<sup>a</sup>ASTM D-97; Reference 54.

<sup>b</sup>Optimal blending condition of 65 : 35 oil : diluent (vol/vol) + 1% pour point depressants (PPD).

**TABLE 11. Cold Storage Stability Test of Vegetable Oils.<sup>a</sup>**

Fluids	Days at -25°C	
	+Diluent <sup>a</sup>	Diluent + PPD <sup>b</sup>
Safflower oil	7 + d	7 + d
High oleic safflower oil	1d	1d
High linoleic safflower oil	7 + d	7 + d

<sup>a</sup>ASTM D-97; Reference 54.

<sup>b</sup>Optimal blending condition of 65 : 35 oil : diluent (vol/vol) + 1% PPD.

and by interfering with the stacking process during cooling. The addition of PPD further lowers the pour point without any particulate precipitation. High oleic and high linoleic oils appear to show a better response in the presence of additive molecules.

In addition to exhibiting good low-temperature behavior, base oils should be stable over extended time at low temperature to qualify for any industrial and automotive applications. Although high oleic oils exhibit good thermal-oxidative behavior and acceptable PPD response, they fail in an industry-specified low-temperature extended storage stability test. Table 11 presents the storage stability test data of selected vegetable oils in the presence of diluents and PPD molecules (68). Using the optimized diluent and PPD concentration, safflower and high linoleic safflower oils showed acceptable fluidity well beyond seven days with some loss in optical clarity.

## 7. CONCLUSIONS

- The widespread use of vegetable oils as lubricant basestocks will depend largely on how well they perform during high-temperature oxidation and low-temperature applications.
- Oxidative stability and low-temperature characteristics of vegetable oils should be improved before they are considered for universal lubricant application.

- The presence of polyunsaturation in the FA chain reduces evaporative loss but accelerates oxidative degradation.
- High oleic vegetable oils perform better than regular vegetable oils in terms of thermal and oxidative stability.
- Diluents and PPD have a positive influence in lowering the pour point and increasing the stability in cold storage of vegetable oils and are dependent on the FA composition of the vegetable oils.

## REFERENCES

1. R. A. Padavich and L. Honary, "A Market Research and Analysis Report on the Vegetable Oil Based Industrial Lubricants," SAE Tech Paper 952077, **13** (1955).
2. S. J. Randles and M. Wright, *J. Syn. Lubr.*, **9**, 145 (1992).
3. N. S. Battersby, S. E. Pack, and R. J. Watkinson, *Chemosphere*, **24**, 1998–2000 (1992).
4. R. Becker and A. Knorr, *Lubr. Sci.*, **8**, 95 (1996).
5. M. H. Brodnitz, *J. Agric. Food Chem.*, **16**, 994 (1968).
6. J. W. Hagemann et al., *J. Amer. Chem. Soc.*, **49**, 118 (1972).
7. D. W. Murray, J. M. MacDonald, and A. M. White, *Proceedings of the 11th World Petroleum Congress on Processing, Transportation and Storage*, **4**, 447 (1982).
8. O. S. Privett and M. L. Blank, *J. Amer. Oil Chem. Soc.*, **39**, 465 (1962).
9. T. P. Labuza and S. Bergquist, *J. Food Sci.*, **48**, 712 (1983).
10. F. Shahidi, *Natural Antioxidants Chemistry, Health Effects and Application*, AOCS Press, Champaign, Illinois, 1997, p. 1.
11. W. Grosch, *J. Agric. Food Chem.*, **27**, 239 (1979).
12. D. Steinberg, S. Parthasarathy, T. E. Carew, J. C. Choo, and J. L. Witztum, *New Engl. J. Med.*, **320**, 915 (1989).
13. D. Harman, *Free Radical in Biology*, vol. 5, Academic Press, New York, 1982, p. 255.
14. J. P. Coates and L. C. Setti, *ASLE Trans.*, **29**, 394 (1986).
15. T. P. Labuza, *Crit. Rev. Food Technol.*, **2**, 365 (1971).
16. D. Swern, *Organic Peroxides*, vol. 1, Wiley/Interscience, New York, 1970, p. 115.
17. American Oil Chemist's Society Official Method Cd-12-57, *Amer. Oil Chem. Soc.*, Champaign, Illinois, 1983.
18. Oxidationsstabilität von Ölen und Fetten-Rancimatmethode, Metrohm AG, Herisau, Application Bulletin Metrohm Nr. 204/1 d, 1994.
19. M. W. Laubli and P. A. Bruttel, *J. Amer. Oil Chem. Soc.*, **63**, 792 (1988).
20. M. Loury, *Lipids*, **7**, 671 (1972).
21. J. M. De Man, F. Tie, and L. De Man, *J. Amer. Oil Chem. Soc.*, **64**, 993 (1987).
22. M. H. Gordon and F. Murshi, *J. Amer. Oil Chem. Soc.*, **71**, 649 (1994).
23. T. A. Jebe, M. G. Matlock, and R. T. Sleeter, *J. Amer. Oil Chem. Soc.*, **70**, 1055 (1993).
24. A. R. Wewala, ed., *Natural Antioxidants. Chemistry, Health Effects and Applications*, AOCS Press, Champaign, Illinois, 1997, p. 351.
25. B. Matthäus, C. Wiezorek, and K. Eichner, *Fat Sci. Technol.*, **96**, 95 (1994).

26. B. Matthäus, C. Wiezorek, and K. Eichner, *Lebensmittelchemie*, **47**, 85 (1993).
27. DGF Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten, Tensiden un verwandten Stoffen, Deutsche Gesellschaft für Fettwissenschaften e.V., Munster, Wissenschaftliche Verlagsgesellschaft GmbH Stuttgart, Method C-IV 6a, 1984.
28. American Oil Chemist's Society Official Method Cd-12b-92, *Amer. Oil Chem. Soc.*, Champaign, Illinois, 1993.
29. M. W. Laubli and P. A. Bruttel, *J. Amer. Oil Chem. Soc.*, **63**, 792 (1986).
30. S. E. Hill and E. G. Perkins, *J. Amer. Oil Chem. Soc.*, **72**, 741 (1995).
31. S. Asadauskas, *Oxidative Degradation of Fluids Based on Synthetic and Natural Esters*, PhD Thesis, Pennsylvania State University, State College, Pennsylvania, 1997, pp. 88–97.
32. B. Kolawski, *Thermochim. Acta*, **213**, 135 (1993).
33. S. Shankwalkar and D. Placek, *Lubr. Eng.*, **50**, 261 (1993).
34. A. Adhvaryu, J. M. Perez, I. D. Singh, and O. S. Tyagi, *Energy Fuels*, **13**, 493 (1999).
35. S. Asadauskas and S. Z. Erhan, *J. Amer. Oil Chem. Soc.*, **76**, 316 (1999).
36. B. Kowalski, *Thermochim. Acta*, **156**, 347 (1989).
37. B. Kowalski, *Thermochim. Acta*, **184**, 49 (1991).
38. A. Adhvaryu et al., *Thermochem. Acta*, **364**, 87 (2000).
39. D. Chapman, *Chem. Rev.*, **62**, 433 (1962).
40. C. W. Hoerr and F. R. Paulicka, *J. Amer. Chem. Soc.*, **45**, 793 (1968).
41. J. W. Hagemann and J. A. Rothfus, *J. Amer. Chem. Soc.*, **60**, 1308 (1983).
42. K. Larsson, "Physical Properties: Structural and Physical Characteristics," in *The Lipid Handbook*, F. D. Gunstone, J. L. Harwood, and F. B. Padley, eds., Chapman & Hall, New York, 1994, pp. 449–457.
43. J. W. Hagemann, "Thermal Behavior and Polymorphism of Acylglycerols," in *Crystallization and Polymorphism of Fats and Fatty Acids*, N. Garti and K. Sato, eds., Marcel Dekker, New York, 1988, pp. 9–95.
44. S. de Jong, T. C. van Soest, and M. A. van Schaick, *J. Amer. Oil Chem. Soc.*, **68**, 371 (1991).
45. V. D'Souza, L. de Man, and J.M. de Man, *J. Amer. Oil Chem. Soc.*, **68**, 907 (1991).
46. Standard Test Method for Pour Point of Petroleum Products D-97, ASTM Standards, American Society for Testing Materials, Philadelphia, Pennsylvania, vol. 05.02, 1991, pp. 57–64.
47. Acryloid EF-100 Series Pour Point Depressants, Rohm and Haas Company, Philadelphia, Pennsylvania, 1995, pp. 1–8.
48. I. S. Rhee, C. Velez, and K. Bernewitz, "Evaluation of Environmentally Acceptable Hydraulic Fluids," TARDEC Tech. Report 13640, Warren, Michigan, 1995, pp. 1–15.
49. A. P. Bentz and B. G. Breidenbach, *J. Amer. Chem. Soc.*, **46**, 60 (1969).
50. V. Antila and J. Finn, *Dairy Sci.*, **27**, 1 (1966).
51. C. K. Cross, *J. Amer. Chem. Soc.*, **47**, 229 (1970).
52. P. Claudy, J. M. Letoffe, B. Neff, and B. Damin, *Fuel*, **65**, 861 (1986).
53. P. Claudy, J. M. Letoffe, B. Chague, and J. Orrit, *Fuel*, **67**, 58 (1988).
54. F. Noel, *Thermochim. Acta*, **4**, 377 (1972).
55. P. Redelius, *Thermochim. Acta*, **85**, 327 (1985).

56. J. C. Hipeaux, M. Born, J. P. Durand, P. Claudy, and J. M. Letoffe, *Thermochim. Acta*, **348**, 147 (2000).
57. Z. Jiang, J. M. Hutchinson, and C. T. Imrie, *Fuel*, **80**, 367 (2001).
58. D. J. Cebula and K. W. Smith, *J. Amer. Chem. Soc.*, **68**, 591 (1991).
59. J. W. Hagemann, W. H. Tallent, and K. E. Kolb, *J. Amer. Chem. Soc.*, **49**, 118 (1972).
60. J. W. Hagemann and J. A. Rothfus, *J. Amer. Chem. Soc.*, **60**, 1123 (1983).
61. A. Adhvaryu et al. *Thermochim. Acta*, **395**, 191 (2003).
62. S. Z. Erhan and S. Asadauskas, *Ind. Crops Prod.*, **11**, 277 (2000).
63. ASTM Standards, v. 05.02, Standard test method for Kinematic Viscosity of Transparent and Opaque Liquids D-445, American Society for Testing Materials, Philadelphia, Pennsylvania, 1991, pp. 169–176.
64. C. J. Lee, E. E. Klaus, and J. L. Duda, *Lubr. Eng.*, **49**, 441 (1993).
65. ASTM Standards, v. 05.02, Standard Test Method for Pour Point of Petroleum Products D-97, American Society for Testing Materials, Philadelphia, Pennsylvania, 1991, pp. 57–64.
66. H. W. Gardner, *Free Rad. Biol. Med.*, **7**, 65 (1989).
67. F. D. Gunstone, “Autoxidation and photo-oxygenation,” in *The Lipid Handbook*, 2nd ed, Chapman and Hall, New York, 1994, pp. 566–571.
68. S. Z. Erhan and J.M Perez, eds., *Biobased Industrial Fluids and Lubricants*, AOCS Press, Champaign, Illinois, 2002, pp. 1.



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## *Vegetable Oils in Production of Polymers and Plastics*

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### **1. INTRODUCTION**

Polymers are a group of materials made up of long covalently bonded molecules, which are obtained either from natural or synthetic sources. Within the life sciences, the study of polymers has helped to foster the emergence of molecular biology through focus on natural macromolecular substances such as proteins, nucleic acids, and polysaccharides. Largely through engineering efforts, a series of commercially synthetic polymers have been successfully used in many applications in modern society.

Polymers, in the form of plastics, are used in making articles of daily use, such as knobs, handles, switches, pipes, heart valves, and so on. An overwhelming percentage of the polymers to make these commodities are synthesized from petroleum sources or natural gas raw materials. The key petrochemicals for polymer synthesis (ethylene, propylene, styrene, vinyl chloride monomer, and others) are produced largely from naphtha, one of the distillation fractions of crude oil or from natural gas. Once synthesized, the polymer materials, such as polyethylene, polypropylene,

polystyrene, and polyvinyl chloride, are passed to major consuming industries. These synthetic polymers are, however, often not environmentally friendly because they typically do not undergo the process of biodegradation and, of course, are dependent on a limited petroleum resource. The urgent need of today is to develop polymers that are biodegradable so that they become environmentally friendly (1–3). The common biodegradable synthetic polymers include novel aliphatic polyesters, such as poly( $\epsilon$ -caprolactone) (PCL), poly( $\beta$ -methyl- $\delta$ -valerolactone), polylactide, and their copolymers. The most important aspect of synthesizing biodegradable polymers relates to their ability to undergo degradation within the biosphere on coming into contact with micro-organisms, enzymes, or under natural environmental conditions.

In addition, the natural resource of petroleum is being exhausted at a fast rate (4). The escalating cost of petrochemicals and the high rate of depletion of this natural resource present a serious challenge to the innovative potential of chemists (5). Scientists are therefore investigating opportunities to prevent economic losses and inevitable crisis of lowered standard of living as a result of oil shortages in the future. They are searching for new raw materials that can be synthesized into environmentally friendly polymers so as to make available the materials needed by various industries at lower costs (6, 7). These new materials are, in fact, not so new; they stem from natural agricultural sources, called “*renewable resources*” (4, 6, 8). By definition, renewable resources are the agricultural products that are synthesized by the use of solar energy (3, 4, 9). Some examples of these resources are polysaccharides, such as cellulose and starch, and glycerol esters of fats and oils (3, 10).

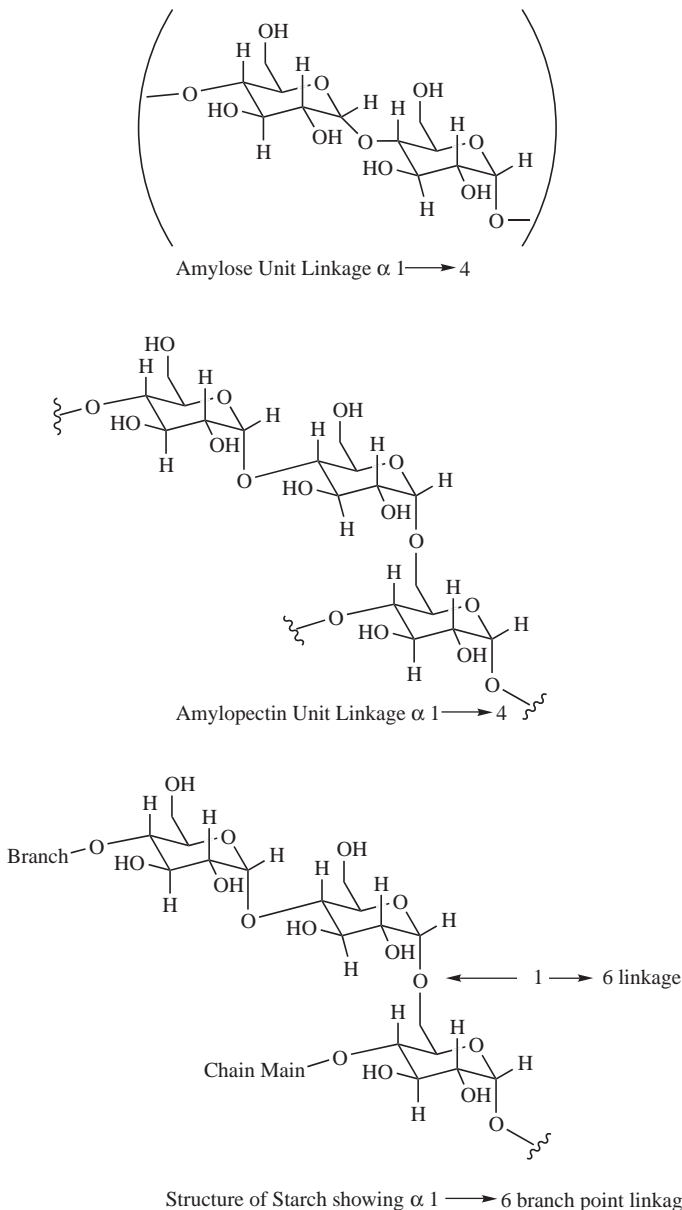
## 2. POLYMERS FROM RENEWABLE RESOURCES

This chapter deals with polymers synthesized from oilseed sources. However, to provide the reader with an appreciation of the area of renewable, biodegradable polymers and the place within this area that polymers from oil seeds occupy in terms of functionality, price, and acceptability, some other polymers from major renewable sources are also discussed. The most well-known and widely used renewable biodegradable polymers are those from polysaccharides. The principal polysaccharides of interest to polymer chemists are starches and cellulose, both of which are polymers of glucose. In addition to these, fibers, polylactic acid (PLA), and triacylglycerols of oils are of particular interest for the development of biodegradable industrial polymers.

### 2.1. Starch

Starch is the most common polymer found in plants. Large amounts of starch can be obtained from tubers such as potatoes, from cereals such as rice, and from seeds such as corn. The starch molecule is heavily hydrated as it contains many exposed hydroxyl groups, which form hydrogen bonds on coming into contact with water. Starch is constituted of linear polymers (amylose) and of branched polymers

(amylopectin) of  $\alpha$ -D-glucose. Amylose consists of long linear chains joined by  $\alpha$  (1  $\rightarrow$  4) linkages. Amylopectin, however, is highly branched in that D-glucose is also joined by  $\alpha$  (1  $\rightarrow$  4) linkages, but the branching point linkage between the two D-glucose molecules have  $\alpha$  (1  $\rightarrow$  6) linkages (11). The structures of amylose, amylopectin, and starch are shown in Figure 1.



**Figure 1.** Structures of amylose, amylopectin, and starch.

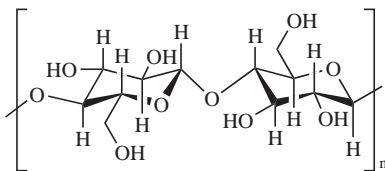
It may be noted from Figure 1 that the starch molecule contains two important functional groups, that is the  $\text{—OH}$  group, which is important for substitution reactions, and  $\text{C—O—C}$ , which is susceptible to chain breakage. As a result of these two important features, starch and its derivatives are used in the synthesis of biodegradable plastics (1).

Starch is generally regarded as a resource that is competitive with petroleum in terms of the preparation of a compostable polymer (12). It is used as an additive to plastics, for cross-linking or bridging to change the structures of plastics into networks, and as fillers for various purposes. Acetylation of starch yields starch acetate, which is considerably more hydrophobic than starch and has an improved solubility so that it can be easily cast into films from simple solvents. Degradation of acetylated starch films occurs when exposed to buffered amylase solution (1). Starch is used as filler in various resin systems to produce films that are impermeable to water but permeable to water vapors. Griffin (13) reported that starch-filled polyethylene films become porous after the extraction of starch. This porous film can then be readily invaded by micro-organisms and rapidly saturated with oxygen, thereby increasing polymer degradation by biological and oxidative methods. Some work has also been reported in which starch is used as a filler in manufacturing polyvinyl chloride (PVC) plastics (1). In addition, the hydroxyl group ( $\text{—OH}$ ) of starch can react with the extremely reactive groups ( $\text{—N=C=O}$ ) of isocyanates spontaneously, (i.e., cross-linking) which can be used to prepare a large number of reactive resins with reduced cost and improved solvent resistance and strength qualities (1, 14).

## 2.2. Cellulose

Cellulose is the most abundantly occurring natural biopolymer. Cellulose is a linear, unbranched homopolysaccharide. It resembles amylose, which is the primary polymeric constituent of starch. However, the major difference is that glucose residues in cellulose have  $\beta$  configuration (11, 12, 15). Cellulose is now receiving greater attention from polymer chemists because of the easy manner in which it undergoes biodegradation by certain micro-organisms. Aerobic soil is rich in floral bacteria and fungi, which will operate cooperatively to degrade polymers. Primarily, cellulose is biodegraded to glucose and cellodextrin. Then, by the action of enzymes, these cellodextrins are converted to glucose. The end products of biodegradation under aerobic conditions are water and carbon dioxide. The final products of biodegradation under anaerobic conditions, on the other hand, are carbon dioxide, hydrogen, methane, hydrogen sulfide, and ammonia (1).

Cellulose is a fibrous, tough, water-insoluble, and crystalline substance. As a result of these characteristics, it is often converted to its derivatives in order to make it more useful. The most commonly used derivatives of cellulose are carboxymethylcellulose, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, cellulose acetate, and cellulose xanthate (12). Among these derivatives, cellulose acetate and cellulose xanthate are cellulose esters, which are now widely used in the manufacturing of fibers, films, and in injection molding thermoplastics.



**Figure 2.** Structure of cellulose.

Cellulose esters represent a class of polymers that have excellent physical properties and are relatively low-cost materials with high market potential (1). The structure of cellulose acetate is shown in Figure 2.

### 2.3. Fibers

In addition to starch and cellulose, a wide variety of organic materials have significant commercial importance to the plastic industry. Fibers, among these, are being currently used as fillers and reinforcing agents in plastic materials. The primary advantages of using renewable lignocellulosic fibers as additives in polymers are that they (16):

1. have low densities
2. are low-cost materials
3. have a nonabrasive nature
4. provide high filling levels
5. require low-energy consumption
6. have high specific properties
7. are biodegradable and environmentally friendly
8. have wide varieties available throughout the world

Wood flour, for example, is a finely ground fibrous material obtained primarily from pine and spruce. Cotton is another plant fiber that is important in thermosetting molding compounds. Cotton fibers are used in the plastic industry in several forms: as flock, cordage, woven fabrics, nonwoven fabrics or felts, and chopped fabrics. The products obtained by using these fibers have reduced cost, increased impact strength, and other mechanical properties, as well as having improved moldability and appearance of finished molded parts. Wood flour; cotton fibers; sisal, another fiber plant; and hemp fiber, obtained from *Cannabis sativa* (12), are all used as filler and act as a reinforcing material for plastics. A lot of work has been done by Mohanty et al. (16–23) using lignocellulosic fibers from jute, pineapple leaf, sisal, and others, in making biopolymers. They observed that lignocellulosic fibers play important roles in modifying physical as well as chemical properties of the polymers (17–19). Rout et al. (20) have studied the use of coir as reinforcement in polymer composites. It was found that the efficiency of fiber-reinforced material

depends on the fiber/matrix interface and the ability of transferring stress from the matrix to the fiber. Mohanty et al. (22) studied the chemical modifications of jute yarns in making biopolymers and found that alkali-treated yarns produce better mechanical properties than the defatted ones because of the improved fiber matrix adhesion of the previous ones. Mishra et al. (16) studied surface modifications and mechanical improvements in pineapple leaf polyesters. It was observed that the surface-modified pineapple leaf fibers were good reinforcing agents for polyester matrices as well as having increased tensile and flexural strength.

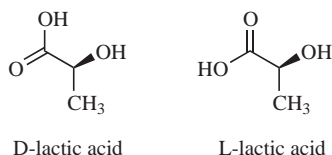
## 2.4. Polylactic Acid (PLA)

Poly(lactic acid) is not a new polymer. It belongs to the family of aliphatic polyesters commonly made from  $\alpha$ -hydroxy acids, which can be synthesized via two major routes. One method involves the removal of water using solvent, under conditions of high temperature and pressure. The polymer yielded using this method may be coupled with isocyanates, epoxides, or peroxides to produce a variety of other polymers. The other method involves the removal of water without solvent under milder conditions to produce a cyclic intermediate dimer referred to as lactide (24). Poly(lactic acid) is one of the few polymers in which the stereochemical structure can easily be modified by polymerizing a controlled mixture of L- or D-isomers (meso forms) to yield high-molecular-weight polymers. The properties of poly(lactic acid) depend entirely on the ratio of these two meso forms of lactic acid. The structures of the two meso forms are shown in Figure 3.

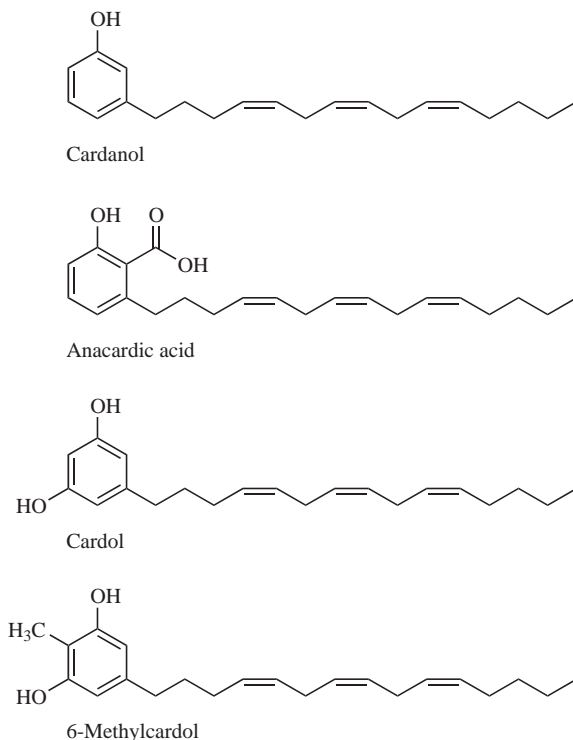
A great variation in the properties of products can be observed by using D, L, and different D/L ratios of poly(lactic acid). A product with high melting point and high crystallinity is obtained by using the L-isomer of lactic acid. On the other hand, an amorphous polymer is obtained by using a mixture of D and L isomers. This feature is very important, particularly in the binder fiber area (24). PLA, furthermore, is environmentally friendly. The products of PLA can completely degrade to carbon dioxide and water (24, 25).

## 2.5. Cashewnut Shell Liquid (CNSL)

Cashewnut Shell Liquid (CNSL) is an agricultural product, which as such, qualifies its inclusion in the category of renewable resource. Major components of CNSL have been characterized by various researchers using different techniques such as ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) spectroscopy,



**Figure 3.** D and L forms of lactic acid.

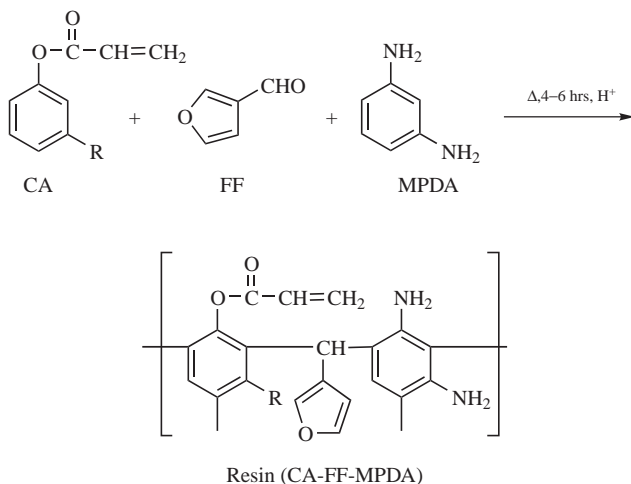


**Figure 4.** Structure of four major components of cashewnut shell liquid (CNSL).

and chromatography. The four major components of cashewnut shell liquid are cardanol, cardol, anacardic acid, and 6-methylcardol (26). Structures of these four components of CNSL are shown in Figure 4.

Several resins have been prepared by Guru et al. (27) using cardanyl acrylate, which is a derivative of cardanol (major constituent of cashewnut shell liquid) and furfural in the presence of an acid catalyst and a selective organic compound. They studied the thermal behavior of these resins as well as their solvent absorptivity using solvents such as toluene and dimethyl formamide. The organic compounds used were thiourea, *o*-hydroxybenzoic acid, *m*-phenylene diamine, and *m*-chlorophenol. The scheme of the reaction of cardanyl acrylate, furfural, and an organic compound is shown in Figure 5. Thermal studies of these resins has shown that the first great loss in weight occurs at 700°C and is a result of segmental fragmentation of cardanyl acrylate and ring unzipping of organic compounds. It was observed that resins obtained from thiourea had a higher thermal stability because of the absence of aromatic rings.

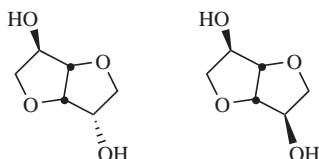
In another study, Nayak et al. (26) prepared CNSL-novalac resins by condensing cardanyl acrylate with *p*-aminobenzoic acid and formaldehyde in the presence of an acid catalyst. Thermogravimetric analysis and degradation studies on these resins have been carried out. It was observed that this resin decomposed with the removal



**Figure 5.** Reaction scheme of polycondensation of cardanyl acrylate (CA) with *m*-Phenylene Diamine (MPDA) and Furfural (FF) in the presence of acid as the catalyst.

of water. Swain et al. (28) have also reported the preparation of a number of resins by condensing diazotized cardanol with formaldehyde and organic compounds in the presence of an acid as a catalyst. They also carried out investigations on the thermogravimetric properties of the resin, which resulted in a weight loss of this resin of about 67–74% at 700°C.

Polyesters and polyurethanes can be prepared by bifunctional monomers like dianhydrohexitols, which are readily available from D-glucose and D-mannose. The structures of dianhydrohexitols are shown in Figure 6. Okada et al. (2) prepared polyesters based on furan rings. The scheme of the polyester formation is shown in Figure 7. They studied the biodegradability of these polyesters by three methods: hydrolysis in a phosphate buffer solution, soil burial degradation, and enzymatic degradation. They observed that the hydrolytic degradability of these polyesters is low, whereas soil burial degradation shows that several spores and hyphae of actinomycetes were grown within 100 days showing erosion of the surface of the film, meaning these polyesters are biodegradable.



**Figure 6.** Structures of dianhydrohexitols.



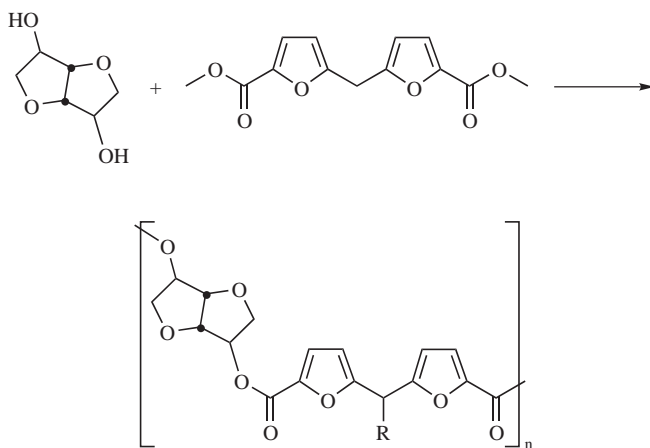
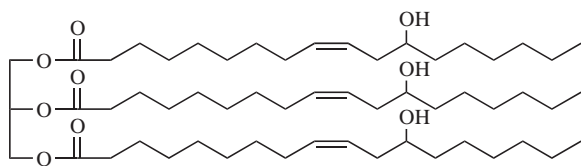


Figure 7. General scheme of polyester formation.

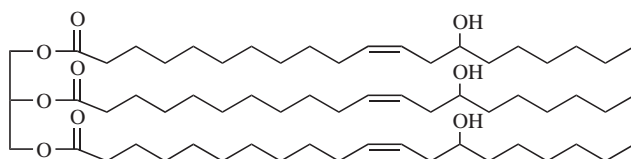
## 2.6. Triacylglycerol Oils

It has been reported above that low-cost biodegradable polymers can be prepared by using polysaccharides, fibers, and polylactic acid. In addition to these renewable resources, naturally occurring triacylglycerol (TAG) oils are also significant starting materials for the production of biodegradable polymers (6, 29). The TAG oils of linseed, tung, lunaria, *Lesquerella gracilis*, and crambe have been used as sources of polymers by virtue of their double bonds; functional groups that can result in polymerization (8). The double bonds of these and other TAG oils can also be epoxidized or converted into hydroxyl groups to increase their reactivity (6). Only a few TAG oils, however, contain naturally occurring special functional groups, i.e., hydroxyl and epoxy groups (30, 31). For example, castor oil and *Lesquerella palmeri* (also called bladder pods or pop weed) contain hydroxyl groups in addition to double bonds. Similarly, vernonia oil contains a natural epoxide functional group (31–33). The structures of the main triacylglycerols of castor, lesquerella palmeri, and vernonia oils are shown in Figure 8. The hydroxyl and epoxide functional groups on these long and complex TAG molecules may be exploited to allow such molecules to be cross-linked, which allows large macromolecules to be formed.

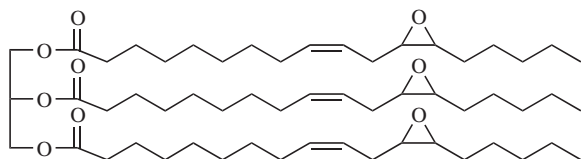
Castor oil from castor beans contains high percentage triacylglycerols (TAGs) of ricinoleic acid (83.6–90%), which is a C-18 fatty acid. Castor oil is unique in that its TAGs contain both double bonds and nonconjugated hydroxyl groups. The trifunctional nature of castor oil contributes toughness to the structure, and the long fatty acid chain imparts flexibility. As a result of its unusual structure, this oil is very versatile in its applications. It is used in making paints, adhesives, and urethane foams (34). *Lesquerella* oil, a C-20 oil, is obtained from a wild plant. It contains fatty acids similar to ricinoleic acid, but also contains two additional



Caster oil (triricinolein)



Lesquerella palmeri oil (trilesquerolic acid)



Vernonia oil (trivernolin)

**Figure 8.** Chemical structures of triricinolein, trilesquerolic, trivernolin, major triacylglycerols, respectively of castor, *Lesquerella palmeri*, and vernonia oils.

—CH<sub>2</sub>— groups on the acid residue. Vernonia oil has a relatively low viscosity because of high epoxide concentration. It is, therefore, mostly used as a diluent for coating applications (34).

Soybean oil, another TAG oil, is being used in the manufacturing of plastics, resins, and adhesives. Soybeans themselves contain 20% oil and 40% proteins; they contain discrete groups of proteins that have unusual adhesive properties (35). Soy-based plastics have many applications, which include their use in the production of parts for agricultural equipment, such as tractors and farming machines, and for the automotive industry. The applications also include civil engineering components for bridges and highways, marine infrastructures such as pipes and offshore equipment, rail infrastructure such as carriages, box cars, and grain hoppers, and in the construction industry such as formaldehyde free particle board, ceilings, and engineered lumbers (36). Different techniques such as injection molding and compression molding are used to prepare soy-protein plastics.

Jiratumnukul and Michael (37) have reported the use of different glycol esters from soybean oil, such as ethylene glycol, propylene glycol, diethylene glycol, and dipropylene glycol, for making new coalescent aids. They investigated the properties of these coalescent aids as related to evaporation rates and Minimum Film

Formation Temperature (MFFT). Based on their observations, soybean oil glycol esters are not classified as volatile organic compounds.

### 3. EXPLOITATION OF THE FUNCTIONAL GROUPS ON TRIGLYCEROL MOLECULES FOR THE PRODUCTION OF POLYMERS

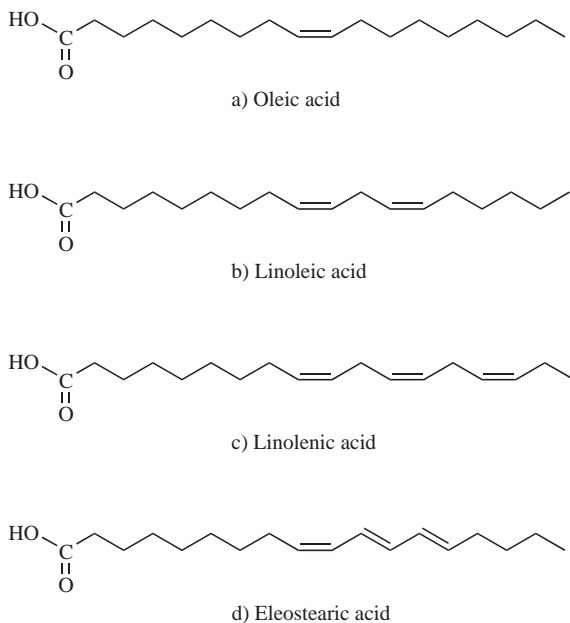
Different functional groups on TAG oils, such as carbon-carbon double bonds and epoxy and hydroxyl groups, play an important part in the formation of polymers. Oils from various sources contain different functional groups. The occurrence of these functional groups in oils from different sources and their significance in the production of polymers is described below.

#### 3.1. Double Bonds

Among the oils that contain carbon-carbon double bonds as the functional groups, linseed, tung, corn, cottonseed, rapeseed, and soybean are more widely used as polymeric sources. Linseed oil is extracted from the seeds of the flax plant (*Linum usitatissimum*). The major constituents of linseed oil are  $\alpha$ -linolenic acid (60%), linoleic acid (29%), and oleic acid (27%). This composition varies with changes in climatic conditions. On the other hand, tung oil, also called china wood oil, is derived from the seeds or nuts of the trees *aleurites fordii* and *A. montana*. The major constituent of tung oil is eleostearic acid (77–82%), whereas the other important components of tung oil are oleic acid (3.5–12.7%) and linoleic acid (8–10%). It is known that the carbon-carbon double bond in oleic acid is at C9, in linoleic acid it is at C6 and C9, and in linolenic acid it is at C3, C6, and C9, whereas eleostearic acid has double bonds at positions C5, C7, and C9.

The structures of oleic, linoleic, linolenic, and eleostearic acids are given in Figure 9. Linseed oil and the tung oil are collectively called “drying oils,” which are defined as liquid oils that dry in air to form a solid film (these oils have iodine values greater than or equal to 150 units). Soybean oil, sunflower oil, and canola oil are semidrying oils, with iodine values between 110 units and 150 units. The drying power of such oils is directly related to the chemical reactivity conferred on the TAG molecules by the carbon-carbon double bonds of the unsaturated acids, which allows them to react with atmospheric oxygen, thus leading to the process of polymerization to form polymeric networks.

Linseed oil, which contains 60%  $\alpha$ -linolenic acid, is an example of a nonconjugated oil, which is rich in polyunsaturated fatty acids. These polyunsaturated fatty acids contain double bonds, which are separated by at least two single bonds. The linolenic acid content in nonconjugated oils plays an important role in the drying process that is generally considered to be the result of a process of autoxidation followed by polymerization when the oil absorbs large amounts of oxygen. The process of autoxidation in the case of nonconjugated oil systems begins with the dehydrogenation of unsaturated fatty acids, such as linolenic acid, by means of atmospheric oxygen. As a result, dehydrogenated radicals are formed and chain



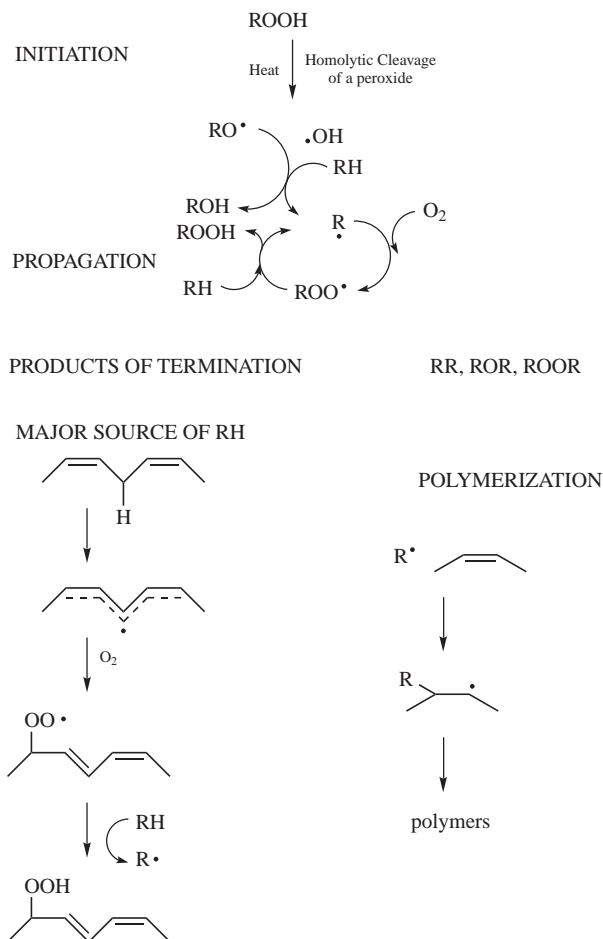
**Figure 9.** Structures of oleic, linoleic,  $\alpha$ -linolenic and eleostearic acid.

polymerization starts with the formation of hydroperoxide. Furthermore, cross-linking takes place to form large molecules. A summary of this process is shown in Figure 10.

Tung oil with eleostearic acid as its major component, on the other hand, is an example of conjugated oil systems. The conjugated double bonds of oils, such as those of tung oil, favor polymerization and oxidation more rapidly than nonconjugated oils. The principal drying component of tung oil is eleostearic acid. As a consequence of this polymerization, the resultant product obtained is highly resistant to water and alkali. Drying of films in the case of conjugated oils consists of the following three steps:

1. Induction: This process begins by the autocatalysis of eleostearic acid, and the oxygen uptake starts increasing slowly.
2. Initiation: The film continues to absorb oxygen from the atmosphere and, as a result of this absorption, the mass of the film increases and the double bonds of eleostearic acid undergo a rearrangement process. On rearrangement, hydroxyl and hydroperoxy groups are formed in the film.
3. Cross-linking: As a result of the above two steps, the number of double bonds decreases due to cross-linking and, thus, larger molecules are formed.

For many applications, tung oils often cure so rapidly that a highly wrinkled surface forms. Therefore, it is necessary to modify the reactivity of tung oil, which is



**Figure 10.** Stepwise drying process of oils with double bond functionality.

possible by reducing the number of double bonds present. The reactivity can be modified by chemical means, such as the Diels-Alder reaction with a reactive dienophile (38) and copolymerization with styrene (39) and diacrylate (40); this reduces the number of double bonds and causes the cure speed of the copolymers to be slower, resulting in a nonwrinkled surface. Also, by controlling comonomer stoichiometry, sufficient residual double bonds should remain so that oxidative cure of the copolymers would be still possible.

On the other hand, the high degree of unsaturation of this type of drying oil has made it a potential monomer for polymerization into useful polymers. More recently, Li and Larock (41) reported the conversion of tung oil to solid polymers by cationic copolymerization with divinylbenzene as a comonomer. The resulting polymers have proven to be thermosetting materials with good mechanical

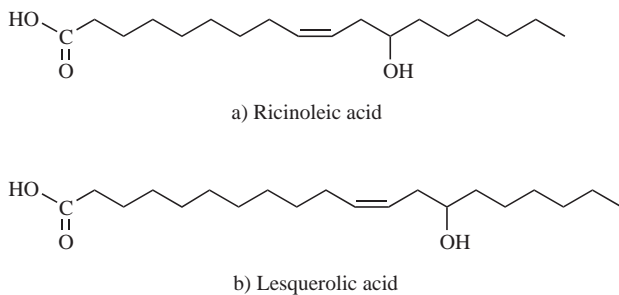
properties and thermal stabilities and may find applications in replacing petroleum-based polymeric materials because of their presumed ability to biodegrade and the low cost of their preparation from renewable natural resources (i.e., vegetable oils).

Furthermore, the multiple double bonds also make tung oil a thermally polymerizable monomer at elevated temperatures. Li and Larock (42) produced a variety of polymers prepared by thermal copolymerization of tung oil, styrene, and divinylbenzene in the temperature range of 85–160°C by varying the stoichiometry, oxygen uptake, peroxides, and metallic catalysts. They found that the stoichiometry and the addition of metallic catalysts greatly affect the mechanical, thermal, and physical properties of the resulting polymers. However, the variations of oxygen uptake and peroxides have little effect. Li and Larock also (43–50) proposed a direct method to convert soybean oil to polymers by cationic copolymerization with divinylbenzene or mixtures of styrene and divinylbenzene initiated by boron trifluoride diethyl etherate or other modified initiators. The polymers obtained can range from soft rubbers to hard plastics, depending on the reagents, stoichiometry, and initiators used in the synthetic process. The resulting polymers exhibit thermal, physical, and mechanical properties that were competitive with those of their petroleum-based counterparts, as well as some other very promising properties, including good damping and shape memory properties.

Knot et al. (51) converted soybean oil to several monomers for use in structural applications. They prepared rigid thermosetting resins by using free radical copolymerization of maleates with styrene. The maleates are obtained by glycerol *trans*-esterification of the soybean oil, followed by esterification with maleic anhydride. They also synthesized several TAG-based polymers and composites and compared their properties. It was found that the moduli and glass transition temperature ( $T_g$ ) of the polymers varied and depended on the particular monomer and the resin composition. They proposed that the transition from glassy to rubbery behavior was extremely broad for these polymers as a result of the TAG molecules acting both as cross-linkers as well as plasticizers in the system.

### 3.2. Hydroxyl Groups

Triacylglycerol oils, such as castor and lesquerella, are naturally occurring oils that have hydroxyl groups on their major TAG molecules. The major component of castor oil is ricinoleic acid (C18), which has a hydroxyl group at C12. On the other hand, the principal component of *Lesquerella palmeri* is lesquerellic acid (C21), having a hydroxyl moiety at C14. The structures of ricinoleic and lesquerolic acids are shown in Figure 11. Based on the TAG molecules of these acids shown in Figure 8, these oils are referred to as trihydroxyl polyols or triols. These polyols are important for the production of cross-linked polymers. The presence of the hydroxyl groups permit reaction with diisocyanates to form polyurethanes. Polyesters are formed when hydroxyl groups react with dibasic acids, such as sebacic acid obtained from castor oil, to form aliphatic polyesters with the removal of a water molecule as byproduct (52). Castor oil derivatives, obtained through alkali pyrolysis can also be useful, such as decanedioic acid (sebacic acid), which is



**Figure 11.** Structures of ricinoleic and lesquerolic acid.

used as a monomer for nylon 610, and undecylenic acid, which forms 11-aminoundecanoic acid, which is the monomer for nylon 11 (34). Nylon is an important polymer that is used for engineering plastics in the automotive and transport industry, for example. Nylon products are also used in powder coating to cast metals that require abrasion, impact, and corrosion resistance.

