Biochemistry & Analytical Biochemistry
ISSN: 2161-1009

Pisoschi and Negulescu, Biochem & Anal Biochem 2011, 1:1
DOI: 10.4172/2161-1009.1000106

Review Article Open Access

Methods for Total Antioxidant Activity Determination: A Review

Aurelia Magdalena Pisoschi[1]* and Gheorghe Petre Negulescu[2]

[1]*University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105, Splaiul Independentei, sector 5, 050097, Bucharest, Romania*

[2]*University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Land Reclamation and Environmental Engineering, 59, Marasti Street, sector 1, 011464, Bucharest, Romania*

Abstract

An overview of the importance and mechanism of action of antioxidants, as well as of the methods of assessment of the antioxidant capacity is presented.

Antioxidants react through free radical or molecular oxygen quenching, being capable to either delay or inhibit the oxidation processes which occur under the influence of molecular oxygen or reactive oxygen species.

Antioxidants are responsible for the defense mechanism of the organism against the pathologies associated to the attack of free radicals, thus the intake of plant derived antioxidants is involved in the prevention of degenerative diseases caused by oxidative stress, such as cancer, Parkinson, Alzheimer or atherosclerosis.

The methods of antioxidant capacity evaluation, including spectrometry, chromatography and electrochemical techniques are detailed with respect to principles and analytical performances.

Keywords: Antioxidants; Oxidative stress; Reactive oxygen species; Antioxidant capacity; Antioxidant assessment

Introduction

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. They are used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals.

Antioxidants are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals.

Endogenous antioxidants are enzymes, like superoxide dismutase, catalase, glutathione peroxidase or nonenzymatic compounds, such as uric acid, bilirubin, albumin, metallothioneins. When endogenous factors cannot ensure a rigurous control and a complete protection of the organism against the reactive oxygen species, the need for exogenous antioxidants arises, as nutritional supplements or pharmaceutical products, which contain as active principle an antioxidant compound. Amongst the most important exogenous antioxidants, vitamin E, vitamin C, β-carotene, vitamin E, flavonoids, mineral Se are well known, but also vitamin D and vitamin K_3.

Exogenous antioxidants can derive from natural sources (vitamins, flavonoids, anthocyanins, some mineral compounds), but can also be synthetic compounds, like butylhydroxyanisole, butylhydroxytoluene, gallates, etc [1].

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of foodstuffs [2].

Health Benefits of Antioxidants

Recently, antioxidants have attracted considerable attention in relation to radicals and oxidative stress, cancer prophylaxis and therapy, and longevity [3]. Phenols and polyphenols are the target analytes in many such cases; they may be detected by enzymes like tyrosinase or other phenol oxidases, or even by plant tissues containing these enzymes [4-18].

The recommendations based on epidemiological studies are such, that fruits, vegetables and less processed staple foods ensure the best protection against the development of diseases caused by oxidative stress, such as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and cataract [19]. The explanation consists in the beneficial health effect, due to antioxidants present in fruit and vegetables [20]. There are numerous antioxidants in dietary plants: carotenoids, phenolic compounds, benzoic acid derivatives, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans, and lignins [21]. Of the 50 analysed food products with high antioxidant content [20], 13 were spices, 8 were fruits and vegetables, 5 were berries, 5 were chocolate-based, 5 were breakfast cereals, and 4 were nuts or seeds. Considering the typical serving sizes, blackberries, walnuts, strawberries, artichokes, cranberries, brewed coffee, raspberries, pecans, blueberries, ground cloves, grape juice and unsweetened baking chocolate were at the top of the classification [20].

Fruit juices, beverages and hot drinks contain high amounts of antioxidants, like polyphenols, vitamin C, vitamin E, Maillard reaction products, β-carotene, and lycopene [22]. The consumption of fruit juices, beverages and hot drinks was found to reduce the morbidity and mortality caused by degenerative diseases [23-28]. Antioxidants are known to play a key role in the protective influence exerted by plant foods [28-32]. Epidemiologic studies that analyse the health

***Corresponding author:** Aurelia Magdalena Pisoschi, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105, Splaiul Independentei, sector 5, 050097, Bucharest, Romania; E-mail: apisoschi@yahoo.com

Received September 15, 2011; **Accepted** October 27, 2011; **Published** October 31, 2011

Citation: Pisoschi AM, Negulescu GP (2011) Methods for Total Antioxidant Activity Determination: A Review. Biochem & Anal Biochem 1:106. doi:10.4172/2161-1009.1000106

implications of dietary components rely on the estimation of intakes of sample populations, which are found in databases that provide the compounds found in commonly consumed foods. Thus, the availability of appropriate and complete food composition data is vital. Due to the diversity of chemical compounds with antioxidant activity present in foodstuffs, complete databases of antioxidant contents are not yet available. In addition, levels of single antioxidants in foodstuffs do not necessarily reflect their total antioxidant potential (TAP) [22]; the total antioxidant potential depends on the synergic and redox interaction among the different molecules present in food [33,34]. Geographical differences in food composition should also be considered when regional surveys are performed.

The total antioxidant potential is a relevant tool for investigating the relationship between dietary antioxidants and pathologies induced by the oxidative stress. This was confirmed by the data obtained from a recent population-based control study, proving that diet TAP resulted in reduced risk of both cardiac and distal gastric cancer [35]. Several analytical methods were recently developed for measuring the total antioxidant capacity of food and beverages: these assays differ in the mechanism of generation of different radical species and/or target molecules and in the way end-products are measured [33,34,36-39].

The consumption of fruits and vegetables, as well as of grains and nuts, has been associated with reduced risk of chronic diseases [40-42]. Among food components fighting against chronic diseases, great attention has been paid to phytochemicals, plant-derived molecules endowed with steady antioxidant power. The cummulative and synergistic activities of the bioactive molecules present in plant food are responsible for their enhanced antioxidant properties. Hence, an appropriate investigation of the role of dietary antioxidants in disease prevention, should be based on a complete database of antioxidant-rich foodstuffs [40].

The evaluation of the total antioxidant capacity (TAC) may be an appropriate tool to determine the additive antioxidant properties of plant foods [43]. The importance of TAC as a novel instrument to estimate the relationship between diet and oxidative stress-induced diseases, is presented in recent studies [44,45] showing a negative association between dietary TAC and the incidence of gastric cancer or the levels of C-reactive protein. In order to assess the overall intake of TAC in population studies, the TAC of 34 vegetables, 30 fruits, 34 beverages and 6 vegetable oils, of varieties most often consumed in Italy, has been analysed using three different assays [43]. Among fruits, the highest antioxidant activities were found in berries, among beverages, coffee had the greatest TAC, followed by citrus juices, which exhibited the highest value among soft beverages [43]. The TAC of spices, dried fruits, sweets, cereals, pulses, and nuts was determined with the aim to complete the Italian TAC database [40]. In fiber-rich foods where phenolics are present in free or bound forms, such as cereals, legumes, and nuts, the contribution of bound antioxidant compounds to the TAC value was evaluated [40].

