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STUDY OF THE PREPARATION PROCESS AND VARIATION OF WALL COMPONENTS IN CHIA (*Salvia hispanica* L.) OIL MICROENCAPSULATION

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Abstract

A study of the microencapsulation process of omega-3 rich oil extracted from chia (*Salvia hispanica* L.) seeds was carried out, which included a comparative analysis of the microcapsules obtained by the spray- and freeze-drying methods using isolated soy proteins and maltodextrin as wall materials at different proportions. Color characterization of the obtained powders was performed and revealed a darker and yellower appearance of the

freeze-dried samples compared with the spray-dried ones. Moisture content was measured for each sample and all presented values around 3.5%. The SEM micrographs revealed that spray-dried microcapsules can occur individually or may form clusters or aggregates, with the particles exhibiting a size range varying from 4 to 10 μm. Encapsulation efficiency was measured, with no significant differences found between drying methods or on varying the proportion of wall components (all samples presented values of aproximately 60%). The oxidative stability of microencapsulated oils under accelerated oxidative conditions revealed protection factors 2-fold higher for all samples. In addition, microencapsulated oil stored under 25 ºC revealed lower hydroperoxide values than those of unencapsulated oil throughout the whole storage test. Finally, oil encapsulated in SPI microcapsules also showed lower HPV values than that of the acceptable limit for virgin and cold-pressed vegetable oils (15 meq. O_2/kg oil) during the storage test, while unencapsulated oil attained the acceptable limit in 62 days. This result represents an increase in the time of the oil shelf life of between 30 and 48%.

Keywords: soy protein; maltodextrin; spray-drying; freeze-drying; microcapsules; chia oil.

Abbreviations:

Microcapsule (MC)

Isolated soy proteins (SPI**)**

Maltodextrin (MD)

Chia oil (CO)

Recovered solid yield (SY)

Scanning electron microscopy (SEM)

Total oil (TO)

Superficial or free oil content (SO)

Encapsulation efficiency (EE)

Hydroperoxide values (HPV)

Induction time (IT)

Protection factor (PF)

1. Introduction

The current food trends reveal a marked rise in consumer interest in food of significant nutritional value that also benefits the physiological functions of the body. Related to this, food rich in unsaturated fatty acids has many positive health effects. The knowledge obtained in recent years about the influence of ingested fatty acids on health having aroused a particular interest in the consumption of polyunsaturated fatty acids, which are grouped into the omega-9, omega-6 and omega-3 series. A diet rich in these compounds decreases the risk of numerous diseases, especially cardiovascular diseases, cancer and metabolic syndrome. Among vegetable sources, chia seeds have 28 – 32 % oil content and are among the richest sources of omega-3 (61 – 70%) fatty acids. The consumption of 1 g of chia oil per day meets the basic requirements of omega-3 fatty acids in a healthy adult. Increased consumption of omega-3 oils can be also achieved by fortifying staple food such as bread, milk and yogurt with omega-3 fatty acids. However, incorporation of omega-3 fatty acids into food is restricted due to their oxidative instability and because of the formation of oxidized products. When exposed to environmental factors, such as air, light and temperature, the chemical and organoleptic conditions of these oils are altered by oxidation reactions [1]. As this behavior reduces the shelf-life of the relevant food from a scientific-technological viewpoint, the microencapsulation of omega-3 and omega-6 rich oils is currently considered to be an interesting protective alternative. Microencapsulation is defined as a process in which tiny particles or droplets are surrounded by a coating wall or embedded in a homogeneous or heterogeneous matrix that forms a barrier between the component in the capsule and the environment [2]. This technology is currently being widely studied due to its ability to prevent oil oxidation, thus maintaining its nutritional

properties. Furthermore, undesirable flavors of some specific food ingredients can also be minimized by using this methodology [3–5]. The microencapsulation of several flavors [3], antioxidants [6,7], vitamins [8], probiotics [9], fish oil [10] and vegetable oils has been reported [11,12].

From the technological point of view, the incorporation of lipophilic compounds, such as oils into many food matrices, especially in water-based carriers, is restricted due to the low solubility and hydrophobicity of these compounds, with the development of hydrophilic powders containing oil being a possible solution for this issue. Of the different wall materials used for microcapsule (MC) preparation, polysaccharides have been some of the most used compounds for this application [13].

The use of vegetable proteins as wall material is currently being investigated, particularly in the nutrition, cosmetic and pharmaceutical fields, as their excellent tensioactive properties make these materials highly suitable for microencapsulation techniques that require preliminary emulsions. For example, isolated soy proteins (SPI**)** have excellent film-forming ability and gas barrier properties, and have been shown to be an excellent wall materials for microcapsule formation due to their natural abundance, low cost, nutritional value and biodegradability [6]. In addition, several new developments involving protein / polysaccharide combinations as wall materials can be found in the literature; in particular, maltodextrin (MD) is commonly used in combination with other components [14]. This material is characterized by its relatively low cost, neutral aroma and taste, low viscosity at high solid concentrations and good protection against oxidation, and can be used as a filler or drying agent in various applications. Although a major problem with this wall material is its low emulsifying capacity, combining this component with SPI overcomes this drawback, and subsequent heating to 85 °C leads to glycosylated compounds via Maillard

reactions [15]. The development of protein-polysaccharide conjugates, made by the Maillard reaction represents an important alternative for applications in foods, medicines, and cosmetics. In recent years, some studies report the existence of risks and benefits on health derived from Maillard reaction products [16]. Important precedents derived from toxicological studies claim that these products cannot be classified as potent dietary mutagens or genotoxins and some Maillard products presents beneficial health effects [17–19]. In addition, these glycosylated proteins have been reported to show good emulsifying [20] and antioxidant properties [21] and a great stability over a greater range of pH compared with the unmodified protein [22] making them more suitable for use in foods. Taking all of these factors into account, Maillard reaction products can be considered a good alternative for the development of new microencapsulated systems [15].