Isocyanates are the derivatives of isocyanic acid ( $\text{H}-\text{N}=\text{C}=\text{O}$ ). The functionality of the isocyanate ( $-\text{N}=\text{C}=\text{O}$ ) group is highly reactive toward proton-bearing nucleophiles, and the reaction of isocyanate usually proceeds with addition to the carbon-nitrogen bond. The reactions of isocyanates fall into two main categories: (1) active hydrogen donor, and (2) nonactive hydrogen reaction. The more significant of these is the first category, where isocyanates react with polyols, which involves reaction with active hydrogen. The second category of reactions involving nonactive hydrogen reactions usually leads to cycloaddition products and linearly polymerized products. Some examples of diisocyanates are: 2,4-toluene diisocyanate, 2,6-toluene diisocyanate, 1,6-hexamethylene diisocyanate, and 1,5-naphthalene diisocyanate, among others. The reactivity of isocyanates depends on their chemical structures. Aromatic isocyanates are usually more reactive than their aliphatic counterparts. The presence of electron withdrawing substituents on isocyanates increases the partial positive charge on the carbon atom and moves the negative charge further away from the reaction site. As a result of this character, the reaction between the donor substance and the carbon atom of isocyanates is fast. Therefore, whenever polyols or triols react with an isocyanate, the resulting polyurethane is cross-linked. The extent of cross-linking affects the stiffness of the polymer. The polymer structure must be highly cross-linked when a rigid foam is required, whereas less cross-linking gives rise to flexible foams. The degree of cross-linking is entirely dependent on the  $\text{NCO}/\text{OH}$  ratio. Branching occurs at the urethane linkage when  $\text{NCO}/\text{OH}$  ratio is low. Low degree of cross-linking allows the molecules freedom of movement resulting in the improvement in strength and creep resistance. However, a slight loss in the soft, flexible, rubbery behavior occurs. On the other hand, when  $\text{NCO}/\text{OH}$  ratio is high, the probability of the formation of urea linkages is greater and, therefore, the branching takes place at the urea linkage points. A high degree of cross-linking, in contrast, immobilizes the polymer molecules and, thus, the resulting polymer becomes a thermoset

plastic. Das and Lenka (53) and Barrett et al. (34) have reported the preparation of polymers by using toluene diisocyanate and hexamethylene diisocyanate. They observed that increased ratio resulted in a highly cross-linked product with high thermal stability.

It is known that increased cross-linking brings the polymer backbone closer together. There is, therefore, a reduction in molecular mobility and an increase in glass transition temperature. The weaker and less stable cross-linkages at high temperatures tend to reopen and revert back to linear structures. Less cross-linked polymers absorb large amounts of solvent and, thus, swell to form soft gels. Highly cross-linked polymers, however, absorb less solvent molecules as a result of less molecular mobility and, thus, cannot move apart to accept solvent molecules.

The preparation of polyols from vegetable oils, such as castor oil, safflower oil, linseed oil, and soybean oil, has been studied by several groups. There are many ways to introduce hydroxyl groups into oils, resulting in different polyol structures and different polyurethanes ranging from elastomers to rigid foams. Polyurethane prepared from castor polyols exhibits a broad range of properties. Low-viscosity urethane polymers have been found extremely useful for potting electrical components, for which fast penetration without air voids and fast dispensing cycles are desirable (54). Meanwhile, very low-viscosity polyurethane systems containing castor polyols have been prepared for use in telephone cable (55). On the other hand, polymerization, whether chemical or oxidative, of castor oil resulted in oils with higher viscosity that were more useful in the polyurethane coating industry than untreated ones (56).

Castor oil and its derivatives have been used in the preparation of rigid, semirigid, and flexible polyurethane foams. Castor oil's resistance to hydrolysis, pigment dispersion ability, and compatibility with polyether polyols has also made it useful as a modifier for polyether-based foam. Castor oil can also be used to formulate commercially acceptable rigid polyurethane foams for use as thermal insulations and structural material (57). Superior rigid polyurethane foams have been prepared from hydroxymethylated polyol esters of castor acids. Frankel et al. (58, 59) prepared castor, safflower, and linseed oil derivatives with enhanced hydroxyl group by hydroformylation with a rhodium-triphenylphosphine catalyst, followed by hydrogenation. The polyurethane foams obtained had good compressive strength and dimensional stability that met the requirements of commercial products.

Recently, Petrovic and coworkers (60–69) developed two technologies to prepare soybean oil-based polyols for general polyurethane use. In the first technology, the oil was first epoxidized using the standard epoxidation procedure, followed by alcoholysis to form the TAG polyol. In the second, the double bonds of the soybean oil were first converted to aldehydes by hydroformylation with either rhodium or cobalt as the catalyst, followed by hydrogenation to alcohols by nickel. The polyols were then reacted with a diisocyanate to yield polyurethanes. The resulting polyurethane can behave as a hard rubber or a rigid plastic, depending on the methods used in the reaction process, such as by controlling the degrees of conversion, using different diisocyanate components, and varying the stoichiometry. They found that the rhodium-catalyzed hydroformylation of soybean oil with high conversion of



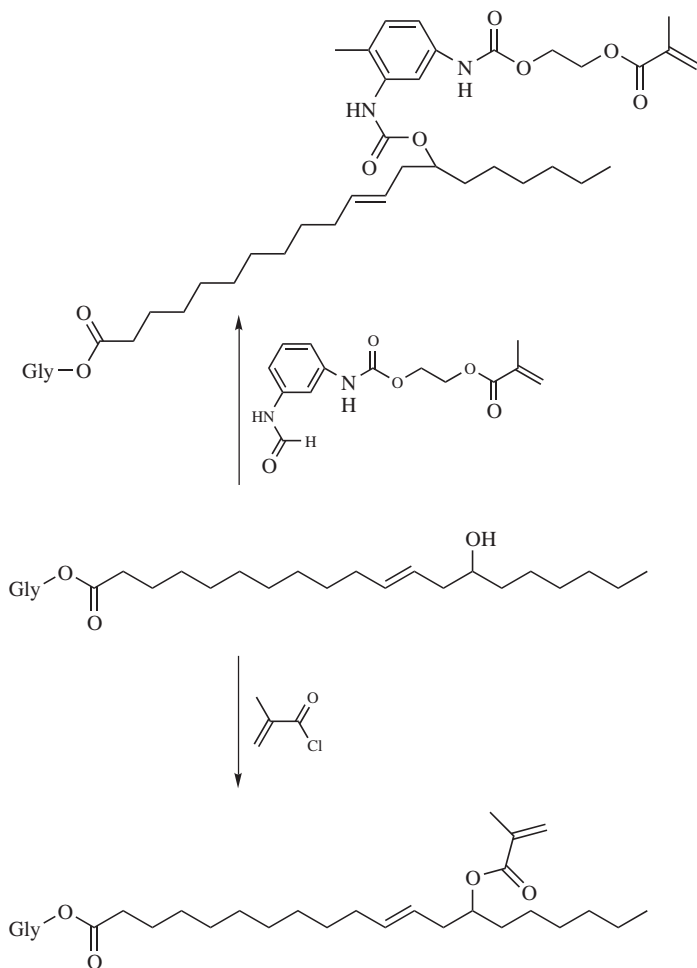
olefins leads to a rigid plastic polyurethane at room temperature, whereas the cobalt-catalyzed hydroformylation with low conversion of olefins gave a hard rubber. The properties of the product can also be controlled by the cross-linking density. Polymers prepared with NCO/OH ratio from 1.05–0.8 were glassy, whereas with the others, less than 0.8 were rubbery. The selection of the diisocyanate component affects the cross-linking and properties of the polyurethane as well. Usually, aromatic isocyanates produce more rigid polyurethanes than their aliphatic counterparts, but their oxidative and ultraviolet stability are lower. Within aromatic isocyanates, 2,4-toluene diisocyanate (TDI) is chosen to obtain the most flexible product. Rigid foams can be made from TDI prepolymers but are most often based on a polymeric diphenylmethane 4,4- diisocyanate (MDI). Aliphatic isocyanates give rubbery polymers with higher elongation at break, higher swelling, and lower tensile strength. The thermal stability of polyurethanes was also investigated. It was found that the thermal stability depended on the functionality of the polyol, i.e., on the number of urethane groups per unit volume, as well as structural differences.

John et al. (70) studied and compared the properties of polyurethane foam obtained from soybean oil-based polyols and synthetic polyols and found that the soybean-based polyols showed enhanced reactivity and that the foaming reactions proceeded in a very similar way to synthetic polyols. It was also found that their properties were sensitive to several variables such as water content, isocyanate index, and catalysts. The reaction rate was mainly controlled by the water and isocyanate content. As the water content increased, the reaction was faster and the amount of the hard segment increased. In addition, MDI yielded more rigid foams than TDI.

Polyesters obtained from lesquerella oil have variations in colors and have low solution viscosity as compared with castor oil polyesters. The viscosity variations are usually associated with the structural differences of the oil. Solution viscosity of castor oil-based polyesters is usually high because ricinoleic acid in castor oil allows extensive hydroxyl hydrogen bonding. Furthermore, lesquerella oil-based polyesters are effectively plasticized because of longer C20 fatty acid. The hydroxyl group of lesquerella oil can be exploited to make acrylates. The scheme for making lesquerella oil-based acrylates is shown in Figure 12. Lesquerella oil acrylates impart excellent gloss to wood, aluminum, and steel and have good adhesive properties. The hydroxyl groups of lesquerella and castor oil also react with cycloethers such as propylene oxide, epichlorohydrin, and ethylene oxide. As a result of these reactions, novel polyhydroxy compounds of much improved reactivity can be obtained. Epichlorohydrin-modified lesquerella oil has increased reactivity characteristics. The coatings developed from epichlorohydrin and lesquerella oil form harder films in shorter dry time. The mechanism is shown in Figure 13.

### 3.3. Epoxides

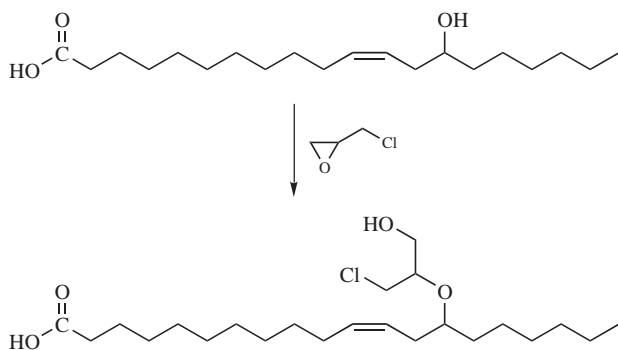
Vernonia oil contains a naturally occurring epoxide group on C12. It is, therefore, important for the productions of polymers. It is also used in making adhesives, plasticizers, industrial coatings, varnishes, and paints (32). Vernonia oil can be



**Figure 12.** Synthesis of lesquerella oil acrylates.

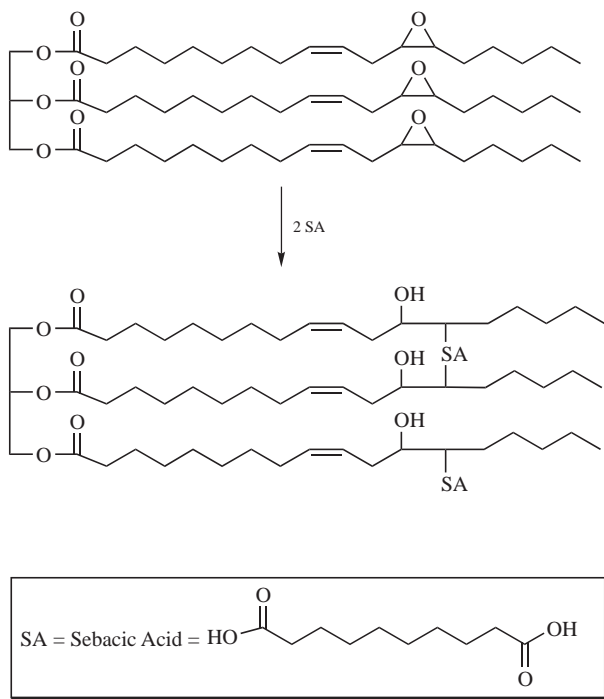
polymerized through a variety of reactions. A natural elastomer can be synthesized by reacting vernonia oil with naturally occurring dibasic acids, such as the sebacic acid derived from castor oil (4). It is known that castor oil is the major source of dibasic acids, such as decanedioic and nonanedioic acids (subaric and azelaic acids, respectively), through pyrolytic decomposition. These acids can also be derived from vernonia oil via an efficient reaction sequence (71). The obtained aliphatic dibasic acids are established industrial raw materials that can be used as plasticizers and impact resistant elastomers as well (30).

On the other hand, the rubbery nature of the polymerized oil may be used as toughening, rigid epoxy materials because it is phase separated into spherical domains when mixed and cured with bisphenol-A epoxy compounds (72–75). A

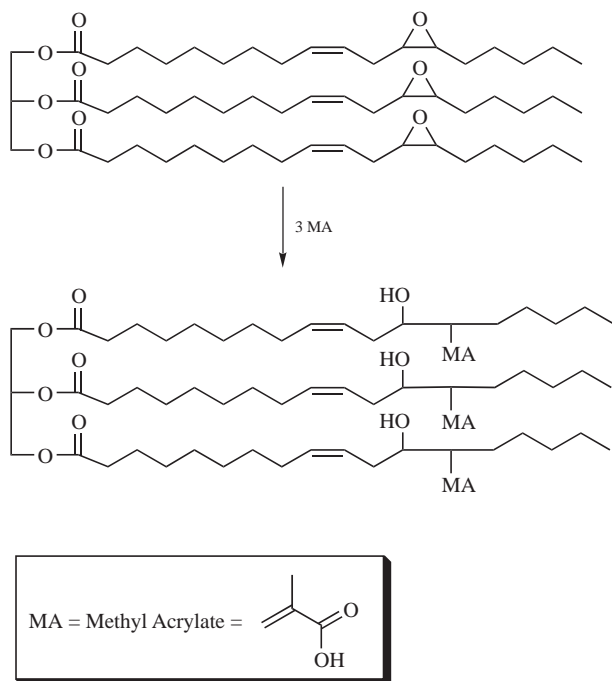


**Figure 13.** Epichlorohydrin modification of lesquerella oil.

reinforced elastomer, however, is formed because the vernonia oil-sebacic acid polyester forms a continuous phase. The reaction is shown in Figure 14. Sperling and Manson (30) compared the glass transition temperature of vernonia oil polyester with that of epoxidized linseed oil polyester. They observed that the glass transition temperature of linseed oil polyester was higher than that of vernonia oil polyester because of dense cross-linking in epoxidized linseed oil. Vernonia oil is



**Figure 14.** Reaction of sebacic acid with vernonia oil.

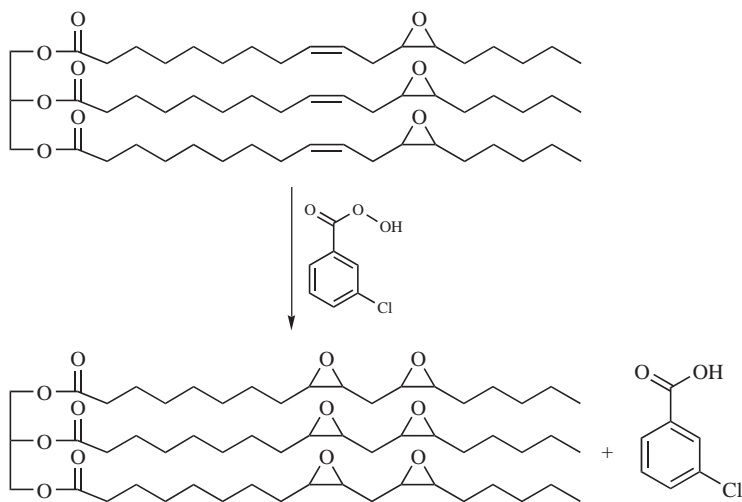


**Figure 15.** Methyl acrylation of vernonia oil.

soluble in many organic solvents and diluents as it contains both oxirane ring and a double bond. These two features are useful in drying and curing mechanisms.

The epoxide moieties of vernonia oil play an important role in making acrylates, groups useful in making UV curing formulations. For instance, the methacrylate ester of vernonia oil is synthesized by reaction with methacrylic acid in the presence of a tertiary amine. The acrylate ester is UV active and is therefore easily polymerized through the acrylate vinyl moieties. The mechanism of making vernonia oil-based acrylates is shown in Figure 15.

Epoxidized soybean and linseed oils are the two main representatives of the industrially produced epoxidized vegetable oils. Both are obtained by epoxidation of the corresponding unsaturated oils, and the degree of epoxidation can be controlled by reaction time. Muturi et al. (76) found that the molecular structures and properties of partially epoxidized soybean and linseed oils are similar to those of vernonia oil. They are suitable for the preparation of low volatile organic compound alkyd and epoxy coatings formulations. However, fully epoxidized oils are not as good as vernonia oil for making reactive diluents for coatings because of their higher viscosities and melting points. Epoxidized soybean oil is widely applied as a polyvinyl chloride (PVC) additive to improve PVC processing, stability, and flexibility (77). In addition, the double bond of vernonia oil can be converted into an oxirane functional group. These “super epoxides” have the potential



**Figure 16.** Epoxidation of vernonia oil.

to serve as the reactive components of epoxide powder coatings. This super epoxidized vernonia oil possesses a higher glass transition temperature than natural vernonia oil. This characteristic is, therefore, useful for excessive plasticization. The epoxidation of vernonia oil is represented in Figure 16.

As these oils contain multiple functionalities, they provide an alternative to petroleum as a chemical feedstock. They are derived from cultivated or wild plants. There is an international interest in using these naturally occurring oils as the starting material for polymer production because excessive quantities of nonrenewable petroleum are now being consumed (34).

#### 4. USE OF NATURALLY FUNCTIONALIZED TRIACYLGLYCEROL OILS IN INTERPENETRATING POLYMER NETWORKS

As mentioned earlier, TAG oils may contain special functional groups that deliver the potential of converting these oils into biopolymers. Such functional groups make these oils particularly important as they can be polymerized with an appropriate bifunctional reactant to form polymers, if they contain a hydroxyl or epoxide functional groups (34, 52). These oils contain hydroxyl as well as unconjugated vinyl groups in their structural backbone. The presence of double bonds can be exploited via a chain-growth mechanism to form polymerized plastics. On account of these two features, these oils have the unique quality to control elasticity as well as plasticity in a single backbone chain structure (78, 79). The cross-linking of hydroxyl groups in a step-growth polymerization gives rise to “Interpenetrating Polymer Networks” (IPN) (79). IPN materials contain “two or more polymers in a network form with at least one polymer that is polymerized or cross-linked in the

immediate presence of the other” (80, 81). Two major IPNs have been explored. These are referred to as sequential and simultaneous IPN. Sequential IPNs involves the preparation of one cross-linked polymer network and a subsequent swelling with the monomer. This is then cross-linked with a second component and polymerized in a network to a form sequential IPN. These two polymer networks are prepared separately before polymerization. On the other hand, simultaneous IPN involve the simultaneous mixing of all the components in one step followed by the formation of both networks via independent reactions proceeding in the same reaction vessel (32, 52, 80, 81).

Polyurethanes and polyurethane-based IPN have gained great importance in recent years. Polyurethanes are divided into several classes. Some polyurethanes are used in foams, whereas others are used for making fibers. Segmented elastomers are perhaps the most interesting class of polyurethanes. This category of polyurethanes is a kind of multiblock copolymer (82). The block copolymer is defined as a type of polymer blend that contains two polymer chains that are linked end to end (81, 83). In addition to various other uses, polyurethane materials are extensively used in the medical field, such as in the production of medical-grade tubing (82).

#### **4.1. Use of Castor Oil-Based Polyurethanes/Polyesters for Making Interpenetrating Polymer Networks**

Several scientists have worked on the production of IPN and their characterization based on a castor oil polyurethane model. Sperling and coworkers (84, 85) have published a number of very interesting papers on IPNs from castor oils. The hydroxyl groups were reacted with 2,4-toluene diisocyanate for polyurethane formation. Styrene and divinylbenzene were polymerized, and thus interpenetrated, to prepare IPN and simultaneous interpenetrating networks (SIN) by using either individually or mixed polyester-urethane. These IPNs and SINS can be tough plastics or reinforced elastomers depending on their compositions, which were shown from modulus-temperature, stress-strain, and impact-resistant studies. Furthermore, the morphology of these IPNs and SINS depended on the synthetic method, such as reaction time, stirring time, and time to pour into the mold. Yenwo et al. (84, 85) synthesized sequential IPNs using castor oil and studied the dynamic mechanical properties of the IPN. The experimental data showed that the molecular mixing of the two networks was extensive but incomplete. Mechanical properties of such systems were related to the degree of phase continuity and inversion.

Siddaramaiah et al. (86) employed an X-ray diffraction method to determine the microstructural parameters of IPNs of castor oil-based polyurethanes and polystyrene and correlated the changes of microstructural parameters to the physical macro changes, such as hardness. They found that the addition of polystyrene (PS) resulted in an increase in hardness that was due to an increase in crystal size.

Nayak and coworkers (7, 29, 87) prepared a series of IPNs by first reacting castor oil with diisocyanate to form a prepolymer then polymerizing with methacrylate, acrylamide, and cardanyl acrylate, respectively, using ethylene glycol

dimethacrylate as cross-linker and benzoyl peroxide as initiator, and studied their high-temperature degradation mechanism and mechanical properties. The thermogravimetric analysis was followed by a computer analysis method for assisting the kinetic mechanism and a new mechanism of degradation was suggested based on these kinetic parameters.

A special case of IPN is the SIN (88). The existence of SINs provides a novel way to control the phase states and mechanical behavior of two-phase materials. As a result of the multiple chemical compositions and synthetic routes involved in the formation of SINs, polymers can be made with very different mechanical behavior. Among the features that control the behavior of the resulting blends, microphase structures and molecular interactions are of greatest importance for the determination of mechanical properties of the two coexisting phases. There are usually two ways of forming a SIN. One involves the simultaneous polymerization of two polymers, whereas the second involves the mixing of two kinds of monomers together, which are polymerized sequentially (88). The two techniques give different products with different morphologies and different mechanical behaviors. Various studies have been done on the properties and structures of the resulting SIN. Devia-Manjarres et al. (88) and Devia et al. (89) studied the morphology and glass transition behavior of SIN based on elastomeric polymers derived from castor oil and cross-linked polystyrene by using electron microscopy and dynamic mechanical spectroscopy techniques, respectively. A two-phase morphology was revealed by electron microscopy. Depending on the synthetic conditions, either phase can be continuous. Morphological details, however, can be determined by compatibility of the polymer networks, speed of stirring, and reaction rates of network formation (80). Dynamic mechanical spectroscopy results showed two glass transition temperatures around the homopolymer glass transition temperatures but shifted inward. These materials proved to be tougher than their corresponding homopolymer networks. The toughness of these SINs increased with a decreasing domain size of the dispersed phase. The use of castor oil-based elastomers in the brittle polymer-polystyrene produces toughened plastic with an enhanced tensile strength and impact properties (89).

Suthar and coworkers (83, 90, 91) investigated the SIN prepared from radical copolymerization of liquid polyester based on castor oil and dibasic acid with polystyrene initiated by benzoyl peroxide in the presence of the cross-linker 1,4-divinylbenzene. This group also reported the preparation and morphology of sequential IPN composed of castor oil polyurethane and various polyacrylates using ethylene glycol dimethacrylate as a cross-linker and benzoyl peroxide as initiator. These materials were characterized in terms of resistance to chemical reagents, the static mechanical properties (tensile strength, Young's modulus, and percent elongation), thermal properties, and morphology (scanning electron microscopy).

Xie et al. (92, 93) synthesized simultaneous IPN from castor oil polyurethane and copolymers of vinyl monomers, including styrene, methyl methacrylate, and acrylonitrile, without cross-linker using a redox initiator at room temperature and both the formation kinetics of cross-linking and grafting on phase separation were examined. It was demonstrated that the resulting materials were mainly grafted IPN

with vinyl or acrylic polymer grafted on the double bonds of castor oil. These IPN exhibited good properties, including high strength, good resilience, solvent resistance, and high abrasion resistance.

#### **4.2. Use of Polyethylene Terephthalate and Castor or Other Naturally Functionalized Triglyceride Oils for Making Semi-Interpenetrating Polymer Networks**

Polyethylene terephthalate (PET) is a semicrystalline thermoplastic (79). Semicrystalline PET is of particular importance because of its properties, which are suitable for the production of engineered plastics. It is a widely used polymer because of its high strength, low price, and good solvent resistance. It is a primary material for the production of bottles, textile industry components and auxiliaries, recording tapes, and packaging films. However, it cannot be used in injection molding as it has a slow crystallization rate. As a result of its slow crystallization rate, the parts molded with PET have poor dimensional stability. PET, in combination with castor or other naturally functionalized triglyceride oils, may be used to improve characteristics such as toughness and faster crystallization rate (32).

As PET is a semicrystalline thermoplastic, the process of synthesizing semi-IPNs based on PET is different from the above-mentioned method. It is known that PET is a condensation product of terephthalic acid and ethylene glycol. Castor oil would polymerize with terephthalic acid in competition with ethylene glycol if SIN formation method were used. On the other hand, PET is aromatic, whereas castor oil is aliphatic in nature; they are, therefore, immiscible, which makes sequential IPN formation impossible. In order to obtain a PET and castor oil network, the two components must be either miscible or well mixed. It was observed that a miscible mixture of PET and castor oil could be obtained by continuously heating the blend together. A bond exchange reaction between ester groups of PET and ester and hydroxyl groups of castor oil occurs during heating. As a result of this interchange, a miscible copolymer mixture is formed and a hybrid semi-IPN structure is obtained (79, 94). The castor oil was shown to improve the crystallization rate of poly (ethylene terephthalate), and after the polymerization of castor oil, it offered a mechanism for toughening the materials.

In addition, PET can be used to form semi-IPNs with other naturally functionalized triglyceride oils, such as vernonia oil (31). The procedures for PET/vernonia semi-IPNs are essentially the same as those of PET/castor ones, but with important differences. For PET/castor mixtures, the diisocyanate cross-linker was added at 240°C and the mixture was poured into the molds rapidly before the castor gel point had been reached. In this case, PET/castor polyurethane semi-IPNs were formed, in which crystallization and gelation occurred simultaneously resulting in a single, broad glass transition temperature. For PET/vernonia, the sebacic acid was added at 280°C, which reduced the temperature to about 250°C, where the mixture was held for another 5 min, then poured into a preheated mold and allowed to cool, during which time the PET crystallizes. In this case, PET/vernonia polyester network was formed, and the PET crystallized prior to network formation because the latter



one took place more slowly. This network displayed two glass transition temperatures. In another case, rather than allowing the mixture to cool, it was kept at 250°C, which is above the crystallization temperature of PET. Under this condition, the network is formed prior to crystallization of PET, therefore, the material was much more rubbery and tough, and it was amorphous. In these semicrystalline semi-IPNs, network formation before or after crystallization or phase separation are factors that affect the crystalline and phase morphology considerably. These factors, in turn, are reflected in the physical properties of the resulting materials. The amounts of bond interchange, phase separation, crystallization of PET, and network formation of cross-linking are factors that control the morphology and properties of materials in this and similar polymer systems.

## 5. CONCLUSIONS

As the world arrives closer to the global realization of our dwindling fossil fuel resources, concern is increasing about continuity of our way of life, as so much of the materials and energy we depend on are sourced from petroleum. As scientists and innovators begin to search in earnest for alternative sources of some of the more ubiquitous materials in our environment, such as plastics, other important factors, such as degradability and environmental sustainability, are being considered. Vegetable oils and other lipids form an important renewable source of such materials, and based on the work reported here by a growing number of researchers, natural and modified triacylglycerol oils can be used to produce polymers with important functional properties. This is an important and growing area of research, and by the time this chapter has been printed, it will almost certainly be dated, given the rate at which research is being performed in this area. However, the material presented here does present a snapshot of the various areas of endeavor to convert TAG oils into polymers.

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## REFERENCES

1. R. Chandra and R. Rustgi, *Prog. Polym. Sci.*, **23**, 1273–1335 (1998).
2. M. Okada, Y. Okada, A. Tao, and K. Aoi, *J. Appl. Polym. Sci.*, **62**, 2257–2265 (1996).
3. J. Rösch and R. Mülhaupt, *Polym. Bull.*, **31**, 679–685 (1993).
4. L. H. Sperling, J. A. Manson, and M. A. Linne, *J. Polym. Mater.*, **1**, 54–67 (1984).

5. H. P. Bhunia, R. N. Jana, A. Basak, S. Lenka, and G. B. Nando, *J. Polym. Sci. Part A: Polym. Chem.*, **36**, 391–400 (1998).
6. M. A. Linne, L. H. Sperling, A. M. Fernandez, S. Qureshi, and J. A. Manson, *J. Polym. Mater.*, **1**, 54–67 (1984).
7. P. A. Nayak, S. Lenka, S. K. Panda, and T. Pattnaik, *J. Appl. Polym. Sci.*, **47**, 1089–1096 (1993).
8. S. S. Nayak, S. K. Das, and S. Lenka, *React. Funct. Polym.*, **40**, 249–254 (1999).
9. S. K. Swain, S. Sahoo, D. K. Mohapatra, B. K. Mishra, S. Lenka, and P. L. Nayak, *J. Appl. Polym. Sci.*, **54**, 1413–1421 (1994).
10. M. J. Donnelly, *Polym. Int.*, **37**, 297–314 (1995).
11. A. L. Lehninger, D. L. Nelson, and M. M. Cox, *Principles of Biochemistry*, 2nd ed., Worth Publishers, New York, 1993, pp. 309.
12. C. J. Weber, *Biobased Packaging Materials for the Food Industry*, The Royal Veterinary and Agricultural University. (2000). Available: <http://www.mli.kvl.dk/foodchem/special/biopack/>.
13. G. J. L. Griffin, “Advances in Chemistry Series 134,” in *Fillers and Reinforcements for Plastics*, American Chemical Society, 1978, Washington, D.C., 159–170.
14. S. R. Sandler and Karo Wolf, in A. T. Blomousist and H. Wasserman, eds., *Polymer Syntheses*, Vol. 1, Academic Press, New York, 1974, p. 196.
15. H. I. Bolker, *Natural and Synthetic Polymers: an Introduction*, Marcel Dekker, Inc., New York, 1974, p. 60.
16. A. Mishra, M. Misra, S. S. Tripathy, S. K. Nayak, and A. K. Mohanty, *J. Reinf. Plas. Comp.*, **20**, 321–334 (2001).
17. A. K. Mohanty, M. Misra, and L. T. Darzal, *Compos. Interface*, **8**, 313–343 (2001).
18. A. K. Mohanty, P. C. Tripathy, M. Misra, S. Parija, and S. Sahoo, *J. Appl. Polym. Sci.*, **37**, 3035–3043 (2000).
19. M. Mishra, M. Misra, S. S. Tripathy, S. K. Nayak, and A. K. Mohanty, *Macromol. Mater. Eng.*, **286**, 107–113 (2001).
20. J. Rout, M. Misra, S. S. Tripathy, S. K. Nayak, and A. K. Mohanty, *Compos. Sci. Technol.*, **61**, 1303–1310 (2001).
21. J. Rout, M. Misra, and A. K. Mohanty, *Polym. Adv. Technol.*, **10**, 336–344 (1999).
22. A. K. Mohanty, M. A. Khan, S. Sahoo, and G. Hinrichsen, *J. Mater. Sci.*, **35**, 2589–2595 (2000).
23. S. Parijia, A. K. Mohanty, M. Misra, and S. K. Nayak, *Polym. Degrad. Stabil.*, **67**, 7–12 (2000).
24. J. Lunt, *Polym. Degrad. Stabil.*, **59**, 145–152 (1998).
25. D. Cam, S. H. Hyon, and Y. Ikada, *Biomaterials*, **16**, 833–843 (1995).
26. S. S. Nayak, D. K. Mishra, P. L. Nayak, and S. Lenka, *Macromol. Rep.*, **32**, 511–521 (1995).
27. B. N. Guru, T. K. Das, and S. Lenka, *Polym. Plast. Technol. Eng.*, **38**, 179–187 (1999).
28. J. R. Swain, S. K. Biswal, and S. Lenka, *Polym. Plast. Technol. Eng.*, **39**, 927–936 (2000).
29. D. Das, S. S. Nayak, S. K. Das, P. L. Nayak, and S. Lenka, *Thermochim. Acta*, **297**, 101–107 (1997).
30. L. H. Sperling and J. A. Manson, *J. Amer. Oil. Chem. Soc.*, **60**, 1887–1892 (1983).

31. L. W. Barrett, G. S. Ferguson, and L. H. Sperling, *J. Polym. Sci. Part A: Polym. Chem.*, **31**, 1287–1299 (1993).
32. L. W. Barrett, O. L. Shaffer, and L. H. Sperling, *J. Appl. Polym. Sci.*, **48**, 953–968 (1993).
33. L. W. Barrett and L. H. Sperling, *Polym. Eng. Sci.*, **33**, 913–922 (1993).
34. L. W. Barrett, L. H. Sperling, and C. J. Murphy, *J. Amer. Oil. Chem. Soc.*, **70**, 523–534 (1993).
35. A. K. Mohanty, M. Misra, and G. Hinrichsen, *Macromol. Mater. Eng.*, **276/277**, 1–24 (2000).
36. R. P. Wool, *Chemtech.*, **29**, 44–48 (1999).
37. N. Jiratumnukul and R. Van De Mark, *J. Amer. Oil. Chem. Soc.*, **77**, 691–697 (2000).
38. D. L. Koplan, ed., *Biopolymer from Renewable Resources*, Springer, New York, 1998, p. 272.
39. K. Brunner and D. R. Tucker, *J. Appl. Chem.*, **1**, 563–568 (1951).
40. D. L., Trumbo and B. E. Mote, *J. Appl. Poly. Sci.*, **80**, 2369–2369 (2001).
41. F. Li and R. C. Larock, *J. Appl. Poly. Sci.*, **78**, 1044–1056 (2000).
42. F. Li and R. C. Larock, *Biomacromolecules*, **4**, 1018–1025 (2003).
43. F. Li and R. C. Larock, *J. Appl. Poly. Sci.*, **80**, 658–670 (2001).
44. F. Li and R. C. Larock, *J. Poly. Sci.: Part B: Polym. Phys.*, **38**, 2721–2738 (2000).
45. F. Li and R. C. Larock, *J. Poly. Sci.: Part B: Polym. Phys.*, **39**, 60–77 (2001).
46. F. Li and R. C. Larock, *Polym. Advan. Technol.*, **13**, 436–449 (2002).
47. F. Li and R. C. Larock, *J. Appl. Poly. Sci.*, **84**, 1533–1543 (2001).
48. F. Li and R. C. Larock, *Polym. Int.*, **52**, 126–132 (2003)
49. F. Li, M.V. Hanson, and R.C. Larock, *Polymer*, **42**, 1567–1579 (2001).
50. F. Li and R. C. Larock, *J. Polym. & Environ.*, **10**, 59–67 (2002).
51. S. N. Knot, J. J. Lascala, E. Can, S. S. Morye, G. I. Williams, G. R. Palmese, S. H. Kusefoglou, and R. P. Wool, *J. Appl. Poly. Sci.*, **82**, 703–723 (2001).
52. L. H. Sperling, C. E. Carraher, S. P. Qureshi, J. A. Manson, and L. W. Barrett, in C. G. Gebelein, ed., *Biotechnology and Polymers*, Plenum Press, New York, 1991, pp. 95–109.
53. S. K. Das and S. Lenka, *Polym. Plast. Technol. Eng.*, **38**, 149–157 (1999).
54. J.M. Arnold, U.S. Patent 4,375,521, March, 1, 1983.
55. W. Dawney, M. Brauer, and J. Chao, U.S. Patent 4,666,968, May, 19, 1987.
56. D. Simone and M. Brauer, U.S. Patent 4,812,533, March, 14, 1989.
57. R. H. J. Leitheiser, C. C. Peloza, and C. K. Lyon, *Cell Plast.*, **5**, 364–372 (1969).
58. C. K. Lyon, V. H. Garret, and E. N. Frankel, *J. Amer. Oil. Chem. Soc.*, **51**, 331–334 (1974).
59. T. H. Khoe, F. H. Otey, and E. N. Frankel, *J. Amer. Oil. Chem. Soc.*, **49**, 615–618 (1972).
60. Z. S. Petrovic, A. Guo, and I. Javni, U.S. Patent 6,107,433, August, 22, 2000.
61. A. Guo, Y. Cho, and Z. S. Petrovic, *J. Polym. Sci: Part A: Polym. Chem.*, **38**, 3900–3910 (2000).
62. A. Guo, I. Javni, and Z. Petrovic, *J. Appl. Polym. Sci.*, **77**, 467–473 (2000).
63. Z. S. Petrovic, A. Guo, and W. Zhang, *J. Polym. Sci: Part A: Polym. Chem.*, **38**, 4062–4069 (2000).
64. I. Javni, Z. S. Petrovic, A. Guo, and R. Fuller, *J. Appl. Polym. Sci.*, **77**, 1723–1734 (2000).

65. A. Guo, D. Demydov, W. Zhang, and Z. S. Petrovic, *J. Polym. & Environ.*, **10**, 49–52 (2002).
66. Z. S. Petrovic, W. Zhang, A. Zlatanich, C. C. Lava, and M. Ilavsky, *J. Polym. & Environ.*, **10**, 5–12 (2002).
67. A. Zlatanich, Z. S. Petrovic, and K. Dusek, *Biomacromolecules*, **3**, 1048–1056, (2002).
68. I. Javni, W. Zhang, and Z. S. Petrovic, *J. Appl. Polym. Sci.*, **88**, 2912–2916 (2003).
69. I. Javni, W. Zhang, and Z. S. Petrovic, *J. Appl. Polym. Sci.*, **90**, 3333–3337 (2003).
70. J. John, M. Bhattacharya, and R. B. Turner, *J. Appl. Polym. Sci.*, **86**, 3097–3107 (2002).
71. F. O. Ayorinde, F. T. Power, L. D. Streete, R. L. Sphepard, and D. N. Tabi, *J. Amer. Oil. Chem. Soc.*, **66**, 690–692 (1989).
72. S. K. Dirlikov, I. Frischinger, and Z. Chen, *Advances in Chemistry Series 252*, American Chemical Society, Washington, D.C., 1996, pp. 95–109.
73. I. Frischinger and S. K. Dirlikov, *Polym. Mat. Sci. Eng.*, **69**, 390–391 (1993).
74. I. Frischinger and S. K. Dirlikov, *Polym. Mat. Sci. Eng.*, **69**, 392–393 (1993).
75. I. Frischinger and S. K. Dirlikov, *Polym. Mat. Sci. Eng.*, **69**, 394–395 (1993).
76. P. Muturi, D. Wang, and S. Dirlikov, *Prog. Org. Coating*, **25**, 85–94 (1994).
77. D. Parida, S. Kenka, and P. L. Nayak, *J. M. S. - Pure Appl. Chem.*, **A32**, 1365–1379 (1995).
78. D. Parida, P. Nayak, D. K. Mishra, S. Lenka, P. L. Nayak, S. Mohanty, and K. K. Rao, *J. Appl. Polym. Sci.*, **56**, 1731–1738 (1995).
79. L. W. Barrett, L. H. Sperling, J. Gilmer, and S. G. Mylonakis, *J. Appl. Polym. Sci.*, **48**, 1035–1050 (1993).
80. N. Devia, J. A. Manson, and L. H. Sperling, *Macromolecules*, **12**, 360–369 (1979).
81. L. H. Sperling and V. Mishra, *Polym. Adv. Technol.*, **7**, 197–208 (1996).
82. V. Mishra, C. J. Murphy, and L. H. Sperling, *J. Appl. Polym. Sci.*, **53**, 1425–1434 (1994).
83. M. Patel, P. Patel, and B. Suthar, *J. Polym. Mater.*, **4**, 147–153 (1987).
84. G. M. Yenwo, J. A. Manson, J. E. Pulido, L. H. Sperling, A. Conde, and N. Devi, *J. Appl. Polym. Sci.*, **21**, 1531–1541 (1977).
85. G. M. Yenwo, L. H. Sperling, J. E. Pulido, J. A. Manson, and A. Conde, *Polym. Eng. Sci.*, **17**, 251–256 (1977).
86. Siddaramaiah, P. Mallu, and R. Somashekar, *J. Appl. Polym. Sci.*, **68**, 1739–1743 (1998).
87. P. Nayak, D. K. Mishra, K. C. Sahoo, N. C. Pati, P. K. Jena, S. Lenka, and P. L. Nayak, *J. Appl. Polym. Sci.*, **80**, 1349–1353 (2001).
88. Devia-Manjarres, J. A. Manson, and L. H. Sperling, *Polym. Eng. Sci.*, **18**, 200–203 (1978).
89. N. Devia, J. A. Manson, and L. H. Sperling, *Polym. Eng. Sci.*, **19**, 878–882 (1979).
90. B. Suthar, K. Jadav, and M. Dave, *Polym. Int.*, **34**, 267–272 (1994).
91. P. Patel and B. Suthar, *Polym. Eng. Sci.*, **28**, 901–905 (1988).
92. H.Q. Xie, C. X. Zhang, and J. S. Guo, *Advances in Chemistry Series 239*, American Chemical Society, Washington, D.C., 1994, pp. 557–570.
93. H. Q. Xie, J. S. Guo, and G. G. Wang, *Eur. Polym. J.*, **29**, 1547–1551 (1993).
94. L. W. Barrett, L. H. Sperling, J. W. Gilmer, and S. G. Mylonakis, *Advances in Chemistry Series 239*, American Chemical Society, Washington, D.C., 1994, pp. 489–516.

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## *Paints, Varnishes, and Related Products*

K. F. Lin

### **1. RELATIONSHIP OF FATS AND OILS TO THE PAINT-COATING INDUSTRY**

Historically, drying oils have been the major film formers of coatings, including paints, varnishes, and inks. Although it is not certain whether linseed oil was used in paints in ancient Egypt, flax was grown and flax seeds were collected at that time. The early Renaissance was probably the real beginning of paints as we know them today in the West. The Van Eyck brothers (1388–1441) are said to be the first to use linseed oil as a binder (1). Whereas in China, tung oil has been used for centuries as waterproofing caulking or as coating for wood objects including boats, houses, and furniture.

The first American paint factory was opened in Boston in 1737 by Thomas Childs (2). The pigment and oil were placed on a granite trough, and a granite ball, known as the Boston Stone, was then rolled over the mixture to make the paint. The ball is now preserved and serves as the symbol for the Federation of Societies for Paint Technology.

The drying oils owe their value as raw materials for decorative and protective coatings to their ability to polymerize and cross-link, or “dry,” after they have been applied to a surface, to form tough, adherent, impervious, and abrasion-resistant

films. Their film-forming properties are closely related to their degree of unsaturation, since it is through the unsaturated centers or double bonds that polymerization and cross-linking take place. With one exception (to be noted later) the oils used in paints varnishes and similar products are relatively high in iodine value. In any given product, there is an optimum degree of reactivity in the oil; the speed with which the oil dries must be balanced against such factors as elasticity and durability in the paint film. In general, however, unsaturation is at a premium in paint and varnish oils, and the oils in greatest demand are those in which drying takes place most readily.

The form in which drying oils are used in coating applications has gone through an evolutionary change over time. The simplest and most primitive way is to use them directly as the film former of a coating. It was discovered that drying oils may be made more useful by altering their natural state. By aging in vats, by heating, or by blowing air through, the viscosity and drying characteristics of the drying oil may be changed enough to improve its general properties for coating applications. In the case of fast-drying oils with conjugated double bonds, such as tung, oiticica, and dehydrated castor oil, heat treatment is necessary to "gas-proof" them, so that the oils do not dry into undesirable wrinkled and/or frosted films. The oils are not necessarily used in their original form of triglycerides for coating applications. It has become a common practice to hydrolyze them first, and the free fatty acids are then used to synthesize coating resins with certain advantages. Therefore, the term *fats and oils* includes fatty acids for the purpose of the discussions in this Chapter, unless otherwise specified. Through progress, people found that sometimes mixtures of different oils could be used to greater advantage and that natural gums could be added; thus oleoresinous varnishes were born. Thereafter, human creativity started to make rapid and diversified progress in the development of new coating materials, many of which have departed completely away from the drying oil base.

When the drying characteristics of oils were relied on as the sole (or major) cause for a varnish-based coating film to dry, those oils belonging to the linolenic or conjugated acid groups, such as linseed, perilla, tung, oiticica, and highly unsaturated winterized fish oils were of the prime interest to coating formulators. Since about the 1950s, with the advent of synthetic resins, particularly, alkyd resins, it has become possible to make considerable use of oils with poorer drying characteristics. Semidrying oils such as soybean oil, safflower oil, and sunflower seed oil have become viable as raw materials for making "drying" paints. Nondrying oils, such as coconut oil, are also used in coating materials. However, their function is primarily that of a plasticizer rather than of an active component for air drying. In addition, castor oil, a nondrying oil, has been converted chemically by dehydration to give excellent drying property.

There is no denying that the once prominent position of fats and oils as the most important raw material for coatings has been greatly eroded by other materials. The total U.S. consumption of drying and semidrying oils in coating and allied applications peaked around 1950 at about 1.2 billion lb. It went down steadily thereafter. According to SRI International (3), the direct use of drying oils accounted for only

about 4% of the total film formers consumed in the United States in 1990, at about 98 million lb, whereas the consumption of alkyds, urethane alkyds, and epoxy esters was estimated at 645, 40, and 12 million lb, respectively (4–6). Very little drying oil is used in paints at present. Drying oils and oxidizing alkyds have been studied as binders for organic inorganic coatings (4). Urethane coatings are the fastest growing sector. Use in 2003 was  $1.6 \times 10^6$ t (5). Epoxy resins are among the most widely used (6).