Various berries and fruit types of less common fruit species are known to contain antioxidants [46]. The intake of high amounts of flavonoids, compounds endowed with antioxidant, antiproliferative and anti-inflammatory activity, may have a positive impact on human health, especially in the prevention of cancer and inflammatory diseases [46].

The Mechanism of Action of Antioxidants

LMWAs (low molecular weight antioxidants) [47] are small molecules that frequently infiltrate cells, accumulate (at high concentrations) in specific compartments associated with oxidative damage, and then are regenerated by the the cell [48]. In human tissues, cellular LMWAs are obtained from various sources. Glutathione (GSH), nicotinamide adenine dinucleotide (reduced form), and carnosine [49] are synthesized by the cells; uric acid (UA) [50] and bilirubin [51] are waste products of cellular metabolism; ascorbic acid (AA) [52], tocopherols and polyphenols are antioxidants obtained from the diet.

Among these LMWAs, a considerable attention was focused on ascorbic acid (AA), known for its reductive properties and for its use on a wide scale as an antioxidant agent in foods and drinks [53]; it is also important for therapeutic purposes and biological metabolism.

Ascorbic acid is an antioxidant with therapeutic properties, which plays an important role in activating the immune response, in wound healing, in osteogenesis, in detoxifying the organism, in iron absorption, in collagen biosynthesis, in preventing the clotting of blood vessels, and in many other metabolic processes [54-56].

Vitamin C can be easily oxidized, its degradation being accelerated by heat, light and the presence of heavy metal cations [57-59]. Thus, due to its content variation, vitamin C represents an important quality indicator of foodstuffs [59] and contributes to the antioxidant properties of food [60-64].

Special attention has been dedicated to the study of antioxidant action mechanism.

The excess free radicals circulating in the body oxidize the low density lipoproteins (LDL), making them potentially lethal; the excess free radicals can also accelerate aging processes and have been linked to other very serious pathologies, such as brain stroke, diabetes mellitus, rheumatoid arthritis, Parkinson's disease, Alzheimer's disease and cancer. Physiologically, the oxygenated free radicals are among the most important radical species. Reactive oxygen species (ROS) comprise species with a strong oxidizing tendency, both of a radical nature (the superoxide radical, the hydroxyl radical) and a non-radical nature (ozone, hydrogen peroxide) [65].

A number of chemical and physical phenomena can initiate oxidation, which proceeds continuously in the presence of (a) suitable substrate(s), until a blocking defence mechanism occurs [66]. Target substances include oxygen, polyunsaturated fatty acids, phospholipids, cholesterol and DNA [67].

The essential features of oxidation via a free radical-mediated chain reaction are initiation, propagation, branching and termination steps [66]. The process may be initiated by the action of external agents such as heat, light or ionizing radiation or by chemical initiation involving metal ions or metalloproteins [68].

Initiation

$$LH + R\cdot \rightarrow L\cdot + RH$$

where LH represents the substrate molecule, for example, a lipid, with R· as the initiating oxidizing radical. The oxidation of the lipid generates a highly reactive allyl radical (L·) that can rapidly react with oxygen to form a lipid peroxyl radical (LOO·).

Propagation

$L\cdot + O_2 \rightarrow LOO\cdot$
$LOO\cdot + LH \rightarrow L\cdot + LOOH$

The peroxyl radicals are the chain carriers of the reaction; they can further oxidize the lipid, producing lipid hydroperoxides (LOOH), which in turn break down to a wide range of compounds [69], including alcohols, aldehydes, alkyl formates, ketones and hydrocarbons, and radicals, including the alkoxyl radical (LO·).

Branching

$LOOH \rightarrow LO\cdot + HO\cdot$
$2\ LOOH \rightarrow LOO\cdot + LO\cdot + H_2O$

The breakdown of lipid hydroperoxides often involves transition metal ion catalysis, in reactions similar to those involving hydrogen peroxide, yielding lipid peroxyl and lipid alkoxyl radicals.

Termination

Termination reactions involve the combination of radicals to form non-radical products:

$LO\cdot + LO\cdot$
$LOO\cdot + LOO\cdot$
$LO\cdot + LOO\cdot$

Primary antioxidants, AH, when present in trace amounts, may either delay or inhibit the initiation step by reacting with a lipid radical or inhibit the propagation step by reacting with peroxyl or alkoxyl radicals [70].

$L\cdot + AH \rightarrow LH + A\cdot$
$LOO\cdot + AH \rightarrow LOOH + A\cdot$
$LO\cdot + AH \rightarrow LOH + A\cdot$

Secondary or preventative antioxidants are compounds that retard the rate of oxidation. This may be achieved in a number of ways, including removal of substrate or singlet oxygen quenching [66, 71].

Methods of Total Antioxidant Capacity Assessment

The various analytical methods [72] of evaluation of the antioxidant capacity fall into distinct categories:

Antioxidant capacity assay	Principle of the method	End-product determination
Spectrometry		
DPPH	Antioxidant reaction with an organic radical	Colorimetry
ABTS	Antioxidant reaction with an organic cation radical	Colorimetry
FRAP	Antioxidant reaction with a Fe(III) complex	Colorimetry
PFRAP	Potassium ferricyanide reduction by antioxidants and subsequent reaction of potassium ferrocyanide with Fe3+	Colorimetry
CUPRAC	Cu (II) reduction to Cu (I) by antioxidants	Colorimetry
ORAC	Antioxidant reaction with peroxyl radicals, induced by AAPH (2,2'-azobis-2-amidino-propane)	Loss of fluorescence of fluorescein
HORAC	Antioxidant capacity to quench OH radicals generated by a Co(II) based Fenton-like system	Loss of fluorescence of fluorescein
TRAP	Antioxidant capacity to scavenge luminol-derived radicals, generated from AAPH decomposition	Chemiluminescence quenching
Fluorimetry	Emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength	Recording of fluorescence excitation/ emission spectra
Electrochemical Techniques		
Cyclic voltammetry	The potential of a working electrode is linearly varied from an initial value to a final value and back, and the respective current intensity is recorded	Measurement of the intensity of the cathodic/ anodic peak
Amperometry	The potential of the working electrode is set at a fixed value with respect to a reference electrode	Measurement of the intensity of the current generated by the oxidation/reduction of an electroactive analyte
Biamperometry	The reaction of the analyte (antioxidant) with the oxidized form of a reversible indicating redox couple	Measurement of the current flowing between two identical working electrodes, at a small potential difference and immersed in a solution containing the analysed sample and a reversible redox couple
Chromatography		
Gas chromatography	Separation of the compounds in a mixture is based on the repartition between a liquid stationary phase and a gas mobile phase	Flame ionisation or thermal conductivity detection
High performance liquid chromatography	Separation of the compounds in a mixture is based on the repartition between a solid stationary phase and a liquid mobile phase with different polarities, at high flow rate and pressure of the mobile phase	UV-VIS (e.g. diode array) detection, fluorescence, mass spectrometry or electrochemical detection

Spectrometric Techniques

Spectrometric techniques [72-91] rely on the reaction of a radical, radical cation or complex with an antioxidant molecule capable to donate a hydrogen atom.