Concerning the encapsulation process, numerous techniques involve making droplets of the active compound and surrounding them by the carrier material in a gas or liquid phase via different physico-chemical processes [23]. The most developed and used processes, on both the small and large scale, are probably those utilizing spray-drying and freeze-drying. Spray-drying is a unit operation by which a liquid product (solution, suspension or emulsion) is atomized in a hot gas (generally air) in order to evaporate the solvent, thereby obtaining powders. Nevertheless, this methodology involves high drying temperatures, which are risky when samples contain polyunsaturated oils. In contrast, the freeze-drying method consists in the elimination of water by freezing and subsequent sublimation, which maintains the preformed structures intact. Furthermore, the very low temperatures and the absence of air prevent product deterioration.

As the characteristics of the products obtained by microencapsulation of chia oil by employing these two methods are not well described in the literature at present, the aim of

this study was the microencapsulation of chia oil by the spray and freeze-drying processes using protein and polysaccharide components as wall materials, in order to obtain high shelf life powders that could be added as bioactive ingredients to several foods. A comparative study of the efficiency of the processes and the physico-chemical and morphological properties of the microcapsules was carried out, with an evaluation of the oil oxidative stability during storage also being conducted.

2. Experimental

2.1. Materials

The following chemicals were used: isolated soy protein SPI SUPRO E with 90% protein on a fat-free, dry-weight basis (The Solae Company, Argentina); maltodextrin (MD) DE15 (Distribuidora NICCO, Argentina); chia oil (CO) was obtained from seeds from the province of Salta, Argentina (Nutracéutica Sturla SRL, Argentina). CO extraction was performed as described below. Briefly, chia seeds were hydrated to obtain 10% (w/w) moisture content. Oil extraction was carried out using a screw press (Komet Model CA 59 G, Germany) at 30 ºC with a 6 mm restriction die and a screw speed of 20 rpm. Hexane (Cicarelli, Argentina), petroleum ether (Cicarelli, Argentina), chloroform (Anedra, Argentina), acetic acid (Anedra, Argentina), sodium hydroxide (Cicarelli, Argentina), potassium iodide (Anedra, Argentina), sodium thiosulfate (Anedra, Argentina) and starch (Anedra, Argentina) were purchased from a local distributor (Proveeduría Científica).

2.2. Emulsion preparation

In order to vary the protein / polysaccharide proportion of the microcapsule wall, the following three different samples were prepared: soy protein 100 % and soy protein / maltodextrin at 1:1 and 1:3 ratios (namely SPI, SPI/MD 1:1 and SPI/MD 1:3, respectively). In all three cases, SPI dispersion (8 %, w/v in dry basis) was prepared in distilled water and stirred at room temperature for 1 h. This dispersion was adjusted to pH 7.0 with 0.2M NaOH and kept overnight at 4 °C for complete protein hydration. Then, 16.5 g and 49.5 g of MD were dissolved in 40 mL of distilled water and added to the SPI to a final volume of 200 mL in order to prepare the 1:1 and 1:3 SPI/MD dispersions. The samples were finally heated in a water bath at 95 °C for 1 h in order to perform Maillard reactions, before being immediately cooled in an ice bath to room temperature. Chia oil was incorporated dropwise into the dispersions at a 2:1 ratio (wall materials : oil) for 15 min at 18000 rpm, using an Ultraturrax homogenizer (IKA T18, Germany). The resulting emulsions (200 mL lots) were stored at 4 °C before starting the drying process.

2.3. Emulsion droplet size

Emulsion droplet size was determined by analysis of microscopic images using the ImageJ Image Processing and Analysis of Java software [24,25]. Approximately 3000 drops of each image were measured, and a frequency analysis was performed to determine the distribution of the droplet diameters. An optical microscope (Nikon Eclipse 90i, Spain) equipped with a digital camera (Leica DFC490, Germany) was used with a 20x magnification.

2.4. Spray-drying process

The spray-drying process was performed in a laboratory-scale Mini Spray Dryer Büchi B-290 (Büchi Labortechnik AG, Switzerland) with a two-fluid nozzle with a cap orifice diameter of 0.5 mm. The process variables were the following: air atomizing pressure: 4.4 L/h; air inlet temperature: 130 °C; air outlet temperature: (80 ± 1) °C; atomization air flow rate: 538 L/h; pump setting: 10 % and aspirator setting: 100% (38 m^3/h).

2.5. Freeze-drying process

The freeze-drying method was initiated by a freezing process. The emulsion was placed into plastic cups and frozen at -80°C for 12 h. The frozen samples were placed in the glass containers which were connected to the freeze-dryer unit. The freeze-drying process was performed using a laboratory bench-top freeze-dryer (Rificor L-T8, Argentina) operated with a condenser at –50°C and a vacuum of 0.1 mbar during 36 h. Glass containers were kept at room temperature (20±2 °C) during the freeze-drying processing.