## 2. A BRIEF OVERVIEW OF THE COATINGS TECHNOLOGY

Modern requirements in protective coatings are extremely diverse and exacting. They go far beyond the mere necessity of protecting the finished surface from weather or from ordinary wear or abrasion. Some coatings (e.g., those employed as electrical insulation) must possess extreme resistance to high temperatures or to penetration by moisture. Others (e.g., marine varnishes and the enamels for coating the interior of cans) must withstand prolonged contact with water or aqueous solutions. Modern assembly-line methods of manufacture produce many particular requirements and have created a special demand for quick-drying finishes. The wide distribution of illustrated journals, the proliferation of advertising matter, and the development of high speed printing processes have greatly elaborated the requirements of users of printing inks. Tung and other conjugated oils are particularly suitable for manufacture of fast-drying finishes, and for a time, the consumption of these oils increased significantly in response to more exacting requirements for specialized finishes. New systems based on epoxy resins, urethane polymers, silicones, and other synthetic intermediates have greatly decreased dependency on tung oil, and use has shrunk significantly.

The complex and diversified requirements of modern industry have to a large extent removed the manufacture of paints and varnishes from the category of an art to that of a science. In most plants, the manufacturing processes are now carried out under careful laboratory control and are freely modified or revised, whenever revision is indicated, in accordance with known scientific principles. As a result, the industry has been able to offer a succession of constantly improved products through periods of fluctuation in the availability of many important raw materials, pressures for solvent replacement to meet emerging air quality standards, and extensive pigment reformulation to replace mercury and lead to conform to new federal regulations on toxicity.

A most important development in the modern paint and varnish industry has been the introduction of synthetic resins as replacements for natural resins in the manufacture of varnishes and enamels. By using synthetic resins it has been possible to produce a variety of coatings that, in many cases, have important points of superiority over any of those compounded from natural resins. The synthetic vehicles are particularly distinguished by their hardness and durability and their high degree of resistance to the action of water, alkalies, and other chemical agents.

New methods for application of paint films and new procedures for curing have placed challenging demands on the resourcefulness of the resin chemist. For years brushing was virtually the only method of application; later, spraying, was used. Now a host of new application and curing techniques are commonplace. These include roller coating, dipping, coil coating, powder coating, electrodeposition, hot spray, fluid bed coating, electrostatic spray, two-component spray, ultraviolet cure, and electron beam cure.

The two foremost reasons for the decline of the direct use of oils, including oleoresinous varnishes, for coating applications are performance and environment. Drying oils by themselves, or even in the form of oleoresinous varnishes, do not give the drying speed and, sometimes, film properties that would satisfy the modern needs. The ease in the application and cleaning of latex paints caused oil-based paint to lose most, if not all, of the trade-sales market. The implementation of Rule 66 in California was the opening salvo for protecting the environment against solvent-based coatings of which practically all oil-based coatings belong. Thus the emphasis has switched to the development and commercialization of other coating materials or systems that are more environment friendly than oil-based materials. Indeed, it is quite remarkable that in the face of such severe odds, oil-based materials have been able to hold ground as well as they have.

There is an extraordinary body of terms used to define various features of protective coatings. Before discussing paints and varnishes and the particular function of fats and oils in coatings, it is desirable to review and define some of the language of the industry.

Protective coatings protect (or decorate) surfaces. Greases, mineral oils, plastic web coats, and mastic compositions may be used for protection, particularly of metal surfaces; but in the usual sense, protective coatings are materials that form durable films adhered to the surface to provide protection.

A varnish is a solvent-thinned combination of a drying oil and a hard resin. Also, a varnish is the clear film obtained using a varnish as a coating vehicle. By extension, vehicles used for clear films are called varnishes although the vehicle may be a true varnish, an alkyd resin solution, a urethane-modified oil, or even a lacquer.

A paint is a pigmented system applied to hide and protect a surface. Paints contain a wide range of ingredients as follows:

The vehicle is the carrier for pigment, consisting of combinations of oils, resins, polymers, and solvents; the nonvolatile portion is commonly called the binder.

Prime pigments are used for their ability to hide or cover the surface. The term *hiding power* is used to describe the relative ability of fixed amounts of different pigments to cover a surface and depends on the difference in refractive indices between the pigment and the binder; thus primary pigments usually have high values in refractive index.

Extender pigments give relatively little hiding, but their cost is lower than that of prime pigments; they provide control of such properties as flow consistency, durability, and adhesion.



Driers are metal salts, especially of cobalt, manganese, zirconium, calcium, and iron, that accelerate the conversion of the liquid film to a solid; lead was commonly used as a primary drier, but due to its toxicity, it is rarely used now.

Solvents or thinners control paint consistency and application properties. Slow solvents evaporate slowly and leave the film “open” (workable) for longer periods than fast solvents, which evaporate rapidly; in water-thinned paints, water is the thinner and there is no control over rate of evaporation.

A variety of other materials may be added for special effects.

Antiskin agents minimize skin formation on the top of the can during use or storage.

Mildewcides protect the applied film from fungus growth.

Wetting agents or griding aids promote wetting of pigment particles by the vehicle.

Antiflood and antifoat agents minimize flooding and floating, deficiencies characterized by separation of colored pigments during drying.

Antisetting agents minimize separation of pigment into a firm or hard mass in the bottom of the can.

Antisag agents minimize sagging or “curtaining” of wet films during application.

Puffing agents, or thixotropic agents, increase the paint consistency and minimize sagging by giving a thixotropic consistency to paint (a type of behavior in which the viscosity of the system decreases when agitated, as under the shear of brushing, and increases when allowed to stand).

Paints are made by grinding pigments in the vehicle. Actually the term *grinding* is somewhat inaccurate. The pigments are received from the manufacturer already as fine in particle size as they will be in the finished paint. The grinding operation is designed to break up the aggregates of pigment particles and to disperse them in the vehicle so that each particle is wetted. Griding is usually carried out in roller mills, in which shear between steel rollers disperses pigments, in ball mills or pebble mills, in which steel balls or pebbles rotating and rubbing against each other in a closed cylinder to produce the shear for dispersing the pigment, or in sand mills, in which agitation of sand causes pigment separation and dispersion. For products in which a fine grind (fine pigment dispersion) is not required, as in barn paints or house paints, high speed rotors may be used to grind the paint. In typical paint manufacture, a paste prepared from all of the pigment and a portion of the vehicle is subjected to the appropriate grinding technique until the desired fineness of grind is attained, and the resultant paste is “let down” (diluted) with the remaining portion of vehicle and solvent.

Fineness of grind is commonly expressed numerically using a grind gauge, which is a shallow wedge cut from polished metal. Paint is filled into the wedge

and a bar is drawn across the surface. With fine grinds, the paint fills the wedge even to the shallowest part. With coarse grinds, the paint is pulled away from the shallow edge of the wedge of the deeper end. The line of demarcation ranging from 0 (very coarse grind) to 8 (ultrafine grind) designated the quality of the pigment dispersion.

An enamel is a paint based on a vehicle that dries to a considerably harder film than paints derived from unmodified drying oils. Paints and enamels are classified by type of finish as follows:

Flat paints or enamels dry to a velvety nonglossy or matte surface.

Semigloss paints or enamels dry to an intermediate gloss range between flat and glossy.

Gloss paints or enamels dry to a highly reflecting surface.

There are many variations in the nomenclature, and films that are called gloss films by one observer might be classified as a semigloss by the individual who demands mirrorlike surfaces. Other designations might be used also such as eggshell (between flat and semigloss) or full gloss to differentiate mirror gloss from normal gloss.

The degree of gloss is measured by a glossmeter, which measures light reflectance at a low angle from the horizontal ( $20^\circ$  gloss) or high angle ( $60^\circ$  gloss). The  $60^\circ$  reflectance is most common, and although ranges of values are not sharply divided, the general consensus is as follows:

Type of Paint	$60^\circ$ Reflectance
Flat	4 or 5 maximum
Eggshell	5–20
Semigloss	20–60 (up to 80)
Low gloss	80 to 90+
High or full gloss	90 to 98+

Since the ability of a surface to reflect light, which gives the gloss measurement, depends on the smoothness of the surface, one can readily visualize that a coating with a greater surplus of binder over its pigment content will have a greater ability to produce a smooth surface, thus high in gloss. Conversely, with a high pigment content, there will be more pigment particles or aggregates at or near the film surface to cause a scattering of light, thus resulting in low gloss. The amount of pigment in the paint is measured by the pigment volume concentration (PVC), i.e., the volume percent of pigments in the dried paint film. In solvent-based systems, products of low PVC (up to 20–25%) are glossy, products in the middle range

(25–50%) have a semigloss finish, and products in the high range (45–70) have a flat or matte surface. Gloss in water-thinned systems does not correspond to the above, particularly in emulsion systems, because the pigment particles are not wetted uniformly by the binder. Solvent-thinned paints containing certain “flattening agents” such as extremely fine silica do not conform to the normal pattern.

Coating systems are divided into two general classifications, depending on the point of application. Trade sales finishes are purchased by the user in a paint store or hardware store (or today even in a drugstore or a supermarket) and are applied by the purchaser, usually by brush or roller. Included are barn paints, house paints, trim paints, varnishes, porch and deck enamels; wall paints, architectural enamels, and similar user-applied finishes. Industrial finishes are applied to objects by the manufacturer, usually by spraying, dipping, roller coating, air knife, or other high speed production application methods, and they are usually force dried by baking. Implement, automotive, appliance, and furniture finishes are typical industrials.

Finishes are also described by the function of the paint. A primer is used to coat the original surface. Its major functional property is good adhesion. Protection against corrosion is an especially important characteristic of metal primers. Hiding is a secondary function.

A sealer is a primer whose major function is covering a porous surface such as plaster, gypsum board, or paperboard with a surface coating that exhibits a minimum penetration into the surface. Good sealing prevents “ghosting,” a film defect in which variation in penetration causes gloss differences and visual color differences in the final coat. A typical ghosting effect is obtained in painting wallpaper in which the pattern can show through multiple coats because of the variability in porosity of the substrate.

A sanding sealer is designed for easy sanding after short dry so that smoother top coats can be attained. Sanding sealers are most important in furniture finishes and industrial finishes such as automotive systems.

An undercoat is another name for primer or sealer, especially as an enamel undercoat, which serves to supply a uniform base for an enamel so that there will not be a wide variation in gloss. Undercoats may be applied over sealers. The top coat or finish coat is the outside layer of paint applied over the primer or sealer.

The specification of products for coating applications involves a large number of factors, including color and color retention of films, rate of setup, bakability, rate of cure of films, hardness of films, adhesion, wetting action in grinding with pigments, flexibility and retention of flexibility on aging, reactivity with pigments, reactivity with driers, water resistance, alkali resistance, solvent resistance, viscosity, viscosity stability in the package as clear products or in pigmented systems, thermoplasticity, durability, compatibility with other film-forming agents, mar resistance, abrasion resistance, stain resistance, performance in the varnish kettle, gloss, gloss retention, etc. Obviously, no single product can be optimum in all of these characteristics, and in each use a compromise must be made to provide the best performance in the intended usage.

### 3. FILM DRYING PROCESS OF OIL-BASED COATING MATERIALS

#### 3.1. Drying of a Nonconjugated System

As mentioned earlier, it is through the unsaturated centers or double bonds of the fatty acids in drying oils that polymerization and cross-linking, i.e., drying, takes place. Hence, oils are conventionally classified, based on their iodine values into three groups: drying, semidrying, and nondrying. The generally accepted demarcations are, respectively,  $>140$ ,  $140-125$ , and  $<125$ . These numbers are, more or less, arbitrarily assigned. When the oil contains conjugated double bonds, the iodine values determined are usually low due to incomplete halogen absorption. Such a classification can only be used for a rough guidance.

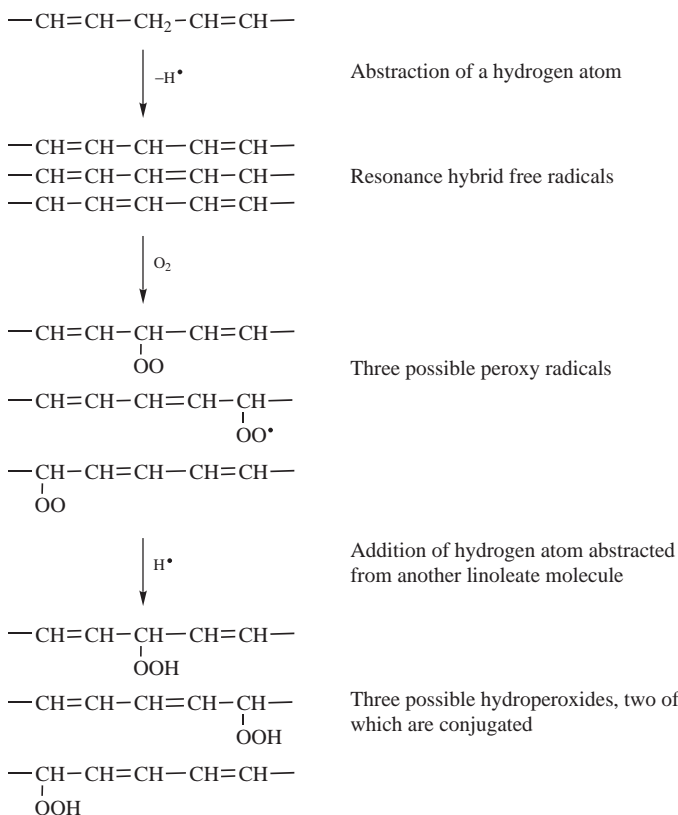
Wicks and Jones (7) suggested that the methylene groups between two double bonds, i.e., the  $\text{CH}_2$  groups allylic to two  $\text{C}=\text{C}$  groups, are much more reactive than those being allylic to only one  $\text{C}=\text{C}$  group and are mostly responsible for the drying of the nonconjugated oil. Thus the average number of such groups  $f_n$  in an oil molecule serves as a better indicator for the drying characteristics of the oil. An oil with an  $f_n$  value of greater than 2.2 is a drying oil, those with  $f_n$  values somewhat less than 2.2 are semidrying, and there is no sharp dividing composition between semidrying and nondrying oils, according to the authors. While such a classification system does have merits over the conventional way (based on iodine value), it does not provide a "rule" for classifying oils with conjugated unsaturation.

The chemical mechanism of drying has been established as an oxidative radical chain reaction process, which has been summarized as follows (8):

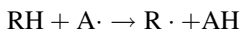
1. A period of induction at the beginning of the reaction during which no visible change in physical or chemical properties in the oil is noticed; natural antioxidant compounds are consumed during this period.
2. The reaction becomes perceptible and oxygen uptake is considerable; discrete interaction of oxygen and olefins takes place followed by the formation of hydroperoxides.
3. Conjugation of double bonds occurs accompanied by isomerization of cis to trans unsaturation.
4. The hydroperoxides start to decompose to form a high free-radical concentration; the reaction becomes autocatalytic.
5. Polymerization and scission reactions begin and yield high molecular weight cross-linked products and low molecular weight carbonyl and hydroxy compounds; carbon dioxide and water are also formed and are present in the volatile products of film formation.

It is now generally believed that the induction is slow at first but is autocatalytic and the rate increases steadily. The rate depends on the reaction conditions such as temperature, light, and traces of heavy metals or inhibitors in the oil or coating (9).

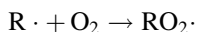
The active sites are the allylic carbon  $\alpha$  to a double bond, especially those  $\alpha$  to two double bonds with one on each side, such as carbon number 11 in a 9,12-octadecadienoic (linoleic) acid and proceeds through the following mechanism:



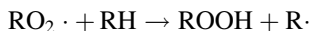
The initial step is believed to be the dehydrogenation from the  $\alpha$ -methylene group to form a radical. Since such a hydrogen extraction would require a considerable amount of energy, a number of investigators proposed that the hydrogen is removed through reaction with a free radical. Thus a radical,  $A\cdot$ , abstracts a hydrogen from a molecule of linoleate,  $RH$ , to form the radical  $R\cdot$ ,



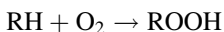
Since the radical is allylic to the double bonds on either side of it, resonance hybrid free radicals are formed resulting in shifting the double bonds to a conjugated position. This is then followed by:



and



The net reaction is hydroperoxide formation:



During the oxidation to form hydroperoxides, the natural *cis,cis* unsaturation of linoleate is converted to *cis*, *trans* and *trans*, *trans* isomers. Privett and co-workers (10) concluded that at least 90% of linoleate hydroperoxide preparations are conjugated. When the oxidation is conducted at 0°C the hydroperoxides are predominantly *cis*, *trans* isomers, but room temperature oxidation produces a large amount of *trans*, *trans* unsaturation (11, 12). Ethyl or methyl linoleate hydroperoxides are relatively low melting and as a result purification by crystallization is difficult. Bailey and Barlow (13) prepared high melting *p*-phenylphenacyl linoleate, oxidized the ester in benzene solution, and isolated virtually pure hydroperoxide by crystallization. Infrared spectra of the 99% purity *p*-phenylphenacyl linoleate hydroperoxide correspond to a *trans*, *trans* conjugated isomer.

The autoxidation of linoleate described above shows the characteristic features of a chain reaction involving free radicals. Materials that decompose to form free radicals catalyze the reaction even when present in very low concentrations to produce high yields of hydroperoxides; initiation of the reaction by light can produce quantum yields much greater than unity and easily oxidized substances that consume free radicals, but do not themselves undergo significant autoxidation, can markedly inhibit the chain reaction.

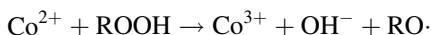
Although there is quite general agreement on the mechanism of the chain propagation reaction, there is much less unanimity of opinion on the primary reaction to produce the radicals (indicated as  $\text{A}\cdot$  above) responsible for the initiation of the chain reaction. Originally, it was proposed that hydroperoxides are the initial products of autoxidation (14, 15). Primarily because of the high energy requirement for rupture of the  $\alpha$ -methylene carbon-hydrogen bond several authors (16–19) almost simultaneously concluded that the initial point of oxidative attack was the double bond and not the  $\alpha$ -methylene group, although some (16) proposed a limited attack at the double bond to produce radicals in sufficient amount to initiate the chain reaction through the  $\alpha$ -methylene carbon.

Kahn (20) questioned the formation of a diradical and proposed direct addition of oxygen to a double bond to form a cyclic transition state, which breaks down to yield the hydroperoxide. The theory of oxidation has received little support, because it does not explain the inhibitory effect of free-radical acceptors in the initial stages of autoxidation.

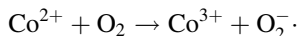
It has been contended that the direct attack of oxygen on the double bond has low thermodynamic probability (21, 22), and it has been considered that trace metal contaminants catalyze the initiation of autoxidation by producing free radicals through electron transfer. Alternative pathways are as follows, using cobalt as an

example of a metal that can facilely shift valence states in oxidation–reduction reactions:

1. Reduction activation of trace hydroperoxides in the system yields free radicals.

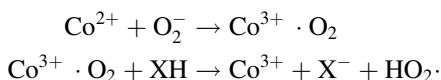


2. Direct reaction of a metal ion with oxygen:

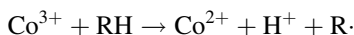


The  $\text{O}_2^- \cdot$  radical ion reacts readily with a proton to form the  $\text{HO}_2 \cdot$  radical, which can initiate the chain reaction of oxidation.

3. Complex reaction of metal compounds with oxygen and subsequent formation of an  $\text{HO}_2 \cdot$  radical.



4. Oxidation by electron transfer of the  $\alpha$ -methylenic group by the metal ion.



According to Uri (21) the kinetic and thermodynamic probabilities for formation of free radicals by the metal-catalyzed initiation reaction are considerably more favorable than the Bolland and Gee (16) proposal of diradicals by direct oxidation of a double bond.

Once hydroperoxides are formed, even in trace amounts, they can play a profound role in the autocatalysis. Monomolecular decomposition yields two free radicals:



A bimolecular reaction, perhaps proceeding through intermediate hydrogen bonding, is more probable:



Either the monomolecular or the dimolecular decomposition serves to feed new radicals into the reaction to initiate the chain reaction of autoxidation. These radicals may further react through different paths. They may follow a radical chain mechanism or other well-known radical reactions, such as coupling or disproportionation.

The reactions may lead to the formation of dimers or polymers or may achieve cross-linking, resulting in an insoluble, infusible film (i.e., drying). Apparently, the dominant reaction path depends on the temperature. At room temperature, mostly C—O—C bonds are produced, whereas C—C bonds are predominantly formed under baking conditions.

The free radicals may also undergo chain cleavage reactions. Low molecular weight by-products, such as water, carbon dioxide, aldehydes, ketones, and alcohols may be formed, which cause the odor and taste of the oils. The strong odor of rancid soybean oil was shown to be caused by 2-pentylfuran found in oxidized oil in storage (23).

Chemically, the air-drying of a nonconjugated oil such as linseed is characterized by the adsorption of 12–16% by weight of oxygen. The reactivity of drying oils is based on the mesomeric stabilization of the radical intermediate: the unpaired electron is delocalized over several carbon atoms, and less energy is required to eliminate the proton as illustrated below (24).

Triglycerides	Mesomers	Activation Energy (kJ/mol)	Relative Rate of Oxidation
Stearate	a	415	0
Oleate	2	335	1
Linoleate	5	289	120
Linolenate	11	168	330

<sup>a</sup>Saturated molecules.

### 3.2. Drying of a Conjugated System

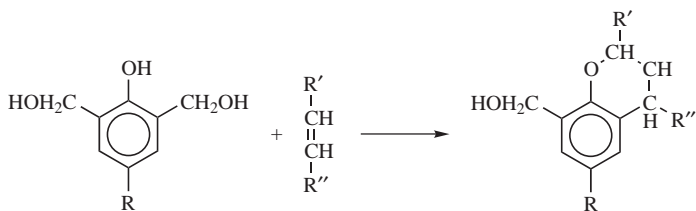
Tung oil, whose dominant feature is the conjugated *cis*, *trans*, *trans*-9,11,13-octadecatrienoic acid,  $\alpha$ -eleostearic acid, dries to a coherent film with absorption of only 5% by weight of oxygen. Privett (25) suggested oxidation through 1,2- or 1,4-addition to the diene system to yield noncyclic peroxides. Faulkner (26) identified 1,6-peroxide in addition and suggested that the autoxidation does not proceed via hydroperoxide groups but rather via cyclic peroxides. It has also been found that the triene content decreased and the diene content increased in proportion to the absorption of oxygen (27, 28). The main reaction is believed to consist of a direct attack by oxygen on the C=C double bonds to form cyclic peroxides and dienes. The peroxides then react with allylic methylene groups or thermally dissociate to give radicals, initiating a radical chain reaction mechanism, forming polymers via C—C or C—O—C bonds (29).

## 4. OLEORESINOUS VARNISHES

As noted, coating systems were advanced from oil-only vehicles to oleoresinous varnishes for improved performances. These are basically oils that have been



“hardened” or modified by treatment with one or more suitable resins, natural or synthetic. The oils and resins are combined, usually by heating together at temperatures of 250°C or above, until a homogeneous mixture is formed. In most cases, it is simply a case of dissolving the resin or resins in the oil. In some cases, chemical reaction may have taken place between the resin and the oil, such as that between the methylol groups of a “heat-reactive” phenolic resin and the double bonds of a drying oil in forming a chroman ring structure as shown below (30):



Chroman ring structure

The major improvements obtained by incorporating resins into drying oils are faster drying, greater film hardness, higher gloss, better water and chemical resistance, and greater durability. The degree of property change depends on the type and the amount of the resin incorporated in the oil. Varnish makers express the oil to resin ratio in terms of oil length, which is defined as the number of gallons of oil used per 100 lb of the resins in the varnish. Varnishes are categorized according to their oil length as short- (5–15 gal), medium- (16–30 gal), and long-oil (30 + gal) varnishes. These demarcations are somewhat arbitrary and not universally agreed.

From oleoresinous varnishes, the coatings industry progressed into alkyd resins. While one might say that this was only an evolutionary change, it nevertheless did open a new horizon for coating technologists and has been responsible for the longevity of oil-based coating materials. It behooves us to take a more comprehensive look at the various aspects of alkyd resins.

## 5. ALKYD RESINS

Alkyd resins have been the workhorse for the coatings industry over the last half century. The term *alkyd* was coined to define the reaction product of polyhydric alcohols and polybasic acids, in other words, polyesters. However, its definition has been narrowed to include only those polyesters containing monobasic acids, usually long-chain fatty acids. Thus thermoplastic polyesters typified by polyethylene terephthalate (PET) used in synthetic fibers, films, and plastics and unsaturated polyesters typified by the condensation product of glycols and unsaturated dibasic acids (which are widely used in conjunction with vinylic monomers in making sheet molding compounds or other thermosetting molded plastics) are not considered as part of the alkyd family and are beyond the scope of the present discussion.

The first appearance of the term *alkyd resin* in the subject index of *Chemical Abstracts* was in 1929, under "resins." It was not until 1936 that *alkyd resins* was listed in its alphabetical place, but still appeared as "see resinous products." The proliferation of literature on alkyd resins peaked in the 1940s through the 1960s. Research activities on alkyds in the United States, as indicated by the number of publications, has apparently tapered off in the last two decades. Readers who are alkyd history buffs can find more detailed historical reviews (31–34).

In spite of the challenges from many new coating resins developed over the decades, alkyd resins, as a family, have maintained a prominent position even until today. There are two major reasons for such sustained popularity. First, alkyds are extremely versatile. An alkyd technologist can choose from a large variety of reaction ingredients and at widely different ratios to tailor the structure and properties of the resin or to obtain similar resin properties from different ingredients, as their availability or cost may sometimes so dictate. For almost any given coating application, from baking enamels for appliances to flat house paints to clear wood finishes, one can design an alkyd resin to meet the property requirements. The second reason is that alkyd resins can be made at relatively low cost. Most of the raw materials are fairly low cost commodity items, and major capital investment and high processing cost are not needed to produce the resins.

## 5.1. Basic Reactions and Resin Structure

The main reactions involved in alkyd resin synthesis are polycondensation by esterification and ester interchange. If one uses the following symbols to represent the basic components of an alkyd resin:  $\text{O}-\underset{\text{O}}{\text{R}}-\text{O}$ , a polyol molecule or radical;

$\text{X}-\text{A}-\text{X}$ , a polybasic acid molecule or radical; and  $\text{X}-\text{F}$ , a mono-basic acid molecule or radical, a schematic representation of the resin molecule can be given (Figure 1). As Figure 1 implies, there is usually some amount of residual acidity along with free hydroxyl groups left in the resin molecules. The structure-property relationship and the principles commonly followed to design the resin structure will be discussed below.

## 5.2. Classification of Alkyd Resins

Alkyd resins are usually referred to by a shorthand description based on a certain way of classification or a combined classification, from which the general properties

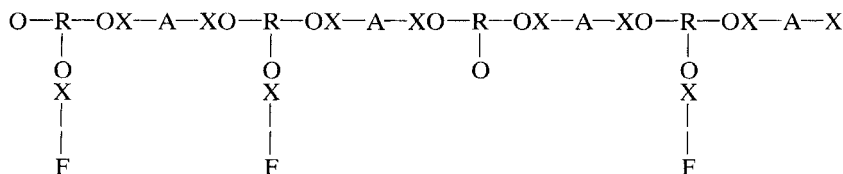


Figure 1. Schematic representation of an alkyd resin molecule.

of the resin become immediately apparent. The commonly used bases for classification are as follows.

*Drying versus Nondrying, and the Specific Source of Fatty Acids.* Alkyd resins can be broadly classified into the drying type and the nondrying type, depending on the ability of their film to dry by air oxidation. This drying ability is derived from the polyunsaturated fatty acids in the resin composition. If drying oils, such as linseed oil, are the sources of the fatty acids for the alkyd, the resin would belong to the drying type and is usually used as the film former of coatings or inks. On the other hand, if the fatty acids come from nondrying oils, such as coconut oil, the resin would be a nondrying alkyd. They are used either as plasticizers for other film-formers, such as in nitrocellulose lacquers, or are cross-linked through their hydroxyl functional groups to become part of the film former. More frequently, an alkyd resin is classified by the source of the fatty acids, e.g., a linseed alkyd, a tung oil-modified soy alkyd, and a coconut alkyd.

*Classified by Oil Length or Fatty Acid Content.* Probably inherited from oleo-resinous varnish practice but with a different way of expression, alkyd resins are also classified by their oil length. For an alkyd resin, the oil length is defined as the weight percent of oil or triglyceride equivalent, or alternatively, as the weight percent of fatty acids in the finished resin, for example, the resin represented in Figure 1. The structure indicates that the molar ratio of these three ingredients is 4 : 4 : 3. Assume that the polyol is glycerol, the polybasic acid is phthalic anhydride, and the fatty acids came from soybean oil with an average molecular weight of 280. The formula weight of the resin would be 1674 and the triglyceride equivalent of the fatty acids would be 878, thus the oil length would be 52.4%. Alternatively, the above resin would be described as one having 50.2% fatty acids. Since the overwhelming majority of alkyd resins are based on phthalic anhydride, it is also customary to describe an alkyd in terms of its phthalic anhydride content in percents based on the finished resin.

By this approach, alkyd resins are classified into four classes:

Resin Class	Percent Oil	Percent Fatty Acids	Percent Phthalic Anhydride
Very long oil	>70	>68	<20
Long oil	56–70	53–68	20–30
Medium oil	46–55	43–52	30–35
Short oil	<45	<42	>35

It should be noted that these demarcations are arbitrary and may vary from author to author. Furthermore, the boundaries are usually not clear-cut.

More frequently, alkyd resins are described by a combined classification in terms of their oil length, the type of fatty acids, and any unusual ingredients. Such descriptions as an isophthalic, very long tall oil alkyd or a medium oil dehydrated castor-PE (the PE refers to pentaerythritol, not polyethylene) alkyd or a short oil lauric-benzoic alkyd would immediately project the general properties of the resin.

### 5.3. Oil Length–Resin Property Relationship

Obviously, the oil length of an alkyd resin has profound effects on the properties of the resin. A few of these effects are discussed below.

*Effect on Solubility.* At long oil lengths, the aliphatic hydrocarbon chains of the fatty acids constitute the major portion of the mass of the resin molecules; therefore, the resin would be soluble in nonpolar aliphatic solvents. Conversely, as the oil length decreases and the phthalic content increases, the aromaticity of the resin molecules increases, and the aromaticity and/or the polarity of the solvent will also need to be increased to dissolve the resin effectively.

*Effect on Drying Characteristics.* Alkyd resin molecules have a comblike structure, with a thermoplastic polyester backbone and dangling fatty acid side chains. Each of these two fractions contributes to the drying, or film-forming, characteristics of the resin. The backbone fraction dries by solvent release, similar to a lacquer material, whereas the side chain fraction dries in a manner similar to the oil from which the fatty acids came. Therefore, short oil alkyds develop a surface dryness relatively quickly due to a faster solvent release, which is often further facilitated by the fact that the solvents used have high volatility. However, their through-dry in air is usually slower, because the fatty acid side chains are fewer in numbers and more scattered in space to cross-link with each other through the action of oxygen, and the dry surface would impede the transportation of air oxygen to reach down into the film. On the other hand, long oil alkyds are relatively slow in reaching the “set-to-touch” stage of surface drying, but the greater abundance of fatty acid side chains and the relative openness of the film surface would facilitate the film to reach through-dry.

Other trends of changing properties (Table 1) would become obvious, considering how the structure of resin molecules would change with oil length. Theoretically, one could design and make alkyd resins at almost any oil length. However, for any given set of starting ingredients, as the oil length goes up, it will reach a point

**TABLE 1 Trends of Property Changes with Oil Length of Alkyd Resins<sup>a</sup>.**

Oil Length	Long	Medium	Short
Requirement of aromatic, polar solvents	—————	—————	—————
Compatibility with other film formers	—————	—————	—————
Viscosity	—————	—————	—————
Ease of brushing	←————	—————	—————
Air dry time, set-to-touch,	←————	—————	—————
Through-dry	←————	—————	—————
Film hardness	—————	—————	—————
Gloss	—————	—————	—————
Gloss retention	—————	—————	—————
Color retention	—————	—————	—————
Exterior durability	←————	—————	—————

<sup>a</sup>Primarily referring to drying-type alkyds.

where the maximum extent of fatty acid modification of the polyester molecules has been achieved, and any additional amounts of fatty acids or oil remain as separate entities, blended with the polyester molecules. Refer again to the resin structure in Figure 1. If the molar ratio is 1 : 1 : 1 among glycerol, phthalic anhydride, and soy acids and the reaction was carried to completion, the resin would have an oil length of 60.5%, or 57.9% of fatty acids. There is no more room in the resin structure to accommodate any additional amount of fatty acid. Therefore, with those three ingredients, if the oil length exceeds 60.5%, the excess amount of oil would only be retained in the resin as a blend. Obviously, the “very long oil” types of alkyd resins would almost certainly be resin–oil blends.

The maximum oil length of an alkyd resin (before it becomes a resin–oil blend) depends on the molecular weight of the ingredients as well as the functionality of the polyol. If the C18 soy fatty acids in the above example is replaced with C12 lauric acid, the transition would be reached at 52.6% oil. On the other hand, if a *tetra*-hydroxyl polyol, such as pentaerythritol, replaces glycerol, the stoichiometry would allow 2 moles of fatty acids, for every 1 mole of phthalic anhydride and pentaerythritol. Thus theoretically, the maximum amount of soy fatty acids that may be chemically combined in the resin structure would be 70.9%, equivalent to a 74.1% oil length.

#### 5.4. Major Ingredients

Each of the three principal components of alkyd resins—the polybasic acids, the polyols, and the monobasic acids—has a large variety to be chosen from. The selection of each one of these ingredients will affect the properties of the resin. As will be shown later, the choice of ingredients may even affect the choice of manufacturing processes. To both the resin manufacturers and the users, the selection of the proper ingredients is a major decision.

*Polybasic Acids and Anhydrides.* The major types of polybasic acids used in alkyd preparation are as follows.

Type	Molecular Weight	Equivalent Weight
Phthalic anhydride	148	74
Isophthalic acid	166	83
Maleic anhydride	98	49
Fumaric acid	116	58
Adipic acid	146	73
Azelaic acid	160	80
Sebacic acid	174	87
Chlorendic anhydride	371	185.5
Trimellitic anhydride	192	64

Phthalic anhydride is by far the most important dibasic acid used in alkyd preparation, because of its low cost and the excellent overall properties it imparts to the

resin. Its anhydride structure allows a fast esterification to form half-esters at relatively low reaction temperatures without liberating water, thereby avoiding the danger of excessive foaming in the reactor. However, since the two carboxyl groups of phthalic anhydride are in the *ortho* position to each other on the benzene ring, cyclic structure may and does occur in the resin molecules. Consequently, the development of chain length of the polymer would be restricted, and the average molecular weight would tend to be low. Phthalic anhydride has a tendency to sublime. (Heat of sublimation: 143 g-cal/g; heat of vaporization: 87.2 g-cal/g.) Therefore, care must be taken to prevent its loss.

Isophthalic acid is the *meta* isomer of phthalic acid. Since the two carboxyl groups are adequately separated, the chances of forming a cyclic structure in the resin molecules are greatly diminished. Therefore, isophthalic alkyds usually attain higher molecular weight and show much higher viscosity than their phthalic counterparts at the same oil length. This is the major motivation for resin manufacturers to use isophthalic acid for the preparation of long oil alkyds. Another major advantage of isophthalic acid is that the resultant alkyd resins show much higher thermal stability than the phthalic type (35). In spite of these advantages, isophthalic acid has not gained the same popularity as phthalic anhydride in alkyds, because the resin making process is much more complicated and difficult than that with phthalic anhydride. Its melting point (350°C) is much higher than that of phthalic anhydride (131°C), and it has a low solubility in the initial alkyd reactants, which causes the reactants to stay as a two-phase solid-liquid system and does not become clear until the reaction is near complete (36, 37). The diacid does not readily form half-esters at relatively low reaction temperature as would the anhydride, and twice as much water will be formed and needs to be removed from esterification. Usually, additional care and equipment are needed for the higher processing temperature required for isophthalic acid.

The *para* isomer terephthalic acid may also be used for making alkyds. The resultant resins showed even better thermal stability than isophthalic alkyds (35). However, it has all the disadvantages of isophthalic acid and is more expensive. It is rarely used in making alkyd resins.

Maleic anhydride is sometimes used for partial substitution of phthalic anhydride in making alkyds. It imparts vinylic unsaturation functionalities in the backbone chain of the resin molecules, which allows the resin to be grafted with styrene, acrylic esters, or other vinyl monomers. The presence of a small amount of maleic anhydride, up to 10% on molar base of the total dibasic acids, in the resin formulation would accelerate the viscosity increase during the resin manufacturing process. The resins usually dry more rapidly and give harder films with improved color, adhesion, water resistance, alkali resistance, and exterior durability. However, the resin cooking process needs to be monitored and controlled with greater care, particularly when it is near the desired end point, to prevent gelation. Fumaric acid, the *trans* isomer of maleic acid, may be used in an equivalent manner.

Maleic and fumaric acid can also be, and are often, incorporated in alkyd resins in the form of the Diels-Alder adduct of rosin. The adducts are tribasic acids. They provide one of the means to impart pendant carboxyl groups in the resin molecules,

which can then be saponified to give ionic and, in turn, water-soluble characteristics to the resin. Alkyds containing maleic–rosin adducts often have poorer color retention, toughness, gloss retention, and exterior durability.

Aliphatic dibasic acids, such as succinic, adipic, azelaic, and sebacic acids have also been used to make alkyd resins. Their linear and flexible chain structure lends higher flexibility and lower viscosity to the resin than the rigid aromatic rings of phthalic acids.

Chlorendic anhydride is the Diels–Alder adduct of maleic anhydride and hexachlorocyclopentadiene. It is also known as hexachloro-endo-tetrahydrophthalic (HET) anhydride. The major interest of the alkyd industry in this material is that the resultant resins contribute to the flame retardancy of the coatings. It has been reported to give a greater reaction rate than phthalic anhydride, such that at 204–210°C (400–410°F) the reaction rate approximates that of phthalic anhydride at a temperature of 238°C (460°F) (38). However, the resins are prone to develop darker color, particularly at high processing temperature. Tetrachlorophthalic anhydride, made by conventional chlorination of phthalic anhydride, would also impart flame retardancy to its alkyds. However, it is appreciably less soluble in the usual processing solvents than is phthalic anhydride and is reported to be of appreciably lower chemical reactivity (39).

Trimellitic anhydride (TMA), 1,2,4-benzenetricarboxylic acid anhydride, has gained greater prominence in recent years due to the greater interest in water-soluble alkyds. A partial substitution of the phthalic anhydride with TMA gives a measured quantity of pendent carboxyl groups for water solubilization with ammonia or other suitable base. The anhydride hydrolyzes to the acid form simply by allowing it to stand in open containers. Premature cross-linking of alkyd resins formulated with a high content of TMA would occur at high acid numbers when large amounts of trimellitic acid are present (40).

*Polyhydric Alcohols.* The major types of polyol used in alkyd synthesis are as follows.

Type	Molecular Weight	Equivalent Weight
Pentaerythritol	136	34
Glycerol	92	31 <sup>a</sup>
Trimethylolpropane	134	44.7
Trimethylolethane	120	40
Ethylene glycol	62	31
Neopentyl glycol	104	52

<sup>a</sup>Since glycerol is usually supplied at 99% purity (1% moisture), its equivalent weight is commonly assumed to be 31 in recipe calculations.

Pentaerythritol (PE) is one of the most important polyols used in alkyd resins. Its molecular structure, four methylol groups (CH<sub>2</sub>OH) surrounding a center carbon atom, is the basis for its many interesting attributes. The four equal and highly

reactive primary hydroxyl groups make it versatile for designing resin structures, and the neopentyl core structure lends stability against heat, light, and moisture. As a result, alkyds based on PE usually are superior to their counterparts based on glycerol in viscosity, drying properties, film hardness, gloss retention, color and color stability, humidity resistance, thermal stability, and exterior durability. On the other hand, its high functionality demands that the resin composition be more carefully designed and the synthesizing process be more carefully monitored and controlled to reduce or eliminate the tendency of gelation. Dipentaerythritol and tripentaerythritol are linear dimer and trimer of PE. They are hexa- and octa-functional polyols, respectively. Technical grades of PE usually contain small or trace quantities of di- and tri-PE that were not completely removed in the manufacturing process. The high functionality of these materials makes them impractical to be considered as the sole or major polyol of an alkyd resin.

Among the triols, glycerol is undoubtedly the most important one in alkyd technology. Natural fats and oils are triglycerides. Therefore, whenever oils are used directly as the source of fatty acids in an alkyd resin, glycerol will automatically be a part of the polyols of the resin. Besides the difference in functionality, the major difference between glycerol and PE is that one of the hydroxyl groups in glycerol is secondary, which has lower reactivity than primary hydroxyl groups. This often manifests itself as if glycerol had a de facto functionality of less than 3. Consequently, a larger excess of glycerol would be required in the resin formulas, which would result in poorer resin properties as a coating material. At high temperatures, the proton on the secondary carbon in glycerol may undergo a dehydration reaction with one of the primary hydroxyl groups on the adjacent carbon atom to give water and acrolein, whereas such reaction is not possible with PE. Glycerol alkyds are more prone to thermally decompose to give color bodies, resulting in darkening of the resin.

Trimethylolethane and trimethylolpropane are synthetic triols. Like PE, they have the neopentyl structure and equivalent primary hydroxyl functional groups. Therefore, they also yield alkyds with better resistance to heat, light, moisture, and alkali than glycerol. They have one less hydroxyl group than PE, and the equivalent weights of these polyols are higher than that of PE. Trimethylolethane has been reported to give alkyds that are faster drying and higher in film hardness than trimethylolpropane (41), whereas trimethylolpropane was claimed to give alkyds with better water and alkaline resistance, color and color retention, and impact resistance than trimethylolethane (42).

Diols such as ethylene glycol, propylene glycol, and neopentyl glycol are sometimes used as part of the polyols in alkyd formulations primarily for the purpose of regulating the functionality of the reaction system. Their relatively low boiling points, (197, 188, and 207°C), respectively, for the above three glycols) require that special precautionary measures be taken during the resin manufacturing process.

Analogous to the use of linear  $\alpha,\omega$ -dibasic acids (such as adipic and sebacic), polyols with long, flexible chains between hydroxyl groups (such as 1,4-butanediol, 1,6-hexanediol, and diethylene glycol) may also be used to impart greater flexibility in the resin.



It should be pointed out that under high temperatures, such as those used for alkyd resin synthesis, and in the presence of high acidity, etherification between the hydroxyl groups of two polyol molecules may condense them into a new polyol with a functionality of  $n + n' - 2$ , where  $n$  and  $n'$  are the numbers of hydroxyl groups of the two original molecules. The introduction of such high functionality polyols plus the net reduction of total available hydroxyl groups can lead to an increased danger of gelation during the poly-condensation process.

*Monobasic Acids.* The overwhelming majority of monobasic acids used in alkyd resins are long-chain fatty acids of natural occurrence. They may be used in the form of oil or free fatty acids. Free fatty acids are usually available and classified by their origin, viz., soy fatty acids, linseed fatty acids, coconut fatty acids, etc. The fatty acid composition of various types of fats and oils that are commonly used in alkyd resins are given in Table 2.

The drying property of alkyd resins reflects directly that of the oil or fatty acids in the resin structure, discussed earlier. It should be pointed out that alkyds based on conjugated unsaturated fatty acids, such as those from tung and oiticica oils, dry so fast that if not properly moderated, the surface layer will dry long before the underlayer, resulting in a wrinkled surface due to the stresses created in the dried surface layer. Therefore, in alkyd resins, tung oil and oiticica oil are primarily used to furnish a minor portion of the fatty acids to improve drying properties. Even so, greater care must be exercised during the manufacturing process to avoid gelation, which is caused by the dimerization of the fatty acid chains through a Diels-Alder addition between the conjugated diene structure on one molecule and a double bond on another molecule. It should be noted that nonconjugated diene groups, such as those in linoleic and linolenic acids, may undergo isomerization to become conjugated. Furthermore, ene-reaction could also occur between two unsaturated fatty acid chains, which leads to gelation.

Rheineck and co-workers (44) have found that linolenic acid is responsible for the high yellowing tendency of alkyds based on linseed oil fatty acids. Therefore, alkyds intended for making white or light color enamels should avoid high linolenic content fatty acids by choosing soy oil, safflower oil, or dehydrated castor oil (DCO). Alkyds made with nondrying oils or their fatty acids have excellent color and gloss stability. They are frequently the choice for white industrial baking enamels and lacquers.

Since the mid-1950s, tall oil fatty acids (TOFA) have become available in good quality and large quantities. Refined grades of TOFA have degrees of unsaturation rivaling that of soy acids. Since it is a year-round by-product from the paper industry, its supply and price are more stable than agricultural products like soy fatty acids. It is used extensively in medium- to long-oil alkyds, virtually as equivalent to soy fatty acids. Although the minor quantities of rosin acids in TOFA may impart some yellowing tendency, its lack of linolenic acid may be more than enough to give as good or even better color retention than soy fatty acids. The typical properties of refined grades of commercial TOFA are given in Table 3.