The DPPH method: [2,73-75] DPPH• (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical, due to the delocalization of the spare electron on the whole molecule. Thus, DPPH• does not dimerize, as happens with most free radicals. The delocalisation on the DPPH• molecule determines the occurence of a purple colour, with an absorbtion band with a maximum around 520nm.

When DPPH• reacts with a hydrogen donor, the reduced (molecular) form (DPPH) is generated, accompanied by the disappearance of the violet colour. Therefore, the absorbance diminution depends linearly on the antioxidant concentration. Trolox is used as standard antioxidant [74, 75].

The spectrophotometric method with DPPH was applied to antioxidant capacity determination in fruit juices [75] and fruit (guava) extracts [74]. The standard curve was linear between 25 and 800mM Trolox [74]. Results are expressed in μM Trolox Equivalents/g fresh mass. Antioxidant activity of guava fruit methanol extracts, as determined by the DPPH method are comprised between 16.2 ± 1.0 and 32.0 ± 5.1μM TE/ fresh mass [74].

The ABTS method: The ABTS cation radical ($ABTS^{\cdot+}$) [76] which absorbs at 743 nm (giving a bluish-green colour) is formed by the loss of an electron by the nitrogen atom of ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)). In the presence of Trolox (or of another hydrogen donating antioxidant), the nitrogen atom quenches the hydrogen atom, yielding the solution decolorization.

ABTS can be oxidized by potassium persulphate [43,74] or manganese dioxide [77], giving rise to the ABTS cation radical

(ABTS•+) whose absorbance diminution at 743 nm was monitored in the presence of Trolox [43,74,77], chosen as standard antioxidant.

The spectrophotometric method based on the absorbance diminution of ABTS cation radical was applied to antioxidant content determination in guava fruit extracts [74], fruit and vegetable extracts, soft beverages, alcoholic beverages, tea and coffee [43]. The standard curve was linear between 25 and 600μM Trolox [74]. The values of the total antioxidant capacity of guava extracts ranged between 22.3 ± 0.9 and 37.9 ± 3.4μM TE/ fresh mass [74].

The antioxidant activity of soft beverages, as determined by the ABTS method is comprised between 0.09 mM Trolox/liter for Cola and 3.30mM Trolox/liter for grapefruit juice [43].

The FRAP (ferric reducing antioxidant power) method: The FRAP (ferric reducing antioxidant power) method relies on the reduction by the antioxidants, of the complex ferric ion-TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine). The binding of Fe^{2+} to the ligand creates a very intense navy blue color. The absorbance can be measured to test the amount of iron reduced and can be correlated with the amount of antioxidants [43,74,78]. Trolox [43] or ascorbic acid [78] were used as references.

The total antioxidant activity of white- and yellow-flesh nectarines [78] was evaluated by FRAP method, the results being expressed as Ascorbic Acid Equivalent Antioxidant Capacity, AEAC). Thea values ranged between 14.4 and 104.5mg/100 of fruit.

The ORAC (oxygen radical absorption capacity) assay: [74,79, 80] the method measures the antioxidant scavenging activity against the peroxyl radical, induced by 2,2'-azobis-(2-amidino-propane) dihydrochloride (AAPH), at 37°C. Fluorescein was used as the fluorescent probe. The loss of fluorescence was an indicator of the extent of the decomposition, from its reaction with the peroxyl radical. Antioxidant activity of guava fruit methanol extracts were determined by the ORAC method. The standard curve was linear between 0 and 50mM Trolox. The obtained results ranged between 18.2 ± 2.3 and 25.5 ± 1.6μM TE/ fresh mass [74].

The HORAC (hydroxyl radical averting capacity) assay: [79,81]: this technique relies on the measurement of the metal-chelating activity of antioxidants, under the conditions of Fenton-like reactions. The method uses a Co(II) complex and hence evaluates the protecting ability against the formation of hydroxyl radical. Fluorescein is incubated with the sample to be analysed, then the Fenton mixture (generating hydroxyl radicals) was added. The initial fluorescence was measured, after which the readings were taken every minute after shaking. Gallic acid solutions were used for building the standard curve.

The TRAP (total peroxyl radical trapping antioxidant parameter) assay: the luminol-enhanced chemiluminescence (CL) [79,82] was exploited to monitor the reactions involving the peroxyl radical. The CL signal is driven by the production of luminol derived radicals, resulted from the thermal decomposition of AAPH. The TRAP value was determined from the duration of the time period during which the sample quenched the chemiluminiscence signal, due to the presence of antioxidants.

The lipid peroxidation inhibition assay: The lipid peroxidation inhibition assay method uses a Fenton-like system (Co(II) + H_2O_2), to induce lipid (e.g. fatty acid) peroxidation [79,83].

α-linolenic acid was chosed as a model substrate. It was mixed with the analysed sample, as well as with the Fenton-like mixture, to induce lipid peroxidation. After the end of the incubation, the concentration of thiobarbituric acid-reactive substances (TBARS) was measured, as the index of lipid peroxidation. Lipid peroxidation was expressed in nmoles of TBARS per 1 ml of mixture α-linolenic acid/analysed sample.

The PFRAP (potassium ferricyanide reducing power) method: [84, 85] An absorbance increase can be correlated to the reducing ability of antioxidants/antioxidant extracts. The compounds with antioxidant capacity react with potassium ferricyanide, to form potassium ferrocyanide. The latter reacts with ferric trichloride, yielding ferric ferrocyanide, a blue coloured complex, with a maximum absorbance at 700nm.

The CUPRAC (cupric reducing antioxidant power) assay: [84, 86] the standard antioxidants or extracts are mixed with CuSO4 and neocuproine. After 30min, the absorbance was measured at 450nm. In the assay, Cu(II) is reduced to Cu(I) through the action of electron-donating antioxidants. Results are expressed in milligrams of Trolox per liter of extract.