2.6. Recovered solid yield

The recovered solid yield (SY) was calculated as the ratio of the powder weight collected after each drying experiment (Wf, dry basis) and the initial amount of components in the prepared emulsions (except water) (Wi, dry basis), as indicated in Equation 1.

$$SY = (Wf / Wi) \times 100 \qquad \text{(Eq. 1)}$$

2.7. Microcapsule characterization

2.7.1. Macroscopic aspect

The freeze-dried and spray-dried microcapsules obtained were photographed with a digital camera (Nikon, Japan) in order to reveal their macroscopic aspects. Color measurements were obtained using a colorimeter (Konica-Minolta CM600d, Japan), working with D65 (day light) and an inclination of 10 °. The color parameters were expressed as L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) values.

2.7.2. Moisture content

Moisture content was measured for each sample with a moisture analyzer with halogen heating (model MB45 OHAUS, United States). The sample moisture content analysis was carried out immediately after the drying process.

2.7.3. Morphological analysis

Particle size and morphology were evaluated by scanning electron microscopy (SEM). Powders were attached to a double-sided adhesive tape mounted on SEM stubs, coated with chrome under vacuum, and examined with a FEG SEM scanning electron microscope (Carl Zeiss - Sigma, Germany).

2.7.4. Total oil determination

Total oil (TO) determination was carried out according to a previous methodology [26] with some modifications. Briefly, 4.0 ± 0.1 g of each sample were placed in a Soxhlet extraction apparatus for 24 h, with 200 mL of n-hexane solvent. The total oil extracted was weighed and expressed as a percentage of oil with respect to the weight (dry basis) of the microcapsules. This assay was performed in duplicate for each sample.

2.7.5. Determination of encapsulation efficiency

The superficial or free oil content (SO) of MC was determined according to Gallardo et al. [14] with some modifications. First, 2.00 ± 0.01 g of the sample were weighed, which were then mixed with 30 mL of petroleum ether, stirred for 1 min and filtered. The resulting solids were washed with 10 mL of petroleum ether and the organic phases were combined. The solvent was then evaporated and the remaining oil was heated at 105 °C in an oven to constant weight. This assay was performed in duplicate and the encapsulation efficiency (EE) was determined by calculating the ratio of the total oil contained in the microcapsules (TO) and the free oil (SO) located on its surface [27], according to Equation 2.

$$EE = [(TO - SO) / TO] \times 100 \qquad \text{(Eq. 2)}$$

2.8. Oil oxidative stability study

2.8.1. Oil oxidative stability analysis under accelerated conditions (Rancimat test)

The microencapsulated and unencapsulated oils were subjected to accelerated oxidation conditions in a Rancimat apparatus (743 Rancimat METROHM, Switzerland) [14]. The samples were exposed to elevated temperatures at constant air flow, and the volatile oxidation products (mostly derived from formic acid) were transferred by the airflow to the solution (distilled water). From continuous recording of the conductivity of this solution, oxidation curves were plotted and their inflection point was defined as the induction time (IT), which was expressed in hours. In this study, the conditions used were: 100 °C, air flow 20 L/h, amount of oil 1 g and quantity of MC 1.5 g. In addition, the protection factor (PF) was defined as the ratio of induction time of the microencapsulated oil and the

induction time of unencapsulated oil. This assay was performed in duplicate for each sample.

2.8.2. Oil oxidative stability analysis under room temperature storage conditions

Bulk oil and microcapsules in 250 mL amber glass bottles were placed in a thermostated chamber at (20 ± 3) ºC and (60 ± 5) % relative humidity. The samples were stored for 3 months, during which 4 g of samples were extracted at different times in order to evaluate their hydroperoxide value (HPV). These measurements were carried out by iodometric titration, following an AOCS methodology [28] with some modifications. Briefly, an extraction of encapsulated oil was made using cold hexane for 24 h. Then, (0.20 ± 0.01) g of the extracted oil was placed in 10 mL vials, and 3 mL of acetic acid: chloroform (3 : 2 % v/v) was added and stirred vigorously until complete dissolution was achieved. Subsequently, 0.5 mL of saturated potassium iodide solution was added and the solution was kept in the dark for 1 min. The reaction was stopped by the addition of 3 mL of distilled water, and 0.5 mL of starch solution (1 %, w/v) was added as an indicator. Finally, solutions were titrated with 0.001N Na_2SO_3 until the brown color disappeared. This assay was performed in duplicate for each sample. The calculation of HPV was carried out using Equation 3 which is expressed in milliequivalents of oxygen/kg oil.

$$HPV = (S - B) \times N \times 1000) / w \qquad \text{(Eq. 3)}$$

where S represents the volume in mL of the sodium thiosulfate solution consumed by the sample, and B is the volume consumed by the blank. N is the normality of sodium thiosulfate solution, and w represents the mass of oil expressed in grams.

To determine the kinetic reaction order of hydroperoxide formation by oxidation of bulk and encapsulated oil, HPV values were plotted vs storage time and these curves were fitted to zero-, first-, and second-order kinetics model using equations (4-6), respectively.

$HPV_t = -k \times t + HPV_0$ (Eq. 4)

$Ln\ HPV_t = Ln\ HPV_0 - k \times t$ (Eq. 5)

$1/\ HPV_t = k \times t + 1/\ HPV_0$ (Eq. 6)

where HPV_0 is the initial peroxide value at day 0; HPV_t is the peroxide value after t (time); k is the hydroperoxide formation rate constant, which was obtained from the slope of the plot of lipid hydroperoxide versus time. The data of the induction period were not taken into account for the determination of k [29].