A number of monobasic acids that are not derived from fats and oils have been used in alkyd resins. However, except in the rare cases of making the so-called

**TABLE 2. Fatty Acid Compositions of Fats and Oils Commonly Used in Alkyd Resins.**

Oil	Iodine Value	Saponification Value	Fatty Acid Composition (Percent by Weight)					Other
			Saturated <sup>a</sup>	Palmitoleic	Oleic	Linoleic	Linolenic	
Castor	85.8	195	2.4	—	7.4	3.1	—	Ricinoleic, 87.0; dihydroxystearic, 0.6
Castor, dehydrated	125–135	191	2.4	—	8.0	86	3.0	About 33% of the linoleic is 9,11-conjugated.
Coconut	8.7	257	76.6	—	5.7	2.6	—	Caprylic, 7.9; capric, 7.2; of the saturated, lauric, 48.0 and myristic, 17.5
Cottonseed	105.0	196	27.2	2.0	22.9	47.8	—	Tetradecenoic, 0.1
Linseed	180	191	9.3	—	19.0	24.1	47.4	Lignoceric, 0.2
Menhaden	148–185	191	24.0	15.0	30.0	—	—	Highly unsaturated C <sub>20</sub> H <sub>2(20-x)</sub> O <sub>2</sub> , 19; and C <sub>22</sub> H <sub>2(22-x)</sub> O <sub>2</sub> , 12. <sup>b</sup>
Oiticica		192	11.3	—	6.2	—	—	Licanic, 82.5
Peanut	93.3	190	13.8	1.7	54.3	26.0	—	Arachidic, 2.4; behenic 3.1: lignoceric, 1.1
Rapeseed	102.3	175	6.1	1.5	12.3	15.8	8.7	Behenic, 0.7; lignoceric, 0.8; eicosenole, 4.8; erucle, 47.8; docosendienole, 1.5
Safflower	136.2	191	6.0	—	32.8	61.1	1	
Soybean	132.6	193	13.4	1.0	23.5	51.2	8.5	Saturated C20-C24, 2.4
Sunflower	130.8	188	7.1	—	34.0	57.5	—	Lignoceric, 0.4
Tung		192	5.0	—	5.0	3.0	—	Eleostearic, 87

<sup>a</sup>Aliphatic monocarboxylic acids, C<sub>12</sub> to C<sub>20</sub>, principally palmitic and stearic.

<sup>b</sup>x = 4 – 10.

Owing to incomplete halogen absorption, iodine values for conjugated acid oils by the usual methods (Wijs, Hanus, etc.) are both low and variable. The true value of fresh tung oil, as determined by special method, is 248–252: that of oiticica oil is 205–220 (43).

TABLE 3. Typical Properties of Refined Tall Oil Fatty Acids<sup>a</sup>.

Characteristic	Grade Designation					
	Pamak 1	Pamak 2	Pamak 4A	Pamak 4	Pamolyn 200 <sup>b</sup>	Pamolyn 300 <sup>a</sup>
Acid number	193	192	191	188	195	196
Iodine number	125	128	130	131	162	156
Total fatty acids, %	96.8	95.9	94.1	91.5	97	97
Saturated acids, % of free fatty acids	2.0	—	—	4.0	<1	<1
Oleic	51.0	—	—	51.0	22.0	21.0
Linoleic, nonconjugated	41.0	—	—	39.0	68.0	39.0
Linoleic, conjugated	6.0	—	—	6.0	10.0	40.0
Linolenic	—	—	—	—	—	—
Rosin acids, %	1.4	1.8	3.5	4.0	1.5	1.5
Unsaponifiables, %	1.8	2.3	2.4	4.5	1.5	1.5
Color, Gardner	3	3	4	6	3+	3
Titer, °C	5	5	5	6	-28	-28

<sup>a</sup>Data from Hercules, Inc. (Wilmington, Del.).

<sup>b</sup>Enriched polyunsaturated fatty acids from highly refined TOFA.

<sup>c</sup>Same as Pamolyn 200, with further treatment to isomerize the nonconjugated linoleic acid.

oil-free alkyds for special purposes, they are used in conjunction with fatty acids to modify resin properties. Rosin acids, primarily in the form of abietic acid, are the most common type of such acids. They may be used in neat form or be brought in as a part of TOFA. Presumably, the fused ring structure of rosin contributes to the film hardness, initial gloss, and water resistance of the alkyd. However, color and color retention, and exterior durability will be adversely affected if the rosin content goes much above 5–6%. The drying rate of alkyds usually appears to be improved with rosin modification. However, since rosin does not participate in the oxidative drying mechanism that applies to polyunsaturated fatty acids, the true drying rate of the alkyd resin would be reduced due to a reduction of the fatty acid unsaturation. Synthetic saturated carboxylic acids (such as pelargonic acid, 2-ethylhexanoic acid, and isoctanoic acid) and aromatic monobasic acids (such as benzoic acid and *p*-alkyl-benzoic acids) can improve color retention, gloss retention, and exterior durability even better than those based on castor or coconut fatty acids. The aromatic acids, similar to rosin, also give higher film hardness and faster apparent drying rate.

## 5.5. The Concept of Functionality and Gelation

The concept of functionality and its relationship to polymer formation was first advanced by Carothers (45) in 1929. Flory (46) greatly expanded the theoretical consideration and mathematical treatment of polycondensation systems. Thus if a dibasic acid and a diol are reacted to form a polyester, assuming there is no possibility of other side reactions to complicate the issue, only linear polymer molecules

will be formed. When the reactants are present in stoichiometric amounts, the average degree of polymerization  $\bar{x}_n$  follows the equation:

$$\bar{x}_n = 1/(1 - p) \quad (1)$$

where  $p$  is the extent of reaction, in fractions. Thus when the reaction is driven to completion, theoretically, the molecular weight would approach infinity and the whole mass would form one giant polymer molecule. Although the material should theoretically be still soluble and fusible, it is considered and defined as a gel, and this would be the only time that difunctional "monomers" could be polymerized to gelation.

The functionality of the system  $f$  is the sum of all of the functional groups, i.e., equivalents, divided by the total number of moles of the reactants present in the system. Thus in the above equimolar reaction system:

$$f = (1 \times 2 + 1 \times 2)/(1 + 1) = 2$$

However, when there are reactants with three or more functionalities participating in the polymerization, branching and the formation of intermolecular linkages (i.e., cross-linking of the polymer chains) become definite possibilities. If extensive cross-linking occurs in a polymer system to form network structures, the mobility of the polymer chains is greatly restricted. Then the system would lose its fluidity and transform from a moderately viscous liquid to a gelled material with infinite viscosity. The experimental results of several such reaction systems reported by different investigators are collected in Table 4.

The data in Table 4 show that when the reactants are present in stoichiometric proportions, gelation occurs before the completion of esterification, and the extent of reaction  $p$  reached at the gel point depends on the functionality of the system. Carothers (47) showed that at the gel point,  $p = 2/f$ . Thus, to avoid premature gelation, the polymerization system should have an average functionality of no more than 2. This can be accomplished by adding low functionality reactants and/or adding an excess amount of one of the reactants, usually the one with high functionality constituents. The latter has the net effect of reducing the functionality of the reactant. For example, if a 20% excess of glycerol over the stoichiometric

**TABLE 4. Gel Points of Polyesterification Reaction Systems with Stoichiometric Reactants (46).**

Polybasic Acid (COOH)	Polyol (OH)	$f$	Percent Esterification at Gel Point
Adipic (0.707) + tricarballic (0.293)	di-EG (1.0) <sup>a</sup>	2.103	0.911
Dibasic (1.0)	glycerol (1.0)	2.400	0.765
Adipic (1.0)	PE (1.0) <sup>b</sup>	2.667	0.578

<sup>a</sup>Diethylene glycol.

<sup>b</sup>Pentaerythritol.

amount required to esterify all of the carboxyl groups present in the formula is added, the glycerol would have an effective functionality of 3/1.2, or 2.5. Frequently, both of these measures are taken to safeguard against premature gelation. Patton (48) showed that for alkyd resins, the extent of the reaction at gel point was

$$p_c = 2/f = 2m_o/e_o$$

where  $e_o$  is the total effective equivalents of all of the reactants present at the beginning of the reaction (i.e., the excess reactants are discounted in the manner discussed above),  $m_o$  is the total number of moles of all reactants at the beginning, and  $f$  is the effective average functionality of the formulation.

## 5.6. Microgel Formation and Molecular Weight Distribution

Bobalek and co-workers (49) observed that the behavior of alkyd resin reaction often deviates from that predicted by the theory of Flory. They proposed a mechanism of microgel formation by some of the alkyd molecules at a relatively early stage of the reaction. The microgel particles would be dispersed and stabilized by smaller molecules in the remaining reaction mixture. As polyesterification proceeds, more microgel particles would be formed, until finally a point is reached at which they could no longer be kept separated. The microgel particles would then coalesce or flocculate, phase inversion would occur, and the entire reaction mass would be "gelled." They showed that the drying capability of an alkyd resin comes primarily from the microgel fraction and, "when the highest molecular weight fraction representing about 20% of the total was removed through fractionation, the residual linoleic alkyd lost all ability to air dry to a hard film."

Solomon and co-workers (50, 51) further elaborated on the microgel theory by proposing the formation of micelles as precursors of microgels. They proposed that when some of the molecules have grown to reach certain fatty acid: polyester ratios, surface activity develops to form micelles. The polyesterification reactivity at the surface of the micelles would be preferentially greater, which would lead to the eventual formation of microgels. From electronmicroscopy evidence, they observed that the size of microgels increased with reaction time, and particle diameters as large as  $2\mu$  have been reported. Functional groups such as OH groups in the microgel particles are believed to be buried in the structure and not available for reactions. Whereas in polyesterification reactions without fatty acids, at all stages of the reaction up until the physical gelation of the reaction mixture, the hydroxyl values corresponded with the calculated values. Furthermore, the oil-free systems showed no sign of microgel formation under electron microscope, and the reaction mixture would undergo a sudden change from a soluble polymer to a gelled mass. The reaction temperature for alkyd preparation in both of the above references was kept at no more than  $200^\circ\text{C}$ , well short of what would normally be required for the bodying of unsaturated oils in the absence of an oxidizing reagent. This indicates that polymerization between unsaturated fatty acids of the resin molecules is not necessary for the formation of microgels.

Kumanotani and co-workers (52–55) further confirmed the formation of microgels by characterizing fractions of alkyd resins from preparative GPC columns. Their results showed that the presence of microgel can be detected even in low molecular weight fractions. Colloidal gel particles up to 10+  $\mu\text{m}$  in diameter were observed in the high molecular weight ( $>10^5$ ) fractions, with or without unsaturated fatty acids in the alkyd formulation. However, the unsaturated fatty acids made a significant contribution to the formation of the colloidal particles. Higher reaction temperature led to higher molecular weight and broader molecular weight distribution. Acid value and hydroxyl value each went through a minimum in the middle fractions (molecular weight about  $10^3$ – $10^4$ ), whereas the polyester: monoacid ratio increased with the molecular weight of the fraction. Cured films from alkyds with greater amount of colloidal fractions gave better thermomechanical properties. Finally, the high molecular weight colloidal fractions were preferentially adsorbed by pigment particles and would thus stabilize the pigment dispersion in the coating formulation.

### 5.7. Basic Principles for the Designing of Alkyd Resins

The process of alkyd resin designing should begin with the following question: what would be the intended application(s) of the resin? The application would dictate property requirements, such as solubility, viscosity, drying characteristics, compatibility, film hardness, film flexibility, acid value, water resistance, chemical resistance, environmental endurance, etc. With these targets in mind, a selection on oil length, and a preliminary list of alternative choices of ingredients can then be made. For commercial production, the raw material list is screened based on considerations in material cost, availability, yield, impact on processing cost, and potential hazard to health, safety, and the environment. The list may be further narrowed by limitations imposed by the production equipment or other considerations. Once the oil length and ingredients are chosen, the first draft of a detail formulation for the resin can then be made.

It would be highly desirable that one could rely on a simple equation or formula to obtain the optimum formulation of the alkyd resin with the chosen ingredients, and several approaches have been proposed for such purpose (56–59). However, the complexity of the alkyd reaction system has rendered these equations to be of no more value than providing a first approximation of a starting formula. The causes of the complexities include the formation of intramolecular cyclic structures, which would reduce the chance of gelation; the etherification of polyols, which would increase the chance of gelation by forming higher functionality materials and reducing the number of hydroxyl groups available for esterification; the cross-linking between unsaturation groups, especially the conjugated double bonds, which would increase the chance of gelation; and the phenomenon of microgel formation.

Except when nondrying alkyds are used strictly as plasticizers for other thermoplastic polymers, alkyd resins do not remain as a thermoplastic material in their ultimate application. The film integrity is largely derived after the resin molecules have been cross-linked, either through the unsaturation functionalities on their fatty

acid side chains or through the reactions of their residual hydroxyl or carboxyl functionalities with such cross-linking agents as amino resins or polyisocyanate materials. In a sense, alkyds are usually made and applied as B-stage resins. Therefore, it is not necessary to build the molecules of alkyd resins to huge molecular weights, as one would for thermoplastic polymers. In fact, too high a molecular weight would lead to poor solubility and high solution viscosity and would be undesirable for practical applications. Most of the published data show that the average molecular weight of alkyds is less than 10,000. Nevertheless, within the practical limits, it is still preferred to have a linear backbone structure and high molecular weight to give the best film-forming and film properties. Alkyd formulations with an equimolar ratio of dibasic acids: polyols tend to have the best chance of achieving a linear molecular structure and high molecular weight.

Thus a simple molecular approach is favored by some of the alkyd chemists for deriving the starting formulation. The basic premise of this approach is that when the total number of moles of polyols is equal to or slightly larger than that of the dibasic acids and the hydroxyl groups are present in an empirically prescribed excess amount, the probability for gelation to occur would be small. Table 5 lists the empirical requirements of excess hydroxyl groups based on the oil (fatty acid) length of the alkyd. The values were developed based on experimental experience (57, 58). With the new understanding that some of the hydroxyl groups would be buried in microgel structures (49–55), such requirements may be better rationalized. The procedure of this method for formulating alkyd resins will be illustrated with examples.

The first example demonstrates the formulations of a 50% soy oil alkyd for baking enamels. The preliminary selection of ingredients would be alkaline refined soy oil, phthalic anhydride (PA), and pentaerythritol. The basis for calculation is 1 mole of PA. From Table 5, the excess OH recommended at 50% oil length is 25%. Therefore, the quantity of PE required, in equivalents, would be

$$E_{PE} = 1 \times 2 \times (1 + 0.25) = 2.5 \text{ eq.} = 0.625 \text{ moles}$$

**TABLE 5. Excess Hydroxyl Content Required in Alkyd Formulations.**

Oil Length, Percent Fatty Acid <sup>a</sup>	Percent Excess OH Based on Diacid Equivalents	Percent Excess OH in Finished Resin
62 or more	0	0
59–62	5	0–5
57–59	10	5–10
53–57	18	10–15
48–53	25	15–20
38–48	30	20–25
29–38	32	25–30

<sup>a</sup>Based on C18 fatty acids with average equivalent weight of 280. If the average equivalent weight of the monobasic acids is significantly different, adjust accordingly.

Since the total polyol is to be equimolar to PA, the glycerol from the soy oil will, therefore, be  $(1 - 0.625) = 0.375$  moles, which gives  $(3 \times 0.375) = 1.125$  moles of soy fatty acids. The ingredients can be listed as follows:

Ingredient	<i>M</i>	COOH	OH	Weight (g)
PA	1.0	2.000		148.0
Soy oil	0.375	1.125	1.125	330.0
Tech-PE	0.625	—	2.500	88.5
Total	2.000	3.125	3.625	566.6
Water	1.0	—	—	(18.0)
Resin	—	—	—	548.5

The above formulation does not meet the test of 50% oil length. The oil content must be reduced. A reduction in oil would cause a corresponding reduction in glycerol, consequently, free glycerol is added to make up the loss. Let  $M_{PE} = X$ ,  $M_{Gly} = Y$ , and  $M_{oil} = Z$ . Since the total polyols is to be equimolar to dibasic acids,  $X + Y + Z = 1$ . The 25% excess OH requirement defines  $4X + 3Y = (2 \times 1.25) = 2.5$ , and the 50% oil length requirement gives the following:

$$880Z / (148 + 141.6X + 93Y + 880Z - 18) = 0.5$$

where 880, 148, 141.6, 93, and 18 are the molecular weights of the oil, PA, PE, glycerol, and water, respectively. When solve the simultaneous equations to find  $X = 0.221$ ,  $Y = 0.539$ , and  $Z = 0.240$ . Thus the “final” formulation is listed as follows:

Ingredient	<i>M</i>	COOH	OH	Weight (g)
PA	1.0	2.000	—	148.0
Soy oil	0.240	0.720	0.720	211.2
Glycerol	0.539	—	1.617	50.1
Tech-PE	0.221	—	0.884	31.3
Total	2.000	2.720	3.221	440.6
Water	1.0	—	—	(18.0)
Resin	—	—	—	422.6

The above formulation meets all of the requirements of the resin design, i.e., equimolar PA and polyols, 25% excess OH, and 50% oil.

The next example shows the formulation of a 50% TOFA alkyd for baking enamels. Assume that PA, PE, ethylene glycol (EG), and refined TOFA with 4% rosin acids are the chosen ingredients. From the given constraints, the



following simultaneous equations can be established. Let  $M_{EG} = X$ ,  $M_{PE} = Y$ , and  $M_{TOFA} = Z$ .

$$X + Y = 1$$

$$2X + 4Y = 2 \times (1 + 0.25) + Z$$

$$295Z \div [148 + 141.8Y + 62X + 295Z - 18 \times (1 + Z)] = 0.5$$

Solve the equations, to find  $X = 0.362$ ,  $Y = 0.638$ , and  $Z = 0.776$ . Therefore, the “final” formulation can be listed as follows:

Ingredient	<i>M</i>	COOH	OH	Weight (g)
PA	1.000	2.000	—	148.0
Tech-PE	0.638	—	2.552	90.3
EG	0.362	—	0.724	22.4
TOFA-4	0.776	0.776	—	228.9
Total	2.776	2.776	3.276	489.6
Water	1.776	—	—	(32.0)
Resin	—	—	—	457.6

The percent excess OH =  $(3.276 - 2.776)/2 = 25\%$ , and the oil length =  $228.9/457.6 = 50\%$  TOFA.

The “final” formulations derived in the above examples are meant to be only the starting formulations. They should be fine-tuned based on small-scale laboratory experiments before being used in plant production.

Since the molecular chain length or the degree of polymerization is a function of the extent of the reaction as shown in equation 1, the alkyd reaction is usually carried to a point short of completion, i.e., to a finite acid number to guard against premature gelation. It has been shown that the esterification of phthalic anhydride was slower and showed higher temperature dependence, i.e., higher activation energy, than that of fatty acids (60–62). Therefore, one may assume that the residual acidity belongs to unreacted dibasic acids, which contributes to the limiting of chain growth. In real practice, an additional safety margin against premature gelation is provided by having a slight molar excess of polyols over dibasic acids in the alkyd formulation. If the molar ratio between the polyols and the dibasic acids is  $r$ , equation (1) may be rewritten as:

$$\bar{x}_n = (1 + 1/r) / [(2/r)(1 - p) + 1 - 1/r] \quad (2)$$

which indicates that a fractional increment in  $r$  and/or a fractional reduction in  $p$  would give a substantial reduction in  $\bar{x}_n$ . Generally, the value of  $r$  is chosen between 1 and 1.05.

## 5.8. Chemical Procedures for Alkyd Resin Synthesis

Different chemical procedures may be used for the synthesis of alkyd resins. The choice is usually dictated by the choice of the starting ingredients.

*Alcoholysis Process.* Cost and availability often dictate that oil, rather than free fatty acids, be used as raw material for alkyd synthesis. Since oil, in the form of triglycerides, is essentially inert and would not participate in the polyesterification reaction, heating the oil with the polyol and the dibasic acid would result in the formation of seedy polycondensates between the polyol and the polybasic acids leaving the oil unreacted. The two phases thus would be incompatible with each other. Therefore, the triglycerides must first react with additional polyol to redistribute the fatty acids among all of the polyols, thereby liberating free hydroxyl groups from the oil for further reaction with the dibasic acids. The reaction is alcoholysis. It is usually catalyzed by basic compounds such as metal oxides, hydroxides, salts, or soaps such as naphthanates. In the past, litharge was the most popular choice as the catalyst. It was found that on a molar basis lead compounds were the most efficient among the 36 that were included in the study (58). In recent years, due to the concern of lead poisoning from the resultant coatings, lithium hydroxide, sodium hydroxide, or calcium oxide have been commonly used. The dosage of these catalysts usually ranges from 0.01 to 0.06% metal based on the weight of the oil for lead and 0.008 to 0.02% for lithium or calcium. The amount of catalyst added should be kept at the minimum required for completing the reaction in an acceptable batch time. They may cause poor color, poor water resistance, or haziness in the final resin.

Ideally, 2 moles of polyol would react with 1 mole of triglyceride to form 3 moles of monoester. In reality, the reaction would reach an equilibrium, whereby some amount of diesters and triesters and neat polyol, including glycerol and the added polyol, would coexist in the reaction mixture. The composition of the alcoholysis product at equilibrium from soy oil and glycerol (1 : 2 mole ratio), and soy oil and monopentaerythritol have been reported as follows (63):

Component	Mole Percent	
	Oil-Glycerol	Oil-Mono-PE
Glycerol		
Monoester	42	20.6
Diester	21	6.2
Triester	3	2.0
Free glycerol	33	14.0
PE		
Monoester		29.0
Diester		16.8
Triester		5.1
Tetraester		Negligible
Free PE		6.3

Diols, such as ethylene glycol, are usually not added during the alcoholysis step. This is because their monoesters have only one remaining hydroxyl group and would function as chain stoppers, thus severely limiting their utility in the structure design of the resin molecules.

In general practice, the oil is first heated to 230–250°C under an inert gas blanket and agitation. The catalyst, usually predispersed in a small quantity of the oil, and the polyol, usually at 2 times the molar quantity of the oil present in the reactor, are added. The batch is reheated to and maintained at the desired temperature, usually in the 230–250°C range. The progress of the reaction is monitored by periodical sampling from the reactor and checking miscibility with anhydrous methanol. This is because triglycerides are not soluble in methanol, whereas monoglyceride is. When a volume of the alcoholysate can tolerate three or more volumes of methanol without becoming turbid, the alcoholysis process is considered complete.

Acidic contaminants are poisonous to the alcoholysis catalysts and must be avoided. If the oil has a high acid number, or there are high acidity residues left in the reactor from the previous batch, such as sublimed phthalic anhydride condensed under the dome of the reactor, the reaction can be severely retarded. A longer batch time or additional amount of the catalyst would then be required. Both are undesirable.

When the alcoholysis step is complete, the polybasic acid(s) and the balance of polyol, if any, are added. The batch is reheated to and maintained at about 250°C to carry out the polycondensation step to the desired endpoint, usually a combination of the acid value and viscosity of the resin.

*Fatty Acid Process.* When free fatty acids are used instead of oil as the starting component, the alcoholysis step is avoided. All of the ingredients can, therefore, be charged into the reactor to start a batch. The reactants are heated together, under agitation and inert blanket, until the desired end point is reached. Chen and Kumanotani (64) reported that alkyds prepared by the fatty acid process have narrower molecular weight distribution and give films with better dynamic-mechanical properties.

A modified form of the fatty acid process, dubbed “high polymer alkyd technique” was reported (65). A portion of the fatty acids is withheld in the first stage of the process to allow the polycondensation between the dibasic acid and the polyol to have a better chance of extending the polyester chain without being terminated by the monoacids. After the acid value of the reactant has reached a desired low level, indicating the completion of the polycondensation, the remaining portion of fatty acids is then added to complete the process. The resins prepared by this technique have more linear backbone chain structure, higher molecular weight, and higher viscosity than the corresponding ones with identical formulation but prepared by the conventional process.

*Fatty Acid–Oil Process.* When oil represents only a minor portion (33% or less) of the total furnish of fatty acids in an alkyd formulation, the alcoholysis step may be avoided. All of the ingredients, dibasic acid, polyol, oil, and free fatty acids may be charged together into the reactor and proceed as in the fatty acid process. Apparently, the oil is incorporated into the resin by ester interchange at the reaction

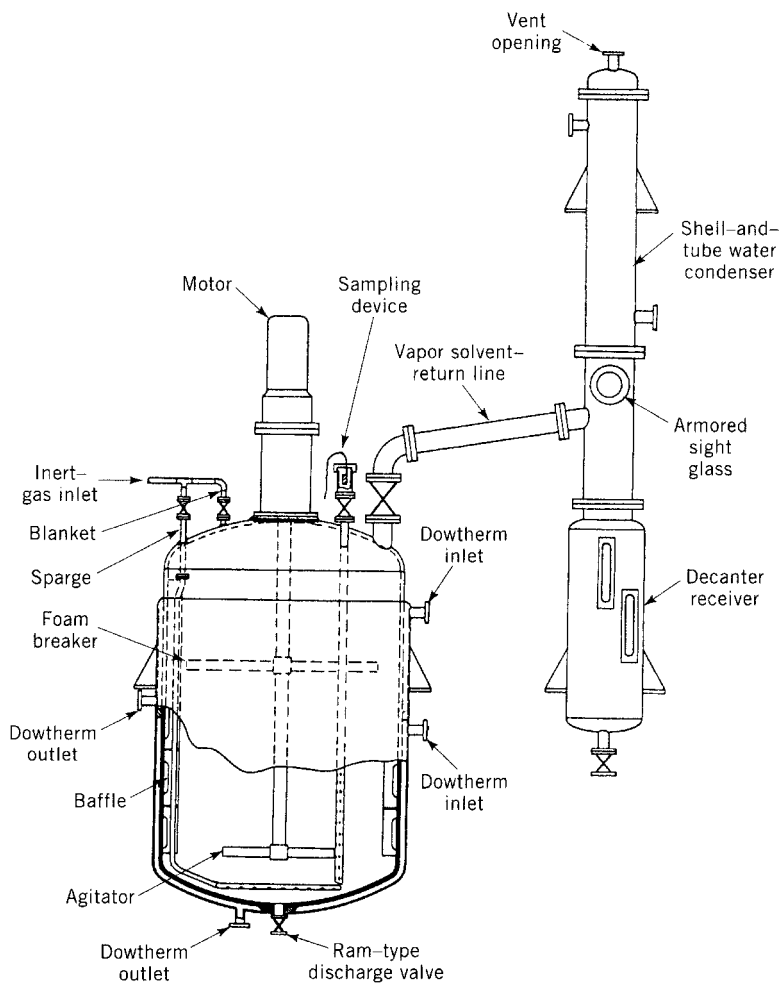
temperature. The resultant resins give higher viscosity (4), faster surface drying, and slower through-dry (3). If the oil content is too high, not enough of it may be incorporated in time, then it, would result in a partial gelation to form "seeds."

*Acidolysis Process.* As mentioned previously, isophthalic and terephthalic acids are difficult to process in ordinary alkyd preparation methods, due to their high melting point and low solubility in the reaction mixture. An acidolysis process was developed for this purpose (6). The dibasic acid is heated together with the oil in the resin formulation under agitation and inert gas blanket to about 280°C, holding for about 40 min. In this reaction, which is self-catalyzed by the acidity of the reaction mixture, an ester interchange occurs. A carboxyl group of the dibasic acid displaces that of a fatty acid on the oil molecule and splits off the fatty acid. The completeness of the acidolysis reaction is determined by a tedious extraction of the oil phase and analysis of its free fatty acid content by titration. The analysis takes several hours to complete. Rapid test methods, comparable to the methanol miscibility test for alcoholysis, that could be used for process control of the acidolysis reaction have yet to be developed. Therefore, the process is normally controlled by reaction time and temperature, based on experience. After acidolysis, the reactant temperature is dropped to about 230°, the polyol is charged and heated back up to the desired temperature to bring the esterification step to the desired end point. The acidolysis process is not suitable for phthalic anhydride or other dibasic acids with a high tendency to sublime.

*Alkyd Resin Production Processes.* Parallel to the above chemical procedures, the processing method may also be varied with different mechanical arrangements to remove by-product water, to drive the esterification reaction toward completion.

*Fusion Process.* In the fusion process, also frequently referred to as fusion cook, inert gas is continuously sparged from the bottom of the reactor to carry away water vapor from the reaction mixture. The exhaust is then either vented away or sent to a fume scrubber, which is frequently a small vessel with water atomizing nozzles. After the reaction is completed, the finished resin may be discharged, filtered, and packaged without solvent. More frequently, it is cooled to a safe temperature, then dissolved with the desired type and amount of solvent in a thinning tank, filtered, and packaged, or pumped to a storage tank. The reactor usually needs to be cleaned by charging a small volume of solvent into the vessel and heated to reflux for an appropriate time period. If deemed necessary, the vessel is further cleaned by digesting with caustic soda solution.

The fusion process has the advantage of simplicity in mechanical arrangement. However, it has several significant disadvantages. Low boiling and/or subliming ingredients, such as glycols and phthalic anhydride, would be lost during the reaction causing the product composition and its properties to deviate from the design. The material loss causes an increase in the cost of the resin. Reactants as well as the product may adhere to the reactor walls above the surface level of the charge, which will contaminate or even become catalyst poisons to the subsequent batches. And the resin produced from a fusion cook is more prone to develop dark colors. For these reasons, most of the manufacturers have discontinued the practice of fusion cook, unless it is dictated by the existing equipment.



**Figure 2.** Equipment for solvent processing of alkyd resins. Courtesy of Hercules Inc. (Wilmington, Del.).

*Solvent Process.* In the solvent process, or solvent cook, the water formed from the reaction is removed from the reactor as an azeotropic mixture with an added solvent, typically xylene. Usually between 3 to 10 weight percent, based on the total charge of the solvent, are added at the beginning of the esterification step. The mixed vapor passes through a condenser. The condensed water and solvent have low solubility in each other, and phase separation is allowed to occur in an automatic decanter. The water is removed, usually to a measuring vessel. The amount of water collected can be monitored as one of the indicators of the extent of the reaction. The solvent is continuously returned to the reactor to be recycled. A typical equipment for this process is shown in Figure 2. The reactor temperature is

modulated by the amount and type of refluxing solvent. Typical conditions are as follows.

Solvent	Weight Percent	Temperature (°C)
Xylene	3	251–260
Xylene	4	246–251
Xylene	7	204–210
High flash naphtha	10	204–210

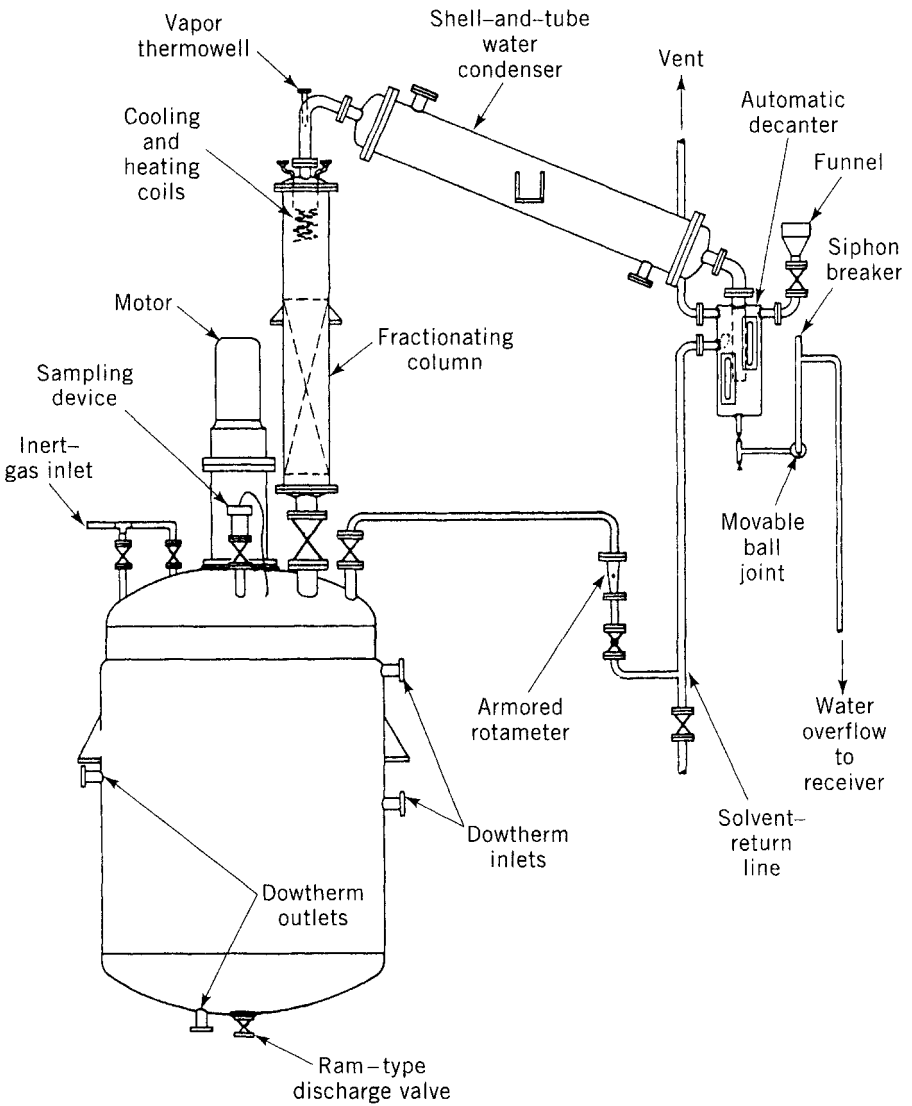
The solvent vapor also serves as a blanket in the reactor. The processing solvent is usually left in the product as part of the dilution solvent.

The refluxing solvent provides a constant wash to the reactor, and brings back the reactants that had escaped out of the reaction mixture. The reaction temperature is better controlled by the constant refluxing, and the viscosity of the reaction mixture is lower, which improves the effectiveness of the agitation. The product usually has better color and is more uniform than those made by the fusion process. Ordinarily, the reactor requires no more than a solvent wash to be clean enough for the next batch. These advantages far outweigh the higher cost of the production facility. Therefore, few would consider building a new alkyd plant without solvent process capability.

When low boiling ingredients such as ethylene glycol are used, a special provision in the form of a partial condenser will be needed to return them back into the reactor. Otherwise, not only would the balance of the reactants be upset and the raw material cost of the resin be increased, they would also become part of the pollutant in the waste water and incur additional water treatment costs. Usually, a vertical reflux condenser or a packed column is used as the partial condenser, which is installed between the reactor and the overhead total condenser (Figure 3). The temperature in the partial condenser is monitored and maintained at a level to effect a fractionation between water, which is to pass through the reactor, and the glycol or other materials, which is to be condensed and returned to the reactor. If the fractionation is poor and water vapor is also condensed and returned, the reaction will be retarded and result in a loss of productivity. As the reaction proceeds toward completion, water evolution slows down, and most of the glycol will have been combined into the resin structure. The temperature in the partial condenser may then be raised to facilitate the removal of water vapor.

### 5.9. Process Control

The progress of the alkyd reaction is usually monitored by periodical determinations of the acid number and the viscosity (solution in a suitable solvent and at an appropriate concentration) of samples taken from the reactor. The frequency of sampling is commonly every half hour. The general practice is to plot the determined values separately against time on semilogarithmic coordinants (Figure 4).



**Figure 3.** Solvent-processing equipment using a partial condenser. Courtesy of Hercules Inc. (Wilmington, Del.).

Toward the end of the reaction, the resin viscosity tends to increase exponentially. Gelation in the reactor is always a threat, due either to what the formulation would theoretically allow by the completion of the polyesterification or to the occurrence of some of the side reactions. After the onset of gelation, it would progress extremely rapidly and would be almost impossible to arrest. Therefore, it is routine to

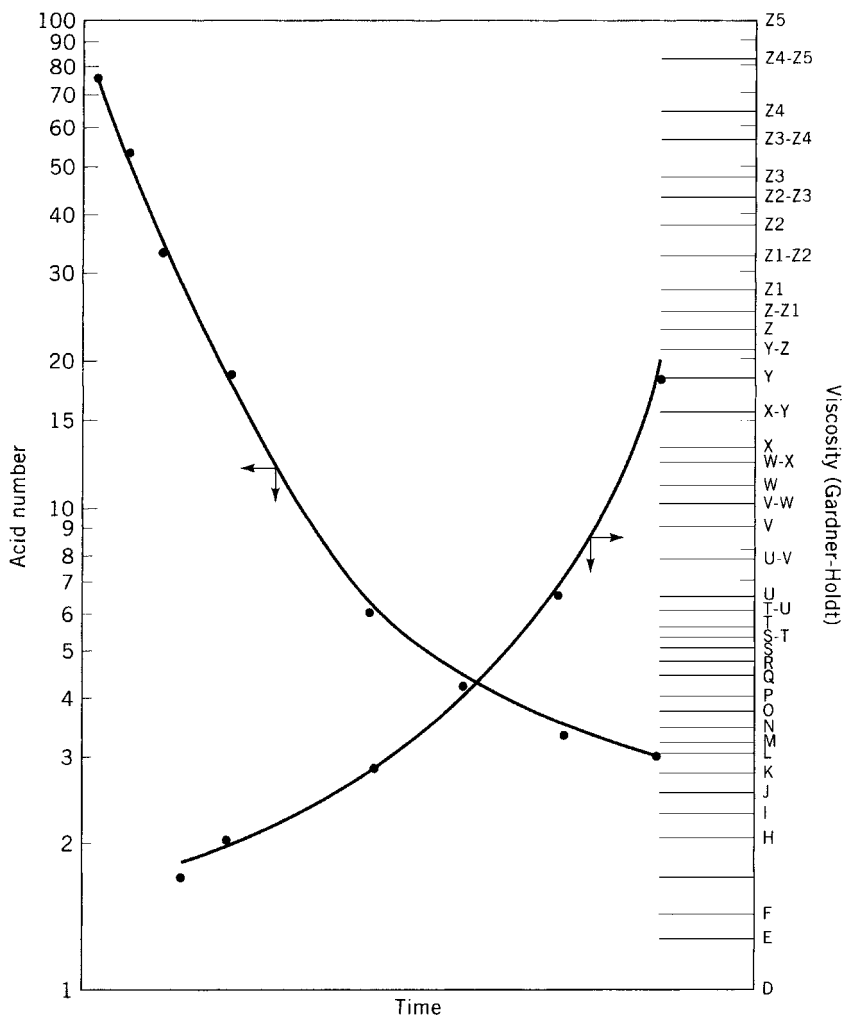


Figure 4. Alkyd reaction control plots.

extrapolate the plots in Figure 4 when predicting the point at which to terminate the reaction in time to prevent gelation. If gelation should occur in the reactor, it would cause not only the loss of product but also significant down time for cleaning the reactor. Some alkyd practitioners have found that a rapid addition of a large quantity of raw oil quenches the runaway gelation, disperses the gel, and significantly eases the cleanup operation. A technique of injecting water or steam during the condensation process to reverse or retard gelation has been reported in the patent literature (66).



## 6. SAFETY AND ENVIRONMENTAL PRECAUTIONS

The manufacturing of alkyd resins involves a wide variety of organic ingredients. While most of them are relatively mild with low toxicity, some of them such as phthalic anhydride, maleic anhydride, solvents, and many of the vinyl (especially acrylic) monomers are known irritants, or skin sensitizers, and are poisonous to humans. Persons involved should be thoroughly familiar with the hazard potential of each and every one of the chemicals by consulting the material safety data sheets provided by the suppliers and practicing the recommended safety precautions in handling the materials. The use of personal safety equipment such as protective goggles, gloves, clothing, and respiratory devices should be diligently observed. Since large quantities of highly flammable solvents are routinely handled in an alkyd plant, fire safety should be the utmost in everyone's mind. Electrical equipment and power circuitry in the plant should conform to all applicable codes, and all equipment should be properly grounded. The areas for the reactors and storage tanks should be separated by fire walls and must be adequately ventilated. The storage tanks should be blanketed by inert gas. A slight positive pressure of inert gas should be maintained in the reactor or storage tanks during discharge of the resin or resin solution to prevent air from being sucked into the vessel to form an explosive mixture with the solvent vapor.

With the ever increasing awareness of the need for environmental protection, the emission of solvent vapors and organic fumes into the atmosphere should be prevented by passing the exhaust through a proper scrubber. The solvent used for cleaning the reactor is usually consumed as part of the thinning solvent. Aqueous effluent should be properly treated before discharge.

## 7. MODIFICATION OF ALKYD RESINS BY BLENDING WITH OTHER POLYMERS

As mentioned earlier, one of the important attributes of alkyds is their good compatibility with a wide variety of other coating polymers. This good compatibility comes from the relatively low molecular weight of the alkyds and the fact that the resin structure contains, on the one hand, a relatively polar and aromatic backbone and, on the other hand, many aliphatic side chains with low polarity. The alkyd resin involved in a blend with another coating polymer may serve as a modifier for the other film former, or it may be the major film former and the other polymer may serve as the modifier for the alkyd to enhance certain properties. The following describes some of these compatible blends.

Nitrocellulose-based lacquers often contain a fair amount of short- or medium-oil alkyds to improve flexibility and adhesion. The most commonly used are short-oil nondrying alkyds. Amino resins or urethane resins with residual isocyanate functional groups may be added to cross-link the coating film for improved

solvent and chemical resistance. The major applications are furniture coatings, top lacquer for printed paper, and automotive refinishing primers.

Amino resins are probably the most important modifiers for alkyd resins. Butylated urea- or melamine-formaldehyde resins are compatible with alkyds. They react with the free hydroxyl groups of the alkyd to effect cross-linking and to impart hardness, mar resistance, chemical resistance, and durability to the coating. Short- or medium-oil alkyds of both drying and nondrying types are frequently used. Color and color retention requirements often dominate the choice of the alkyd. Many industrial baking enamels, such as those for appliances, coil coatings, and automotive finishes (especially refinishing enamels), are based on alkyd-amino resin blends. Some of the so-called catalyzed lacquers for finishing wood substrate require low bake or no bake at all.

Chlorinated rubber is often used in combination with medium-oil drying type alkyds. The alkyd gives better toughness, flexibility, adhesion, and durability, and the chlorinated rubber contributes to faster dry and better resistance to water and chemicals. The major applications are highway traffic paint, concrete floor, and swimming pool paints.

Vinyl resins of the type that are the copolymers of vinyl chloride and vinyl acetate and that contain a fair amount of hydroxyl groups (from the partial hydrolysis of vinyl acetate) and/or carboxyl groups (e.g., from copolymerized maleic anhydride) may be formulated with alkyd resins to improve application properties and adhesion. The blends are primarily used in making marine topcoat paints.

Synthetic latex house paints sometimes contain emulsified long-oil or very long oil drying alkyds to improve adhesion to chalky painted surfaces.

Silicone resins with high phenyl contents may be used with medium- or short-oil alkyds as blends in air-dried or baked coatings to improve heat and weather resistance, whereas the alkyd component contributes to adhesion and flexibility. Major applications include insulation varnishes, heat-resistant paints, and marine coatings.

## 7.1. Chemically Modified Alkyd Resins

While blending with other coating resins provides a variety of ways to improve the performance of alkyds, or vice versa, chemically combining the desired modifier into the alkyd structure would eliminate the compatibility problem and give a more uniform product. Several such chemical modifications of the alkyd resins have gained commercial importance, and are described below.

Vinylated alkyds are alkyd resins that have been incorporated with a significant amount (20–60% by weight) of vinyl monomers (such as styrene, vinyl toluene, and methyl methacrylate) by grafting the monomers through a free-radical mechanism onto unsaturated reaction sites in the resin molecules. The modified resin embodies the good attributes of ease of application, good wetting, and adhesion from the alkyd as well as fast solvent release, hardness, and weather resistance from the vinyl modification. The common objective of such a modification is for achieving a drying rate comparable to that of lacquer materials. The reaction sites on alkyd resin molecules are primarily the allylic carbons on unsaturated fatty acid chains

and the double bond of  $\alpha,\beta$ -unsaturated dibasic acids. Free radicals, generated from the thermolysis of such free-radical initiators as benzoyl peroxide, dicumyl peroxide, and di-*t*-butyl peroxide are usually required to kick off the reaction. Ideally, the initiating species would attack the active sites on the resin molecules and all of the added monomers would be evenly distributed in grafted side chains. In reality, it is inevitable that part of the monomers would engage in homopolymerization, and some of the resin molecules would remain unmodified. The presence of a large amount and high molecular weight homopolymer of the vinyl monomer would lead to incompatibility and result in a hazy product. Methods that have been used to minimize homopolymerization include using fatty acids having conjugated diene structure, using maleic anhydride as part of the dibasic acids for the alkyd, choosing initiators such as peroxides or hydroperoxides that tend more to extract allylic protons than to add to double bonds, avoiding initiators that would decompose at very low temperatures, adding the monomer gradually along with an appropriate amount of the initiator, choosing monomers that would have a more favorable tendency to copolymerize with the active species on the alkyd resin, and properly maintaining the reaction temperature. Chain transferring is the preferred mechanism for terminating chain growth from the addition polymerization of the monomer. Usually, the solvent, the fatty acid chain, and the monomer are effective chain-transfer agents. If an additional transferring agent is used, care must be exercised, or too much of it could cause the formation of a large amount of very low molecular weight homopolymers, and would result in poor film properties. Occasionally, vinylation is first performed on the fatty acids or the oil before the alkyd reactions.

It should be emphasized that the presence of a large amount of either conjugated fatty acids or maleic anhydride in the alkyd formulation gives rise to a high degree of probability of premature gelation during the alkyd reaction. An allowance must be made in the alkyd formulation, and the polyesterification is frequently terminated at a relatively high acid number (about 15), to avoid gelation. It has been reported that the optimum amount of maleic anhydride in the alkyd is an amount having a maleic group in one-third of the resin molecules (17).

A common procedure for the preparation of vinylated alkyds is as follows: first, a base alkyd resin is brought to the desired end point. The resin is then cooled to about 160°C and often diluted with aromatic thinner. Next, the desired monomer is added, usually at about 20–60% based on the final product, followed by an appropriate amount of a free-radical initiator. Alternatively, a premix of the monomer and the initiator is added at a controlled rate over most of the reaction. Then the reaction is brought to monomer reflux, until the residual monomer content has dropped below a specified level. The residual monomer, if any, is stripped away before the product is diluted in a solvent, filtered, and packaged.