Fluorimetry: fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength. In most cases, emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation. Fluorescence emission occurs when an orbital electron of a molecule relaxes to its ground state, by emitting a photon of light after being excited to a higher quantum state by some type of energy. Fluorescence assay has been used to antioxidant content determination [87-91].

The fluorescence spectroscopy has been applied for the determination of phenolic compounds in oils [87]. A method based on fluorescence is proposed to quantify the butylhydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) antioxidant concentration in biodiesel produced from sunfower and soybean oils. Fluorescence and excitation spectra of the solutions were recorded at room temperature using a spectrofluorimeter. The emission spectra were obtained under excitation at about 310 nm and fluorescence in the 320-800nm range was evaluated. Biodiesel samples without BHA and TBHQ showed fluorescence band at about 420nm, which can be attributed to tocopherols, inherent to the vegetable oils used in the biodiesel production. The addition of BHA and/or TBHQ is responsible for the occurence of a fluorescence band around 330nm. It was verified that the fluorescence intensity around 330nm increases linearly as function of antioxidant concentration with correlation coefficient of about 1, regardless of the oil source and antioxidants.

Fluorimetric methods of ascorbic acid determination are based on dehydroascorbic acid reaction with o-phenylene diamine [88,89]. This technique requires a strict control of pH, as the fluorescence intensity depends strongly of the pH value.

A fluorescence method was developed [90], to examine how membrane sterol lateral organization affects the potency of antioxidants. This information was used to evaluate possible adverse effects of lipid-soluble antioxidants, which was reported in recent clinical studies. In the presence of an antioxidant, the lag time produced during free radical-induced sterol oxidation in lipid vesicles reflects the potency of the antioxidant. The obtained data suggest that while ascorbyl palmitate is a more efficient antioxidant than its water-soluble counterpart as judged by the lag time, it can easily perturb sterol lateral organization by insertion into membrane bilayers, which could impose detrimental effects on cells.

Another fluorescence assay [91] measured the rate and extent of sterol oxidation in lipid bilayers. Dehydroergosterol (DHE), a

fluorescent cholesterol analog, is used as a probe and at the same time, as a membrane component. The assay can also be performed on bilayers containing a mixture of sterols including DHE and nonfluorescent sterols, such as cholesterol and ergosterol. The fluorescence intensity of DHE decreases on oxidation, so the rate and extent of free radical- or enzyme-induced sterol oxidation can be measured as a function of temperature and membrane composition. In agreement with the sterol regular distribution model, it is found that both free radical- and enzyme-induced sterol oxidation vary with membrane sterol content in a well defined alternating manner [91].

Electrochemical techniques

Electrochemical techniques were also applied to antioxidant content and antioxidant capacity determination. Cyclic voltammetry and biamperometry are the most broadly used.

Cyclic voltammetry: Cyclic voltammetry is a type of potentiodynamic electrochemical measurement. In cyclic voltammetry experiments the working electrode potential is ramped linearly versus time. In cyclic voltammetry, the potential of a working electrode is linearly scanned from an initial value to a final value and back, while recording the respective current intensity.

When the value of a set potential is reached, the working electrode's potential ramp is inverted. This inversion can happen multiple times during a single experiment. The current at the working electrode is plotted versus the applied voltage to give the cyclic voltammogram.

The important parameters obtained from a cyclic voltammogram are the intensities of the cathodic and anodic peaks *Ia, Ic*, the anodic oxidation potential (*Ea*), and the cathodic oxidation potential (*Ec*). All these values can be readily obtained from the voltammogram. In the case of a reversible system, the values of the intensities of the cathodic and anodic peaks are equal. For irreversible system, only the presence of one peak is noticeable on the voltammogram. Cyclic voltammetry (CV), shown to be a convenient methodology, has been validated for the quantitation of the Low Molecular Weight Antioxidant capacity of blood plasma, tissue homogenates, and plant extracts. Analysis of the CV tracing yields the values of (i) the biological oxidation potential, E and $E_{1/2}$, which relate to the nature of the specific molecule(s); (ii) the intensity (*Ia*) of the anodic current; and (iii) the area of the anodic wave (S) [47]. The sensitivity of the method, as given by the slope of the calibration graph versus vitamin C was 15.175μA/mM ascorbic acid [47]. The antioxidant capacity of dry vegetal extracts [65] (expressed as mg of ascorbic acid equivalents) was determined by cyclic voltammetry performed at a glassy carbon working electrode. Green tea, black tea, rosemary and coffee, were selected and analysed to test the total antioxidant capacity of respective dry extracts. For the three extracts obtained from each matrix the antioxidant capacity was determined by the measurement of the anodic area of a cyclic voltammogram.

Of the substances tested, those in which dry methanol extracts display the highest total antioxidant capacity are: Green Tea > Black Tea > Rosemary > Arabica Coffee> Herb Tea > Acerola > Quality Tea > Acai. On the other hand, ofthe substances tested, those for which dry aqueous extracts display the highest total antioxidant capacity are: Green Tea >Black Tea > Arabic Coffee > Herb Tea > Rosemary > Acerola >Quality Tea > Acai. This implies that these two solvents do not always have the same extractive capacity for all the antioxidant substances contained in the various vegetal samples.

Cyclic voltammetry results of antioxidant capacity determination in buckwheat products showed good correlation with the data obtained by spectrophotometry [92]. Cyclic voltammograms of analysed buckwheat extracts were useful for evaluation of the antioxidant capacity. The total charge below the anodic current waveform was correlated with the data obtained by the spectrophotometric method with $ABTS^{+\cdot}$ and $DPPH^{\cdot}$. The changes in the antioxidant capacity of buckwheat and its products followed the changes in flavonoid composition.

The amperometric method: The amperometric method involves the measurement of the intensity of the current that flows between a working electrode and a reference electrode, at a fixed (applied) value of potential. The current is generated by the oxidation/reduction of an electroactive analyte. The value of the potential is maintained at a set value with respect to a reference electrode [93-95].

The amperometric determination of the antioxidant activity [96] was based on the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH•) at the glassy carbon electrode. All the experiments were performed in a three-electrode electrochemical cell at 140 mV vs. Hg_2Cl_2 | 3M KCl using ethanolic solution (40%) and 0.033M KCl in 0.033 M phosphate buffer, pH=7.4. The linear range obtained for Trolox in 100μM DPPH ethanol-water solution was up to 30μM, with a limit of detection of 0.05μM. The developed method was applied for the evaluation of antioxidant activity of some water or ethanol soluble pure antioxidant compounds and of several samples of tea, wine and some other beverages. The good correlation of results (R^2=0.9993) expressed as Trolox equivalents, was obtained between the proposed amperometric method and the classic spectroscopic method [96].