2.9. Statistical analysis

The analytical determinations were the average of several measurements from independent samples, with statistical differences being estimated by the ANOVA test at the 95 % level ($p \leq 0.05$) of significance. Whenever ANOVA indicated a significant difference between means, a Tukey test was used for comparing the mean values, with differences between means being considered significant when $p \leq 0.05$. In the presented tables, any two means in the same column followed by the same letter are not significantly ($P \geq 0.05$) different.

3. Results and discussion

3.1. Characterization of emulsions

The formation of emulsions is one of the most important and critical steps in the edible oil microencapsulation process. A wall material:oil ratio of 2:1 was previously used in order to

optimize the maximum encapsulation efficiency [30]. In fact, the emulsion stability and droplet size of the formed emulsion play an important role in both the encapsulation efficiency and the morphology of the microcapsules [31]. Here, the micrographs revealed rounded drops, without any coalescence signs. All emulsions had droplet diameters between 1 and 22 μm, with a marked rise in droplet size of the studied emulsions resulting from an increase in the amount of maltodextrin added. Through a frequency analysis, it was found that 58, 55 and 52% of the droplets had sizes smaller than 2.34 μm for SPI, SPI/MD 1:1 and SPI/MD 1:3, respectively. This increase in the oil droplet size in MD-containing emulsions may have been due to a reduction in the emulsion capacity when MD was incorporated into the SPI systems. In another investigation, Rodea-González and co-workers reported a narrower range in droplet size of between 2.97 and 9.01 μm in diameter in emulsions containing chia oil and mixtures of whey protein concentrate and arabic gum as a wall material at different concentrations [32]. This data is similar to that reported by Escalona-García and co-workers for emulsions based on chia oil and whey protein concentrate with mesquite gum (67:33 w/w ratio) as wall components [29].

3.2. Preparation and characterization of microcapsules

In order to obtain chia oil microcapsules containing ~30% of CO, emulsions were dried through the spray-drying and freeze-drying methods. Through macroscopic analysis, it was observed that samples obtained by spray-drying resembled fine powder, while those obtained by freeze-drying were more compact with flakes. Milling of the product was avoided in order to prevent capsule rupture. Table 1 summarizes the obtained results

derived from the color determinations of the microcapsules obtained using these two processes.

Table 1.

Color, particle size and shape of food ingredients are particularly important properties, since they affect the aspect of food products. Color characterization of the microcapsules is important considering their potential food application as a component of premix bakery products rich in omega-3 fatty acids. In this study (Table 1), the main color differences were due to the microcapsules being obtained by different drying methods. Thus, although all samples showed high L* values (white and luminous), for freeze-dried microcapsules, the L* values decreased and b* values increased, revealing a darker and yellower appearance than that of the spray-dried microcapsules. These differences might be attributed to the drying process and the powder size. Related to this, it has previously been demonstrated that the larger the particle size, the less scattered the light is, and consequently the sample appears to be more colored. Conversely, smaller particles scatter and reflect more incident light, and the sample therefore appears to be lighter [33]. Maltodextrin significantly increased the a* coordinate, revealing a higher redness intensity. Moisture content was measured for each sample after the drying process (Table 2), but this did not vary significantly with respect to the different drying processes. Similar values have also been reported for other protein microcapsules [32,34].

SEM images were used to examine differences between the microcapsules obtained by the two drying methods. Anwar and Kunz showed that particle microstructure is an important cause of oxidation, in addition to the coating material and heat treatment [5]. In our study,

micrographs of spray-dried samples revealed that microcapsules can occur individually or may form clusters or aggregates, with the particles exhibiting a size range varying from 4 to 10 µm. All samples presented a continuous and homogeneous surface structure with low roughness and no apparent pores or fractures. The SPI microcapsules revealed a deflated sphere shape with depressions on their surfaces, while the SPI / MD microcapsules had a more spherical shape. This latter effect was more noticeable when the proportion of MD increased. Similar morphologies have been reported in the literature for other spray-dried materials [30]. For example, Frascarelli and co-workers found comparable morphologies for gum Arabic microcapsules, with these authors affirming that this morphology is a typical characteristic of spray dried powders [36]. In addition, Kalab suggested that the depressions are a result of particle shrinkage during drying and cooling [37]. In another investigation, Ré reported that the surface depressions are related to the collapse suffered by the droplets during the initial stages of drying [38].

The freeze-dried samples, on the other hand, had markedly different shapes. As stated above, macroscopically these samples resembled flakes, which on microscopic observation exhibited surface flakes with individual microcapsules forming part of the surfaces. This structure was also reported in the literature for both these and other materials prepared by freeze-drying [4,39]. Furthermore, we observed the presence of depressions corresponding to the detachment of the microcapsules, with the flakes probably being formed by a conglomerate of microcapsules. A similar flake-like but porous structure has been described in the literature for similar materials [5,40], although no pores or fissures were found in our materials. Related to this, the absence of porosity is an important cause of oxidation preservation [41]. The SEM micrographs are shown in Figure 1.

Figure 1.

An evaluation of the drying processes was carried out through the determination of the recovered solid yields (SY). It could be observed that the SY of the spray-dried samples were significantly lower than those of freeze-dried samples (Table 2), due to a considerable amount of solid remaining attached to the walls of the drying chamber. This may have been a result of the relatively low drying temperature selected to preserve the CO chemical quality. It should be noted that spray-drying is a continuous method that is utilized over long time periods. However, in this case, the drying step was particularly short (45 min approximately) since only portions of 300 mL were dried. For longer drying periods, higher SY values would be expected. Moreover, SY values are also known to depend on the scale and type of the drying equipment.