Silicone alkyds are etherification products of alkoxy-polysiloxane oligomers and the free hydroxyl groups of alkyd resins (67, 68). The property improvements and applications are similar to those of the alkyd-silicone blends, with the added advantage of incorporating the stable  $-\text{Si}-\text{O}-\text{C}-$  structure into the alkyd molecules. The preferred silicone oligomers are those with high phenyl contents, and

the alkyds at long- or medium-oil length based on polyols with primary hydroxyls. To improve the thermal stability effect imparted by the silicone modification further, one can use isophthalic alkyds rather than the phthalic type. The etherification reaction may be carried out on an alkyd resin designed for the purpose or on the polyol before it is used in alkyd preparation. The silicone content of the modified alkyd lies usually between 20 and 60% of the total product.

Urethane alkyds, or uralkyds, are alkyds with a part or even all of the dibasic acids replaced by diisocyanates. The isocyanate group,  $-\text{N}=\text{C}=\text{O}$ , reacts with the hydroxyl group of a polyol, at low temperature, to form a urethane linkage,  $-\text{NHC}(\text{O})\text{O}-$ , without spitting out water as a by-product. Toluene diisocyanate (TDI) is commonly used for such modification. It is commercially supplied as an 80:20 mixture of 2,4- and 2,6-isomers. The  $-\text{NCO}$  group *para* to the methyl group has about 8 times greater reactivity than the one on the *ortho* position, which aids greatly the control of the reaction. Since the NCO group is reactive with labile protons, water must be excluded from the reaction system. The esterification reaction of the base alkyd must be brought to the desired end point with the by-product water removed, and the temperature lowered to about  $100^{\circ}\text{C}$  to prevent any continuation of the esterification before the introduction of the NCO reactant. TDI is highly toxic and is usually handled in a closed system under a dry inert gas blanket. Metallic soaps, such as dibutyltin dilaurate, stannous octoate, and calcium naphthate, are used as reaction catalysts. The reaction is vigorous and exothermic. Therefore, the reaction temperature is maintained under  $135^{\circ}\text{C}$ , and great care must be exercised to bring the reaction under proper control.

Uralkyds have superior adhesion, hardness, abrasion resistance, durability, and chemical resistance to the unmodified alkyds. They find major applications in wood floor finishes, marine coatings, metal primers, and maintenance paints.

Phenolic resins are well known for their contribution in improving hardness, gloss, and water and chemical resistance in oleoresinous varnishes. Those based on *p*-alkyl-substituted phenols and with heat-reactive methylol groups have also been incorporated into alkyd resins. The reaction has not been well studied. Presumably, the methylol group would react with the unsaturation functionality on the fatty acid chain to form the chroman structure, similar to what is believed to have occurred in the varnish. Etherification between the methylol group and free hydroxyl of the alkyd resin, catalyzed by the residual acidity in the resin, would be another possible reaction.

Polyamide modified alkyds show a special rheological behavior—they are thixotropic (69, 70). Typically, the polyamide resin would be of the type based on dimer acids, i.e., dimerized unsaturated fatty acids, and aliphatic diamines, such as ethylene diamine. These would react to form polyamide resins with low acid and amine values. The alkyd resin would be a medium- or long-oil drying alkyd. The reaction products from the polyamide and the alkyd are gel-like materials that undergo a time-dependent shear thinning and recover to the gel-like state after the shearing action is stopped. This allows the preparation of “no-drip” paints, which are easy to brush and can be applied at high film thickness from a single coat with little or no danger of sagging. Pigment settling during storage of the paint is also

minimized. The major applications are flat oil-based architectural paints and maintenance paints. Generally, up to 10% of the polyamide based on the weight of the alkyd is added to the alkyd and heated at normal alkyd reaction temperature under agitation. Ester interchange reaction takes place, and fragments of the polyamide resin become chemically bonded to the alkyd. The modified alkyd serves as a compatibilizer for the mutually insoluble unreacted polyamide and alkyd to form a gellike structure. The stiffness of the structure decreases with the increasing amount of the compatibilizer. If the reaction is allowed to continue, and there is no unreacted polyamide left in the system, little or no thixotropy will be exhibited. Therefore, the reaction must be precisely controlled to give the desired degree of thixotropy. Aromatic solvents such as xylene tend to destroy the thixotropic structure. Therefore, they must be reduced to less than 0.5% in the product. Polyamide modified alkyd resins are available commercially to be used as additives for making thixotropic alkyd paints.

## 7.2. High Solids Alkyds

There has been a strong trend in recent years to increase the solids level of all coating materials, including alkyds, to reduce solvent vapor emission. To raise the solids level and still maintain a manageable viscosity, the molecular weight of the resin must be reduced. Consequently, film integrity must be developed through further chain extension and/or cross-linking of the resin molecules during the “drying” step. A high cross-linking density necessitated by the lower molecular weight of the resin would build a high level of stress in the film, and cause it to be prone to cracking. Therefore, adequate flexibility should be designed into the resin structure. This means that the distance between the hydroxyl groups of the polyol and the carboxyl groups of the dibasic acid would need to be lengthened by linear linkages. Thus long-chain diols, polyether polyols, and linear  $\alpha,\omega$ -dibasic acids would not only build in more flexibility but also reduce the viscosity for high solids alkyds, due to the greater spacing of polar ester groups and the reduction of aromaticity in the resin structure. In addition to the manipulation of resin molecular structure for increasing coating solids, the use of more active, though more expensive, oxygenated solvents also serves to reduce the viscosity of resin solutions.

Chain extension and cross-linking of high solids alkyd resins are typically achieved by the use of polyisocyanato oligomers or amino resins. An adequate amount of excess hydroxyl groups must be designed into the alkyd structure to provide reaction sites for these modifiers. To limit the molecular weight of the alkyd resin, the molar ratio between polyols and dibasic acids should be greater than 1. The hydroxyl functionality of the formulation should be controlled by a careful selection of polyols to avoid an overpresence of free hydroxyl groups in the product, which would adversely affect water resistance and other properties of the coating film. Most of the high solids alkyd systems are used in industrial baking finishes. For air drying applications, higher doses of driers are usually needed to achieve acceptable drying rate (71).

### 7.3. Water-Reducible Alkyds

Replacing solvent-borne coatings with water-borne coatings would not only reduce solvent vapor emission but also improve safety against the fire and health hazards of organic solvents. Alkyd resins may be rendered water-reducible by either converting the resin into an emulsion form or by incorporating “water-soluble” groups in the molecules. The latter will be the subject for further discussion.

The most common approach for imparting water solubility in an alkyd resin is to leave enough pendent carboxyl groups in the resin and to neutralize them with a fugitive base, such as ammonia or low molecular weight amines, to build ionic characteristics into the resin. Nonfugitive base materials, such as caustic soda, would leave the salt in the coating film and damage its water- and corrosion-resistance. Trimellitic anhydride (TMA) is the most frequent choice of ingredient to provide the pendent carboxyl groups. It was reported that glycerol gives resins with poor hydrolytic stability (72). Therefore, polyols with primary hydroxyl groups are preferred for the preparation of water-soluble alkyds.

The recommended procedure (37) for the preparation of water-soluble alkyds is to hold off the TMA in the initial stage of the alkyd reaction so that the high functionality of TMA would not be a cause of gelation. When the reaction has progressed to a desired low acid number, i.e., the building of the polymer chain is completed, the temperature is lowered to 180°C; the TMA is then added and maintained at that temperature until a desired acid number, usually about 50–60, is reached. At such a temperature, only the anhydride group of the TMA would react to form half esters, and the remaining two carboxyl groups would essentially remain unreacted. If one desires to have the TMA participating in the backbone structure of the resin, a part of the TMA is charged in the beginning of the alkyd reaction, often with the presence of an appropriate amount of a monohydric alcohol, such as benzyl alcohol, to balance the functionality of the system. The remaining TMA is then added in the same manner as described to “end cap” the resin and to provide pendent carboxyl groups for water solubilization.

The finished resins are usually dissolved in oxygenated coupling solvents, such as glycol ethers, to improve the solubilization of the resin in aqueous media and the handling of the resin. Water and the base are premixed and added to the resin solution when needed. The coupling solvents usually have higher boiling points than water. During the drying process, the solvent would be enriched in the coating film as water evaporates preferentially. The resin molecules would become better solubilized, i.e., molecular chains would be extended, and result in better formation and integrity of the film.

## 8. ECONOMIC ASPECTS

Alkyd resins as a family have remained the workhorse of the coatings industry for decades. In the United States, the total consumption of alkyds increased from about 200,000 t in the mid-1950s to more than 300,000 t in the mid-1960s. It peaked in

1973 at about 345,000 t, constituting about 33% of all synthetic coating resins. In 1980, alkyds still accounted for 30% of the 1,090,000 t of all resins consumed for coatings. From 1987 to 1989, although the consumption maintained at about 300,000 t/year, its market share among all coating resins was reduced to 26% in 1987 and 25% in 1989. At present, 55–60% of the alkyd resins consumed in the United States are used for architectural coatings. A decline in consumption is expected because of regulations involving VOCs (volatile organic emissions). California and the Northeastern United States are expected to adopt regulations that will severely restrict the use of solvent-borne coatings (73).

The overall demand in Europe is expected to decrease at the rate of 3%/yr over the next five years. Environmental regulations are expected by 2007. Japan's consumption has declined, but has stabilized because high performance alternatives have already replaced the coatings in question.

The industry was hard hit in 2003–2004 with higher prices for raw materials such as linseed and soybean oil. Alkyd producers are already developing new water-borne products (73).

Other uses for alkyds are in general industrial coatings such as machinery and metal furniture. Alkyd resin-chlorinated rubber based coatings are used in traffic paints, but use is declining because of VOC concerns. Some alkyds are still used in refinish paints for automobiles. Uralkyds are used as a vehicle for urethane varnishes for the do-it-yourself market.

## 9. FUTURE PROSPECTS

Stemming from the drive by the coatings industry to reduce solvent emission, there has been a clear trend of gradual decline in the market share of alkyd resins. However, their versatility and low cost will undoubtedly continue to keep alkyds as major players in the coatings arena. Alkyds are much more amenable to move toward higher solids than most other coating resins. Great strides in the development of water-borne types have also been made in recent years. There is one more good reason to remain optimistic about alkyds for the future—a significant portion of their raw material, fatty acids, is renewable.

## REFERENCES

1. A. E. Rheineck, *J. Paint Technol.* **44**(566), 35–54 Apr. 1972.
2. *A History of Paint and Color*, Pittsburgh Plate Glass Co., 1951.
3. *An Overview of the Paint and Coatings Industry*, SRI International, Aug. 1992.
4. D. Deffar and M. D. Soucek, *J. Coat. Technol.* **73** (919), 95 (2001).
5. *Urethane Surface Coatings*, SRI Consulting, Oct. 2004.
6. *Epoxy Surface Coatings*, SRI Consulting, Oct. 2004.
7. Z. W. Wicks, Jr. and F. N. Jones in Z. W. Wicks, F. N. Jones, and S. P. Pappas, eds., *Organic Coatings Science & Technology*, Vol. 1, John Wiley & Sons, Inc., New York, 1992, Chapt. 9.

8. A. E. Rheineck and R. O. Austin in R. R. Meyers and J. S. Long, eds., *Treatise on Coatings*, Vol. 1, Part 2, Marcel Dekker, Inc., New York, 1968, Chap. 4.
9. D. N. Rampley and J. A. Hasnip, *J. Oil Colour Chem. Assoc.* **59**, 356–362 (1976).
10. O. S. Privett, W. O. Lundberg, N. A. Khan, W. E. Tolberg, and D. H. Wheeler, *J. Am. Oil Chem. Soc.* **30**, 61, (1953).
11. S. A. Harrison and D. H. Wheeler, *J. Am. Chem. Soc.* **76**, 2379 (1954).
12. O. S. Privett and C. J. Nickell, *J. Am. Oil Chem. Soc.* **33**, 156 (1956).
13. W. J. Bailey and G. L. Barlow, *J. Paint Technol.* **42**(544), 287 (1970).
14. E. H. Farmer and A. Sundralingam, *J. Chem. Soc.* **1942**, 121.
15. E. H. Farmer and D. A. Sutton, *J. Chem. Soc.* **1942**, 139.
16. J. L. Bolland and G. Gee, *Trans. Faraday Soc.* **42**, 236, 244 (1946).
17. E. H. Farmer, *Trans. Faraday Soc.* **42**, 228 (1946).
18. E. H. Farmer, *Trans. Inst. Rubber Ind.* **21**, 122 (1945).
19. F. D. Gunstone and T. P. Hilditch, *J. Chem. Soc.* **1946**, 1022.
20. N. A. Khan, *Can. J. Chem.* **32**, 1149 (1954).
21. N. Uri in W. O. Lunberg, ed., *Autoxidation and Antioxidants*, Vol. 1, Wiley-Interscience, New York, 1961, Chapt. 2.
22. F. W. Heaton and N. Uri, *J. Lipid Res.* **2**, 152 (1961).
23. T. H. Smouse and S. S. Chang, *J. Am. Oil Chem. Soc.* **44**, 509 (1967).
24. H. P. Kaufmann, *Fette Seifen Anstrichmit.* **59**, 153–162 (1957).
25. O. S. Privett, *J. Am. Oil Chem. Soc.* **36**, 507 (1959).
26. R. N. Faulkner, *J. Appl. Chem.* **8**, 448–458 (1958).
27. R. R. Allen and F. A. Kummerow, *J. Am. Oil Chem. Soc.* **28**, 101 (1951).
28. A. E. Rheineck and S. C. G. Peng, *Official Digest* **36**, 878 (1964).
29. L. A. O'Neill, *Chem. Ind.* **1954**, 384–387.
30. K. Hultsch, *J. Prakt. Chem.* **158**, 275 (1941).
31. R. H. Kienle and C. S. Ferguson, *Ind. Eng. Chem.* **21**, 349 (1929).
32. R. H. Kienle, *Ind. Eng. Chem.* **41**, 726 (1949).
33. R. G. Mraz and R. P. Silver in *Encyclopedia of Chemical Technology*, 2nd ed., Vol. 1, John Wiley & Sons, Inc., New York, 1963, pp. 851–880.
34. Z. W. Wicks, Jr., in *Encyclopedia of Chemical Technology*, 5th ed., Vol. 2, John Wiley & Sons, Inc., Hoboken, New Jersey, 2004, pp. 147–169.
35. C-D-I-C Society for Paint Technology, *Official Digest* **430**, 1477 (Nov. 1960).
36. Bulletin IP4, Amoco Chemicals Corp., Chicago, Oct. 1960.
37. Bulletin IP-65b, Amoco Chemicals Corp., Chicago, 1990.
38. Technical Bulletin No. 524-5, Velsicol Chemical Corp., Chicago.
39. R. I. Stirton in *Encyclopedia of Chemical Technology*, 1st ed., p. 595, Interscience Publishers, New York, 1953.
40. Bulletin TMA 27, Amoco Chemicals Corp., Chicago.
41. *IMC Polyols: A Complete Guide*, International Minerals & Chemicals Corp., Des Plaines, Ill., 1980, p. 9.
42. *Trimethylolpropane in Alkyd Coating Resins*, Celanese Chemical Co., New York, 1961, p. 4.



43. L. Klee and G. H. Benham, *J. Am. Oil Chem. Soc.* **27**, 130–133 (1950).
44. A. E. Rheineck, F. D. Williamson, and D. DeClerk, *Am. Chem. Soc. Div. Org. Coat. Plast. Chem.* **22**(1), 24–34 (1962).
45. W. H. Carothers, *J. Am. Chem. Soc.* **51**, 1548 (1929).
46. P. J. Flory, *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, N.Y., 1953, Chapt. 9.
47. W. H. Carothers, *Trans Faraday Soc.* **32**, 43 (1936).
48. T. C. Patton, *Alkyd Resin Technology, Formulating Techniques and Allied Calculations*, Wiley Interscience, New York, 1962.
49. E. G. Bobalek, E. R. Moore, S. S. Levy, and C. C. Lee, *J. Appl. Polym. Sci.* **8**, 625–657 (1964).
50. D. H. Solomon, B. C. Loft, and J. D. Swift, *J. Appl. Polym. Sci.* **11**, 1593–1602 (1967).
51. D. H. Solomon and J. J. Hopwood, *J. Appl. Polym. Sci.* **10**, 1893–1914 (1966).
52. J. Kumanotani, H. Hata, and H. Masuda, *Org. Coat.* **6**, 35–54 (1984).
53. H. Kiryu, K. Horiuchi, K. Sato, and J. Kumanotani, *Shikizai Kyokaiishi* **57**(11), 597–601 (1984).
54. H. Hata, H. Tomita, Y. Nishizawa, and J. Kumanotani, *Fatiproc Cong.* **15**(3), 111–485/509 (1980).
55. H. Hata, J. Kumanotani, Y. Nishizawa, and H. Tomita, *Fatiproc Cong.* **14**, 359–364 (1978).
56. N. M. Wiederhorn, *Am. Paint J.* **41**(2), 106 (1956).
57. R. P. Silver, *Alkyd Report No. 8*, Hercules Inc., Wilmington, Del.
58. R. P. Silver, *Alkyd Report No. 1.2*, Hercules Inc., Wilmington, Del.
59. T. C. Patton, *Off. Digest Fed. Paint Varnish Prod. Clubs* **430**, 1544 (1960).
60. Y. Tanizaki and T. Yoshida, *Shikizai Kyokaiishi* **45**(2), 83–87 (1972).
61. T. Nagata, *J. Appl. Polym. Sci.* **13**, 2601–2619 (1969).
62. T. Yoshida, *Kobunshi Kagaku* **22**, 769 (1965).
63. *Alkyd Report No. 1.5*, Hercules Inc., Wilmington, Del.
64. L. W. Chen and J. Kumanotani, *J. Appl. Polym. Sci.* **9**, 3649–3660 (1965).
65. W. M. Kraft, G. T. Roberts, E. G. Janusz, and J. Weisfeld, *Am. Paint J.* **41**(28), 96 (1957).
66. W. B. Callahan and B. F. Coe (to E. I. du Pont de Nemours & Co.), U.S. 4,045,932 (Aug. 30, 1977).
67. A. F. Karim, B. Golding, and R. A. Morgan, *J. Chem. Eng. Data* **5**, 117 (1960).
68. *Silicone Notes 7-701, 7-702a, 7-703*, *Technical Bulletin*, Dow Corning Corp., Midland, Mich.
69. A. G. North, *J. Oil Color Chem. Assoc.* **39**(9), 696 (1956).
70. P. Oldring and G. Hayward, eds., *Resins for Surface Coatings*, SITA Technology, London, 1987.
71. J. Larson, *Am. Paint Coat. J.*, 56–61 (Apr. 4, 1984).
72. M. Lerman, *J. Coat. Technol.* **48**(12), 37–42 (1976).
73. Alkyd/Polyester Surface Coatings, SRI Consulting, Jan. 2005.

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## *Leather and Textile Uses of Fats and Oils*

Paul Kronick and Y.K. Kamath

### 1. GENERAL USE OF FATS AND OILS IN LEATHER

Although most of the 20-odd steps of leathermaking use fats and oils as detergents, by far the majority of these materials are used as additives to soften leather. Oils are usually applied in aqueous emulsions while the leather is still wet from tanning. Several oils are conventionally used, often made self-emulsifying by partial sulfation or sulfonation by treating with sulfuric acid. These oils are called *fatliquors*. Heavy grades of leather are also softened by the addition of neat melted fat after the leather has been dried. This “hot stuffing” process involves softening the leather by milling it in the presence of the warm molten fat. Although the milling by itself is sufficient to soften the leather, the fat dispersed among the fibers allows the leather to remain soft if it is dampened and dried again. The amount of these materials that are used annually has not been compiled, but it is over 20 million pounds per year.

Aside from softening, a smaller amount of oils and fats as detergents are also used in preliminary cleaning of the raw skins and hides; to suspend lime particles, buffer the alkaline solution that removes the epidermis and hair, and suspend these materials when they come off; to remove grease; to tan certain types of leather; and to control the penetration of dyes.

## 2. SOFTENING OF LEATHER

### 2.1. Drying Skin or Hide

The skin or hide of an animal is an organ whose complex structure can be simplified for purposes of analysis into two layers: a thin (<1 mm) epidermis and a thicker (up to 1 cm) dermis. The epidermis, bearing the hair, is removed early in the steps of leathermaking. The dermis comprises very long fibrils, 100 nm in diameter, composed mostly of the protein collagen type I. The fibrils are organized into interwoven bundles. Among the fibrils is a concentrated solution or network of glycoproteins and, immediately after slaughter, blood plasma. There are also blood vessels and nerves, which are removed during leathermaking before the tanning (collagen crosslinking) step, which is the last step before the addition of fatliquor.

If skin or hide is dried, even minus the hair and epidermis, it becomes a hard material, like horn. If the skin is thin, this product can be used as a tough parchment, but little else. This behavior is attributed to the proteins and proteoglycans found among the fibers of the hide, which act as a glue when they dry.

If the skin is agitated with lime-buffered water (pH = 12.5), it swells enormously, and most of the glue-like material is extracted. On neutralization, however, the fibers become flaccid and, on drying, stick together to again form a hard mass. Before being dried for leather, however, the extracted skin is tanned: the collagen protein of the fibers is crosslinked to make them stiffer. The resulting product can be dried to a leathery material that is stiff, but much more flexible than that dried without tanning. To soften tanned skin, one can mill it by lifting and dropping it in a large rotating drum or in a staking machine, which pounds it with small hammers. The result is not satisfactory, however, for a number of reasons, including the effect on the integrity of the surface of the leather and the inability to rewet the leather without making it become stiff again.

### 2.2. Use of Oil or Fat

If, after tanning, a soluble resin is added and the leather is dried and milled in the presence of melted grease, a flexible but resilient product is obtained that can be rewet, within limits, and dried again to the same esthetically pleasing product. This hot-stuffed leather is actually a commercial product used for the upper parts of shoes. The product is not stable, however, to dry cleaning, which removes the grease. The grease removed from this dry-cleaned leather must be replaced.

It is not necessary to depend on mechanical milling to make soft leather. Usually, after the hide is tanned, it is tumbled in a drum with an oily emulsion (fatliquor). On drying, the emulsion droplets coalesce among the fibers, producing a soft leather containing 10–15% oil on basis of dry weight. Although this leather can be wet again and dried without becoming stiff, the organic solvents used in dry cleaning can extract the oils as they do to the fats in hot-stuffed leather.

There are other drawbacks to conventionally fatliquored leather. When it is used in automobile upholstery, it is often heated by sunlight in the unstirred air of the parked vehicle to temperatures high enough to sublime or distill the fat or oil onto the windows. This deposited material is troublesome to remove and causes some lots of leather to be rejected by the automobile manufacturer. Another drawback is flammability, which can be obviated by a judicious choice of oil in the emulsion. Further, the oil sometimes tends to migrate to the surface of the leather, causing an unpleasant film, or spew, to form. Under some conditions it can cause separation of the finish or coating from the leather substratum.

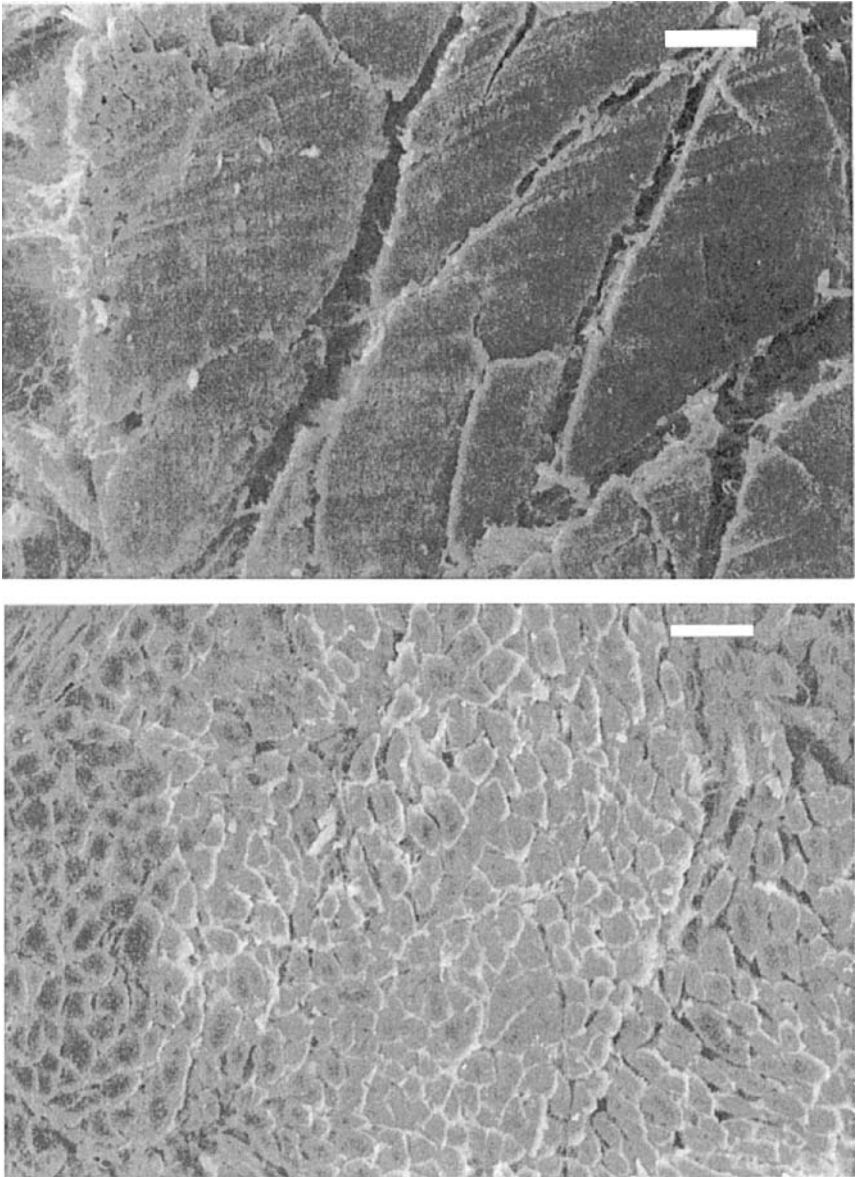
### 3. SOFTENING WITH FATLIQUOR

#### 3.1. Physical Mechanism

It is not clear exactly why oil, added as a fatliquor emulsion when the leather is wet, causes the leather to dry to a soft product. The most cogent hypothesis derives from the observations of von Fuchs (1), who noticed that the oil could be removed from the dried leather with solvent without causing it to become stiff. One concludes that the fatliquor prevents the fibers from sticking together during the drying but afterward is not needed. There is uncertainty, however, about what comprises a fiber. We have evidence that it is a bundle of the unitary 100-nm collagen fibrils, perhaps 10  $\mu\text{m}$  in diameter (2) (Figure 1). The interaction of collagen and sulfated oils has been studied (3).

The emulsion of a fatliquor should not be too stable. The leathermaker wants to control its depth of penetration into the leather by the tendency of the surfactant to adsorb to the protein of the hide. This adsorption depletes the micelles of surfactant, causing the emulsion to break before it permeates the interior of the hide, releasing the oil somewhat superficially. The untreated interior thereby retains its stiffness, but with only a small mechanical moment. The product is then firm but resilient (not like cardboard). The released oil, with the surfactant tightly bound to the fibers, also resists being washed out of the leather by water. Suede leather, on the other hand, requires full penetration of the fatliquor and, therefore, an emulsifier that does not bind tightly to the protein.

As noted, when a leather is dried without fatliquor, the fibrils adhere and form irregular fiber bundles with a linear cross-sectional dimension of about 100  $\mu\text{m}$ . When the leather is still wet in the fatliquoring step, however, the emulsion is distributed among the fibrils. As the fatliquored leather dries, the fibrils still tend to adhere to each other but with weakened adhesive force because of the presence of the oil. We believe that the lateral forces, due to shrinkage of the bundles of fibrils as water evaporates, causes them to rupture into the 10- $\mu\text{m}$  fibers. What is important is that the eventual softness of the leather would depend on the topology of the fragments rather than directly on the rheological or chemical properties of the oil. The same geometric effect should be achieved by mechanical milling of the dried leather in the presence of grease or oil, as in hot stuffing.



**Figure 1.** Effect of fatliquor on the structure of fibers in leather after drying. (Top) The 100- $\mu\text{m}$  fiber bundles in the leather dried without fatliquor are intact. (Bottom) They are broken into fragments when dried with fatliquor, making the leather compliant. Bar = 10  $\mu\text{m}$ .

### 3.2. Components and Formulation

In formulating a fatliquor, one is concerned with the chemical interactions with the substance of leather fibers, the dyes, and the tanning agent, mostly hydrated chromic oxides. Mostly, these interact with the emulsifier. With the likely introduction of substitutes for chromium, such as aluminum or vanadium, the reactions of the emulsifiers will have to be adjusted. The surface charges of the new inorganic materials will be different from that of chromium, affecting the tendencies of various emulsions to coalesce. This chemistry can be adjusted by changes in pH and perhaps will lead to other requirements on lengths of the fatty chains.

The neutral portion of the fatliquor can be almost any oil. Triglycerides are most common, but wool grease and mineral oil are also used. The oil is chosen to be nonvolatile (for permanence and to prevent the fogging effect described above), minimally soluble in water, and liquid at about 40°C (and so emulsifiable). Longer carbon chains (above 14) are better. This has been attributed to retention of the oil among, rather than within, the fibrils (4). Mixtures of different oils are usually used. Esthetic properties are resistance to discoloration and hardening and lack of unpleasant odor. An important chemical property for an oil is that it should be easily sulfonated, sulfated, or oxidized so that it can be the starting material for its own emulsifier. In fact, the most commonly used fatliquors are partially sulfonated oils, adjusted to the correct degree of sulf(on)ate by addition of more base oil. One of the most common oils is castor oil, which can be both sulfated and sulfonated. Others commonly used are fish oils, especially from cod; neatsfoot oil, rendered from the bones of cattle; rice bran oil; soy oil; coconut oil; linseed oil; and rapeseed oil.

Sulf(on)ation is not the only method used to prepare fatliquor from oil. Simple oxidation of fish oil with air or nitric acid is another, again causing a portion of the oil to become amphiphathic. The products are often referred to as "moellons" or "degras."

Leather softeners that are technically satisfactory can also be made from jojoba oil (5) and even butadiene rubber (6). Waste animal fat obtained from scraping the flesh from the fresh hide, before the leathermaking process is begun, can be used after sulfation or ethoxylation.

Although neat fats and greases can be added to the leather after it has been dried, oils must be emulsified if they are to be used during the wet end of the leathermaking process. Fortunately, most oils can usually be sulf(on)ated or oxidized easily, making them amphiphilic; the usual degree is 50% of the triglyceride molecules, so that an oily emulsion can be prepared with excess neutral oil. By artful formulation, the properties of the emulsion are carefully controlled with regard to the viscosity and size of the particles and their affinity for the leather fibers and the chromic oxide tanning agent. These properties affect the ability of the emulsion particles to penetrate the leather. Castor oil, unlike fish oil, yields the sulfated as well as the sulfonated product when it is treated with sulfuric acid; its emulsions have a greater tendency to break in the presence of electrolytes. When used as fatliquors, its emulsions tend to break in the superficial regions of the leather, releasing their oils there

rather than deeper inside. This allows the leathermaker a means to control deposition of oil “stratigraphically.” As already described, this permits adjustment of the mechanical properties and leather esthetics.

A balance of properties of the product can be obtained by formulations based on natural oils to which sulf(on)ated oils are added. The mixtures can then be optimized statistically with respect to the mechanical properties (7).

Aside from sulf(on)ates, carboxylates (soaps) and phosphates can also be used in the surfactant. An emulsion based on alkyl phosphate emulsifier is reported to give an especially soft leather (8). Phosphates are reviewed by Fricke and Haeussler (9). There is also experience with sulfosuccinic acid amides (10) and other surfactant formers.

Before the growth of organic chemistry and techniques for sulfonating fats and oils, tanners emulsified oils for leather softeners with soap and protective colloids. These mixtures were prepared as water-in-oil emulsions, called “mayonnaise” in the trade and added to the water in the drum with the leather. They are still used but not nearly as frequently as sulf(on)ated oils. On the other hand, salts of naphtheneic acid are used with hydrocarbon oils (e.g., 11) to make the reverse forms of these.

Egg yolk was once commonly used, probably because of its lecithin, to emulsify oils in leathermaking. Lecithin itself is effective as a fatliqour emulsifier (12). Non-ionic emulsifiers have the advantage of forming emulsions that are stable to electrolytes, so they do not interact electrostatically with the chromium or aluminum tanning ions. Nonionic–anionic combinations (e.g., 1% nonionic–99% anionic) are now used to suppress the accumulation of fatty deposits or extrusions at the surface of the leather. These systems are under active development.

Cationic surfactants are used mainly to treat the surface of the leather, making it waxy. These are typically C-16 to C-18 alkylbenzyl quaternary ammonium compounds (e.g., 13). Also effective are cationic fatliquors from aminated and quaternized castor oil; other vegetable oils are also used (14).

Oils are frequently chlorinated in addition to being sulf(on)ated or oxidized. The reaction is extended to both the neutral oil and the surfactant, to make them less volatile and more flame resistant. Fish oil is the usual substrate for this treatment, but it has been extended to fatliqour from mineral oil and paraffin wax (e.g., 15).

#### 4. OTHER SOFTENING MATERIALS

Leather is also softened, after it is dried, by the addition of grease at temperatures at which it is fluid. These “stuffing compounds” are made from wool grease or high-melting (230–266°C, 110–130°F) mixtures of mineral waxes and fatty acids. For reasons that are not understood, they must be used at much higher levels than fatliquors—usually about 30% of the weight of the leather, instead of 10–15%.

The development of new softening materials is considered to be a high priority by the international leather industry. Research to find materials that give softer leather for garments without the drawbacks of greasy feel, odor, and cost that attend

increasing the amounts used is proceeding in many laboratories in many countries (16). Research on leather softeners is driven by the same considerations everywhere: the need to find new sources of oils and greases that are of constant quality, inexpensive, convertible to emulsifiers, color-fast, and preferably colorless, odorless, fixed well in the leather, and ecologically acceptable.

Examples of such developments are sulfation of transesterified rapeseed oil phosphatides (17) and the use of bicontinuous microemulsions obtained by the addition of aliphatic alcohols (18, 19). The stability of these systems promises to be more reliable than those in present use. Oils from wood (tall oil) can be sulfonated for self-emulsifying fatliquors (20). Polymerizable oils have been used in fatliquors (21). We expect more progress on the use of high polymers, such as the already commercial alkyl acrylate esters developed by Hodder et al. (22–24), and material based on elastomers (6). We anticipate the development of novel systems to be encouraged by the demand for leather in washable garments and automobile upholstery.

## 5. EVALUATING EFFECTS OF FAT IN LEATHER

The amount of residual natural fat and added fatliquor is usually determined by chemically analyzing hexane extracts, following ASTM Standard D-3495 (25). The leather is extracted in a Soxhlet apparatus and the amount dissolved in the hexane is determined gravimetrically. Analyses for specific fatty components are described in (26).

Fatliquor affects mostly the mechanical properties, measured also by a collection of ASTM methods given in (25). In addition, the sounds emitted by leather when it is deformed are greatly suppressed by the presence of fat, supporting a method of assay by means of the acoustic emission test (27).

## 6. GENERAL USE OF FATS AND OIL FOR TEXTILES

Consumer textiles are produced from natural and synthetic fibers. To be converted into useful goods, such as apparel and home furnishings, fibers have to go through a series of processes such as spinning, weaving, and dyeing. The economics of production of these materials demand relatively high speeds of processing. Under these conditions, fibers and yarns coming into contact with other surfaces undergo frictional heating and abrasive damage and often break, impairing the efficiency of the process. Sticking of the abraded material to the processing machinery makes this situation worse.

In addition to abrasion, triboelectric charging is a serious problem in the production and processing of synthetic fibers. Charged fibers, because of interfiber repulsion, do not form a coherent yarn, and it is difficult to wind such yarns on bobbins to be transferred to other locations for further processing. To overcome these problems, combinations of oils, fats, and their derivatives are used extensively as



lubricants and antistatic agents, known in the trade as spin finishes. Mechanisms of their action in the fiber and yarn-forming processes are discussed. Oils and fats in the form of their surface-active derivatives are also used in scouring, dyeing, and softening. It is important to note that synthetic oils have replaced most of the natural oils in the processing of textiles. Natural oils are used mainly as their fatty acid derivatives.

## 7. COMMON TEXTILE FIBERS

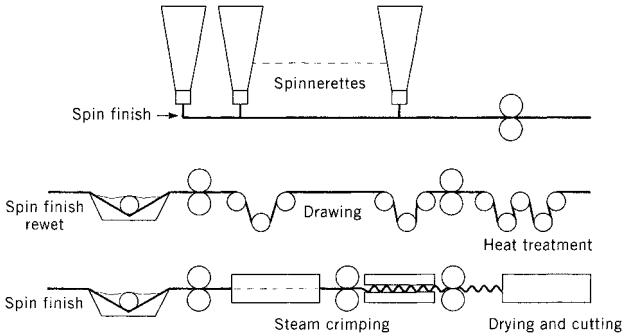
With the exception of silk, which is extruded as a continuous filament by the silk worm, most natural fibers, such as cotton, occur in the form of short filaments called staple fibers. Synthetic fibers like nylon, polyester, and poly(propylene), are melt spun (28) in the form of continuous filaments. High-melting acrylic fibers, on the other hand, are produced by a process known as wet spinning (29) in which fibers are regenerated by precipitating the polymer in the fiber form in a suitable nonsolvent. Some of the less common fibers are sometimes produced by solution spinning, in which fibers are regenerated by evaporating the solvent. All manufactured fibers can be converted into the staple form by cutting the continuous filaments to the required length.

## 8. PROCESSING OF FIBERS

### 8.1. Spinning

In the textile trade, spinning refers to the production of yarns by twisting staple fibers, as well as the production of yarns from synthetic polymers by the extrusion of continuous filaments through spinnerettes. In the spinning of staple fibers, yarns are produced at relatively high speeds by the alignment, drawing, and twisting of fibers in automatic machines. Stout (30) has studied the role of lubrication in spinning of staple fibers. Open-end spinning (31), used mainly for short staple fibers, is a more recent spinning method that eliminates the intermediate step of fiber alignment. In open-end spinning, fibers are brought to a twisting location in a chamber by air turbulence. The stability and the efficiency of this process depends on good fiber cohesion, which prevents yarn breakage. Treating the staple fiber with an oil promotes fiber cohesion, in addition to lubrication against hard surfaces of the processing machines to prevent abrasive damage.

Melt or solution spinning of synthetic fibers is a marvel of modern technology. Fiber lubrication, which is of the utmost importance in this high-speed process, is achieved by the application of spin finish—a combination of oils and surfactants. A typical spin line for the production of polyester staple fiber is shown schematically in Figure 2. The number of filaments, which can vary from tens to thousands, come into contact with various parts of the machinery, some of which are heated for proper fiber modification. To replenish the lost finish and to ensure adequate lubrication,



**Figure 2.** Schematic of a typical spin line for the production of staple fibers.

the finish is applied more than once at strategic locations along the spin line. Spin finishes are also applied to solution spun fibers, after the regeneration step. As these finishes protect the fibers in subsequent processing, an even distribution at low add-on is an important requirement. Methods of determining the distribution of finishes on fibers and yarns have been developed (32).

## 8.2. Texturing

As a result of crimp, natural fibers form relatively bulky yarns. These produce soft fabrics that are preferred by the consumer. Texturing is a process that imparts bulk to melt-spun continuous filament yarns, by introducing crimp in the individual filaments. False-twist friction texturing is one such process (33). The heated yarn is passed between ceramic friction discs rotating in opposite directions, which introduces a temporary twist in the yarn. The twist is undone as the yarn leaves the discs, leaving a permanent crimp in the individual filaments. The mechanism of texturing has been presented (34). Application of a proper finish is critical for the success of this process in which the interfiber friction should be low and the fiber-disc friction should be high. Formulating a finish that achieves these two opposing requirements at a high temperature without degradation is a challenge (35). The effect of volatilization of a lubricant on yarn friction has been investigated (36). The nature of the finish plays an important role in the uniformity of the textured yarn and consequently in the quality of the fabric.

## 8.3. Weaving and Knitting

Solid fats, like tallow, are sometimes used as lubricants on sized warp yarns in shuttle looms. Without such lubricants, warp yarns can unravel and break as a result of the abrasion of the size by the shuttle and the heddle. This would introduce defects in the fabric and will also affect the efficiency of the weaving process. In knitting, the lubrication of the yarns is also important. Lack of lubrication results in nonuniform yarn movements that affect the quality of the fabric.

## 9. PHYSICAL EFFECTS OF OILS AND FATS ON FIBERS AND YARNS

### 9.1. Fiber Lubrication

Surfaces of materials possess different degrees of roughness depending on the way they are produced. When two solid surfaces are pressed together with a force acting normal to them, they make contact at the tips of the asperities in the two surfaces. In the case of soft metals and polymers, because of yielding, these points of contact form adhesive junctions. The minimum force required to slide one surface over the other is the force of friction ( $F$ ) and is given by (37)

$$F = As, \quad (1)$$

where  $A$  is the area of real contact (much smaller than the geometrical area) at the asperities and  $s$  is the shear strength of the junctions. This is shown schematically in Figure 3. When a liquid film of a low-shear strength material is introduced at the interface, some of the solid–solid junctions are replaced by the liquid film. The new friction force is given by (38)

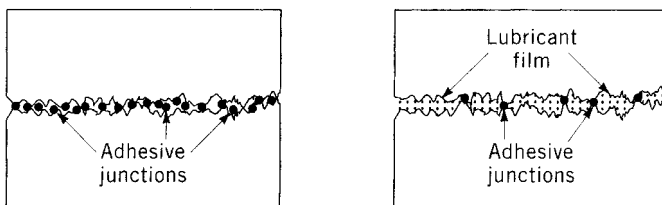
$$F' = A[\alpha s + (1 - \alpha)s_l], \quad (2)$$

where  $\alpha$  is the fraction of the surface with solid–solid junctions and  $s_l$  is the shear strength of the liquid. It should be noted that  $F' \ll F$  because  $\alpha \ll 1$  and  $s_l \ll s$ .

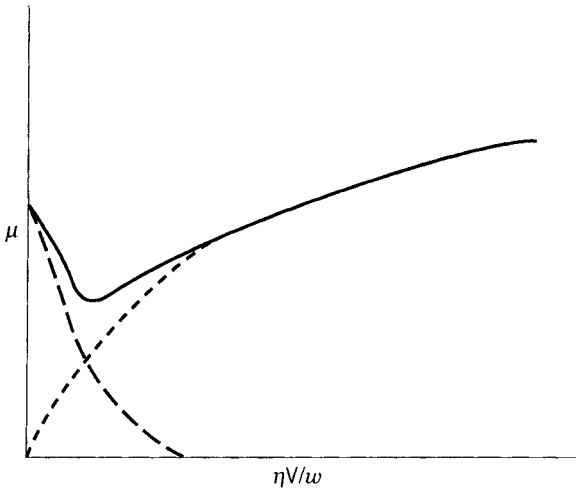
Adhesion of the lubricant film to the solid surface is an important requirement. Lack of adhesion will lead to the squeezing of the lubricant film out of the interface and the re-establishment of the solid–solid junctions (39, 40). This will render the lubricant less effective. The work of adhesion ( $W$ ) between a liquid lubricant and the solid surface is given by (41)

$$W = \sigma(1 + \cos \theta), \quad (3)$$

where  $\sigma$  is the surface tension of the liquid and  $\theta$  is the contact angle, which is a measure of the interaction between the liquid and the solid. As, for a given solid



**Figure 3.** Schematic of the effect of a lubricant film on the formation of adhesive junctions at a solid–solid interface.



**Figure 4.** Schematic of the effect of viscosity and sliding speed on hydrodynamic friction.  $W$  is the normal load (44).

surface,  $\cos \theta$  is inversely related to the surface tension of the liquid (42), proper formulation of a lubricant to maximize  $W$  by adjusting  $\sigma$  and  $\cos \theta$  is important. The solid-liquid interaction parameter,  $\cos \theta$ , also plays an important role in the spreading and penetration of the liquid lubricant into the yarn (43). This type of friction at low sliding speed is known as boundary friction, and lubrication in this regime is known as boundary lubrication (38).

A different frictional behavior is observed in the lubrication of fibers and yarns at high speeds (44). Friction appears to increase with the sliding speed. Hydrodynamic origin of this behavior was first explained by Reynolds (45) based on the lubrication of bearings rotating at high speeds. According to this theory, at high rates of shear, hydrodynamic pressure is generated in the lubricant film, which supports the normal load at the interface. The friction coefficient is found to be a function of the product of viscosity ( $\eta$ ) and the sliding speed ( $V$ ). This relationship between the friction coefficient ( $\mu$ ) and  $\eta V$  is seen in the hydrodynamic region of Figure 4, at high values of  $\eta V/W$  (44). In the boundary friction regime, at low values of  $\eta V/W$ , high friction results from the solid-solid junctions. Under these conditions, abrasive wear is the result. As the formation of adhesive junctions is a time-dependent phenomenon, at high sliding speeds, fewer adhesive junctions are formed, and therefore, friction decreases with an increase in sliding speed. Stick-slip phenomenon, which is characteristic of boundary friction, disappears (46). In the hydrodynamic region, on the other hand, high coefficients of friction need not be indicative of abrasive wear. In this friction regime, under the conditions of good adhesion, a lubricating finish film is always present at the solid-solid interface.