The biamperometric method: The biamperometric method is based on the measurement of the current flowing between two identical working electrodes polarized at a small potential difference and immersed in a solution containing a reversible redox couple. Indirect biamperometric measurement relies on the reaction of the analyte with the indicating redox couple, its selectivity depending on the specificity of the reaction involving the oxidized or reduced form of the redox pair and the analyte. Fe^{3+}/Fe^{2+}, I_2/I^-, $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ are redox couples commonly used in biamperometric measurements [97].

A common redox pair chosen in biamperometric studies was DPPH•/DPPH. Antioxidants react with DPPH• (radical form) generating DPPH (reduced form), the intensity of the resulted current being proportional to the residual concentration of DPPH•, after its reaction with the analyte (antioxidant) [75, 98].

Two identical Pt [75] or glassy carbon [98] electrodes were used, where the reduction of the DPPH• radical and the oxidation of the reduced form (DPPH) take place as follows:

Electrode 1: DPPH• + e^- → DPPH

Electrode 2: DPPH → DPPH• + e^-

The reduction of DPPH•at electrode 1 gives rise to a cathodic current, while the oxidation of DPPH at electrode 2 generates an anodic current. In biamperometry, the controlled parameter is the potential difference between the two identical working electrodes. The potential values of the two electrodes are not controlled with respect to a reference electrode. The biamperometric detector response is linear with respect to that constituent of the redox couple which is present in lower concentration. Working conditions were chosen for a DPPH• (oxidized form) concentration smaller than DPPH concentration. Each antioxidant addition in a solution containing the redox couple DPPH•/DPPH decreases the concentration of the oxidized (radical) form and increases the concentration of the reduced form, thus generating a

current proportional to the concentration of antioxidant. In the case of the proposed method, the cathodic current is limited by the lower concentration of DPPH• (radical form) in the indicating mixture. The DPPH•/DPPH method was applied to the determination of the total antioxidant capacity in fruit juices [75], tea, wine and coffee [98]. The sensitivity of the method was 20.1 nA/μM of Trolox, while the limit of detection accomplished by the used measuring device was 0.05 μM [98].

Another redox couple used in biamperometric antioxidant capacity assay is $ABTS^{+}$/ABTS [99,100]. The ABTS cation radical was enzymatically produced by peroxidase in a tubular flow-through reactor. The performance of the bioreactor was tested at different concentrations of immobilized enzyme, ABTS and hydrogen peroxide. Interdigitated array microelectrodes were used as electrochemical sensors for the biamperometric determination. The results of antioxidant activity were determined using Trolox as a standard. The applied interdigitated electrode (IDE) detector accomplished a good sensitivity of 0.3 nA/μM Trolox and offered linear range between 20 to 500μM Trolox. Real samples like juices, tea and wine were analysed [99].

The ABTS cation radical was also produced bienzymatically, by using glucose oxidase and peroxidase. The linearity of IDE detector was tested in the range 20μM-2000μM and a good sensitivity of 0.165 nA/μM for Trolox solutions was obtained [100]. The interdigitated gold electrodes were used for biamperometric determination of antioxidant capacity of alcoholic beverages (wine and spirits) [100].

Biosensors method

Oxidoreductases are the most oftenly used in biosensor applications because of their electron transferring properties during catalysis. These enzymes offer the advantages of being stable and in some situations do not require coenzymes or cofactors. There are several reviews and books referring to antioxidant and antioxidant capacity determination by biosensors [72, 101-104].

Potential applications of biosensors for evaluation of antioxidant status include monitoring of superoxide radical (O^{2}•−), monitoring of nitric oxide (NO), monitoring of glutathione, monitoring of uric acid, ascorbic acid or phenolic compounds [104].

A carbon paste DNA-based biosensor for the electrocatalytical evaluation of total antioxidant capacity was constructed [105]. The method was based on the partial damage of a DNA layer adsorbed on the electrode surface by OH• radicals, generated by Fenton reaction and the subsequent electrochemical oxidation of the intact adenine bases, to generate an oxidation product that was able to catalyse the oxidation of NADH. The presence of antioxidant compounds scavenged hydroxyl radicals, leaving more adenine molecules unoxidized, and thus, increasing the electrocatalytic current of NADH measured by differential pulse voltammetry. Using ascorbic acid as a model antioxidant species, the detection of amounts as low as 50 nM ascorbic acid in aqueous solution was possible [105].

Frequently, polyphenols are the main contributors to the antioxidant capacity of several plants which contain them. Several amperometric biosensors for the detection of phenolic compounds have been developed, on the basis of enzymes, such as tyrosinase, laccase or peroxidase [106-109]. Biosensors for phenolic compounds were constructed by immobilizing polyphenol oxidase (PPO) into conducting copolymers prepared by electropolymerization of pyrrole with thiophene capped polytetrahydrofuran [108].

These enzyme-based biosensors allow the evaluation of the "total phenol content". Since tyrosinase acts on the hydroxyl groups of phenolic compounds, the total amount of -OH groups in red wines was obtained through activity determination by enzyme electrodes. Results are reported in Gallic Acid Equivalent (GAE) as mg/l [108,110]. For polyphenol determination in vegetable extracts [111], an amperometric horseradish peroxidase-based biosensor was employed.

The biosensors were used for the determination of antioxidant capacity in wines, the results being consistent with those obtained by spectrophotometry [112,113] or in orange juices, by biosensors based on screen-printed electrodes [114]. For the analysis of commercial red wines, a multi-walled nanotube ionic liquid electrode with immobilized tyrosinase was used [113]. The sensing ranges were 0.01-0.08 mM in a phosphate buffer solution.

Chromatographic methods

Chromatographic methods were often applied to antioxidant separation and detection, and used before spectrophotometrical or electrochemical assessment of the total antioxidant capacity.

Gas chromatography: Gas chromatography (GC) is a common type of chromatography used for separating and analysing compounds that can be vaporized without decomposition. The process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas mobile phase. The mobile phase is usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support. The comparison of retention times is what gives GC its analytical usefulness. The most common detectors are the flame ionization detector and the thermal conductivity detector.

The antioxidant capacity of turmeric oil (responsible for its antimutagenic capacity) was also determined by chromatographic methods [115]. Turmeric oil and its fractions were analysed by gas chromatography with flame ionisation detector and gas chromatography coupled with mass spectrometry. Turmeric oil and its fractions were then tested for antioxidant activity using the carotene-linoleate model system and the phosphomolybdenum method. The quantitative antioxidant capacity of the turmeric oil and its fractions were measured spectrophotometrically through the phosphomolybdenum method, which is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex with a maximum absorption at 695 nm. The method using the carotene-linoleate [115, 116] model was based on the continuous measurement of the optical density, until the colour of β-carotene disappeared. Butylated hydroxyanisole (BHA) was used for the blank.