Total oil and encapsulation efficiency of the microcapsules obtained by the freeze- and spray-drying processes were determined and the obtained results can be observed in Table 2.

Table 2.

The results of total oil (TO) obtained revealed that soy protein-based wall materials were effective retention agents (wall material:oil ratio of 2:1) during the freezing, sublimation and spray-dehydration processes, since TO values where similar to the amount of initially added oil (0.33 g/gsolids).

EE determines the degree of protection of the chia oil, which depends on numerous factors including the nature of the material to be encapsulated, composition of the wall material, distribution of the droplet size in the emulsion and the drying conditions.

Numerous authors have suggested that an increase in the total solid concentration leads to a higher viscosity and smaller sized of droplets being formed in the emulsion, which minimizes the circulation of oil inside particles, thereby preventing their migration to the surface, and consequently, improving oil encapsulation [42].

In the present work, no differences in EE values were observed between spray-dried and freeze-dried microcapsules or among the wall component proportions (Table 2). It has been reported in the literature that the combination of proteins with polysaccharides, depending on the nature of the biopolymers and the degree of complexing, can increase oil-water emulsion stability and lead to a higher encapsulation efficiency [43]. In this study, however, no significant effect of MD incorporation on EE was found. Similar results were also obtained by Martinez-Bustos et al. for modified-starches and MD microcapsules, with values between 61% and 73% being reported by these authors [44]. Higher EE values were also observed after the variation of the protein or the oil concentration. For example, Karaca and co-workers reported EE values ranging from 55% to 83% for flaxseed oil microcapsules based on chickpea or lentil proteins with MD at various concentrations [40].

3.3. Oil oxidative stability study

The microencapsulated and unencapsulated oils were subjected to accelerated oxidative conditions using the Rancimat test. In fact, this methodology is widely used to determine the oxidative stability of microencapsulated oils after the drying process. Although different oxidative conditions and sample amounts were evaluated, temperature was the only

variable that significantly influenced induction time (IT). The same effect was observed by Gallardo et al for linseed oil [14]. Table 3 shows the IT and PF values for SPI and SPI / MD 1:1and1:3 samples prepared by the spray- and freeze-drying methods.

Table 3.

Microencapsulated oils had IT values between 2 to 3 times greater than those of unencapsulated oils (PF values between 2 and 3). These results suggested that the components of the microcapsule wall appear to be an effective barrier against CO oxidative degradation, as the wall matrix probably reduces heat and oxygen flow to the oil droplets. In addition, no significant differences between IT values of encapsulated oils were observed by varying the proportion of wall components or changing the drying method. Microcapsules prepared by spray-drying and freeze-drying were stored in a thermostated chamber at (20 ± 3) ºC and (60 ± 5) % relative humidity. Figure 2 shows the oxidation patterns obtained during the storage of bulk and encapsulated CO for 90 days.

Figure 2.

Figure 2 shows that hydroperoxide values (HPV) gradually increased with storage time. On fitting the HPV vs storage time curves to equations (4-6), it could be observed that all oxidation curves followed first-order kinetics ($R^2 \geq 0.950$) [29]. Oxidation curves slopes determine the apparent reaction rate constants (k) of hydroperoxide formation [45]. For our study, the kinetic parameters (R^2 and k) are shown in table 4.

Table 4.

Regarding the bulk and microencapsulated CO, there was a significantly faster rise in HPV in bulk CO than in microencapsulated CO, with an oxidation constant rate value (k) of 0.760 meqO_2/Kg oil per day being determined for unencapsulated oil, while values between 0.174 - 0.311 meqO_2/Kg oil per day were determined for encapsulated oils. These k values are similar to those reported in the literature by Escalona-García and co-workers for chia oil microcapsules with whey protein concentrate and mesquite gum (67:33 w/w ratio) as wall materials stored at 25 °C. These authors reported values between 0.7 and 0.32 meqO_2/Kg oil per day for bulk and encapsulated oil, respectively, on varying the water activity in the storage chamber [29].

Returning to our work, the unencapsulated oil reached the end point for rancidity or the acceptability limit for virgin and cold-pressed vegetable oils (15 meq. O_2/kg oil) [46] in a markedly shorter time than that of encapsulated oils (around 20 to 30 days before). This result represented an increase in the time of the oil shelf life of between 30 and 48%. Ixtaina and co-workers reported HPV values of unencapsulated CO showing a shelf-life of approximately 40 days, considering a maximum HPV of 10 meqO_2/kg oil, with CO microencapsulated with sodium caseinate and lactose and dried by spray-drying presenting a shelf-life greater than 55 days, depending on the homogenization conditions and drying temperature.