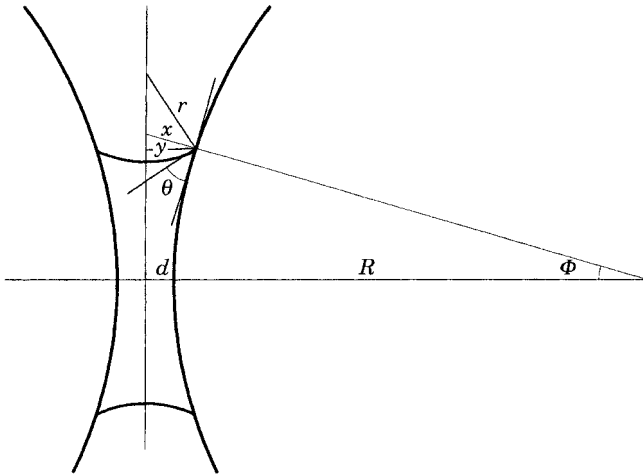


Figure 5. Geometry of a liquid bridge between two filaments.

### 9.2. Fiber Adhesion

Adhesion between the filaments of a yarn is an important requirement in processing of fibers. Twist contributes significantly to yarn cohesion. In the case of low-twist continuous filament yarns, filament adhesion can be improved considerably by the application of a liquid finish. Liquid bridges are formed by the applied finish, and the capillary pressure of these liquid bridges is responsible for the increase in cohesion. This is shown schematically in Figure 5 (47). The force per unit length of the fibers is given by

$$\Delta F = 2\sigma \sin(\varphi + \theta) + \frac{2R\sigma \sin \varphi \cos(\varphi + \theta)}{R(1 - \cos \varphi) + d}, \tag{4}$$

where  $R$  is the fiber radius,  $2d$  is the distance between the fibers,  $\varphi$  is the half-angle subtended by the liquid bridge at the center of the fibers,  $\theta$  is the contact angle, and  $\sigma$  is the liquid surface tension. The first term on the right-hand side is the attraction due to the component of surface tension parallel to the line joining the fiber centers. The second term is the Laplace pressure of the liquid bridge, whose magnitude depends on  $\theta$ . It is at a maximum when  $\theta = 0$ , and is negative when  $\theta > \pi/2$ . Therefore, repulsion between the fibers is possible if the second term in Equation 4 becomes negative and is numerically larger than the first. Physically, this means liquid–air interface of the bridge is convex rather than concave. It is important to note that the presence of liquid bridges in the yarn makes a small but significant contribution to the strength of the yarn (47).

## 10. OILS AND FATS IN TEXTILE PROCESSING

### 10.1. Natural vs. Synthetic Oils

Although natural oils can be used as lubricants and processing aids in textiles, they have some serious drawbacks. As a result of unsaturation, these molecules adsorb strongly on fiber surfaces and are not completely washed off the finished goods. The residues form resinous products by autoxidation in the presence of oxygen leading to “yellowing” (48). Oils with conjugated double bonds in the fatty acids are even worse in this regard. Storage stability of these goods is poor in warehouses and in the presence of ultraviolet light. As a result of this, in modern textile processing, synthetic oils are preferred over their natural counterparts. Saturated fatty acids from natural oils and fats find their way into surfactants in scouring and dyeing and as antistatic agents in spin-finish formulations. Uses of these surfactants in the textile industry have been reviewed extensively (49,50).

### 10.2. Spinning

The commercial importance of spin finishes can be realized from the sheer volume of synthetic fibers produced. For the year 2000, the production figures of the three major synthetic fibers, e.g., polyester, polyamide (nylon), and poly(acrylonitrile) were approximately 18, 4, and 3 million tons, respectively (51). If spin finishes are applied at 0.25–0.5% level, the annual requirement of these finishes will be in the range of 70–140 thousand tons. This has given rise to industries that supply spin-finish components and completely formulated spin finishes for specific applications (52). An experimental nonproprietary spin finish formulation supplied to TRI/Princeton by Henkel Corp. follows:

UCON 50 HB 660 (lubricant),	66.0%
Butyl stearate,	8.5
Oleic acid,	8.5
KOH (45% aqueous),	2.8
POE (6) nonyl phenol,	10.0
POE (2) ethyl hexyl K <sup>+</sup> salt,	4.2

UCON is a random copolymer of ethylene oxide (EO) and propylene oxide (PO) and can be considered as a synthetic oil. POE is poly(oxyethylene), which is a homopolymer of EO. Random EO-PO copolymers are typical of synthetic oils used as lubricants in textile processing (53, 54). PLURONICS (BASF) are block copolymers of EO and PO. Both of these copolymers can be produced with viscosities ranging from that of a thin oil to that of a thick paste. Oils of appropriate viscosity give hydrodynamic lubrication in the high-speed spinning of synthetic fibers. The hydrophile–lipophile balance (HLB) in these polymers can be varied, by changing the EO–PO composition, to suit the aqueous solubility requirements.

The other major synthetic oils used in textile processing are the silicone oils, based on the polymers of dimethyl siloxane,  $(\text{CH}_3)_3\text{Si}-\text{O}-(-\text{Si}(\text{CH}_3)_2-\text{O}-)_n-\text{Si}(\text{CH}_3)_3$  (55).

Similar to the copolymers of EO/PO, these polymers can also be produced with a range of molecular weights and varying viscosities. In contrast to the EO-PO copolymers, these have much lower surface energies and spread easily on a surface to give very thin films. Therefore, they make rather poor boundary lubricants. However, because of the low surface tension and high wettability with other surfaces, they make good hydrodynamic lubricants (42). For the same reasons, these oils are often used in soil and water-repellent treatments of fabrics (56, 57). More recently silicone oils have been combined with POEs and EO-PO copolymers to produce surfactants with varying HLB numbers and are used as lubricants and emulsifying and spreading agents (58). In general, silicones are known to be better lubricants than the EO-PO-based copolymers. The brief nature of this review precludes detailed discussion of the rather voluminous literature available in this area.

Mineral oils of different viscosity are used in the spinning of natural and synthetic fibers (59). These are paraffins of varying molecular weights, byproducts of the petroleum industry.

Apart from lubricants, spin finishes contain antistatic and spreading and emulsifying agents. These are generally alkyl sulfates, alkylbenzene sulfonates, sulfonated fats and oils and poly(ethylene glycol)-modified fatty acids, fatty acid amides, fatty alcohols, and fatty amines. Potassium alkyl phosphates are extensively used in the production of polyester staple fibers.

### 10.3. Scouring

Here oils and fats are used principally as detergents and wetting agents in the cleaning of natural fibers, to remove, for example, fats and waxes from cotton and wool (60) and in removing finishes used as processing aids in the manufacture of synthetics. Generally, anionic surfactants are used in this process. As most natural and synthetic fibers have negative charges on the surfaces, anionics will not adsorb strongly and therefore can be rinsed out easily. Among the nonionic surfactants, alkyl, alkylphenol, fatty acid, and fatty-acid-amide-modified POEs are more common. The pH of the bath is an important consideration in the selection of the proper surfactants.

### 10.4. Dyeing

In dyeing, surfactants are used as wetting agents. They are also used in the formulation of disperse dyes. A stable suspension of the dye is prepared with the help of an anionic surfactant, such as alkyl or aryl alkyl sulfonate. It has been shown that dyeing takes place through the aqueous phase by the slow dissolution of the solid dye particles (61, 62). The presence of the surfactant helps the dissolution of the dye.

In special cases, cationic surfactants are used as dye-leveling agents (63, 64). Cationic dyes with temperature-sensitive diffusion coefficients often yield non-uniform shades, because of the difficulty of maintaining constant temperatures in large

industrial dye baths. Cationic surfactants used in these dye baths diffuse into the fiber ahead of the dye molecules and occupy some of the sites. This, in general, reduces the rate of dyeing, but improves uniformity. Overall dye uptake is not affected because at the end of the process the surfactant molecules are displaced by the dye molecules. Quaternized long-chain surfactants are generally used for this purpose.

### 10.5. Softening

Fabrics often feel rough after scouring and washing. Ionic as well as nonionic surfactants are used to “soften” such fabrics (65). Several mechanisms have been suggested for the softening effect. In the case of low-molecular-weight ionic and nonionic surfactants, softening has been attributed to the moisturizing and lubrication of the fibers in the fabric. When the interfiber friction is low, the fabric deforms easily by the slippage of fibers over one another. This results in a softer “hand.” As a result of low molecular weight and lack of substantivity, these effects tend to be temporary, and the fabric needs to be treated after every wash. A more permanent softening is achieved by the surface deposition of an elastomeric polymer, which forms interfiber bonds. Surface-deposited silicones (66) are known to be superior in this respect. A principal drawback of these polymeric softeners is that they interfere with the water absorbency of the fabric because of their hydrophobic nature. A rather unusual application of silicones is in the shrink proofing of wool garments (67). In this instance, the interfiber bridges prevent unidirectional fiber migrations that otherwise would lead to the felting of the fabric.

## 11. CONCLUDING REMARKS

The foregoing discussion shows the importance of oils and fats in the processing of textiles. As a result of oxidative stability, synthetic oils have superseded their natural counterparts. However, fatty acids from natural oils still play an important role in the processing of textiles. There is considerable pressure on the textile industry to reduce the amounts of these additives, or to eliminate them completely, because of the environmental implications of the effluents (68). In response to this, considerable effort is being made in the industry to develop additive-free processes.

## REFERENCES

1. G. H. von Fuchs, *J. Am. Leather Chem. Assn.*, **52**, 550 (1957).
2. M. Komanowsky, P. H. Cooke, W. C. Damert, P. L. Kronick, and M. D. McClintick, *J. Am. Leather Chem. Assn.*, **90**, 243–257 (1995).
3. P. L. Kronick and P. Cooke, *J. Pol. Sci Part B*, **36**(5), 805–813 (1998).
4. E. Heidemann, *Fundamentals of Leather Manufacture*, Eduard Roether KG, Darmstadt, 1993.
5. J. Pore, *Ind. Cuir*, **20** (June–July 1987).



6. I. A. Sal'nikova, N. K. Baramboim, and D. A. Kutsidi, *Izv. Vyssh. Zaved., Tekhnol. Legk. Prom-sti.*, **29**, 32 (1986).
7. T. E. Gadzhiev and A. G. Danilkovich, *Kozh.-Obuvn. Prom-sti.*, **34**, 19 (1991).
8. K. Sato, *Kikaku Kagaku*, **35**, 15 (1989).
9. B. Fricke and K. Haeussler, *Das Leder*, **11**, 488 (1976).
10. H. H. Friese, U. Ploog, and W. Prinz (to Henkel), German Patent DE 3,620,780, 1987.
11. L. P. Trishina, L. D. Vdovina, and S. K. Kurmanov (to Alma-Ata Fur Combine), Russian Patent SU 1,293,233, 1987.
12. J. Pore, *Ind. Cuir.*, 13 (1987).
13. E. R. Stoica, E. V. Maurer, A. C. Popa, L. Dumitru, G. Drutsch, and V. Burghilea, Romanian Patent RO 78,708, 1982 (to Intreprinderea de Detergenti).
14. F. Hou and R. Fan (to Dahua Leather Chemical Plant), Chinese Patent CN 1,046,561 A, 1990.
15. J. C. Barandiaran, A. Norman, and D. Squarisi, *Ind. Quim.*, **290**, 12 (1988).
16. R. Komforth and A. Lauton (to Ciba Specialty Chemicals Corp.), U.S. Patent 6,033,590, March 7, 2000.
17. Q. Li, Y. Yin, and Y. Shi (to Zhejiang Grain Science Inst.), Chinese Patent CN 1,043,091, 1990.
18. J. Pore, *Cuoio, Pelli, Mater. Concianti*, **66**, 194 (1990).
19. J. Pore, *AQEIC Bol. Tec.*, **41**, 491 (1990).
20. L. P. Zaichenko, V. B. Nekrasova, L. A. Iona, and G. A. Zakharova (to Leningrad Technol. Inst.), USSR Patent 1,370,143, 1988.
21. P. L. Kronick (to USA), U.S. Patent 5,853,427, December 29, 1998.
22. J. J. Hodder, A. El A'mma, P. M. Lesko, and T. Stewart, *Przegl. Skorzany*, **46**, 131 (1991).
23. J. J. Hodder, A. El A'mma, P. M. Lesko, and T. Stewart, *J. Am. Leather Chem. Assn.*, **86**, 22 (1991).
24. P. M. Lesko, T. Stewart, and A. El A'mma (to Rohm and Haas Co.), European Patent EP 372,746 A2 (1990).
25. *ASTM Annual Book of Standards*, Part 21, ASTM, Philadelphia, 1982.
26. A. Decastro and C. E. Retzch, in F. O'Flaherty, W. T. Roddy, and R. M. Lollar, eds., *The Chemistry and Technology of Leather*, Vol. IV, Reinhold Publishing Co., New York, 1965, pp. 178–193.
27. P. Kronick, A. Page, and M. Komanowsky, *J. Am. Leather Chem. Assn.*, **88**, 178 (1993).
28. J. E. McIntyre in M. Lewin and E. Pearce, eds., *Fiber Chemistry*, Marcel Dekker, Inc., New York, 1985, p. 21.
29. B. G. Frushour and R. S. Knorr, in Ref. 28, p. 248.
30. H. P. Stout, *Wear*, **15**, 149 (1970).
31. J. Lunenschloss and K. J. Brockmanns, *Chemiefasern/Textil Industrie*, **33/85**, 570 (1983).
32. Y. K. Kamath, C. J. Dansizer, S. B. Hornby, and H.-D. Weigmann, *J. Appl. Polym. Sci.: Appl. Polym. Symp.*, **47**, 281 (1991).
33. G. D. Wilkinson, ed., *Textured Yarn Technology*, Vol. 2, Monsanto Co., 1967, p. 5.
34. J. J. Thwaites, *J. Text. Inst.*, **60**, 116 (1970).

35. A. Nagahara and E. Otsubo, Japanese Patent 92,126,874, Toray Industries Inc., Apr. 27, 1992; *Chem. Abst.*, **117**, No. 18, Abst. No. 173334 (1992).
36. M. J. Schick, *Textiles Res. J.*, **43**, 198 (1973).
37. K. V. Shooter, *Proc. Roy. Soc.*, **212**, 488 (1952).
38. D. Tabor, *Proc. Roy. Soc.*, **212**, 498 (1952).
39. S. C. Cohen and D. Tabor, *Proc. Roy. Soc.*, **291**, 186 (1966).
40. M. J. Schick, *Textile Res. J.*, **43**, 342 (1973).
41. N. K. Adam in R. F. Gould, ed., *Contact Angle, Wettability and Adhesion, Advances in Chemistry Series*, Vol. 43, American Chemical Society, Washington, D.C., 1964, p. 52.
42. W. A. Zisman, in Ref. 42, p. 1.
43. Y. K. Kamath, S. B. Hornby, H.-D. Weigmann, and M. F. Wilde, *Textile Res. J.*, **64**, 33 (1994).
44. W. W. Hansen and D. Tabor, *Textile Res. J.*, **27**, 300 (1957).
45. O. Reynolds, *Phil. Trans.*, **177**, 157 (1886).
46. M. J. Schick, *Textile Res. J.*, **43**, 103 (1973).
47. J. H. Brooks, U. K. Das, and L. J. Smith, *Textile Res. J.*, **59**, 382 (1989).
48. E. K. C. Park, in Ph.D. Dissertation, Cornell University, Ithaca, New York, 1991.
49. G. C. Johnson, *AIChE National Meeting, 1984 Winter*, AIChE, New York, 1984.
50. T. J. Proffitt and H. T. Patterson, *J. Amer. Oil Chem. Soc.*, **65**, 1682 (1988).
51. *Int. Fiber J.*, **15**(3), 8 (2000).
52. *Ullmann's Encyclopedia of Industrial Chemistry*, 6th ed., Wiley-VCH Verlag, New York, 2002.
53. H. S. Koenig and G. M. Bryant, *Textile Res. J.*, **50**, 1 (1980).
54. K. Park, C. G. Seefried, and G. M. Bryant, *Textile Res. J.*, **44**, 692 (1974).
55. Organofunctional Silanes, Product Bulletin, OSi Specialties, Union Carbide Chemicals and Plastics Co. Inc. Danbury, Connecticut, 1991.
56. L. Tokarzewski, H. Gega, and J. Ossowski, *Textilveredlung*, **15**, 434 (1980).
57. E. M. Abdel-Bary and A. M. Hassanien, *Indian J. Text. Res.*, **5**, 37 (1980).
58. Silwet Surfactants, Product Bulletin, OSi Specialties, Union Carbide Chemicals and Plastics Co. Inc. Danbury, Connecticut, 1992.
59. R. K. Rhodes, U.S. Patent 04082679, Witco Chemical Corp., 1978.
60. E. C. Hansen, in J. E. Lynn and J. J. Press, eds., *Advances in Textile Processing*, Vol. 1, Textile Book Publishers, New York, 1961, p. 251.
61. C. L. Bird, F. Manchester, and P. Harris, *Disc. Faraday Soc.*, **16**, 85 (1954).
62. C. L. Bird, *J. Soc. Dyers Colour*, **70**, 68 (1954).
63. C. L. Zimmerman and A. L. Cate, *Text. Chem. Col.*, **4**, 150 (1972).
64. H. Kellet, *J. Soc. Dyers Colour*, **84**, 257 (1978).
65. R. Steele and J. T. Taylor, in Ref. 32, p. 311.
66. K. O. Jang and K. Yeh, *Textile Res. J.*, **63**, 557 (1993).
67. J. R. Cook, B. E. Fleischfresser, A. M. Wemuss, and M. A. White, *J. Text. Inst.*, **76**, 57 (1985).
68. D. Krishna, P. J. Reddy, D. Gajghate, and T. Nandi, *Indian J. Environ. Prot.*, **11**, 927 (1991).

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## *Edible Films and Coatings from Soybean and Other Protein Sources*

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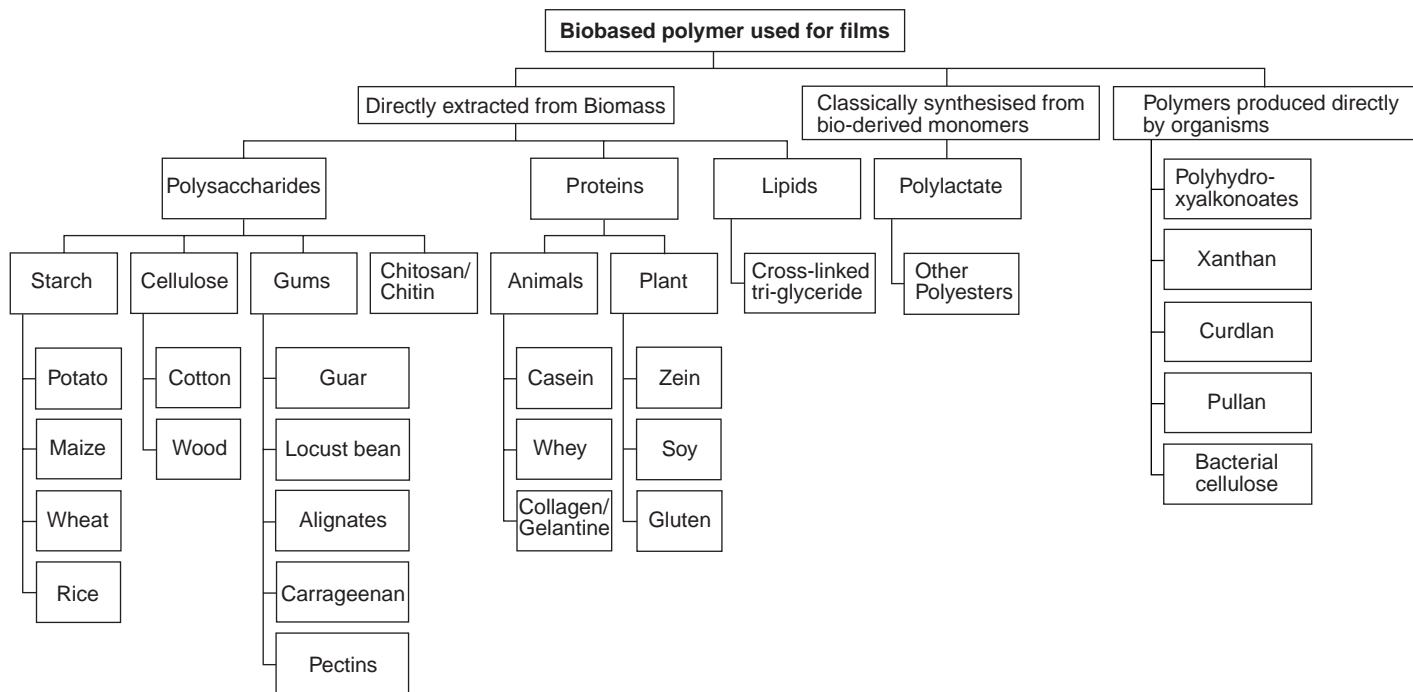
Biobased materials, which are diverse in nature, chemistry, and properties, expand the possibilities to tailor-made films for packaging in the food industry. Biobased materials used in the production of films are shown in Figure 1 (1).

### **1. EDIBLE FILMS AND COATINGS**

Oilseed proteins in the meal after the extraction of oil from soybean, corn, cotton seed, peanut, sunflower seed, rapeseed, and sesame seed are rich sources of plant proteins and suitable for the production of edible films. Protein content of oil seeds varies from 20–40% on dry weight basis. Meals have been used as animal feed. A majority of oilseed proteins are globulins. Soybean meal contains 44–50% protein and is mainly composed of 2S (alpha-conglycinin), 7S (beta- and gamma-conglycinin), 11S (glycinin), and 15S (glycinin polymers) globulins (2). Rapeseed meal contains

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**Figure 1.** Biobased materials used in the film preparation grouped based on origin/method of production [adapted from Weber (1)].

40–45% protein, which is predominantly 2S albumin (napin) and 11S globulin (cruciferin) (3). Peanut meal contains 45–50% protein and has 2S (conarachin I), 8S (conarachin II), and 14S (arachin) globulins (4). Peanut protein film has been reported to have a bland flavor, and could be used to coat intermediate moisture foods (5). Sunflower seed meal has 40–50% protein, mainly consisting of albumins and globulins that are highly soluble in water (6). Cottonseed proteins make up 30–40% of seed kernel and have good film-forming properties (7). Sesame seed meal contains 40–50% protein and is suitable for human consumption (8, 9). Zein is an alcohol soluble prolamine fraction of corn proteins and has higher amounts of non-polar amino acids such as leucine, proline, and alanine (10). Corn zein films are tough, glossy, and brittle, with better moisture barrier ability than other protein films (11). Corn zein has been used commercially in the coating of candy, shelled nuts, and pharmaceutical tablets (12).

Animal proteins, such as milk casein, whey, albumin, collagen, gelatin, keratin, and myofibrillar, are also proposed as raw materials to form edible films (13–15). Extended structures formed by unfolding of protein molecules are required for film formation. Amorphous three-dimensional structures formed by noncovalent interactions among protein chains stabilize the films. At high water content, films are produced by “casting” of viscous solutions, and at low water content, films are produced by extrusion using thermoplastic properties of proteins (13).

Edible films from polysaccharides, proteins, and/or lipid compounds are used in various food products to control gas transfer (15–17).

Edible coatings of food materials have been studied for their ability to provide a barrier to mass transfer of moisture and gases and to improve mechanical integrity of foods (18). Wax was the first edible coating used to coat fruits. Edible coatings can function as primary packaging (1). Controlling the oxygen and carbon dioxide transfer in and out of the product can extend shelf life of food products. Oxygen takes part in many degradation reactions in food such as fat and oil rancidity, microbial growth, enzymatic browning, and vitamin loss. However, for fresh fruits and vegetables, some permeability to oxygen and especially to carbon dioxide is essential, and moderate barrier packaging is more appropriate. Carbon dioxide should be partially eliminated to maintain the quality of fruits and vegetables. Some produce, for example, strawberries, cherries, raspberries, broccoli, and mushrooms, can tolerate and even benefit from carbon dioxide concentrations up to 20% because of a decrease in respiration rate, whereas others such as pears, some apple varieties, lettuce, celery, and tomatoes will be injured by carbon dioxide concentrations above 2% (19). Films with the appropriate permeability are selected to establish a suitable equilibrium modified atmosphere (20). Park et al. (21, 22) reported zein coating on tomato delay color change, weight loss, and maintained firmness during storage. Apart from barrier and antimicrobial properties, edible coating should be organoleptically and functionally compatible with the product (23). Edible coating should not have any impact on sensory characteristics of the food product (1). Edible coatings have to fulfill some basic requirements such as good sensory qualities, high barrier and mechanical efficiencies, sufficient biochemical, physical and microbial stability, and safe for consumption (23).

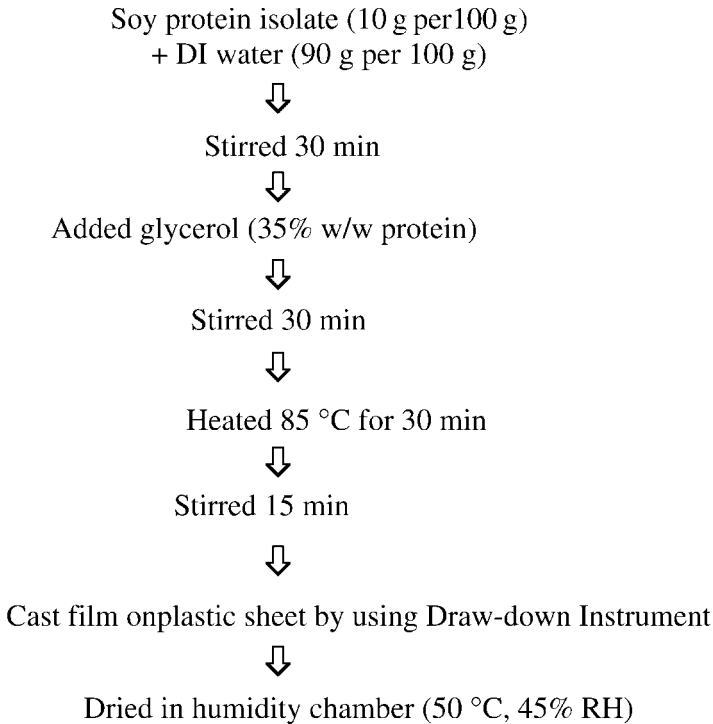
## 2. PROTEIN FILMS

In native state, proteins exist as either fibrous or globular form. Protein should be denatured and unfolded to produce an extended chain structure to form film. Extended protein chains can interact through hydrogen, ionic, and hydrophobic bonds to form a three-dimensional structure (24). Protein films are excellent gas barriers but poor moisture barriers because of their hydrophilic nature. Mechanical properties and gas permeability depend on the relative humidity (1). Al-ameri (25) studied the antioxidant and mechanical properties of soy, whey and wheat protein, and carrageenan and carboxymethyl cellulose films with incorporated tertiary-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), fenugreek, and rosemary extracts. Armitage et al. (26) studied egg albumin film as a carrier of natural antioxidants to reduce lipid oxidation in cooked and uncooked poultry.

### 2.1. Soy Protein Film

The protein content of soybean ranged from 38% to 44%, which mainly consist of globulins 2S, 7S, 11S, and 15S (27). Amino acid asparagine and glutamine are high in soy protein (24). Film formation is a result of polymerization of unfolded proteins by disulfide and hydrogen bonds and hydrophobic interactions (28). Soy protein films are usually formed by the polymerization of 11S and 7S protein by disulfide linkages (29). Polymerization is favored by heating a solution above 60 °C to unfold the protein and a pH between pHs 6.2 and 10.2 to promote sulfhydryl-disulfide interchange reaction. The pHs of the soy protein film-forming solutions influence the properties of films (24). Surface films prepared from 11S protein are smooth, opaque elastic, and have high tensile strength, whereas 7S protein are translucent and have creases (30).

**2.1.1. Plasticizers** Plasticizer is necessary to produce protein- or polysaccharide-based films to have mechanical strength for handling (31). The most common plasticizers used in films are polyols (glycerol, sorbitol, polyethylene glycol), mono-, di-, and oligo-saccharides and lipids (monoglycerols, phospholipids). Surfactants, such as sodium dodecyl sulfate or glycerol monostearate, also have received attention as potential plasticizers (17, 32–34). Plasticizers are small poly alcohol (OH) molecules that can increase chain mobility probably by disrupting the hydrogen bond between neighboring protein strands and by reducing interchain attractive forces, which thereby increases flexibility and reduces brittleness of the film (15, 16). With increased chain mobility, the diffusion coefficient for gas and water vapor also increases. Lower molecular weight and higher hydrophilicity of the plasticizer increase the plasticizing effect. Hydroxyl groups of the plasticizer are believed to replace polymer–polymer interactions by developing polymer–plasticizer hydrogen bonds. Large amounts of plasticizer increase the extensibility and flexibility and decrease the elasticity, mechanical resistance, and barrier properties of the films. Plasticizers chosen for polymer films must be readily soluble in the film solvent and

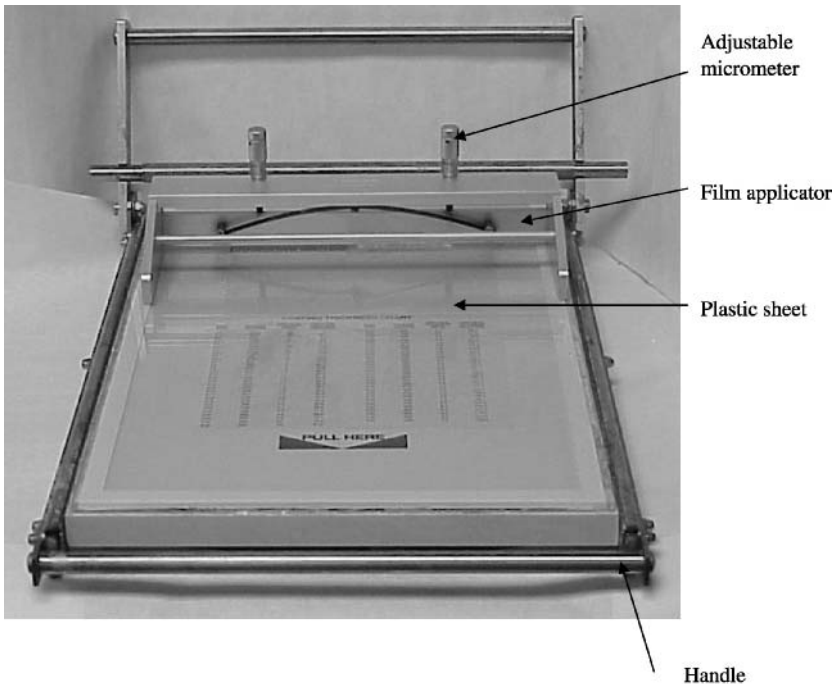


**Figure 2.** Soy protein film preparation.

miscible with polymer. Plasticizers affect mechanical and permeability properties of film, thus, they have to be optimized to reduce adverse effect on film properties (15). Plasticizers also lower water activity, thereby limiting microbial growth (1). Plasticizer concentrations should be optimized to produce films with desirable mechanical strength.

**2.1.2. Film Casting** Edible films are produced by two different mechanisms, dry and wet processing. In the dry process, films are produced at a lower water content by extrusion using the thermoplastic properties of polymers. In the wet process, polymers are dispersed or solubilized into a liquid phase, cast, and dried. The wet process is preferred to apply coatings in liquid form directly onto food products by dipping, brushing, or spraying (13). Wet processed films reported in the literature are produced by pouring a measured amount of film-forming solutions, at different concentrations, onto a surface. This process will result in thicker film and inconsistency in the thickness of film at different positions. A flow diagram for the preparation of a soy protein film-forming solution is shown in Figure 2.

Rhim et al. (35, 36) used soy protein isolate at a concentration of 5 g/100 mL, heated at 70 °C for 20 min and poured the film-forming solution onto a Teflon-coated glass plate. Brandenburg et al. (37) used a soy protein isolate 5 g/100 g

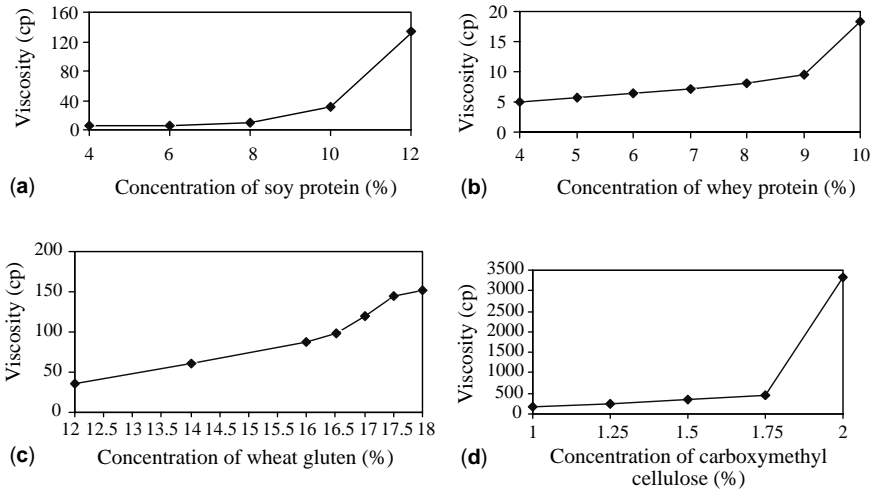


**Figure 3.** Drawdown instrument for casting films.

and heated at 60 °C for 10 min to produce a film-forming solution and poured the film-forming solution onto a Teflon-coated glass plate. Park et al. (38) used a soy protein isolate 5 g/100 mL and heated at 90 °C for 20 min to produce a film-forming solution and poured the film-forming solution onto weighing boats. Rangavajhyala et al. (39) used a soy protein isolate at a concentration of 5 g/100 g and heated at 70 °C for 30 min to produce a film-forming solution and poured the film-forming solution onto a Teflon-coated glass plate.

The thickness of the film will determine the physical properties, including mechanical strength, water, and gas permeability, and any inconsistency in the thickness of film will result in high variation in the physical properties. To represent the coated films, model films should also have uniform and low thickness, which cannot be achieved by casting film in the existing method. Thin films with uniform thickness can be cast with a Drawdown instrument (Figure 3) by using an adjustable micrometer film applicator to spread the film-forming solutions uniformly. The Drawdown instrument is widely used to study the properties of film coating in non-food applications such as paint, varnish, and lacquer. Unlike pouring film-forming solutions, the films produced by casting with the Drawdown instrument need to be optimized for polymer concentrations to have good leveling behavior. Film-forming solution should withstand an increase in surface area without dripping in a coating application. In the edible film studies, variations in the





**Figure 4.** Optimization of concentration of polymer for film-forming solution based on viscosity [adapted from Eswaranandam and Hettiarachchy (40)].

concentration of the polymer are used to produce film-forming solutions that make the comparisons less useful. A film-forming solution can neither be a solution nor be a gel. Extended structures formed by the unfolding of protein molecules are required for film formation. Amorphous three-dimensional structures formed by noncovalent interactions among protein chains stabilize the films (13). The distance between polymer molecules (concentration) will determine the solution and the gel stage. The transition point between solution and gel was expected to be ideal for the film-forming solution. The concentrations of the polymers have to be optimized to get this transition point.

Eswaranandam and Hettiarachchy (40) selected the concentration of soy protein based on the turning point of the slope of concentration versus viscosity to optimize concentration to cast films. To produce the best films, polymer molecules should be in a distance that is not too far to form noncovalent bonds and not too close to form a gel network. The optimum concentration to cast film by the Drawdown instrument is the point of the changing slope of viscosity versus the concentration curve. The turning point of the slope of concentration versus the viscosity curve will express this optimum distance between polymer molecules. The turning point in viscosities is 10.0/9.0/16.5/1.75 g 100-g solution for a soy protein/whey protein/wheat gluten/carboxymethyl cellulose film-forming solution (Figure 4) (40). Table 1 shows the concentrations of soy, whey, wheat proteins, and carboxymethyl cellulose used in the optimization of the film-forming solutions for casting with a Drawdown instrument (40).

Glycerol (plasticizer) concentration used in the literature varied from 35 to 60 g/100-g soy protein (35–39). A lower amount of plasticizer will cause drying out and cracking of the film. The intermolecular disulfide bonds resulting from the denaturing of protein and intermolecular interactions between the protein chain

**TABLE 1. Concentrations of Polymer and Glycerol used in Optimization of Film-Forming Solutions and Their Optimized Concentrations.**

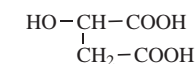
Polymer	Polymer Concentrations used in Optimization	Optimum Polymer Concentration	Glycerol Concentrations used in Optimization	Optimum Glycerol Concentration
	(g/100-g solution)		(g/100-g polymer)	
Soy protein	4.0, 6.0, 8.0, 10.0, 12.0	10.00	25, 30, 35, 40, 45	35
Whey protein	4.0, 5.0, 6.0, 7.0, 8.0, 9.0 10.0	9.00	25, 30, 35, 40, 45	35
Wheat gluten	12.0, 14.0, 16.0 16.5, 17.0, 17.5 18.0	16.5	10,15, 20, 25, 30, 35, 40, 45	15
Carboxymethyl cellulose	1.00, 1.25, 1.50, 1.75, 2.00	1.75	10,15, 20, 25, 30, 35, 40, 45	15

Adapted from Eswaranandam and Hettiarachchy (40).

from hydrogen bonding hydrophobic interactions and the electrostatic forces will result in the brittleness of the film. Higher amounts of plasticizer increase the flexibility and decrease the elasticity, mechanical resistance, and the barrier properties of the films. Plasticizers affect the mechanical and permeability properties of films. The level of plasticizer has to be optimized to reduce the adverse effect on film properties (15).

The optimum level of plasticizer will reduce the protein chain-to-chain interaction and will increase the flexibility of the film (41). Different concentrations of polymers, glycerol, and conditions were required to produce these films with desirable physical and mechanical properties.

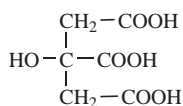
Low-molecular-weight organic acids with one or more OH groups also have a plasticizing effect on films (Figure 5). Krull and Inglett (42) and Cagri et al. (43) reported the plasticizing effect of lactic acid, which increases elongation of wheat gluten and whey protein films. Organic acids are either naturally present in fruits and vegetables or synthesized by microorganisms as a result of fermentation. Acetic, citric, succinic, malic, tartaric, benzoic, and sorbic acids are major organic acids that naturally occur in fruits and vegetables (44).



DL-Malic acid (MW 134.09, EQ W 67.05)  
pK<sub>a1</sub> 3.40, pK<sub>a2</sub> 5.10



Lactic acid (MW 90.08, EQ W 90.08)  
pK<sub>a</sub> 3.86



Citric acid (MW 192.13, EQ W 64.04),  
pK<sub>a1</sub> 3.13, pK<sub>a2</sub> 4.76, and pK<sub>a3</sub> 6.40



Tartaric acid (MW 150.09, EQ W 75.03)  
pK<sub>a1</sub> 3.20, pK<sub>a2</sub> 4.80

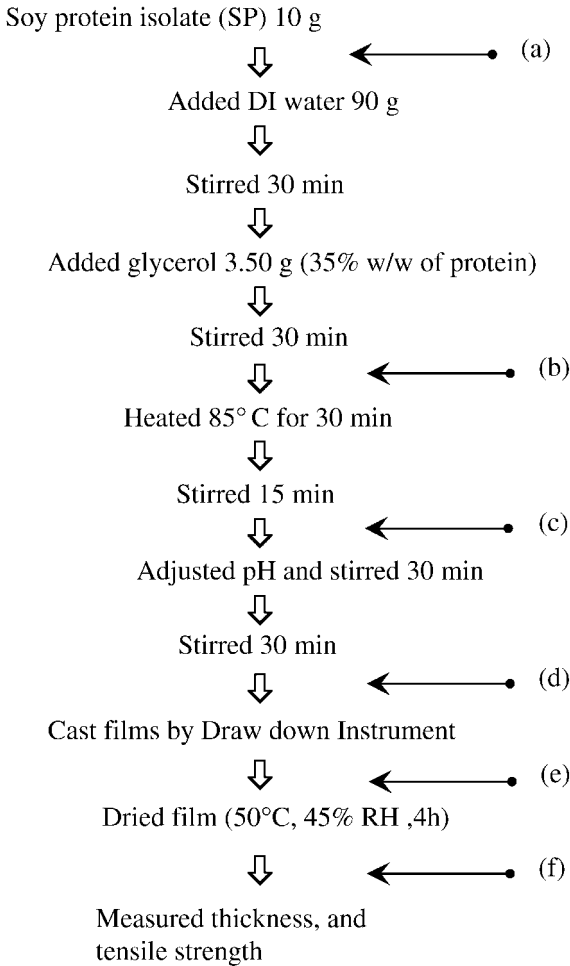
**Figure 5.** Structure, molecular and equivalent weights, and pKa (at 25 °C) of organic acids.

**2.1.3. Ultraviolet or  $\gamma$ -Irradiation and Cross-Linking** The mechanical strength of the film can be improved by cross-linking of the polymers by means of ultraviolet (UV)/ $\gamma$ -radiation, enzymes, and chemicals. UV treatment ( $51.8 \text{ J/m}^2$ ) decreased puncture strength and increased tensile strength of soy protein film (36). Gennadios et al. (45) reported that the application of UV irradiation ( $103.7 \text{ J/m}^2$ ) to soy protein film increased the tensile from 3.7 to 6.1 MPa; furthermore, tensile strength was shown to increase linearly with UV irradiation dosage. Gennadios et al. (45) reported that UV irradiation has weaker energy than does ionizing radiation, which is less likely to cause adverse effects but linearly increases tensile strength ( $R^2 = 0.96$ ) and linearly decreases percentage elongation ( $R^2 = 0.92$ ) of soy protein film with the UV dosage up to  $103.7 \text{ J/m}^2$ .

Ultraviolet or  $\gamma$ -irradiation can be used to cross-link protein film. The efficiency of UV radiation on the mechanical properties of film depends on the amino acids composition and the molecular structure of the protein. A UV irradiation of  $0.0104 \text{ J/cm}^2$  increased the tensile strength (65%) and decreased elongation (31%) of soy protein film but did not influence wheat gluten and pea protein films. The aromatic amino acids tyrosine and phenylalanine in soy protein may participate in cross-linking reactions when irradiated by UV light (45–47). The net results of irradiation could be cross-linking or molecular degradation, which depend on the nature of protein and irradiation dose (48). Vachon et al. (49) reported that  $\gamma$ -irradiation induced cross-links in calcium caseinate film. They also reported that a combination of irradiation and thermal treatments increased the puncture strength of calcium caseinate and whey proteins films. Brault et al. (50) reported that cross-links formed by  $\gamma$ -radiation increased the mechanical strength and water resistance of calcium caseinate film. Ionizing  $\gamma$ -radiation affects proteins by causing conformational changes, aminoacid oxidation, free radical formation, covalent bond breaking, recombination, and polymerization reactions (51). At the dosage of 5–30 kGy,  $\gamma$ -radiation did not affect the tensile strength of soy protein film but increased the tensile strength of soy protein and polyethylene oxide composite film (52).

Gennadios et al. (45) treated soy protein films (soy protein 5 g, glycerol 2.5 g per 100-mL water, pH 10.0) with UV light after drying and peeling from the plates. The UV dosage used in their study was  $0.01037 \text{ J/cm}^2$  ( $0.6 \text{ mW/m}^2$  for 48 h), and reported tensile strength increased linearly with the UV dosage. Rhim et al. (35) also showed an increase in tensile strength of UV-irradiated egg albumin, gluten, and zein films compared with control nonirradiated films. The higher dose of UV irradiation used in this study explores the benefits of UV irradiation other than increasing tensile strength.

The effect of UV treatment on the tensile strength depended on the step of film preparation (Figure 6, Table 2) (53). UV treatment of soy protein isolate and during film preparation steps before casting (d), before drying (e), and after drying (f) increased tensile strength of the film at pH 7.0. UV treatment before heating (b) was not significantly different from that of the control with no UV treatment. Although UV treatment decreased elongation of the film before break, the differences are not significant ( $p > 0.05$ ).



**Figure 6.** UV treatment ( $7.56 \text{ J/cm}^2$ ) at different steps of soy protein film solution preparation (arrows point to the steps of UV treatment) [adapted from Eswaranandam (53)].