HPLC (high performance liquid chromatography): HPLC (high performance liquid chromatography) typically utilizes different types of stationary phases, a pump that moves the mobile phase(s) and analyte through the column, and a detector to provide a characteristic retention time for the analyte. The detector (usually a diode array detector) may also provide additional information related to the analyte, (i.e. UV/Vis spectroscopic data for analyte if so equipped).

A pump provides the higher pressure required to move the mobile phase and analyte through the densely packed column. The increased density arises from smaller particle sizes. This allows a better separation on columns of shorter length and ensures higher velocity. Normal-phase HPLC uses a polar stationary phase and a non-polar, non-aqueous mobile phase, and works effectively for separating analytes readily soluble in non-polar solvents. Reversed phase HPLC has a

non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily.

The antioxidant activity using a HPLC system with post-column on-line antioxidant detection, based on 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid radical scavenging activity. The method was applied to the determination of antioxidant content of coffee [117]. Following separation of the coffee samples on the HPLC column, the eluate was directed to a PDA (photodiode array) detector and then mixed with a stabilised solution of the ABTS cation radical and the solution was directed to a detector monitoring absorbance at 720 nm. The ABTS cation solution has a deep blue colour, and any quenching of the radical results in a loss of colour indicated by a negative peak on the HPLC trace. The antioxidant contributions of individual HPLC peaks were added to give the total HPLC-derived antioxidant activity. The total antioxidant capacity of the green coffee determined with the on-line HPLC system was 760 ± 2.5μmol Trolox/l and 984 ± 25.8μmol Trolox/l for the roasted coffee [117].

A HPLC method with fluorescence detection [118] was developed for the determination of propyl gallate, nordihydroguaiaretic acid, butylated hydroxyanisole, tert-butylhydroquinone and octyl gallate in edible oils and foods. The HPLC separation was performed on a C18 column using a mixture of 5% acetic acid-acetonitrile-methanol as the mobile phase and monitored by using a fluorescence detector. Sample peaks were identified by comparison of the fluorescence spectra with those of antioxidant standards. Average recoveries of fortified antioxidants at 100 micrograms/g were 72.1-99.6%. Coefficients of variation were 0.7-7.2% [118].

The antioxidant activity of the extracts from root barks of adult plant and root of seedlings extracts [119] was evaluated by HPLC coupled to electrochemical detection (HPLC-ED).

The EICD electrochemical detector was composed of a glassy carbon working electrode, an Ag/AgCl reference electrode and a Pt electrode. The separation of the analytes was carried on a Gemini C18 column, using the isocratic mode and acetonitrile/water mixture containing acetic acid as mobile phase. The optimal potential of the standards was obtained on a hydrodynamic voltammogram, from the evaluation of peak areas vs applied potential vs Ag/AgCl [119].

The advantages and shortcomings of the antioxidant assessment methods were presented by Prior and colab [120]. Several articles highlighten the advantages and disadvantages of in vivo and in vitro analysis [121-128].

The methods are chosen as a function of the nature of the sample and the comparison is valid only on the same sample types. The advantages of the analytical techniques can reffer to the complexity of the necessary tools, to the simplicity of the applied procedure, to the duration of the analysis, to the biological relevance and the performances of the method (sensitivity, precision, accuracy, detection limit).

Determinations relying on photometric measurements (DPPH, ABTS and FRAP assays) are simple and rapid and need only a UV-Vis spectrophotometer to perform, which probably explains their widespread use in antioxidant screening.

Most methods can be rapidly automatized and some can be applied in vivo (e.g. ABTS assay). Nevertheless, the analytical signal is sometimes difficult to measure and does not account for all antioxidants.

TRAP assay has been criticized as being based on an unphysiological oxidative stress (water-soluble peroxyl radicals), the FRAP assay does not measure thiol antioxidants, such as glutathione. The DPPH assay was considered as not based on a competitive reaction, because DPPH· is both radical probe and oxidant. Interpretation is complicated when the tested compounds have spectra that overlap DPPH· at 515nm. The FRAP assay is characterized by a fast kinetics (4-6 min) but in fact this is not always true. Some polyphenols react more slowly and require longer reaction times for detection, for example, 30min. Copper has advantages over iron for antioxidant assay, in that all classes of antioxidants, including thiols, are detected with little interference from reactive radicals, and the copper reaction kinetics are faster than in case of iron. CUPRAC assay is complete in minutes for ascorbic acid, uric acid, gallic acid, and quercetin, but requires 30-60 min for more complex molecules. ORAC method is based on a temperature-sensitive reaction. Hence, temperature control is essential.

Regarding the complexity of the analytical instruments, the photometric methods are the simplest, followed by voltammetric and chromatographic methods.

Voltammetry offers low detection limits, even when compared to more expensive techniques. It requires little sample preparation. This technique provides us with the advantage of a fast analysis as well as with the easiness and rapidity of the standard addition method application. Because of the low cost of the required equipment, as well as simplicity of the employed procedures, voltammetry appears to offer an attractive alternative to the titrimetric or instrumental methods, in particular in food quality control. It does not require complicated, expensive equipment and well-qualified personnel like chromatography, nor is it laborious or time consuming like the previously mentioned instrumental technique [59].

Conclusions

The increasing interest gained by antioxidants is due to the health benefits provided mainly by natural sourced (exogenous) low molecular weight antioxidants. This consists in preventing the occurence of oxidative-stress related diseases, caused by the attack of free radicals on key biocomponents like lipids or nucleic acids.

Various methods and analytical tools are employed for antioxidant content and total antioxidant capacity evaluation: spectrometry, electroanalytical methods, chromatography.

These techniques are able to offer a complete profile of the antioxidant content of foodstuffs.

Acknowledgements

This work was co-financed from the European Social Fund through the Sectoral Operational Programme Human Resources Development 2007-2013, project number POSDRU/89/1.5/S/63258 "Postdoctoral school for zootechnical biodiversity and food biotehnology based on the eco-economy and the bio-economy required by ecosanogenesis".

References

1. Litescu SC, Sandra AV, Eremia SAV, Diaconu M, Tache A, et al. (2011) Biosensors Applications on Assessment of Reactive Oxygen Species and Antioxidants. Environmental Biosensors. In Tech Rijeka Croatia.
2. Molyneux P (2004) The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 26: 211-219.
3. Kalcher K, Svancara I, Buzuk M, Vytras K , Walcarius A (2009) Electrochemical sensors and biosensors based on heterogeneous carbon materials. Monatsh Chem 140: 861-889.

4. Ly SY (2008) Voltammetric analysis of DL-α-tocopherol with a paste electrode. J Sci Food Agric 88: 1272-1276.

5. Kong YT, Imabayashi SI, Kano K, Ikeda T and Kakiuchi T (2001) Peroxidase-based amperometric sensor for the determination of total phenols using two-stage peroxidase reactions. Am J Enol Vitic 52: 381-385.