In contrast with data reported by Serfert et al. [47] , immediately after the spray-drying process, no increase in lipid oxidation products was found in the oil samples, with the HPV ranging from 0.7 to 1.9 meqO_2/kg oil and no significant differences ($p \leq 0.05$) being observed between spray-dried samples and unencapsulated oils at day 0. Likewise,

relatively low HPV were reported for microencapsulated CO samples with sodium caseinate and lactose as wall components at day 0 by Ixtaina et al [48]. In our research, an inlet air temperature of 130 °C was used since greater temperatures provide more available energy for the lipid oxidation process, thereby favoring the formation of peroxides according to Tonon and co-workers [49]. Our results revealed that the components of the microcapsule wall seemed to be an effective barrier, which reduced CO oxidative degradation. These results are in contrast, however, with other studies which reported a pro-oxidative effect by the polysaccharide microcapsule wall on the encapsulated oils. For example, Martinez and co-workers reported an increase in HPV values for encapsulated CO with maltodextrin in combination with hydroxypropyl methylcellulose as wall components and dried in a spray-dryer with respect to bulk chia oil in storage tests [35].

By comparing the results obtained for the different samples, it could be observed that no significant variations were found between the samples undergoing spray-drying and freeze-drying. Accordingly, it can be stated that the temperature reached for the encapsulated oil in the drying chamber of the spray-drying equipment did not significantly contribute to the first steps of the oxidation process, since no marked variations were observed in relation to the freeze-dried samples. On the other hand, significant differences ($p \geq 0.05$) were observed in the variation of the wall components. For both drying methods, a decrease in oxidative stability was determined for the MD-containing samples since larger HPV were measured at all recorded times. On this basis, it can be stated that the incorporation of MD produced an opposite effect to the intended one. Furthermore, on analyzing the effect produced by incorporation of MD, it can be inferred that the structural rigidity produced by cross-linking the two components limited the protein chain flexibility, thereby decreasing its emulsification capacity. This effect is known to be related to the film-forming ability

and gas barrier properties. In addition, the increase in oil droplet size in the prepared emulsion indicated a lower emulsion capacity of SPI/MD compared to that of the SPI systems, revealing a lower level of oil protection. However, it should be noted that during the 90-day storage period the oil encapsulated in SPI microcapsules without MD still remained suitable for consumption, since values lower than 15 meq. O_2/kg oil were detected [46].

4. Conclusions

The microencapsulation processes of chia (*Salvia hispanica* L.) oil described in this study were able to produce omega-3-rich powders that can be used as ingredients for enriched foods. A comparative analysis of the microcapsules obtained by the spray- and freeze-drying methods was carried out. In addition, different wall materials used as isolated soy proteins and maltodextrin added at different proportions were evaluated in order to identify any differences between the products obtained. In this investigation, the physical and morphological properties were determined and differences were observed at both the macro and micro scales. Chia oil obtained by cold pressing displayed an acceptable chemical quality with minimal levels of hydroperoxides. The encapsulation quality was evaluated through total oil and encapsulation efficiency, with no significant differences being revealed between drying methods or when varying the proportion of wall components. Induction times of microencapsulated chia oil were between 2 and 3 times higher than those of uncapsulated oil under accelerated oxidative conditions. Finally, the oxidative stability observed during storage of bulk and encapsulated chia oil for 90 days demonstrated that microencapsulated oil produced a protective effect generated from the wall matrix. These results imply an increase in the shelf life of the microencapsulated chia oil of between 30

and 48% during the storage period. In addition, SPI powders containing around 30% of chia oil and stored 90 days showed HPV values below the Codex limit (15 meq/Kg oil). Thus, the present study provides important new information about incorporating this oil into processed foods, particularly in baked products.

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Figure captions

Figure 1. SEM micrographs of SPI (A), SPI / MD 1:1(B) and SPI / MD 1:3 (C) microcapsules obtained by spray-drying and SPI (D), SPI / MD 1:1 (E) and SPI / MD 1:3 (F) obtained by freeze-drying methods.

Figure 2. Hydroperoxides values **(**HPV) at different storage times for SPI, SPI / MD 1:1 and SPI / MD 1:3 samples dried by spray-drying (A) and SPI, SPI / MD 1:1 and SPI / MD 1:3 samples dried by freeze-drying (B).

Table captions

Table 1. L*, a* and b* color values of SPI, SPI / MD 1:1 and SPI / MD 1:3 samples prepared by spray- and freeze-drying.

Table 2. Moisture content, solid yield (SY), total oil (TO) and encapsulation efficiency (EE) of SPI and SPI / MD samples obtained by spray- and freeze-drying processes.

Table 3. Induction time (IT) and protection factor (PF) values obtained for SPI and SPI / MD 1:1 and 1:3 samples obtained by spray- and freeze-drying processes.

Table 4. R-squared (R^2) and apparent reaction rate constants (k) of oxidation curves of bulk and encapsulated oils.