At pH 3.0, UV treatment significantly increased maximum tensile strength and tension at break of the film at steps a, d, and e (soy protein isolate, before casting, and before drying). UV treatment at steps b, c, and f (before heating, before adjusting pH, and after drying) did not show a significant effect on maximum tensile strength and tension at break of the film ( $p > 0.05$ ). UV radiation is absorbed by aromatic amino acids in soy protein, resulting in conformational modifications and cleavage of S—S bonds (52). UV irradiation at  $1.0 \text{ J/cm}^2$  had minimum/no effect on tensile properties of wheat gluten and pea protein films (48, 54). A high amount of tyrosine and phenylalanine in soy protein that participates in the cross-linking reactions induced by UV irradiation is responsible for the increase in tensile strength (46). Drastic conformational changes of protein molecules during heating

**TABLE 2. Effect of UV Treatment (7.56 J/cm<sup>2</sup>) at Different Steps\* (a-f) of Film Preparation on the Tensile Strength and Elongation of the Soy Protein Film.**

pH	Steps of UV Treatment in Film Preparation	Maximum Tension (MPa)	Tension at Break (MPa)	Elongation %	Thickness μm
pH 7.0	Control (no UV)	16.6 ± 2.3 <sup>c**</sup>	15.3 ± 2.1 <sup>b</sup>	65.2 ± 7.9 <sup>a</sup>	27.1 ± 3.4 <sup>a</sup>
	(a) Soy protein isolate	22.7 ± 1.1 <sup>ab</sup>	20.2 ± 1.4 <sup>a</sup>	43.8 ± 13.2 <sup>a</sup>	27.9 ± 0.4 <sup>a</sup>
	(b) Before heating	19.9 ± 1.5 <sup>bc</sup>	17.6 ± 1.4 <sup>ab</sup>	37.7 ± 12.2 <sup>a</sup>	27.9 ± 0.6 <sup>a</sup>
	(d) Before casting	22.8 ± 1.4 <sup>ab</sup>	19.1 ± 1.2 <sup>ab</sup>	45.2 ± 9.8 <sup>a</sup>	29.7 ± 2.3 <sup>a</sup>
	(e) Before drying	22.7 ± 1.6 <sup>ab</sup>	20.0 ± 1.3 <sup>a</sup>	29.5 ± 7.9 <sup>a</sup>	28.2 ± 1.3 <sup>a</sup>
	(f) After drying	27.3 ± 1.8 <sup>a</sup>	22.5 ± 1.4 <sup>a</sup>	52.4 ± 13.2 <sup>a</sup>	28.6 ± 1.2 <sup>a</sup>
	pH 3.0	Control (no UV)	11.7 ± 0.3 <sup>de</sup>	11.7 ± 0.3 <sup>cd</sup>	32.7 ± 8.4 <sup>ab</sup>
(a) Soy protein isolate		14.2 ± 0.6 <sup>a</sup>	14.1 ± 0.6 <sup>a</sup>	26.9 ± 3.4 <sup>b</sup>	38.4 ± 1.6 <sup>b</sup>
(b) Before heating		10.7 ± 0.4 <sup>e</sup>	10.7 ± 0.4 <sup>d</sup>	32.2 ± 7.4 <sup>ab</sup>	41.1 ± 0.9 <sup>a</sup>
(c) Before adjusting pH		11.2 ± 0.3 <sup>cd</sup>	11.1 ± 0.3 <sup>cd</sup>	24.4 ± 4.8 <sup>bc</sup>	39.4 ± 0.5 <sup>ab</sup>
(d) Before casting		13.3 ± 0.6 <sup>abc</sup>	13.2 ± 0.6 <sup>ab</sup>	19.4 ± 5.1 <sup>bc</sup>	37.6 ± 0.5 <sup>bc</sup>
(e) Before drying		13.8 ± 1.0 <sup>ab</sup>	14.2 ± 0.9 <sup>a</sup>	7.0 ± 1.8 <sup>c</sup>	40.3 ± 0.3 <sup>ab</sup>
(f) After drying		12.1 ± 0.3 <sup>cde</sup>	12.0 ± 0.3 <sup>bcd</sup>	46.5 ± 5.6 <sup>a</sup>	38.6 ± 0.9 <sup>ab</sup>

\*See Figure 6 for details.

\*\*Values are means ± SEM of triplicate analyses. Means followed by the same superscript in a column are not significantly different (*p* > 0.05).

Adapted from Eswaranandam (53).

and adjusting pH may affect the cross-links formed during UV irradiation at the steps b and c (before heating, before adjusting pH). Soy protein isolate can be UV irradiated to increase the tensile strength of the film at neutral and acidic pHs.

## 2.2. Film Properties

**2.2.1. Film Thickness** Several problems associated with thick edible coating including anaerobic respiration in apples and banana (55), rapid weight loss in tomatoes (21, 22), the elevated level of core flush in apple (56), and increased incidence of decay in cucumber have been reported (57). Thin film should have adequate mechanical strength and extensibility to maintain integrity and withstand external stress that would occur during processing, handling, and the storage of the product (58). The distance within polymer molecules and between polymer and plasticizer molecules and the level of dryness will determine the mechanical strength of the film.

Soy protein films with the thickness of 63.3–93.5 μm (35, 36), 84–128 μm (38), and 156.4 μm (59) have been reported. However, thick films are not suitable for applications including coatings of food products. Table 3 shows the thickness of soy, whey and wheat protein, and carboxymethyl cellulose films produced by casting (40). Wheat gluten film had the greatest thickness (44.1 μm) followed by soy protein (27.5 μm) and whey protein films (13.1 μm). Carboxymethylcellulose films also can be produced with low thickness (20.3 μm). The high molecular weight of wheat gluten (monomeric gliadins 30–80 kDa, polymeric glutenins 30–120 kDa) and soy proteins (7S globulin 168 kDa, 11S globulin 300–380 kDa) compared with whey protein (β-lactoglobulin 18.3 kDa, α-lactalbumin 14.2 kDa) may have contributed to the greater thickness of these films. For the applications of food

**TABLE 3. Thickness and Mechanical Properties of Films.**

Polymer Film	Thickness $\mu\text{m}$	Tensile Strength (MPa)	Tension at Break (MPa)	Elongation %	Puncture Strength (N)
SPI	27.5 <sup>b</sup>	2.89 <sup>c</sup>	2.86 <sup>c</sup>	4.99 <sup>a</sup>	0.47 <sup>b</sup>
WPI	13.1 <sup>a</sup>	20.13 <sup>b</sup>	18.51 <sup>b</sup>	11.24 <sup>a</sup>	0.64 <sup>b</sup>
WG	44.1 <sup>c</sup>	23.28 <sup>ab</sup>	22.63 <sup>ab</sup>	7.56 <sup>a</sup>	3.54 <sup>a</sup>
CMC	20.3 <sup>b</sup>	27.26 <sup>a</sup>	27.26 <sup>a</sup>	13.78 <sup>a</sup>	3.54 <sup>a</sup>

<sup>a</sup>Values are means of triplicate analysis. Means with same superscript in each column are not significantly different ( $p > 0.05$ ).

Adapted from Eswaranandam and Hettiarachchy (40).

coating, low thickness of film is preferred. Other investigators produced films with higher thickness. Homogenous film by pouring results in films with high thickness. Homogenous film by casting with the Drawdown instrument can produce films with low thickness. The optimized film-forming solution produced for casting with the Drawdown instrument with low thickness will resist dripping from the surface when applied on the surface of food products.

**2.2.2. Color** The color of the film can affect the consumer acceptance of coated produce (60). Five different color systems XYZ, Yxy,  $L^*a^*b^*$ ,  $L^*C^*H^0$ , and Hunter Lab are used to express absolute chromaticity. Color system XYZ is measured as tristimulus values of color and forms the basis for most calculations. In the color system Yxy, Y is a lightness factor expressed as a percentage based on a perfect reflection of 100%. x and y are chromaticity coordinates defined by the following equations:

$$x = \frac{X}{X + Y + Z}, \quad y = \frac{Y}{X + Y + Z}.$$

Color  $L^*a^*b^*$  system closely represents the color sensitivity of the human eye.  $L^*$  is the lightness variable, and  $a^*$  and  $b^*$  are color coordinates defined by the following equations:

$$L^* = 116 \left( \frac{Y}{Y_n} \right)^{\frac{1}{3}} - 16, \quad a^* = 500 \left[ \left( \frac{Y}{X_n} \right)^{\frac{1}{3}} - \left( \frac{Y}{Y_n} \right)^{\frac{1}{3}} \right], \quad b^* = 200 \left[ \left( \frac{Y}{Y_n} \right)^{\frac{1}{3}} - \left( \frac{Z}{Z_n} \right)^{\frac{1}{3}} \right],$$

where X, Y, and Z are measured tristimulus values of the sample.

$X_n$ ,  $Y_n$ , and  $Z_n$  are tristimulus values of the light source used (for  $D_{65}$ ,  $X_n = 95.045$ ,  $Y_n = 100.00$ , and  $Z_n = 108.892$ )

The red color never contains green components, the blue color never contains yellow components, and the white color never contains black components.

The total color difference  $\Delta E^*_{ab}$  is defined by following equation:

$$\Delta E^*_{ab} = \sqrt{(L^* - L_t^*)^2 + (a^* - a_t^*)^2 + (b^* - b_t^*)^2},$$

where  $L_t^*$ ,  $a_t^*$ , and  $b_t^*$  are target colors.

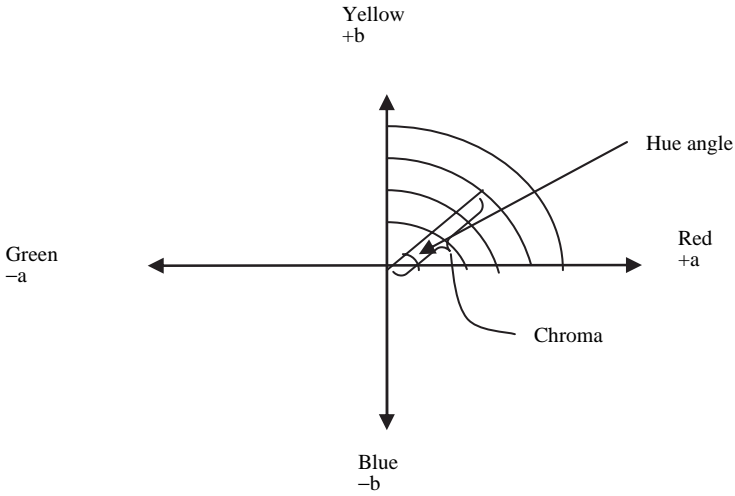


Figure 7. LCH Color coordinates.

The  $L^*C^*H^\circ$  color system uses the same coordinates (Figure 7) as the  $L^*a^*b^*$  system, but it uses cylindrical coordinates instead of Cartesian coordinates. In this system,  $L^*$  represents lightness,  $C^*$  represents chroma where  $C^* = \sqrt{(a^*)^2 + (b^*)^2}$ , and  $H^\circ$  represents Hue angle defined by

$$\begin{aligned}
 H^\circ &= \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad \text{when } a^* > 0 \text{ and } b^* > 0, \\
 H^\circ &= 180 + \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad \text{when } a^* < 0, \\
 H^\circ &= 360 + \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad \text{when } a^* > 0 \text{ and } b^* < 0.
 \end{aligned}$$

In the Hunter Lab color system,  $L$  is the lightness variable and  $a$  and  $b$  are chromaticity coordinates defined as follows:

$$\begin{aligned}
 L &= 100\left(\frac{T}{Y_n}\right)^{\frac{1}{2}}, \\
 a &= 175\left[\left(\frac{0.0102X_n}{Y/Y_n}\right)^{\frac{1}{2}}\right]\left[\left(\frac{X}{X_n}\right) - \left(\frac{Y}{Y_n}\right)\right], \\
 b &= 70\left[\left(\frac{0.00847Z_n}{Y/Y_n}\right)^{\frac{1}{2}}\right]\left[\left(\frac{Y}{Y_n}\right) - \left(\frac{Z}{Z_n}\right)\right],
 \end{aligned}$$

where  $X, Y,$  and  $Z$  are tristimulus values of the sample.

**TABLE 4. Color L\*a\*b\*, Chroma Hue Angle and  $\Delta E$  of Films.**

	L	a	b	Chroma	Hue Angle	$\Delta E^*$
SPI	96.69 <sup>a</sup>	-0.62 <sup>b</sup>	6.69 <sup>b</sup>	6.71 <sup>b</sup>	95.23 <sup>a</sup>	4.90 <sup>b</sup>
WPI	96.17 <sup>b</sup>	0.15 <sup>a</sup>	2.15 <sup>c</sup>	2.15 <sup>c</sup>	86.14 <sup>c</sup>	1.00 <sup>c</sup>
WG	94.52 <sup>c</sup>	-0.61 <sup>b</sup>	10.01 <sup>a</sup>	10.02 <sup>a</sup>	93.44 <sup>b</sup>	8.56 <sup>a</sup>
CMC	96.52 <sup>ab</sup>	0.17 <sup>a</sup>	2.05 <sup>c</sup>	2.05 <sup>c</sup>	85.25 <sup>c</sup>	0.62 <sup>c</sup>

Values are means of triplicate analysis. Means with same superscript in each column are not significantly different ( $p > 0.05$ ).

L, \* Lightness index from black (0) to white (100).

<sup>a</sup>Greenness (-80) to redness (+100).

<sup>b</sup>Blueness (-80) to yellowness (70).

<sup>c</sup>Chroma.

$\Delta E$ , Color difference from white standard plate (L, 97.10; a, -0.13; b, 1.88).

Adapted from Eswaranandam and Hettiarachchy (40).

$X_n$ ,  $Y_n$ , and  $Z_n$  are tristimulus values of the light source used (for  $D_{65}$ ,  $X_n = 95.045$ ,  $Y_n = 100.00$ , and  $Z_n = 108.892$ )

The color characteristics of the film can be affected by various treatments. Rhim et al. (35) reported that the transparent greenish-yellow color ( $L = 92.9$ ,  $a = -3.27$ , and  $b = 15.4$ ) of soy protein film increased lightness (L) and decreased yellowness (a) and greenness (b) by the addition of polyethyleneglycol alginate. Rhim et al. (36) also reported color changes of heat-cured (90 °C 24 h) and UV-treated (51.8 J/m<sup>2</sup>) soy protein film. Both heat-cured and UV-treated films had lower lightness (L) and greater yellowness (b) than control. Yellowish coloration (+b) increased linearly with the UV-irradiation dosage up to 103.7 J/m<sup>2</sup> (46). The color of the wheat gluten film changed drastically by heat curing. When the temperature of heat curing of wheat gluten film increased from 20 °C to 140 °C, lightness (L) decreased by 20% and redness (a) and yellowness (b) increased by 1137% and 307%, respectively (48).

Table 4 shows the color lab chroma and hue angle of soy, whey and wheat protein, and carboxymethyl cellulose films (40). Wheat gluten film was darker (Lower  $L = 94.52$ ) than all other films, which is in agreement with the L value (94.73) of wheat gluten film reported by Micard et al. (47). Soy protein film had higher L value (96.69) than other films. Film color is influenced by thickness and pH of the film. Rhim et al. (36) reported an L value of 93.0 for soy protein film at a pH of 10.0. Wheat gluten and soy protein films are more yellowed (higher b, 10.01 and 6.69, respectively) than whey protein and CMC film, although the yellow color was visually unnoticeable in thin films. Micard et al. (47) also reported a higher b value (11.15) of wheat gluten film. Rhim et al. (36) reported a higher b value (14.40) for soy protein film at a pH of 10.0. The hue angle of the films was high (85.25–95.23) because of the lower a value and the higher b value. The yellow color of wheat gluten and soy protein films resulted in a higher color difference ( $\Delta E$ ) from the standard white color plate, and the transparent character of whey protein and CMC films resulted in a lower color difference ( $\Delta E$ ) from the standard white color plate. Thin films with low  $\Delta E$  will not mask the original color of the product and are preferred for coating.



Thin film with high tensile and puncture strength, low color difference, and low water vapor permeability is more suitable for application as coating, the carriers of antimicrobials, and nutraceuticals than thick film produced by pouring film-forming solution.

**2.2.3. Mechanical Properties** Edible films should have adequate mechanical strength and extensibility to maintain integrity and withstand external stress that would occur during processing, handling, and storage (58). Tensile and puncture strengths are used to express the mechanical property of the film. Mechanical strength and barrier properties of the film can be increased by cross-linking. Protein can be cross-linked by irradiation or enzymes, such as transglutaminase, peroxidase, or chemicals, such as glutaraldehyde, glyceradehyde, formaldehyde, gossypol, and tannic and lactic acids and divalent cations (61). Yildirim and Hettiarachchy (62) studied the properties of transglutaminase cross-linked whey protein and 11S globulin film. Transglutaminase cross-linking increased tensile strength and decreased solubility of the film.

Incorporation of additives other than cross-linking agents will result in lower tensile strength and higher elongation because these molecules form hydrogen bonds with an amide group of proteins (15). Yildirim and Hettiarachchy (62) reported that transglutaminase cross-linking increased tensile strength and puncture strength of whey protein and 11s globulin composite film by two times the control. Rhim et al. (35) reported that incorporation of polyethyleneglycol alginate increased tensile strength and decreased percentage elongation of the soy protein film. Rhim et al. (36) also reported that puncture strength of the soy protein film was lower in acidic medium and higher in alkaline medium than in water. Were et al. (63) reported high tensile and puncture strength with added cysteine of soy protein and wheat gluten (4:1) film at a pH of 7. Wu and Zhang (64) reported a decrease in tensile strength of soy protein film from 18.5 MPa to 3.8 MPa and an increase in percent elongation from 20% to 230% by the addition of 20–70-g ethylene glycol per 100-g soy protein. Galietta et al. (34) reported increasing glycerol concentration from 25% to 40% decreased mechanical strength, and incorporation of formaldehyde up to 9-g/100 g dry matter increased mechanical strength of whey protein film. An increase in tensile strength (75%) and stiffness (Young's module) (314%) and a decrease in elongation (36%) with aging of wheat gluten films for 5 days was reported by Micard et al. (47), because of the formation of a disulfide bond by thiol oxidation. Perez-Gago and Krochta (65) reported an increase in tensile strength, Young's modulus, and elongation with increased heat denaturation temperature from 70 °C to 100 °C and time from 5 to 20 min. Divalent cations such as calcium can cross-link between negatively charged groups of protein molecules and decrease mobility of protein chains that contributes to an increase in tensile strength and barrier properties (66).

Table 3 shows the thickness and tensile and puncture strength, tension at break, and percent elongation of soy, whey and wheat protein, and carboxymethyl cellulose films (40). Wheat gluten film showed higher tensile (23.28 MPa) and puncture strengths (3.54 N). Micard et al. (47) reported a very low tensile strength (2.1 MPa)

for wheat gluten. The differences observed may be from high glycerol content (23%) and low heating temperature (50 °C ) of the film-forming solution, which is not enough to open the structure of protein molecules (48). The soy protein film showed lower tensile (2.89 MPa) and puncture strengths (0.47 N). Tensile and puncture strengths of the films depend on pH and thickness of the film. Brandenburg et al. (37) reported the tensile strength of soy protein films (thickness 63.8 μm) to be 3.13, 4.33, 5.23, and 4.54 MPa at a pH of 6.0 to 8.0, and 10.0 and 12.0, respectively. Park et al. (38) reported the tensile strength of soy protein film to be 5.5 MPa at a pH of 9.0, and Rhim et al. (36) reported the tensile strength of 8.2 MPa at a pH of 10.0. Thin homogenous protein films produced by casting had higher tensile and puncture strength than thick pouring films. Drawdown cast film has the advantage of high mechanical strength even at low thickness compared with poured film because of the uniform thickness.

Carboxymethyl cellulose film showed higher tensile (27.26 MPa) and puncture strengths (3.54 N). A tensile strength of 10 to 20 MPa was reported for methylcellulose film (67). Thin homogenous cellulose films produced by casting with the Drawdown instrument had higher tensile and puncture strength than thick pouring films. There is no significant difference ( $p > 0.05$ ) in percent elongation soy, whey and wheat protein, and carboxymethyl cellulose films.

**2.2.4. Barrier Property—Water Vapor Permeability (WVP)** Protein films are excellent gas barriers but poor moisture barriers because of their hydrophilic nature. Mechanical properties and gas permeability depend on the relative humidity (1).

Water vapor permeability of edible film quantifies the efficiency to reduce moisture transfers between food and its surrounding medium. Transport of water vapor through polymer films proceeds through (1) adsorption on to the surface, (2) solution into polymer matrix, (3) diffusion through polymer matrix, and (4) desorption of water vapor from another surface of polymer film (68). Water flux varies nonlinearly with water vapor pressure difference so that the WVP is not an inherent property of hydrophilic films (69). The water vapor transmission rate increased with increasing moisture content of the film and relative humidity of the two sides of the film with a constant difference between two sides (70). Protein films are poor water vapor barriers because of their hydrophilic nature, which is a major challenge for many food applications (1). Water vapor barrier properties can be improved by incorporating hydrophobic lipid material or by developing bilayer protein-lipid film (71). Water molecules also affect barrier and other properties such as plasticization, swelling, and solubilization of hydrophilic films by causing sorption and swelling, and by changing the structure of the film. But the water barrier property of hydrophobic films, such as polyethylene, depends only on the water vapor pressure difference on both sides of the film (72). Diffusivity and solubility of water molecules in the film matrix influence the water vapor transmission through protein film. Water vapor diffusivity increases by plasticizer molecules, which increase inter-chain spacing between polymers (17).

Water vapor permeability can be measured by the gravimetric method (ASTM E96-00e1) (73) for a hydrophobic film. For hydrophilic films, the method is

corrected to include partial pressure gradient in the stagnant air layer between the mounted film and the water and thickness of the film. When the stagnant air gap is > 14-mm air convection will occur due to the effect of evaporative cooling (69).

Were (74) studied the effect of cysteine-added, soy protein–wheat gluten, composite film. Incorporation of cysteine increased tensile strength of soy:gluten 4:1 film at a pH of 3.0 from 3.68 to 4.94 MPa. Park et al. (38) studied the effect of calcium salts and glucano- $\delta$ -lactone on the mechanical properties of soy protein film. Calcium sulfate 0.3% (w/w of soy protein) increased the tensile strength and puncture strength of soy protein film from 5.5 and 5.9 to 8.6 and 9.8 MPa, respectively. Glucano- $\delta$ -lactone 0.3% (w/w of soy protein) increased the tensile strength and puncture strength to 8.3 and 8.8 MPa, respectively.

High moisture content (38.5–41.7%) and lower moisture adsorption (3.1%) during the WVP determination may favor the initial adsorption, solution, diffusion, and desorption, which caused higher WVTR of soy protein film. Wheat gluten films had the lowest WVTR (48.96 g/h m<sup>2</sup>). Low moisture content (15.0-17.0%) and lower moisture adsorption (2.0%) during the WVP determination may hinder the moisture transmission through the wheat gluten film. Water vapor permeability of soy protein film (1.01 g mm/kPa h m<sup>2</sup>) were higher than carboxymethyl cellulose films (0.76 g mm/kPa h m<sup>2</sup>) and whey protein films (0.69 g mm/kPa h m<sup>2</sup>). Wheat gluten film (0.48 g mm/kPa h m<sup>2</sup>) has lower WVP. Higher WVPs were reported for films with greater thickness. Park et al. (38) and Rhim et al. (35) reported that soy protein films with the thickness of 84  $\mu$ m and 63  $\mu$ m have a WVP of 8.86 and 9.00 g mm/kPa h m<sup>2</sup>, respectively. Anker et al. (75) reported that whey protein films with the thickness of 175  $\mu$ m had a WVP of 14 g mm/kPa h m<sup>2</sup>. Yildirim and Hettiarachchy (62) reported a WVP of 2.71 g mm/kPa h m<sup>2</sup> for whey protein film with the thickness of 74  $\mu$ m. Thin film produced by casting had lower water vapor permeability than films prepared by pouring. Water vapor permeability of 0.49 g mm/kPa h m<sup>2</sup> was reported for wheat gluten film (48).

Table 5 shows the WVP and WVTR of soy, whey and wheat protein, and carboxymethyl cellulose films (40). Lower thickness of the films might result in lower WVTR and WVP in all films. McHugh et al. (69) reported a direct correlation

**TABLE 5. Water Vapor Barrier Property of the Films.**

Polymer Film	Moisture Content of the Film (%)		Moisture Adsorption (%)	WVTR (g/h m <sup>2</sup> )	WVP (g mm/kPa h m <sup>2</sup> )
	Before WVP Determination	After WVP Determination			
SPI	38.5 <sup>a</sup>	41.7 <sup>a</sup>	3.1	61.08 <sup>a</sup>	1.01 <sup>a</sup>
WPI	26.8 <sup>b</sup>	38.1 <sup>b</sup>	11.3	48.96 <sup>b</sup>	0.69 <sup>b</sup>
WG	15.0 <sup>c</sup>	17.0 <sup>c</sup>	2.0	34.50 <sup>c</sup>	0.48 <sup>c</sup>
CMC	26.3 <sup>b</sup>	34.3 <sup>b</sup>	8.1	52.69 <sup>b</sup>	0.76 <sup>b</sup>

<sup>a</sup> Values are means of triplicate analysis. Means with same superscript in each column are not significantly different ( $p > 0.05$ ). Water vapor partial pressure under ( $P_2$ ) and over ( $P_3$ ) side of the film are 2.64 and 0.78 kPa, respectively.

Adapted from Eswaranandam and Hettiarachchy (29).

between thickness and WVP. The WVTR of soy protein film (61.08 g/h m<sup>2</sup>) was higher than CMC film (52.69 g/h m<sup>2</sup>) and whey protein film (48.96 g/h m<sup>2</sup>).

The physical and mechanical properties indicate that edible films have a potential application in agricultural and biomedical fields. The potential applications of edible film for commercial application to extend shelf-life and improve microbial safety in whole and minimally processed fruits and vegetables as well as poultry meat and sea food products seems promising. Furthermore, these edible films can be tailored to incorporate antimicrobials, antioxidants, phytonutrients, nutraceuticals, nutrients, flavor, colors, and food additives. These areas need extensive research.

## REFERENCES

1. C. J. Weber. (2000). *Bio-based Packaging Materials for the Food Industry Status and Prospectives*. Frederiksberg C, Denmark. The Royal Veterinary and Agricultural University. Available: [www.mli.kvl.dk/foodchem/special/biopack/](http://www.mli.kvl.dk/foodchem/special/biopack/).
2. G. Robbelen, R. K. Downey, A. Ashri, *Oil Crops of the World, Their Breeding and Utilization*, McGraw-Hill, New York, 1989, p. 553.
3. M. R. B. M. Salleh, N. Maruyama, M. Adachi, N. Hontani, S. Saka, N. Kato, Y. Ohkawa, and S. Utsumi, *J. Agric. Food Chem.*, **50**, 7380–7385 (2002).
4. V. Prakash and M. S. N. Rao, *CRC Crit. Rev. Biochem.*, **20**, 265–363 (1986).
5. D. S. Cha and M. S. Chinnan, *Crit. Rev. in Food Sci. and Nutri.*, **44**, 223–237 (2004).
6. S. G. Pe  rez, K. B. Merck, J. M. Vereijken, G. A. Van Koningsveld, H. Gruppen, and A. G. J. Voragen, *J. Agric. Food Chem.*, **50**, 1713–1719 (2002).
7. J. Grevellec, C. Marquie, L. Ferry, A. Crespy, and V. Vialettes, *Biomacromolecules*, **2**, 1104–1109 (2001).
8. K. Bandyopadhyay and S. Ghosh, *J. Agric. Food Chem.*, **50**, 6854–6857 (2002).
9. M. Sen and D. K. Bhattacharyya, *J. Agric. Food Chem.*, **49**, 2641–2646 (2001).
10. P. R. Shewry and B. J. Mifflin, *Advances in Cereal Science and Technology*, American Association of Cereal Chemists, Inc., Minnesota, 1985, pp. 1–83.
11. A.F. Pomes, *Encyclopedia of Polymer Science and Technology: Plastics, Resins, Rubbers, Fibers*, Interscience Publishers, New York, 1971, pp. 125–132.
12. D. S. Cha and M. S. Chinnan, *Crit. Rev. in Food Sci. and Nutri.*, **44**, 223–237 (2004).
13. B. Cuq, N. Gontard, and S. Guilbert, *Cereal Chem.* **75**, 1–9, (1998).
14. A. Gennadios, T. H. McHugh, C. L. Weller, and J. M. Krochta, *Edible Coatings and Films to Improve Food Quality*, Technomic Publishing Co., Lancaster, PA, 1994, pp. 201–277.
15. S. Guilbert, *Food Packaging and Preservation, Theory and Practice*, Elsevier Applied Science, London, 1986, pp. 371–394.
16. J. J. Kester and O. Fennema, *Food Tech.*, **40**, 47–59 (1986).
17. S. Guilbert, and N. Gontard, *Foods and Packaging Materials-Chemical Interactions*, The Royal Society of Chemistry, Cambridge, U.K., 1995, p. 231.
18. M. B. Perez-Gago and J. M. Krochta, *J. Food Sci.*, **64**, 1034–1037 (1999).

19. F. Riquelme, M. T. Pretel, G. Martinez, M. Serrano, A. Amoros, and F. Romojaro, *Food Packaging and Preservation*, Blackie Academic, Glasgow, 1994, pp. 141–157.
20. D. Zagory and A. A. Kader, *Food Tech.*, **42**, 70–77 (1988).
21. H. J. Park, M. S. Chinnan, and R. L. Shewfelt, *J. Food Sci.*, **59**, 568–70 (1994).
22. H. J. Park, M. S. Chinnan, and R. L. Shewfelt, *J. Food Pro. Pre.*, **18**, 317–331 (1994).
23. F. Debeaufort, J. A. Quezada-gallo, and A. Voilley, *Crit. Rev. Food Sci.*, **38**(4), 299–313 (1998).
24. J. M. Krochta, *Food Proteins and Their Applications*, Marcel Dekker, New York, 1997, pp. 529–550.
25. F. M. Al-ameri, M.Sc. thesis, University of Arkansas, Fayetteville, AR, 2001.
26. D. B. Armitage, N. S. Hettiarachchy, and M. A. Monsoor, *J. Food Sci.*, **67**(2), 631–634 (2002).
27. W. J. Wolf and A. K. Smith, *Food Tech.*, **15**, 12 (1961).
28. D. Fukushima and J. Van Buren, *Cereal Chem.*, **47**, 687–696 (1970).
29. D. R. Briggs and W. J. Wolf, *Arch. Biochem. Biophys.*, **72**, 127–129 (1957).
30. S. Okamoto, *Cereal Foods World*, **23**, 256–260 (1978).
31. J. A. Torres, *Protein Functionality in Food Systems*, Marcel Dekker, New York, 1994, pp. 467–507.
32. A. Gennadios, C. L. Weller, and R. F. Testin, *Trans. Amer. Soc. Agric. Eng.*, **36**, 465–470 (1993).
33. P. F. Fairley, J. Monahan, J. B. German, and J. M. Krochta, *J. Agric. Food Chem.*, **44**, 438–443 (1996).
34. G. Galietta, L. D. Gioia, S. P. Guilbert, and B. Cuq, *J. Dairy Sci.*, **81**, 3123–3130 (1998).
35. J. W. Rhim, A. Gennadios, D. Fu, C. L. Weller, and M. A. Hanna, *Lebensm-Wiss Technol. (Food Sci. Technol.)* **32**, 129–133 (1999).
36. J. W. Rhim, A. Gennadios, A. Handa, C. L. Weller, and M. A. Hanna, *J. Agric. Food Chem.*, **48**, 4937–4941 (2000).
37. A. H. Brandenburg, C. L. Weller, and R. F. Testin, *J. Food Sci.*, **58**, 086–1089 (1993).
38. S. K. Park, C. O. Rhee, D. H. Bae, and N. S. Hettiarachchy, *J. Agric. Food Chem.*, **49**, 2308–2312 (2001).
39. N. Rangavajhyala, V. Ghorpada, and M. Hanna, *J. Agric. Food Chem.*, **45**, 4204–4208 (1997).
40. S. Eswaranandam and N. S. Hettiarachchy, *J. Agric. Food Chem.*, Manuscript in review.
41. R. Sothornvit and J. M. Krochta, *J. Food Eng.*, **50**, 149–155 (2001).
42. L. H. Krull and G. E. Inglett, *Cereal Sci. Today*, **16**, 232–236 (1971).
43. A. Cagri, Z. Ustunol, and E. T. Ryser, *J. Food Sci.*, **66**, 865–870 (2001).
44. L. R. Beuchat, *Food Safety Issues*, FSA/WHO, Food Safety Unit/World Health Organization, 1998, p. 42.
45. A. Gennadios, J. W. Rhim, A. Handa, C. L. Weller, and M. A. Hanna, *J. Food Sci.*, **63**, 225–228 (1998).
46. K. Tomihata, K. Burezak, K. Shiraki, and Y. Ikada, *Polymer Preprints*, **33**, 534–535 (1992).
47. V. Micard, R. Belamri, M. H. Morel, and S. Guilbert, *J. Agric. Food Chem.*, **48**, 2948–2953 (2000).

48. W. M. Urbain, *Radiation Chemistry of Major Food Components*, Elsevier Scientific, Amsterdam, 1977, pp. 63–130.
49. C. Vachon, H. L. Yu, R. Yefsah, R. Alain, D. St-Gelais, and M. Lacroix, *J. Agric. Food Chem.*, **48**, 3202–3209 (2000).
50. D. Brault, G. D'Aprano, and M. Lacroix, *J. Agric. Food Chem.*, **45**, 2964–2969 (1997).
51. J. C. Cheftel, J. L. Cuq, and D. Lorient, *Food Chemistry*, 2nd ed., Marcel Dekker, New York, 1985, pp. 245–369.
52. V. M. Ghorpade, A. Gennadios, M. A. Hanna, and C. L. Weller, *Cereal Chem.*, **72**, 559–563 (1995).
53. S. Eswaranandam, Ph.D. dissertation, University of Arkansas, Fayetteville, AR, 2004.
54. J. Gueguen, G. Viroben, P. Noireaux, and M. Subirade, *Industrial Crops Prod.*, **7**, 149–157 (1998).
55. N. H. Banks, *J. Exper. Bot.*, **35**, 127–129 (1984).
56. S. M. Smith and J. R. Stow, *Ann. Appl. Biol.*, **104**, 383–386 (1984).
57. R. H. Risse, D. Chun, R. E. McDonald, and W. R. Miller, *Hort Sci.*, **22**, 274–279 (1987).
58. L. Yang and A. T. Paulson, *Food Research Int.*, **33**, 571–578 (2000).
59. S. Ko, M. E. Janes, N. S. Hettiarachchy, and M. G. Johnson, *J. Food Sci.*, **66**, 1006–1011 (2001).
60. L. F. Kunte, A. Gennadios S. L. Cuppett, M. A. Hanna, and C. L. Weller, *Cereal Chem.*, **74**, 115–118 (1997).
61. E. R. Lieberman and S. G. Gilbert, *J. Polym. Sci.*, **41**, 33–43 (1973).
62. M. Yildirim and N. S. Hettiarachchy, *J. Food Sci.*, **63**, 248–252 (1998).
63. L. Were, N. S. Hettiarachchy, and M. Coleman, *J. Food Sci.*, **64**(3), 514–518 (1999).
64. Q. X. Wu and L. N. Zhang, *Indust. Eng. Chem. Res.* **40**, 1879–1883 (2001).
65. M. B. Perez-Gago and J. M. Krochta, *J. Food Sci.*, **66**, 705–710 (2001).
66. J. M. Krochta, A. E. Pavlath, and N. Goodman, *Engineering and Food*, Elsevier Applied Science, New York, 1990, pp. 329–340.
67. F. Debeaufort, A. Voilley, and P. Meares, *J. Agric. Food Chem.*, **45**, 685–689 (1997).
68. F. Debeaufort, A. Voilley, and P. Meares, *J. Membr Sci.*, **91**, 125–133 (1994).
69. T. H. McHugh, R. Avena-Bustillos, and J. M. Krochta, *J. Food Sci.*, **58**, 889–903 (1993).
70. S. L. Kamper and O. Fennema, *J. Food Sci.*, **49**, 1482–1485 (1984).
71. A. Gennadios and C. L. Weller, *Cereal Foods World*, **36**, 1004–1009 (1991).
72. V. Morillon, F. Debeaufort, M. Capelle, G. Blond, and A. Voilley, *J. Agric. Food Chem.*, **48**, 11–16 (2000).
73. ASTM. E96-00e1, *Annual Book of American Standard Testing Methods*, ASTM, Philadelphia, PA, 2003.
74. L. M. Were, M.Sc. thesis, University of Arkansas, Fayetteville, AR, 1998.
75. M. Anker, M. Stading, and A. Hermansson, *J. Agric. Food Chem.*, **47**, 1878–1886 (1999).

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## *Pharmaceutical and Cosmetic Use of Lipids*

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### **1. INTRODUCTION**

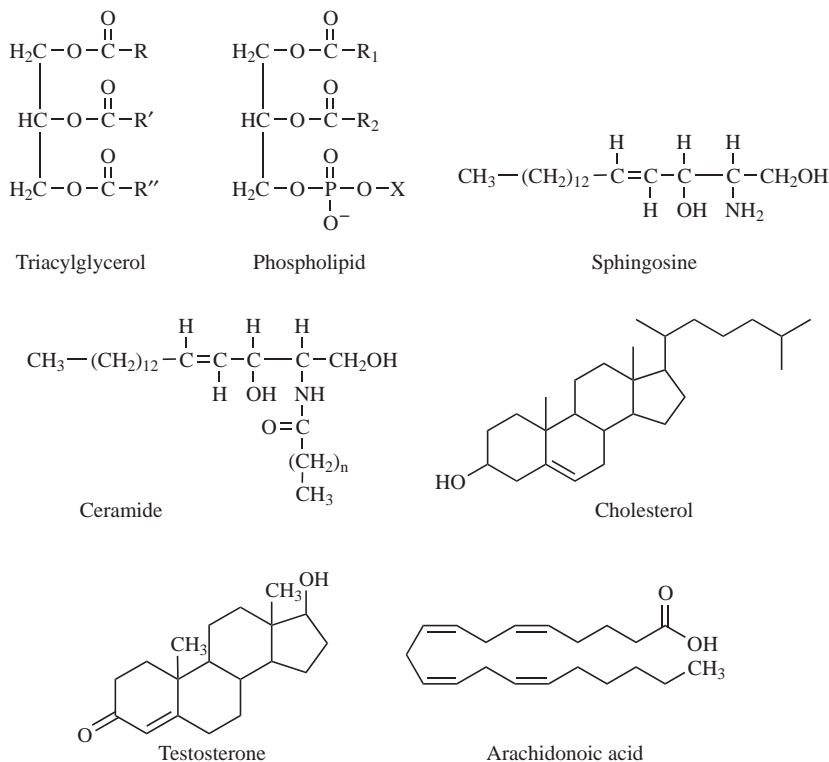
Lipids are a major source of storage energy, are important precursors in the body's metabolic processes, and are essential components of cell membranes and other biological structures. Lipids also play important roles in absorption of fat-soluble nutrients. They function as essential fatty acids (omega-3 and omega-6), fat-soluble vitamins, cellular transport components, and food/medical supplements. Besides nutrition and biological functions, lipids play an essential role in processing, quality, and the organoleptic and texture properties of food products (1, 2). Fatty acids are important components of cell membrane processes and play a major role in the formation of eicosanoids. Lipids come mainly from one of two sources, ingested foods or are synthesized in the body, primarily in the liver.

The metabolic role of lipids and the manner in which they operate in cellular structures is increasingly better understood. This understanding has allowed for the developments of a diverse number of applications both in the pharmaceutical and cosmetic fields. The major types of lipids that are present in the human body and/or play major roles in metabolic processes are triacylglycerols (TAGs), free

**TABLE 1. Metabolic Fatty Acids.**

Carbon number	Common Name	Structure	Comments
14:0	Myristic acid	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$	Found in plasma membrane-associated cytoplasmic proteins
16:0	Palmitic acid	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$	End product of mammalian fatty acid synthesis
16:1 $\Delta^9$	Palmitoleic acid	$\text{CH}_3(\text{CH}_2)_5\text{C}=\text{C}(\text{CH}_2)_7\text{COOH}$	Found in storage lipids
18:0	Stearic acid	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$	Found in storage lipids
18:1 $\Delta^9$	Oleic acid	$\text{CH}_3(\text{CH}_2)_7\text{C}=\text{C}(\text{CH}_2)_7\text{COOH}$	Most abundant in adipose lipids
18:2 $\Delta^{9,12}$	Linoleic acid	$\text{CH}_3(\text{CH}_2)_4\text{C}=\text{CCH}_2\text{C}=\text{C}(\text{CH}_2)_7\text{COOH}$	Essential fatty acid
18:3 $\Delta^{9,12,15}$	Linolenic acid	$\text{CH}_3\text{CH}_2\text{C}=\text{CCH}_2\text{C}=\text{CCH}_2\text{C}=\text{C}(\text{CH}_2)_7\text{COOH}$	Essential fatty acid
20:4 $\Delta^{5,8,11,14}$	Arachidonic acid	$\text{CH}_3(\text{CH}_2)_3(\text{CH}_2\text{C}=\text{C})_4(\text{CH}_2)_3\text{COOH}$	Precursor for eicosanoid synthesis





**Figure 1.** Structures of major bioactive lipids.

fatty acids, phospholipids, sphingolipids, bile salts, steroids and sterols, cholesterol, eicosanoids, and fat-soluble vitamins. Table 1 and Figure 1 show a more detailed list of these lipids and their properties.

Lipid nutritional supplements have been in use before the term *nutraceutical* was coined. Products such as fish oils, shark cartilage, shark liver oil, and vitamins have been in the market since the beginning of the twentieth century. Some of the health claims of these products lacked strict scientific documentation in the past, and their curative properties were mostly anecdotal. However, today there is a better understanding of the biological properties of lipids and their application has extended to combined pharmaceutical and cosmetic fields such as disease prevention and treatment, excipients and coadjuvants, *trans*-dermal carriers, and skin emolliency agents. This has led to the development of bioactive cosmetic and pharmaceutical products whose name has recently been coined as *cosmeceuticals*.

## 2. LIPIDS IN DISEASE PREVENTION AND TREATMENT

There is currently a wide range of vegetable oils and fats with diverse nutritional, pharmaceutical, and cosmetic properties. The metabolism of triacylglycerol lipids

start after ingestion, where they are typically hydrolyzed by lipases in the digestive tract into monoacylglycerols and free fatty acids and then absorbed in the upper segment of the small intestine. These are then synthesized back into TAGs in the mucosal epithelial layer and enter the bloodstream as chylomicrons through the lymphatic system. These chylomicrons are then transported via the bloodstream to the peripheral tissues and organs to perform diverse functions (3).

## 2.1. Essential Fatty Acids

It has been widely documented that essential fats, or lack of them, play an essential role regarding the onset or control of many diseases such as inflammation, arthritis, and diabetes. Omega-3 fats are commonly used to improve growth and development of infants, treatment of disease in adults, and other disease prevention measures (4–8). Fish oil has been studied for the treatment of inflammation caused by excessive pro-inflammatory eicosanoids and cytokine production. Fish oil has been suggested for the prevention and therapy of rheumatoid arthritis and other chronic diseases (9, 10). The main sources of omega-3 essential fatty acids are marine oils and oilseeds. Oils of plant origin with a high content of omega-3 fatty acids include flax and perilla oils that contain over 50% alpha-linolenic acid.

## 2.2. Fat-soluble Vitamins

Vitamin A, derived from carotenoids and retinoids, is required for eye development and prevention of vision disease. Retinol and retinoic acid also play an important role in the proper function of mucose-secreting cells. Vitamin A influences the modulation of gene expression related to cell differentiation and cell adhesion (11). Intake requirements for Vitamin A are approximately 1.4 mg/day, and the main source of this vitamin is animal products and fats and oils. One of the primary roles of Vitamin E is to control the peroxidation of polyunsaturated fatty acids (PUFA). Vitamin E has been reported to inactivate disease-causing free radicals and control oxygenase activity. Vitamin E and antioxidants in general are known to minimize peroxidation at the cellular level and thus help prevent premature cellular aging and tissue degeneration. The daily recommended intake of Vitamin E is 200–400 IUs (12). Currently, the main source of Vitamin E or alpha tocopherol is vegetable oils. Vitamin D is reported to regulate calcium and phosphorous metabolism as well as to modulate secretion of polypeptides and hormones. Milk is the most important source of Vitamin D, and it is generally acknowledged as the main provider of this nutrient for the U.S. population, with an adequate daily intake of 5–10 mg (13). Vitamin K is a nutrient required for the biological activity of several coagulation factors in the bloodstream. Vitamin K has also been reported to play a role in other physiological processes and may affect other parameters of bone metabolism, such as calcium hemostasis. This vitamin is commonly found in green vegetables, and its supply does not seem to present a problem (14).

## 2.3. Sterols and Steroids

Phytosterols are found in most common vegetable oils. For example, soybean oil, one of the most commonly consumed oils, is reported to contain approximately 0.36% sterols and 0.124% tocopherols (15). The effect of phytosterols on the reduction of serum cholesterol has been attributed to the possible inhibition of intestinal reabsorption of circulating cholesterol. The saturated version of sterols, stanols, is reported to be more readily metabolized, and this has led to the development of nutritional supplements and it is used commercially in food products such as margarines. The main stanol reported for this application is sitostanol, a saturated derivative of sitosterol. Sterol and cholesterol are used by the human body to synthesize important hormones such as testosterone and progesterone, which are used also in many pharmaceutical applications.

## 2.4. Structured Lipids (SLs)

Structured lipids consist of triacylglycerols containing a mixture of short-, medium-, and long-chain fatty acids produced through chemical or enzymatic modification. This modification is usually done by interesterification. In application in the food and medical fields, SLs are normally synthesized through lipase-catalyzed interesterification reactions (16, 17). An example of a medical application of structured lipids products is short- and medium-chain triacylglycerols as a source of rapid energy for preterm infants and patients with fat malabsorption-related diseases, as well as for cancer and AIDS patients. The main source of these SLs is from fats like coconut oil and dairy fats through interesterification and fractionation (17). Medium-chain triacylglycerols (MCTs) are basically transported directly via portal circulation instead of being absorbed via the lymphatic system after micellar transport at the intestinal wall. Another structured lipid suggested as a treatment to lower serum triacylglycerols is diacylglycerols (18). More recently, conjugated linoleic acid (CLA) has been introduced into the supplement foods market either in pure form or as a structured lipid and many medical application studies have been reported (19–21). CLA is actually a group of double-bond positional isomers of naturally found linoleic acid. CLA is naturally present in low concentrations in some animal fats of ruminants. The CLA isomers that have been found with more bioactive properties are the *cis*-9, *trans*-11, and *trans*-10, *cis*-12 linoleic acids. The reported physiological effects of CLA include anticarcinogenic, antiatherogenic, reduced fat to lean body mass, improvement of type II diabetes mellitus, and immunomodulating properties (22). CLA has been found to exhibit consistent anti-tumor properties at levels as low as 0.25–1.0% of total fats. CLA currently available commercially at nutritional supplement outlets is usually in synthetic form (23).