6. Mena ML, Carralero V, Gonzalez-Cortes A, Yanez-Sedeno P, Pingarron JM (2007) Bioelectrochemical evaluation of the total phenols content in olive oil mill wastewaters using a tyrosinase–colloidal gold–graphite–Teflon biosensor. Int J Environ Anal Chem 87: 57-65.

7. Granero AM, Fernandez H, Agostini E and Zon MA (2008) An amperometric biosensor for trans-resveratrol determination in aqueous solutions by means of carbon paste electrodes modified with peroxidase basic isoenzymes from brassica napus. Electroanalysis 20: 858-864.

8. Zoulis NE and Efstathiou CE (1996) Preconcentration at a carbon-paste electrode and determination byadsorptive-stripping voltammetry of rutin and other flavonoids. Anal Chim Acta 320: 255-261.

9. Volikakis GJ and Efstathiou CE (2000) Stripping voltammetry using nujol-graphite and diphenylether-graphite paste electrodes. Talanta 51: 775-785.

10. Korbut O, Buckova M, Labuda J, Gruendler P (2003) Voltammetric detection of antioxidative properties of flavonoids using electrically heated DNA modified carbon paste electrode. Sensors 3: 1-18.

11. Cummings EA, Mailley P, Linquette-Mailley S, Eggins BR, McAdams ET, et al. (1998) Amperometric carbon paste biosensor based on plant tissue for the determination of total flavanol content in beers. Analyst 123: 1975-1980.

12. Eggins BR, Hickey C, Toft SA and Zhou DM (1997) Determination of flavonols in beers with tissue biosensors. Anal Chim Acta 347: 281-288.

13. Cummings EA, Linquette-Mailley SC, Mailley P, Cosnier S, Eggins BR, et al. (2001) A comparison of amperometric screen printed carbon electrodes and their application to the analysis of phenolic compounds in beers. Talanta 55: 1015-1027.

14. Busch JLHC, Hrncirik K, Bulukin E, Boucon C, Mascini M (2006) Biosensor measurements of polar phenolics for the assessment of the bitterness and pungency of virgin olive oil. J. Agric. Food. Chem. 54: 4371-4377.

15. Labuda J, Buckova M, Heilerova L, Caniova-Ziakova A, Brandsteterova E, et al. (2002) Detection of Antioxidative Activity of Plant Extracts at the DNA-Modified Screen-Printed Electrode. Sensors 2: 1-10.

16. Kim HJ, Chang SC and Shim YB (2002) Cyclodextrin modified screen printed graphite electrodes for detection of phenols. Bull Korean Chem Soc 23: 427-431.

17. Capannesi C, Palchetti I, Mascini M and Parenti A (2000) Electrochemical sensor and biosensor for polyphenols detection in olive oils. Food Chem 71: 553-562.

18. Romani A, Minunni M, Mulinacci N, Pinelli P, Vincieri FF, et al. (2000) Comparison among differential pulse voltammetry, amperometric biosensor, and HPLC/DAD analysis for polyphenol determination. J Agric Food Chem 48: 1197-1203.

19. Halvorsen BL, Holte K, Myhrstad MCW, Barikmo I, Hvattum E, et al. (2002) A systematic screening of total antioxidants in dietary plants. J Nutr 132: 461-471.

20. Halvorsen BL, Carlsen MH, Phillips KM, Bohn SK, Holte K, et al. (2006) Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. Am J Clin Nutr 84: 95-135.

21. Lindsay DG and Astley SB (2002) European research on the functional effects of dietary antioxidants—EUROFEDA. Mol Aspects Med 23: 1-38.

22. Ramadan-Hassanien MF (2008) Total antioxidant potential of juices, beverages and hot drinks consumed in Egypt screened by DPPH in vitro assay. Grasas y aceites 59: 254-259.

23. Gillman MW, Cupples LA, Gagnon D, Posner BM, Ellison RC, et al. (1995) Protective effect of fruits and vegetables on development of stroke in men. J Am Med Assoc 273: 1113-1117.

24. Rimm EB, Aschiero A, Giovannucci E, Spiegelman D, Stampfer MJ, et al. (1996) Vegetable, fruits and cereal fiber intake and risk of coronary heart disease among men. J Am Med Assoc 275: 447-451.

25. Cohen JH, Kristal AR and Stanford JL (2000) Fruit and vegetable intakes and prostate cancer risk. J Natl Cancer Inst 92: 61-68.

26. La Vecchia C, Altieri A and Tavani A (2001) Vegetables, fruit, antioxidants and cancer: a review of Italian studies. Eur J Clin Nutr 40: 261-267.

27. Terry P, Terry JB and Wolk A (2001) Fruit and vegetable consumption in the prevention of cancer: an update. J Intern Med 250: 280-290.

28. Rodríguez-Bernaldo de Quirós A and Costa HS (2006) Analysis of carotenoids in vegetable and plasma samples: A review. J Food Compos Anal 19: 97-111.

29. Gey KF (1990) The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms. Biochem Soc Trans 18: 1041-1045.

30. Gey KF, Puska P, Jordon P and Moser UK (1991) Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. Am J Clin Nutr 53: 326-334.

31. Willett WC (1991) Micronutrients and cancer risk. J Am Med Assoc 53: 265-269.

32. Liyana-Pathirana CM, Shahidi F and Alasalvar C (2006) Antioxidant activity of cherry laurel fruit (Laurocerasus officinalis Roem.) and its concentrated juice. Food Chem 99: 121-128.

33. Ramadan MF, Kroh LW and Moersel JT (2003) Radical scavenging activity of black cumin (Nigella sativa L.), coriander (Coriandrum sativum L.) and niger (Guizotia abyssinica Cass.) crude seed oils and oil fractions. J Agric Food Chem 51: 6961-6969.

34. Ramadan MF and Moersel JT (2007) Impact of enzymatic treatment on chemical composition, physicochemical properties and radical scavenging activity of goldenberry (Physalis peruviana L.) juice J Sci Food Agric 87: 452-460.

35. Serafini M, Bellocco R, Wolk A and Ekstrom AM (2002) Total antioxidant potential of fruit and vegetables and risk of gastric cancer. Gastroenterology 123: 985-999.

36. Wang H, Cao G and Prior RL (1997) Oxygen radical absorbing capacity of anthocyanins. J Agric Food Chem 45: 304-309.

37. Benzie IFF and Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power. Meth Enzymol 299: 15-27.

38. Benzie IFF and Szeto YT (1999) Total antioxidant capacity of teas by ferric reducing/antioxidant power assay. J Agric Food Chem 47: 633-636.