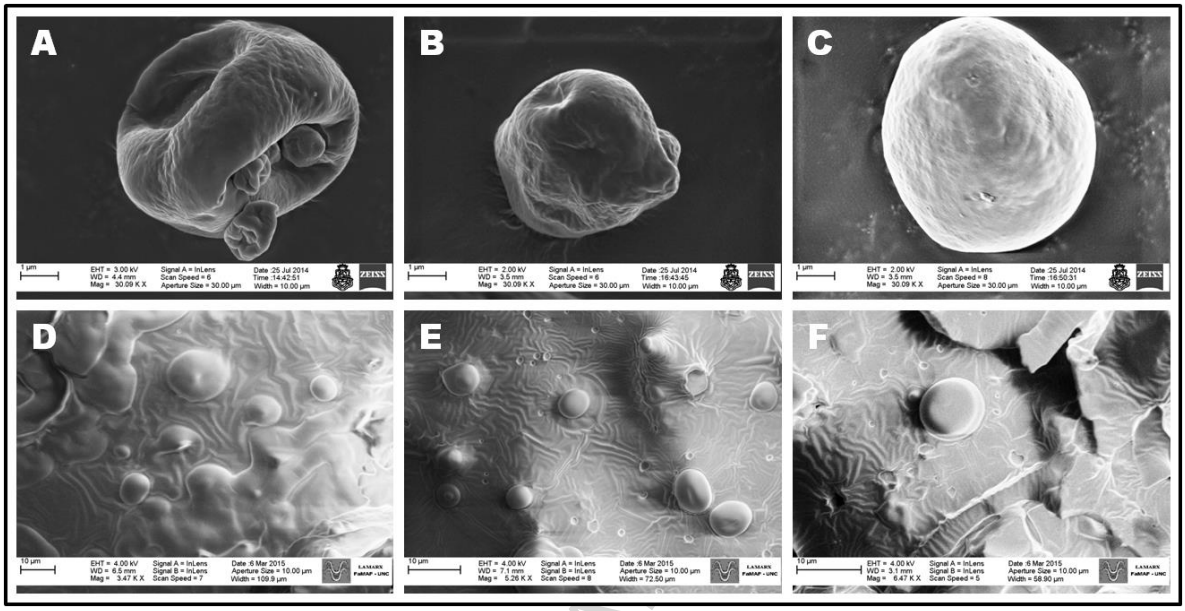


Figure 1

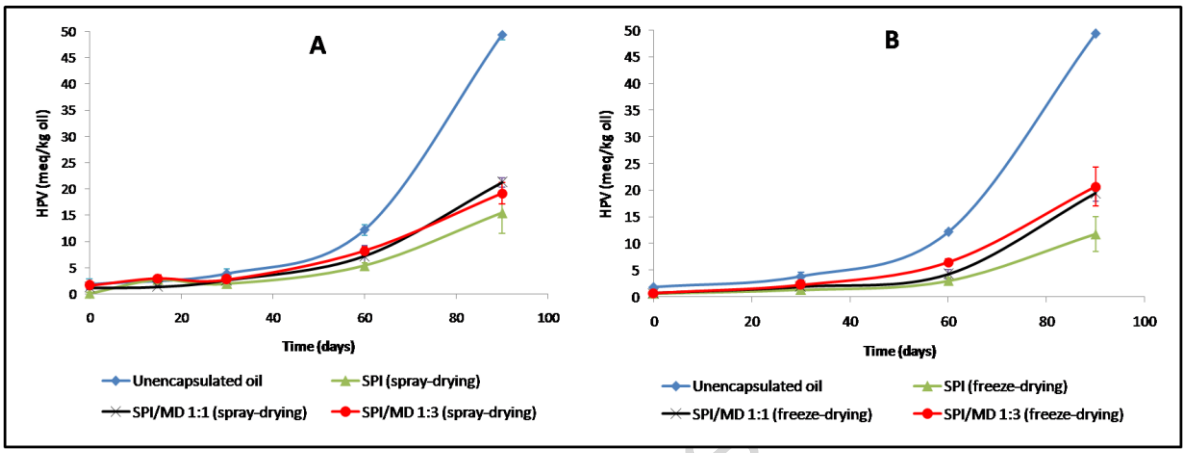


Figure 2

Table 1

Drying process	Sample	L*	a*	b*
	SPI	89.72 ± 0.69 ^{A}	-0.04 ± 0.15 ^{A}	12.96 ± 0.80 ^{A}
Spray-drying	SPI / MD 1:1	90.24 ± 0.56 ^{A}	-0.01 ± 0.13 ^{A}	15.65 ± 0.33 ^{A}
	SPI / MD 1:3	89.80 ± 0.65 ^{A}	0.52 ± 0.16 ^{BC}	14.35 ± 0.34 ^{A}
	SPI	82.18 ± 1.5 ^{B}	0.22 ± 0.30 ^{AB}	17.43 ± 0.63 ^{B}
Freeze-drying	SPI / MD 1:1	81.94 ± 0.67 ^{B}	0.67 ± 0.12 ^{C}	17.64 ± 0.42 ^{B}
	SPI / MD 1:3	82.94 ± 0.93 ^{B}	1.26 ± 0.13 ^{D}	17.95 ± 0.81 ^{B}

Any two means in the same column followed by the same letter are not significantly ($P \geq 0.05$) different.

Table 2

Drying process	Sample	Moisture content (%)	SY (%)	TO (g / g $_{solids}$)	EE (%)
Spray-drying	SPI	3.3	50.4 ± 5.7 ^{A}	0.26 ± 0.04 ^{A}	57.3 ± 2.9 ^{A}
	SPI / MD 1:1	3.9	46.6 ± 7.8 ^{A}	0.25 ± 0.05 ^{A}	60.2 ± 3.9 ^{A}
	SPI / MD 1:3	3.1	38.7 ± 5.9 ^{A}	0.23 ± 0.03 ^{A}	52.5 ± 4.9 ^{A}
Freeze-drying	SPI	3.1	97.9 ± 1.8 ^{B}	0.31 ± 0.02 ^{A}	61.1 ± 10.1 ^{A}
	SPI / MD 1:1	3.7	96.6 ± 1.0 ^{B}	0.26 ± 0.04 ^{A}	59.6 ± 9.9 ^{A}
	SPI / MD 1:3	3.4	98.1 ± 2.2 ^{B}	0.35 ± 0.05 ^{A}	65.5 ± 1.1 ^{A}

Any two means in the same column followed by the same letter are not significantly ($P \geq 0.05$) different.