## 2.5. Phospholipids

Phospholipids are well-known natural emulsifiers, wetting and dispersing agents, as well as liposome formers. They already have many food and pharmaceutical

applications and are well known for their synergistic functional and physiological properties, which make them very useful in a diverse number of food and medical formulations. In the digestive system, phospholipids play an important role in the metabolism of lipids and other digestive fluids. Their emulsifying properties allow them to play important roles in metabolic systems at the cellular level, such as membrane structure and cell membrane transport systems. This makes them very efficient as transport coadjuvants. They in fact ensure miscibility at liquid–liquid and liquid–solid interfaces in many biological pathways (24). The most common types of phospholipids are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), and phosphatidylserine (PS). PS has been shown to enhance neuronal membrane function and has been prescribed to older persons to improve cognitive function (25). PS is found at levels of up to 1% in commercial lecithin from vegetable oils and 3% in commercial lecithin from eggs. The Food and Drug Administration (FDA) authorized two health claims for PS on February 24, 2003. The first claim was “Phosphatidylserine (PS) may reduce the risk of cognitive dysfunction in the elderly,” and the second claim was “Phosphatidylserine (PS) may reduce the risk of dementia in the elderly.” Phospholipids, as structured phospholipids, are also used to enhance the biological activity of other functional lipids such as conjugated linoleic acid (26).

### 3. LIPIDS IN DRUG DELIVERY

The fact that most vegetable oils and fats are nontoxic allows them to be used as reliable excipients or carriers in many pharmaceutical formulations. Vegetable oils and fats have been approved as excipients to facilitate delivery of bioactive compounds, to act as fillers, binders, lubricants, solubilizers, emulsifiers, and emollients in a variety of delivery forms including tablets, capsules, suppositories, emulsions (enteral/parenteral), ointments, creams, and lotions. Other nondirect applications include artificial blood, gene delivery, diagnostic imaging, and medical devices (27).

Use of controlled release of drugs and bioactive substances is rapidly growing, and lipids are playing a major role in the formulation of new pharmaceutical products. Controlled release delivery systems can allow the pharmacologist to improve drug efficacy and better determine patient tolerance (28). Applications include delivery systems for cancer treatments, bacterial and fungal infections, and respiratory disease. In some cases, SLs, structured or otherwise, are now being used as both active pharmaceutical ingredients (APIs) and as excipients. Delivery of drugs is done mainly intravenously, orally, and transdermally. The transdermal and oral application of drugs is rapidly developing and lipids are playing a very important role.

#### 3.1. Lipids in Trans-Dermal Applications

*Trans*-dermal drug delivery has become one of the fastest growing systems for some disease treatments. It has been demonstrated that the skin can be used as a

portal of entry for drugs, for both localized and systemic treatment. However, the outer layer of the skin can be a difficult barrier to penetrate. Therefore, permeation-enhancing agents are in some cases necessary to allow intake of some drugs. There are several advantages reported for transdermal drug delivery: (1) bypassing of gastrointestinal and hepatic metabolism, (2) the potential for enhanced efficacy, (3) lower side effects through a more controlled delivery rate, and (4) increased patient compliance through easier application and dosing frequency (27).

Basically, the main purpose of human skin is to serve as a primary defense against outside contaminants like ultraviolet radiation, toxic agents, and microorganisms. It also serves as sensory and excretory organ and a critical regulator of body temperature. It is generally recognized that the upper layer of the skin or stratum corneum has limited permeability, which makes it difficult for drug transport. The human skin is composed of layers of differentiated strata (Stratum corneum, Stratum lucidum, Stratum Iranolosum, Stratum spinosum, Stratum basale). The upper layer of the skin or stratum corneum is the main barrier to permeation of outside elements. It basically consists of a structure of proteins and lipids (29). Even though no comprehensive model explains the structure and function of the mammalian skin barrier, the lipid composition and distribution in stratum corneum has been used to elucidate and model some of the behavior of transdermal transport (30). Modeling of the behavior of the stratum corneum is actively pursued to help provide a basis for skin disease permeability, treatment, topical drug administration, cosmetic formulations, and skin protection (31).

The stratum corneum basically contains a mixture of cholesterol, free fatty acids, and ceramides, placed in multilayers. They mediate both the epidermal permeability barrier and the transdermal delivery of both lipophilic and hydrophilic molecules. Studies have shown that each of the three key lipid classes is required for normal barrier function (32). These reports also show the potential of certain inhibitors of lipid synthesis to enhance the trans-dermal delivery of drugs like lidocaine or caffeine. Thus, the modulation of stratum corneum lipids is an important determinant of the barrier permeability to both hydrophobic and hydrophilic compounds transport and drug penetration. It has been reported that an inverse correlation exists between solute penetration and stratum corneum lipid content (33).

Modulation of epidermal lipid biosynthesis has been reported to boost drug delivery. It has also been suggested that it is both the hydrophobic nature of the lipids as well as their tortuous, extracellular localization that are responsible for the restriction in the transport of most molecules across the stratum corneum. The function of this barrier depends on three key lipids: cholesterol, fatty acid, or ceramides. Delays of synthesis ceramides in the epidermis have been reported as means of barrier perturbation. Inhibitors of lipid synthesis were used to enhance the transdermal delivery of lidocaine or caffeine. Alteration of barrier function was produced by either the fatty acid synthesis inhibitor 5-(tetradecyloxy)-2-furancarboxylic acid, the cholesterol synthesis inhibitor fluvastatin, or the cholesterol sulfate, which resulted in a further increase in lidocaine absorption (33).

The major components of sebaceous lipids in the skin are 45–60% triacylglycerols, 25% wax and sterol esters, 12–15% squalene and 10% free fatty acids (34).

Some fatty acids, especially unsaturated fatty acids, are well-known skin penetration enhancers. The addition of PC to dermal dosage forms has been reported to increase percutaneous absorption. Lipid disperse systems (LDSs) containing polar lipids, such as PC and glycosylceramide, are also useful for increasing the percutaneous permeation of drug through rat abdominal skin in both *in vitro* and *in vivo* systems.

Liposomes have widely reported an effective means of *trans*-dermal transport system. The use of liposomes has been reported in applications for topical and intravenous uses (35). Although the mechanism of this effect is not fully explained, it has been suggested that liposomes pass intact through the lipid-rich outer layer of the skin to the dermis, where they become localized. The suggestion has been made that the follicular pathway contributes to the liposomal delivery of drugs into the deeper skin strata (36).

Liposomal systems also can form an effective drug reservoir in the upper layers of the skin. This is particularly useful for local skin therapy. Ethosomal carriers composed of phospholipid vesicular systems with alcohols are also effective at enhancing *trans*-dermal delivery of both lipophilic and hydrophilic compounds. The use of these ethosomes has been used in the delivery of minoxidil to the pilosebaceous section of the skin with better results than conventional liposomes. Similar results are reported in clinical studies with acyclovir in a topical therapy treatment of recurrent herpes labialis. Other application reports with ethosomes are patches containing testosterone (37).

Topical applications in the form of spray also have been reported as vehicles for enhanced *trans*-dermal delivery of drugs such as testosterone, estradiol, progesterone, and norethindrone acetate. More effective drug penetration was reported with enhancers padimate and octyl salicylate and compared with laurocapram and oleic acid (38). Other methods reported for enhanced percutaneous drug absorption include iontophoresis (39), ultrasound or sonophoresis (40), and electroporation (41).

### 3.2. Lipids in Oral Drug Delivery

Macromolecules are part of a growing group of new drugs with many clinical applications. They are mostly administered parenterally because oral application may cause degradation or may have limited absorption in the digestive tract. Other approaches to promote the noninvasive delivery of these drugs are transmucosal and oral delivery of macromolecular drugs using lipid-based delivery agents.

Lipids are already used in a wide variety of oral drug delivery applications. Studies have recently reported the use of lipidic excipients and the functionality of the apical efflux pump and enterocyte-based metabolism. Basically, some lipophilic drugs can be made more bioavailable by traveling through the processes of the digestive tract (42). It is widely documented that the addition of fatty acids can improve the bioavailability of drugs that are poorly absorbed by the gastrointestinal tract by increasing their uptake into the lymphatic system (43, 44). The addition of fatty acyl groups also enhances permeability across intestinal mucosa (45) as well as other epithelial barriers such as buccal, nasal, and pulmonary mucosae (46). The

use of triacylglycerol esters for delivery of peptides across the blood-brain barrier was also reported (47).

One example of this application is Halofantrine hydrochloride, a new phenanthrenemethanol antimalarial with high activity against drug-resistant strains of *Plasmodium falciparum*. It has been documented that the bioavailability of this drug increased approximately three-fold when co-administered with food. In this case, the solubilization of the drug with the bile salts was reported as being responsible for the activity increase. The formation of association of the drug with chylomicrons appreciably contributed to the transport through the lymphatic system (48). A review on delivery agents for oral absorption of macromolecular drugs, particularly oral delivery, of heparin has also appeared (49).

Self-emulsifying formulations, known as self-emulsifying drug delivery systems (SEDDS), have been reported to produce stable microemulsions as they dissolve in a compatible dilution system. A SEDDS formulation normally contains a lipid, surfactants, and cosolvents. The main objective is to facilitate the subsequent assimilation of a drug in the digestive tract without getting inactivated by the pH environment or in the bile salt micellar structures. Also, it is desired that the emulsified drug be independent of the ingested food.

Besides emulsifying properties, another useful characteristic of solid fats are their malleable melting points profiles. Fat crystals have the structure-forming properties that allow the formation of a matrix for suspension of drug particles. When used simply as carriers, some lipids can provide a matrix that can keep the bioactive components uniformly distributed and released in a timely manner. The properties of the crystals such as melting time can be manipulated by blending solid fats with different ratios of liquid oils (50).

A lipid system of biosomes composed of phosphatidylcholine from soybean and medium-chain monoacylglycerol and low-molecular-weight heparin (LMWH), used in the treatment and prevention of thromboses, was reported (51). Heparin is known as having poor oral absorption. The system is applicable for oral and parenteral administration as well as for enhancing dermal, rectal, and nasal absorption of other drugs.

Lipid suspensions in the nanoparticle size range has been reported in parenteral nutrition. Using high-pressure homogenization, the particle size range between 50 nm and 1000 nm has been reported (52). This technique is reported to allow long-term stability (over 3 years), a controlled release, and sterilization. To investigate the release and entrapment behavior of prednisolone, a medication used in eye irritation treatments, was used as the drug carried in the lipid nanoparticles with good results.

The behavior of submicron emulsion is now better understood, especially in relation to the charge of the emulsion. Their therapeutic application has been expanded to intravenous, ocular, and oral administration. It has been reported that positively charged submicron emulsions interact desirably with biological membranes that are negatively charged. The positive charge of submicron emulsions results from cationic excipients such as lipids, polymers, and surfactants in formulations. This difference in charge results in an increased drug uptake and can be

designed for site-specific targeting. Use of this property has been shown in enhanced drug permeation through cornea and drug targeting of the lung after intravenous injection (53). This also suggests a new therapy for lung cancer chemotherapy.

Tris, another product, has been used by the pharmaceutical industry in many applications. This compound is regarded as relatively inexpensive, nontoxic, and readily available. Tris is favored for having structural similarities to glycerol and has the advantage of being symmetrical and avoids structural isomerization. Several drugs can be readily attached to the amine group. It also allows for the synthesis symmetric tri-fatty acyl esters, which is not possible with glycerol. Initially, the technology was applied to the delivery of vaccines, but it has since been used to link various drugs to fatty acids. It has also been used to generate a novel class of cationic lipid cytofectins (54). The synthesis of fatty acid conjugates of morphine, indomethacin (a nonsteroidal anti-inflammatory drug), and AZT (an antiviral drug and methotrexate and chlorambucil (antineoplastics) was reported (55).

Intravenous administration of microemulsions, oil in water, that are pharmaceutically acceptable have also been reported. An example of this microemulsion is composed of MCT, soybean phosphatidylcholine and poly(ethylene glycol)(660)-12-hydroxystearate (12-HSA- EO15) as amphiphiles, and poly(ethylene glycol) 400 (PEG 400) and ethanol as cosolvents (56). The mean droplet diameter of the oil-in-water emulsion reported was between 60 nm and 200 nm. It was concluded that it is possible to administer it without producing any significant effect on acid-base balance, blood gases, plasma electrolytes, mean arterial blood pressure (MAP), and heart rate (HR) (concentrations of up to 0.5 mL/kg of the microemulsion).

#### 4. LIPIDS IN COSMETIC APPLICATIONS

A wide range of vegetable and animal oils and fats are currently being used as bases and bioactive ingredients in many cosmetic applications. Typical applications of lipids in cosmetics include emollients and specific ingredients for skin care and treatment, hair care, and make-up/decorative products. The type of lipids commonly used in cosmetics include TAGs, emulsifiers, waxes, and structured lipids. The cosmetics industry was more than \$25 billion in 2000, and new products are continually introduced in the market.

The typical properties assigned to cosmetic products include skin moisturization, emolliency and spreadability and coadjuvants to help maintain skin pliability and softness (57). Moisturization is usually considered to be simply occlusivity and humectancy. The role of cosmetics, especially lipid cosmetics besides moisturization, has expanded to multiple effects such as modulation of barrier function, increasing corneodesmolysis and facilitating corneocyte envelope maturation. Specific examples of lipids of interest are listed in Table 2. Examples of lipids used in cosmetics include products derived from olive oil, shea butter oil, mango kernel oil, borage oil, nut oils, and palm and coconut oils.



**TABLE 2. Percent Fatty Acid Composition of Several Vegetable and Animal Fats and Human Skin.**

Fat	14:0	16:0	18:0	18:1	18:2
Human Adipose	3	25	8	46	10
Whale Blubber	8	12	3	35	10
Butter	10	25	10	25	5
Corn Oil	1	8	4	46	42
Olive Oil	1	5	5	83	7
Peanut Oil	—	7	5	60	20
Soybean Oil	—	7	4	34	53
Lard	1	25	15	50	6

The general rationale to use certain oils in cosmetics is usually based on the belief that TAG oils with fatty acids similar in composition to the human skin will have beneficial properties. This belief is not entirely true; the major components of sebaceous lipids are 45–60% TAG, 25% wax and sterol esters, 12–15% squalene, and 10% free fatty acids (34).

However, TAGs and fatty acid are found abundantly in the human skin along with other components such as cholesterol and ceramides, more specifically in the human stratum corneum (outer layer of the skin) (58), which has been used to explain the high emolliency of some oils. Following this same rationale, blends of other oils rich in oleic, linoleic, and palmitic acids such as olive, corn, and palm oils are also widely used in cosmetics.

Besides the common application in cosmetics such as skin and body care, lipids have been used as vehicles or carriers for penetration enhancement in dermatologic delivery systems specifically for applications such as skin repair. In some specialized cases, the use of oils in either cosmetic or pharmaceutical applications becomes blurred. For example, in addition to emolliency, some products are marketed as having anti-wrinkle and skin enhancement effects. Medium-chain monoacylglycerols have been reported as having mild antimicrobial agents and proven to be effective against micro-organisms found on the skin. Phytosterols are shown to have positive impact on irritated human skin. The term *cosmeceutical* has been coined to define cosmetic products with medical properties.

Oils rich in essential fatty acids have been reported to suppress human granulocyte elastase, a tissue-degenerative enzyme that is released when tissue inflammation occurs (59). Oils rich in essential fatty acids, especially from animal origin, have been reported to suppress inflammation as well. Emu oil, for example, has been reported to have superior moisturizing and cosmetic properties in double-blind studies (60). It has also been reported that emu oil increases the proliferation of cells and the growth of hair follicles in laboratory rats (61). As is the case in pharmaceutical applications, the cosmetic properties of lipids synergistically increase when used in combination with phospholipids. Other oils used in cosmetics such as palm, sesame, safflower, borage, and coconut have been reported to increase the transdermal properties when used in combination with emu oil. The blend of ethyl

salicylate, isopropyl salicylate, and oil of eucalyptus was found to have anti-inflammatory and anti-arthritis activity when tested in laboratory rats with induced polyarthritis (59).

Some fatty acids, especially unsaturated fatty acids, are well-known skin penetration enhancers. It is also known that many fatty acids possess antimicrobial activity. The topical activity of the anti-viral drug acyclovir is hampered by its inadequate permeability through the skin barrier. Some reports have shown that fatty acid extract of cod liver oil as well as the extract in the form of an ointment show effective antiviral properties against herpes simplex virus (HSV-1) (37).

The introduction of cosmetic products with bioactive ingredients is constantly increasing. For example, cosmetic blends of natural tocopherols with MCT are also being commercialized. These products are reported to have quick skin penetration, a high oxidative stability, and solubilization. One of the most common fatty acids used in cosmetics is oleic acid; it is highly stable to oxidation and is reported to have high emolliency. The use of structured lipids has also been increasing in the pharmaceutical and cosmetics industries. The combination of short-chain fatty acids (caprylic and capric acid) and long-chain polyunsaturated fatty acids (such as long chain omega-3 fatty acids) allows for the design of synthetic lipids for specific applications (62).

As mentioned above, the most abundant lipids in the stratum corneum intercellular space are cholesterol and ceramides. Cholesterol is the most abundant lipid species in the stratum corneum intercellular space (see Table 3). It has been

**TABLE 3. Human Stratum Corneum Lipid Composition from Different Sources (wt% of Total Extracted Stratum Corneum Lipid), Adapted from Wertz and Norlén, 2003.**

Ref.	N	Technique	Cholest/ Ceramides	TG	Cholest.	FFA total	FFA unsat.	FFA sat.	Chol.	Cer.	Chol. suff.
(34)	4	TLC	0.8	25.2		19.3	9.5	9.7	14.0	18.1	1.5
(30)	6	TLC	0.7	0.0	10.0	9.0	0.7	8.3	27.0	41.0	1.9
(76)	6	TLC	1.1	23.0	17.0	11.0			23.0	21.9	4.5
(77)	22	HPLC/LSD	0.5	0.0	18.0	11.0	0.0	11.0	24.0	47.0	
(78)	6	TLC	0.6	0.0	12.9	19.4	10.9	8.5	16.1	29.0	3.2
(79)	11	TLC	0.7	13.7	5.0	17.5			11.0	16.0	1.0
(80)	6	TLC	0.9	9.1	3.1	8.0			14.9	16.8	3.2
(81)	20	TLC	0.5						42.7	48.1	9.2
(82)	10	TLC	0.4			64.0			10.0	26.0	
(82)	6	TLC	0.9			30.0			33.0	37.0	
(83)	5	TLC	0.3			21.6			17.3	61.1	0.9
(84)	10	TLC	0.6			36.8	13.0	23.8	23.7	39.5	
Median (wt./wt)			0.6	9.1	11.5	19.3	9.5	9.7	17.3	29.0	1.9
Range (wt./wt)			0.3–0.9	0–25	3–18	8–64	0–13	8–24	10–33	16–61	1–5
Max/Min			3.0		6.0	8.0		3.0	3.3	3.8	5.0

N: Number of subjects.

TG: Triacylglycerols.

FFA: free fatty acids.

TLC: thin layer chromatography.

HPLC/LSD: High-performance liquid chromatography/light scattering detection.

reported that cholesterol is likely to be a key component that determines the barrier capacity for stratum corneum. It has also been reported that cholesterol gives plasticity and increases chain mobility of lipids in the gel and crystalline states (72, 73). Basically, by stabilizing single and coherent gel phases, cholesterol seems to promote the desirable effects of protecting the barrier capacity of stratum corneum against the sudden change in environmental conditions.

Dry skin, for example, has been reported to be caused by disturbances in stratum corneum biology that need to be corrected. Changes in the fragile corneocyte envelope and rigid corneocyte envelope levels occur in dry skin, where the fragile corneocyte envelope predominates. Ceramides are the other most abundant lipid in the stratum corneum. Insufficient ceramides in the layers of the skin have also been connected to damaged or dry skin. More specifically, the lowered levels of ceramides, especially phytosphingosine-containing ceramide and ceramide-1-linoleate, have been associated with dry skin. Also, changes in the corneocyte envelope are reported to be related to the reduction in the level of the enzyme, transglutaminase, which normally cross links the corneocyte envelope proteins and attaches lipids to the corneocyte envelope. Defficient corneodesmolysis is related to scaling and flaking of the skin (74).

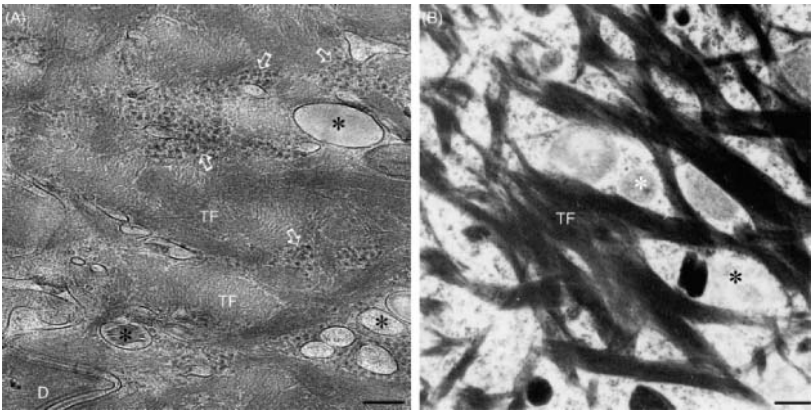
Glycerol is a widely used humectant and moisturizer in cosmetics, and considerable research has been done on its effect on the stratum corneum functionality (75). Glycerol was identified as an effective agent in the formation of lipid crystals under conditions of low humidity. The use of alpha- and beta-hydroxy acids on skin desquamation is now well established and widely practiced (76). New lipophilic variants of salicylic acid are reported to affect corneodesmolysis by acting on the whole structure of the corneodesmosomes. Alpha-hydroxy acids fractionate the corneodesmosomes only (77).

The better understanding of the metabolic processes in the skin has resulted in the development of new techniques to enhance and modulate barrier function of lipid and biosynthesis enhancers. The perturbation of the epidermic barrier has been used to increase the synthesis of some lipid species. For example, the sterol regulatory element binding protein 2 (SREBP-2) has been used to increase enzymes activity responsible for cholesterol and fatty acid biosynthesis (74). It is shown that when epidermal sterol levels decrease, the precursor of SREBP-2 is proteolytically cleaved and the resulting N-terminal fragment diffuses into the epidermis to activate gene transcription of HMGCoA reductase and acetyl-CoA carboxylase. In the case of aged skin, it has been shown that the contents of all lipid species decrease. A sharper decrease in the synthesis of cholesterol in the dynamic barrier is reported and, to a lesser degree, the biosynthesis of ceramides and/or fatty acids. The use of various lipid blends including cholesterol have been used to help the recovery of the skin barrier (78). Other alternatives reported to increase ceramide synthesis and improve barrier function include alpha-hydroxy acids, not so much as desquamatory agents, but as stimulants of lipid biosynthesis. Lactic acid has also been reported to increase ceramide biosynthesis and thus improve barrier functionality. Presumably, lactic acid achieves this increase by acting as a general lipid precursor (79).

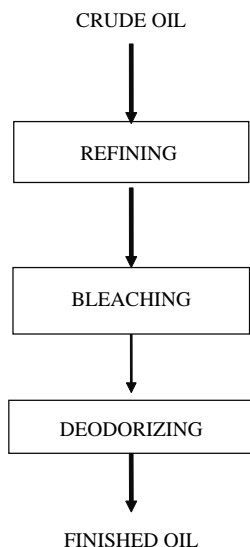
Another forefront technique to improve the function of the stratum corneum and enhance barrier repair in dry skin is the use of epidermal differentiation. A number of hormone receptors for epidermal differentiation have been identified. This family of receptors includes retinoic acid receptors, the steroid receptors, the thyroid receptors, the Vitamin D receptors, the peroxisome proliferator-activated receptors, the farnesol-activated receptors, and the liver-activated receptors. It is reported that these transcription factors bind their respective ligands and regulate many of the aspects of cellular proliferation and differentiation. Examples of ligands for the last three transcription factors are fatty acids for the peroxisome proliferator-activated receptor, farnesol for the farnesol-activated receptor, and hydroxylated cholesterol derivatives for the liver-activated receptor. The stimulation of epidermal differentiation stimulated the synthesis of involucrin, filaggrin, and the enzymes of the ceramide synthesis pathway (74).

## 5. PROCESSING OILS FOR PHARMACEUTICAL AND COSMETICS APPLICATIONS

As a result of the nature of most cosmetic oils and the required specifications for finished products, processing techniques of these materials require special



**Figure 2. Human epidermis.** Medium-magnification transmission electron micrographs of the cytoplasmic space at the midportion of the viable part of human epidermis. (A) Cryo-electron micrograph of vitreous sections. (B) Conventional electron micrograph of resin-embedded sections. In the vitreous cryo-fixed epidermis (A), cellular as well as intercellular space appears densely packed with organic material, whereas in the conventionally fixed epidermis (B), the distribution of biomaterial is characteristically inhomogeneous. Loss of biomaterial appears to have taken place in (B). In (B), the loose tonofilament (TF) networks omnipresent in (A) are aggregated into distinct bundles. Furthermore, the rich variety of interfilament membrane structures, organelles (black asterisk), and “polyribosome-like complexes” (open white arrows), present in (A) are, for the major part, absent in (B). Open white arrows (A): “polyribosome-like complexes” (strongly folded rough endoplasmatic reticulum (cubic-like?) with a small (~25 nm) lattice parameter?); black asterisk (A): different “organelles”; D: desmosome (A); black asterisk (B): artefactual “empty cytoplasmic space”; white asterisk (B): organelle remnants. Section thickness ~100 nm (A). Scale bars 100 nm (A and B). [Norlén (58)].



**Figure 3.** Typical processing of vegetable oils.

considerations. The contaminants such as free fatty acids, peroxides, and unpleasant odors and other impurities must be removed before the oil can be acceptable as an ingredient in cosmetic products. In some cases, the specifications required in cosmetics may be more stringent than those used in food applications, which makes it necessary to further process the oil to make it fit for use. For example, the presence of metals in oils causes rapid development of rancidity in crude or finished oils. Properly refined crude oils contain less than 0.05% free fatty acids, which is adequate for food applications; however, for some cosmetic applications, values of less than 0.03% are needed to avoid skin irritation. Figure 3 shows the typical steps followed in processing of vegetable oils (80).

Oils used in cosmetics are usually light in color or are colorless; therefore, it is necessary to conduct a bleaching step with adsorbents to remove colored materials as well as other impurities such as peroxides, metals, soaps, and protein residues. Once the oil has been properly refined and adsorbent-treated, it can be deodorized by sparging pure steam under high-vacuum. Physical refining or nonchemical removal of free fatty acids and odors by high vacuum distillation can also be accomplished; however, care should be exercised to ensure that contaminants such as metals, soap, and proteins are effectively removed prior to the distillation step.

The free fatty acids formed further oxidize and produce peroxides that decompose into odorous materials. Decomposition of protein-containing materials, particularly if the bacterial count is high, also generates obnoxious smells. This decomposition makes it necessary to follow some or all of the following refining treatments in order to bring the oil to the desired specifications of a final product. To what extent the oils should be refined will depend on its initial quality and on its final use, i.e., cosmetics, pharmaceuticals, or food.

The operation of refining is normally carried out to remove free fatty acids from the crude oil. Free fatty acids are not harmful *per se*. However, they are precursors of peroxides and compounds that are responsible for unwanted odors and rancidity in fats, and their removal is usually the first step in the refining process. This removal is usually done by neutralizing the free fatty acids with a weak caustic solution (10–15%) and adding a slight excess to ensure complete neutralization. The neutralized free fatty acids are then easily removed by centrifugation as saponified matter. In some cases, the removal free fatty acids is done in the deodorization step by distillation through heat and high vacuum.

Another method to refine oils using milder silicate refining agent was recently reported. This process combines neutralization and adsorption and eliminates the use of centrifuges to remove free fatty acids from crude oils (it involves the addition of sodium silicate instead of sodium hydroxide, which allows for the removal of free fatty acids and protein-like materials by adsorption and filtration and avoids the use of more expensive centrifuges). The combined agglomerating and adsorptive characteristics of silicates allow for the removal of these impurities (81).

Bleaching is usually practiced to remove unwanted colored compounds and other contaminants in vegetable oils. Adsorptive clays normally used for bleaching also serve as a catalyst to decompose or remove peroxides and metals that may still be present in the oil. The absorbents used in bleaching effectively remove these contaminants. The next step is usually deodorization, and if any metals are present in the oil during deodorization, it may in fact worsen the quality of the final product.

Deodorizing is generally conducted to remove odors, peroxides, and other compounds from the bleached oil. In some cases, this step is used to remove free fatty acids as well; in which case, it becomes a physical refining operation. If the starting oil is of good quality, the neutralization step with caustic solution may be avoided, and the deodorizer can be used also as a physical refiner. Deodorization is usually done by injecting pure steam into the heated oil (300–450°F) under high vacuum (0.002–4 mm Hg).

At the end of the deodorizing step, it is common to add an antioxidant to the final product such as butylated hydroxytoluene (BHT) or tocopherols, which may retard spoilage and allow a longer shelf life. Metal scavengers such as citric acid (0.01%) are also added during the cooling period of the deodorization process.

### 5.1. Quality of Cosmetics Oils

The ultimate quality of a refined cosmetics oil will depend strongly on the characteristics of the starting material. The quality of a processed oil is usually evaluated by the following factors:

- Free fatty acids (%)
- Peroxide value

**TABLE 4. Common Lipids Used in Cosmetics.**

Product Name	Chemical Description	Properties and Applications
Olive oil	Liquid triacylglycerol oil	Emollient, moisturizing, body care, hair care
Vegetable oils	Liquid triacylglycerols	Emollient, moisturizing, skin and body care products
Hydrogenated vegetable oil	Solid triacylglycerol	Emollient, moisturizing, stick products, skin care
Cacao butter	Solid triacylglycerol	Emollient, skin and body care
Shea butter	Solid triacylglycerol	Emollient, absorbs UV light, skin and body care.
Coconut oil	Triacylglycerol semi solid.	Emollient, quick meltin on skin, skin, body and hair care
Palm oil	Triacylglycerol solid	Emollient, skin, body care
Oil acylglycerols	Ethoxilated mono- and diacylglycerol	Water dispersable, skin conditioning and moisturizing, skin, body and hair care
Medium chain triacylglycerols	Caprylic, capric triacylglycerol	Quick skin penetration, emollient, sunscreen solvent, skin, body and hair care
Polyglyceryls dimers	Polyglycerol esters	Nonionic emulsifier, w/o emulsions
Sodium lactylates	Sodium stearyl lactylate	Ionic emulsifiers, o/w emulsions
Acylglycerol citrates	Citric acid esters of vegetable oils	Ionic emulsifiers, o/w emulsions
Medium chain mono- and diglycerides	Caprillic, capric acid glycerides	Moisturizing, bacteriostatic, pigment dispersant, skin and body care.
Hydroxy fatty acids	Alpha- and beta-hydroxy fatty acids	Skin treatment, desquamation

- Anisidine value
- Color
- Flavor
- Metals (Fe, Ca, Mg, P)
- Water Content
- Results from gas chromatographic analysis

Gas chromatographic analyses are useful not only to determine if an unusual amount of mono- and diacylglycerol sexists, but also if contaminants or adulterants are found in the oil.

## 5.2. Specifications for Oil Quality

As previously mentioned, free fatty acids are precursors for peroxides and carbonyl compounds such as aldehydes and ketones that give rancid smell to the oil. A combination of factors accelerate the spoilage of an oil such as the presence of

**TABLE 5. Cosmetics Toxicity Evaluation. Tier I. (83).**

Study	Recommended Species
Acute oral toxicity	Rat and/or in vitro method
Acute dermal toxicity	Rat and/or in vitro method
Primary skin irritation	Rabbit and/or in vitro method
Primary eye irritation	Rabbit and/or in vitro method
Skin sensitization	Guinea pig or LLNA <sup>a</sup>
Ames assay (gene mutation)	Bacteria

<sup>a</sup>LLNA = Local lymph node assay conducted in mice.

metals, water, and high storage temperatures. The following are typical quality and trading specifications used by the edible oil industry of a final product that will have a good shelf life (82):

Free fatty acids = 0.03–0.05%

Peroxide value = 0.0–0.5 meq/kg

Moisture < 0.05%

Fat stability test, AOM hrs > 15

Basically, the quality of oils and fats and other lipids used in both the cosmetics and pharmaceutical industries has to be equal or superior to that of edible products. With regard to cosmetics, it is up to the manufacturer to ensure that the cosmetics products are safe and will not harm consumers, thus making the cosmetic industry self-regulating. Some exceptions for some cosmetic color and a few prohibited ingredients exist. Table 5 shows a typical tier of tests used by the cosmetics and pharmaceutical industries to ensure the safety and good quality of cosmetic products, and this includes lipid products (83). Special emphasis is placed on the microbial and allergen safety of packaged products. With regard to allergenicity, vegetable oils in general are not a concern and in fact are used for prevention of certain types of dermatitis (84).

## REFERENCES

1. J. Dupont, *Am. J. Clin. Nutr.*, **63**(6), 991S–993S (1996).
2. J. E. Kinsella, *Food Technol.*, **42**(10), 124–42 (1988).
3. J. F. Mead, *Lipids: Chemistry, Biochemistry, and Nutrition*. New York: Plenum Press, 1986.
4. V. M. Sardesai, *J. Nutr. Biochem.*, **3**(4), 154–166 (1992).
5. A. Ferretti, *Nutr. Biochem.*, **9**(2), 88–92 (1998).
6. D. R. Uauy and A. Valenzuela, *Progr. Food Nutr. Sci.*, **16**(3), 199–243 (1992).
7. J. Karlsson, G. Hesselius, B. Nygard, and R. Ronneberg, *J. Nutr. Environ. Med.*, **8**(1), 25–34 (1998).
8. A. P. Simopoulos, *Food Rev. Int.*, **13**(4), 623–631 (1997).



9. D. Volker and M. Garg, *Journal of Clinical and Biochemical Nutrition*, **20**, 83–97 (1996).
10. D. Clissold, in J. H. P. Tyman, ed., *The Potential for prostaglandin pharmaceuticals, In Lipids in Health and Nutrition*, Royal Society of Chemistry, Cambridge, United Kingdom, 1999.
11. J. A. Olson, *Am. J. Clin. Nutr.*, **45**(4), 687–692 (1987).
12. P. M. Farrell, in V. Young and M. Shils, eds., *Modern Nutrition in Health and Disease*, Lea and Fabiger Press, Philadelphia, Pennsylvania, 1988.
13. H. De Luca, in V. Young and M. Shils, eds., *Modern Nutrition in Health and Disease*, Lea and Fabiger Press, Philadelphia, Pennsylvania, 1988.
14. J. M. Conly, *Prog Food Nutr Sci.*, **16**(4), 307–43 (1992).
15. X. Pellerier, *Ann. Nutr. Metab.*, **39**, 291–295 (1995).
16. B. Fitch-Haumann, *INFORM*, **8**(10), 1004–1011 (1997).
17. W. M. Willis, R. W. Lencki, and A. G. Marangoni, *Crit. Rev. Food Sci. Nutr.*, **38**(8), 639–674 (1998).
18. K. Yamamoto, H. Asakawa, K. Tokunaga, H. Watanabe, N. Matsuo, I. Tokimitsu, and N. Yagi, *J. Nutr.*, **131**, 3204–3207 (2001).
19. G. P. McNeill, C. Rawlings, and A. C. Peilow, *J. Amer. Oil Chem. Soc.*, **76**, 443–450 (1999).
20. H. S. Garcia, J. A. Arcos, D. J. Ward, and C. G. Hill, *Biotechnol. Bioeng.*, **70**, 587–591 (2000).
21. A. Rocha and E. Hernandez, *J. Amer. Soc. Oil Chem.*, **81**(7), 685–689 (2004).
22. C. Ip, J. A. Scimeca, and H. J. Thompson, *Cancer*, **74**, 1050–1054 (1994).
23. S. Gnadig, R. Rickert, and J. L. Sebedio, *J. Lipid Sci. Tech.*, **103**, 56–61 (2001).
24. G. Cevc and F. Paltauf, eds., *Phospholipids: Characterization, Metabolism, and Novel Biological Applications*. AOCS Press, Champaign Illinois, 2003.
25. T. H. Crook, J. Tinklenberg, J. Yesavage, W. Petrie, M. G. Nunzi, and D. C. Massari, *Neurology*, **41**(5), 644–649 (1991).
26. M. Hossen and E. Hernandez, *Lipids*, **39**(8), 777–782 (2004).
27. W. N. Charman, *J. Pharm. Sci.*, **45**(3–4), 316–327 (1996).
28. R. Neubert, W. Wohlrab, and C. Bendas, *Skin Pharmacol.*, **2**, 119–129 (1995).
29. P. M. Elias and D. S. Friend, *J. Cell Biol.*, **65**, 180–191 (1975).
30. P. W. Wertz, D. C. Swartzendruber, C. M. Kathi, and D. T. Downing, *J. Invest. Dermatol.*, **89**, 419–424 (1987).
31. C. R. Harding, *Dermatol. Ther.*, **17**(s1), 6–15 (2004).
32. H. Chan, B. C. Finnin, and S. A. Charman, *J. Biomed. Mater. Res.*, **51**(1), 96–106 (1996).
33. J. C. Tsai, R. H. Guy, C. R. Thornfeldt, W. N. Gao, K. R. Feingold, and P. M. Elias, *J. Pharm. Sci.*, **85**(6), 643–648 (1996).
34. M. A. Lampe, M. L. Williams, and P. M. Elias, *J. Lipid Res.*, **24**, 131–140 (1983).
35. K. Egbaria and N. Weiner, *Adv. Drug Del. Rev.*, **5**, 287–300 (1990).
36. T. Ogiso, N. Niinaka, and M. Iwaki, *J. Pharm. Sci.*, **85**(1), 57–64 (1996).
37. E. Touitou, B. Godin, and C. Wiess, *Drug Develop. Res.*, **50**, 406–415 (2000).
38. T. M. Morgan, B. L. Reed, and B. C. Finnin, *J. Pharm. Sci.*, **87**(10), 1213–1218 (1998).
39. R. H. Guy, *J. Pharm. Pharmacol.*, **50**, 371–374 (1998).

40. S. Mitragotri, D. A. Edwards, D. Blankschtein, and R. A. Langer, *J. Pharm. Sci.*, **84**, 697–706 (1995).
41. J. C. Weaver, T. E. Vaughan, and Y. Chizmadzhev, *Adv. Drug Delivery Rev.*, **35**, 21–39 (1999).
42. W. N. Charman, C. J. H. Porter, S. Mithani, and J. B. Dressman, *J. Pharm. Sci.*, **86**(3), 269–282 (1997).
43. A. Garzon-Arburbeh, J. H. Poupaert, M. Claesen, and P. Dumont, *J. Med. Chem.*, **29**, 687–691 (1986).
44. C. J. Porter, *Crit. Rev. Ther. Drug Carrier Syst.*, **14**, 333–393 (1997).
45. M. Hashizume, T. Douen, M. Murakami, A. Yamamoto, K. Takada, and S. Muranishi, *S. J. Pharm. Pharmacol.*, **44**, 555–559 (1992).
46. J. C. Verhoef, H. E. Bodde, A. G. de Boer, J. A. Bouwstra, H. E. Junginger, F. W. Merkus, and D. D. Breimer, *Eur. J. Drug Metab. Pharmacokinet.* **15**, 83–93 (1990).
47. D. Patel, B. D. McKinley, T. P. Davis, F. Porreca, H. I. Yamamura, and V. J. Hruby, *Bioconjug. Chem.*, **8**, 434–441 (1997).
48. A. J. Humberstone, A. F. Cowman, J. Horton, and W. N. Charman, *J. Pharm. Sci.*, **87**(2), 256–258 (1998).
49. A. Leone-Bay, D. R. Paton, and J. J. Weidner, *Med. Res. Rev.*, **20**(2), 169–186 (2000).
50. C. J. H. Porter and W. N. Charman, in R. T. Borchardt, P. L. Smith, and G. Wilson, eds., *Models for Assessing Drug Absorption and Metabolism*. Plenum, New York, 1996, pp. 85–102.
51. K. Bohlinder, B. Olsson, G. Ragnarsson, S. K. Singh, G. Stjernström Öhman, T. Wadsten, A. Carlsson, and B. Herslöf, *Eur. J. Pharm. Sci.*, **2**(4), 271–279 (1994).
52. R. H. Müller, C. Freitas, A. zur Mühlen, and W. Mehnert, *Eur. J. Pharm. Sci.*, **4**(1), S75 (1996).
53. S. C. Yang and S. Benita, *Drug Develop. Res.*, **50**(3–4), 476–486 (2000).
54. F. H. Cameron, M. J. Moghaddam, V. J. Bender, R. G. Whittaker, and T. J. Lockett, *Biochim. Biophys. Acta*, **1417**, 37–50 (1999).
55. X. E. Wells, V. J. Bender, C. L. Francis, H. M. He-Williams, M. K. Manthey, M. J. Moghaddam, W. G. Reilly, and R. G. Whittaker, *Drug Develop. Res.*, **46**(3–4), 302–308 (1999).
56. C. von Corswant, P. Thorén, and S. Engström, *J. Pharm. Sci.*, **87**(2), 200–208 (1998).
57. A. F. Bucalo, *DCI*, **162**(6), 26–31 (1998).
58. L. Norlén, *Inte. J. Cosmet. Sci.*, **25**(5), 209–226 (2003).
59. P. Ghosh, M. Whitehouse, M. Dawson, and A. G. Turner, U.S. Patent 5,431,924, 1995.
60. A. Zemstov, M. Gaddis, and V. M. Montalvo-Lugo, *Austr. J. Dermatol.*, **37**, 159–162 (1996).
61. M. F. Holick and J. F. Kinney, *Drug Cosmet. Ind.*, **58**, 36–41 (1996).
62. V. Jennings, V. F. Thünemann, and S. H. Gohla, *Int. J. Pharm.*, **199**(2), 167–177 (2000).
63. J. L. Lévêque, J. de Rigal, D. Saint-Léger, and D. Billy, *Skin Pharmacol.*, **6**, 111–115 (1993).
64. L. Norlén, I. Nicander, B. Lundh-Rozell, S. Ollmar, and B. Forslind, *J. Invest. Dermatol.*, **112**(1), 72–77 (1999).
65. F. Bonté, A. Sunois, P. Pinguet, and A. Meybeck, *Arch. Dermatol. Res.*, **289**, 78–82 (1997).

66. F. Bonté, P. Pinguet, A. Saunois, A. Meybeck, S. Beguin, M. Ollivon, and S. Lesieur, *Lipids*, **32**(6), 653–660 (1997).
67. A. Yamamoto, S. Serizawa, M. Ito, and Y. Sato, *Arch. Dermatol. Res.*, **283**, 219–223 (1991).
68. A. Di Nardo, P. Wertz, A. Gianetti, and S. Seidenari, *Arch. Dermatol. Venerol. (Stockh.)*, **78**, 27–30 (1998).
69. O. Bleck, D. Abeck, J. Ring, U. Hoppe, J. P. Vietzke, R. Wolber, O. Brandt, and V. Schreiner, *J. Invest. Dermatol.*, **113**(6), 894–900 (1999).
70. A. Weerheim and M. Ponc, *Arch. Dermatol. Res.*, **293**, 191–199 (2001).
71. J. Rogers, C. Harding, A. Mayo, J. Banks, and A. Rawlings, *Arch. Dermatol. Res.*, **288**, 765–770 (1996).
72. B. de Kruyff, P. W. M. van Dijk, R. A. Demel, A. Schuijff, F. Brants, and L. L. M. van Deenen, *Biochim. Biophys. Acta*, **356**, 1–7 (1974).
73. X. Xu and E. London, *Biochemistry*, **39**(5), 843–849 (2000).
74. A. Rawlings, *Cosmet. Toiletries*, **118**(9), 42–46, 48, 50–52 (2003).
75. J. Mattai, C. L. Froebe, L. D. Rhein, A. Simion, D. T. Ohlmeyer, and S. E. Friberg, *J. Soc. Cosmet. Chem.*, **44**, 89–100 (1993).
76. A. W. Johnson, in J. J. Leyden and A. V. Rawlings, eds., *Skin Moisturization* New York: Marcel Dekker, 2002, p. 323.
77. J. L. Leveque and D. Saint-Leger, in J. J. Leyden and A. V. Rawlings, eds., *Skin Moisturization* New York: Marcel Dekker, 2002, p. 353.
78. P. M. Elias and R. Ghadially, *Germ. Dermatol.*, **18**, 103–120 (2002).
79. A. V. Rawlings, A. Davies, M. Carlomusto, S. Pillai, K. Zhang, R. Kosturko, P. Verdejo, C. Feinberg, L. Nguyen, and P. Chandar, *Arch. Dermatol. Res.*, **288**, 383–390 (1996).
80. D. R. Erickson, *Practical Handbook of Soybean Processing and Utilization*, AOCS Press, Champaign, Illinois, 1995.
81. E. Hernandez and S. Rathbone, U.S. Patent 6,448,423, 2002.
82. R. O'Brien, *Fats and Oils. Formulating and Processing Applications*, Technomic Publishing, Lancaster, Pennsylvania, 1998.
83. W. F. Salminen, Jr., *Int. J. Cosmet. Sci.* **24**(4), 217–224 (2002).
84. S. Schliemann-Willers, W. Wigger-Alberti, P. Kleesz, R. Grieshaber, and P. Elsner, *Contact Dermatitis*, **46**(1), 6–12 (2002).

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in cocoa butters	4:161t
in common feed animal fats, fish oils, and vegetable oils	4:338t
composition in selected animal fats, greases, and vegetable oils	1:163t
in evening primrose, black currant, and hemp oils	3:74t
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## Fatty acid 22:0 br4

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## Fatty acid 22:1

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in common feed animal fats, fish oils, and vegetable oils	4:338t		
composition in selected animal fats, greases, and vegetable oils	1:163t		
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in major commercial marine oils	4:339t		
percentage in selected vegetable oils compared	2:64t		
positional distribution in blubber fat	3:266t		
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in sunflower oil	2:665t		
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composition in selected animal fats, greases, and vegetable oils	1:163t		
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distribution in butter fat	2:7t
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## Fatty acid 24:1

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composition in selected animal fats, greases <sup>6</sup> and vegetable oils	1:163t
in pelagic species	3:290t
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in sunflower oil	2:665t

## Fatty acid 24:1n-9

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## Fatty acid 25:0

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