Table 3

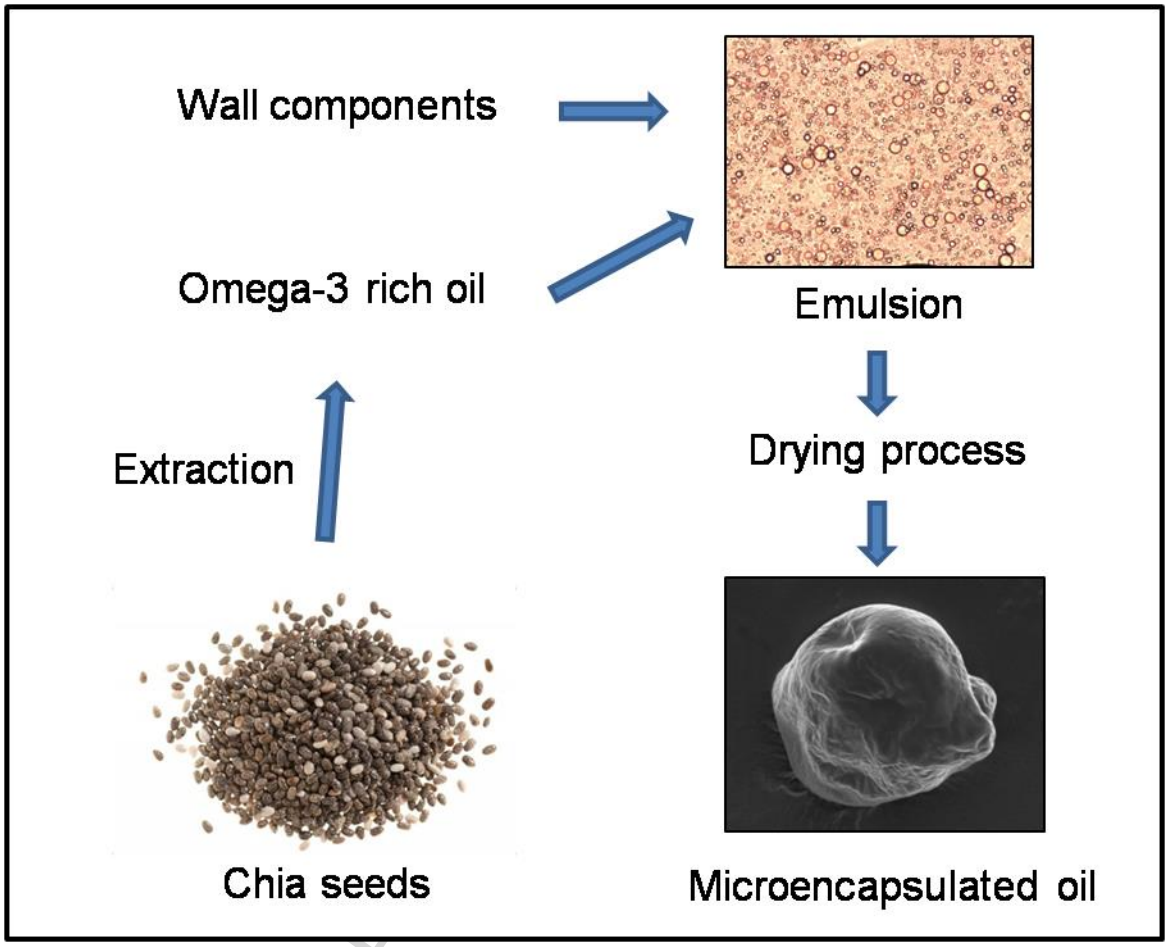
Drying process	Sample	IT (h)	PF
----------	Unencapsulated chia oil	2.40 ± 0.38 ^{A}	----------
Spray-drying	SPI	6.40 ± 0.01 ^{B}	2.67
	SPI / MD 1:1	5.96 ± 0.04 ^{B}	2.48
	SPI / MD 1:3	6.20 ± 0.20 ^{B}	2.58
Freeze-drying	SPI	6.24 ± 0.24 ^{B}	2.60
	SPI / MD 1:1	5.22 ± 0.05 ^{B}	2.18
	SPI / MD 1:3	5.97 ± 0.76 ^{B}	2.49

Any two means in the same column followed by the same letter are not significantly ($P \geq 0.05$) different.

Table 4

Drying process	Sample	R^2	k ($meqO_2$/kg oil per day)
----------	Unencapsulated oil	0.974	0.760 ± 0.011^{A}
Spray-drying	SPI	0.952	0.224 ± 0.012^{C}
	SPI / MD 1:1	0.991	0.311 ± 0.011^{B}
	SPI / MD 1:3	0.978	0.274 ± 0.015^{B}
Freeze-drying	SPI	0.960	0.174 ± 0.009^{D}
	SPI / MD 1:1	0.971	0.292 ± 0.021^{B}
	SPI / MD 1:3	0.999	0.307 ± 0.014^{B}

Any two means in the same column followed by the same letter are not significantly ($P \geq 0.05$) different.



Graphical abstract

Highlights

- A study of the microencapsulation process of omega-3 rich oil was developed.
- Microcapsules presented a spherical morphology without pores and fissures.
- RE and EE were not altered with the variation of wall components and drying method.
- Protected oil showed lower hydroperoxide values than bulk oil in the storage test
- Chia oil powders stored for 90 days presented IP values below the codex limits.