39. Pellegrini N, Simonetti P, Gardana C, Brenna O, Brighenti F, et al. (2000) Polyphenol content and total antioxidant activity of vini novelli (young red wines). J Agric Food Chem 48: 732-735.

40. Pellegrini N, Serafini S, Del Rio SD and Bianchi M (2006) Total antioxidant capacity of spices, dried fruits, nuts, pulses, cereals and sweets consumed in Italy assessed by three different in vitro assays. Mol Nutr Food Res 50: 1030–1038.

41. Hu FB (2003) Plant-based foods and prevention of cardiovascular disease: an overview. Am J Clin Nutr 78(3): 544-551.

42. McCullough ML, Robertson AS, Chao A, Jacobs EJ, Stampfer MJ, et al. (2003) A prospective study of whole grains, fruits, vegetables and colon cancer risk. Cancer Cause Control 14: 959-970.

43. Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, et al. (2003) Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. J Nutr 133: 2812–2819.

44. Serafini M, Bellocco R, Wolk A and Ekstrom AM (2002) Total antioxidant potential of fruit and vegetables and risk of gastric cancer. Gastroenterology 123: 985-991.

45. Brighenti F, Valtueña S, Pellegrini N, Ardigo D, Del Rio D, et al. (2005) Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high-sensitivity C-reactive protein in adult Italian subjects. Br J Nutr 93: 619-625.

46. Gazdik Z, Krska B, Adam V, Saloun J, Pokorna T, et al. (2008) Electrochemical determination of the antioxidant potential of some less common fruit species. Sensors 8: 7564-7570.

47. Chevion S, Roberts MA, Chevion M (2000) The use of cyclic voltammetry for the evaluation of antioxidant capacity. Free Radic Biol Med 28: 860-870.

48. Halliwell B, Gutteridge JMC (1989) Free radicals in biology and medicine. Clarendon Press, Oxford.

49. Chance PA, Sies H and Boveris A (1979) A hydroperoxide metabolism in mammalian organs. Physiol Rev 59: 527-605.

50. Ames BM, Cathcart R, Schwiers E, Hochstein P (1981) Uric acid produces an antioxidant defense in humans against oxidant and radical-caused aging and cancer: a hypothesis. Proc Natl Acad Sci USA 73: 6858-6862.

51. Stocker R, Yamamoto Y, McDonagh A, Glazer AN and Ames BN (1987) Bilirubin is an antioxidant of possible physiological importance. Science 235: 1043-1045.

52. Frei B, England L and Ames BN (1989) Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci USA 86: 6377-6381.

53. Raoof JB, Ojani R and Beitollahi H (2007) Electrocatalytic determination of ascorbic acid at chemically modified carbon paste electrode with 2, 7-bis (ferrocenyl ethynyl) fluoren-9-one. Int J Electrochem Sci 2: 534-548.

54. Tomita IN, Manzoli A, Fertonani FL and Yamanaka H (2005) Amperometric biosensor for ascorbic acid. Eclet Quím 30: 37-43.

55. Voet D and Voet J (1995) Biochemistry. (2ndedn), John Wiley & Sons, New York.

56. Mello LD and Kubota LT (2007) Biosensors as a tool for the antioxidant status evaluation. Talanta 72: 335-348.

57. Bhagavan NV (2002) Medical Biochemistry. Elsevier, Amsterdam.

58. Mohora M (2006) Biochimie medicala. Editura Niculescu, Bucuresti

59. Wawrzyniak J, Ryniecki A and Zembrzuski W (2005) Application of voltammetry to determine vitamin C in apple juices. Acta Sci Pol Technol Aliment 42: 5-16.

60. Glevitzky M, Pop M, Brusturean G A, Bogdan I, Calisevici M, et al. (2008) Efficent use of antioxidants to preserve fruit juice. Rev Chim (Bucharest) 59: 1291-1295.

61. Popa CV, Danet AF, Jipa S and Zaharescu T (2010) Determination of total antioxidant activity of wines using a flow injection method with chemiluminescence detection. Rev Chim (Bucharest) 61: 11-16.

62. Pisoschi AM, Danet AF and Kalinowski S (2008) Ascorbic acid determination in commercial fruit juice samples by cyclic voltammetry. JAMMC 8.

63. Pisoschi AM, Negulescu Gh P and Pisoschi A (2010) Ascorbic acid determination by an amperometric ascorbate oxidase-based biosensor. Rev Chim (Bucharest) 61: 339-344.

64. Pisoschi AM, Pop A, Negulescu Gh P and Pisoschi A (2011) Determination of ascorbic acid content of some fruit juices and wine by voltammetry performed at Pt and carbon paste electrodes. Molecules 16: 1349-1365.

65. Campanella L, Martini E, Rita E and Tomassetti M (2006) Antioxidant capacity of dry vegetal extracts checked by voltammetric method. J Food Agric Environ 4: 135-144.

66. Antolovic M, Prenzler PD, Patsalides E, McDonald S and Robards K (2002) Methods for testing antioxidant activity. Analyst 127: 183–198.

67. Ming-Hua Y and Schaich KM (1996) Factors affecting DNA damage caused by lipid hydroperoxides and aldehydes. Free Radical Biol Med 20: 225-236.

68. Kanner J, German JB and Kinsella JE (1987) Initiation of lipid peroxidation in biological systems. Crit Rev Food Sci Nutr 25: 317-364.

69. Cheeseman KH and Slater TF (1993) An introduction to free radical biochemistry. Br Med Bull 49: 481-493.

70. Madhavi DL, Deshpande SS and Salunkhe DK (1996) Food Antioxidants: Technological, Toxicological and Health Perspectives. Marcel Dekker, New York.

71. Frankel EN and Meyer AS (2000) The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. J Sci Food Agric 80: 1925-1941.

72. Giardi MT, Rea G, Berra B (2010) Bio Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors. Landes Bioscience and Springer Science+Business Media.

73. Brand-Williams W, Cuvelier ME and Berset C (1995) Use of a free radical method to evaluate antioxidant activity. Lebensm-Wiss u-Technol 28: 25-30.

74. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L and Byrne DH (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19: 669–675.

75. Pisoschi AM, Cheregi MC and Danet AF (2009) Total antioxidant capacity of some commercial fruit juices: electrochemical and spectrophotometrical approaches. Molecules 14: 480-493.

76. Marc F, Davin A, Deglène-Benbrahim L, Ferrand C, Baccaunaud M, et al. (2004) Studies of several analytical methods for antioxidant potential evaluation in food. Med Sci 20: 458-463.

77. Su L, Yin JJ, Charles D, Zhou K, Moore J, et al. (2007) Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. Food Chem 100: 990–997.

78. Gil MI, Tomas-Barberan FA, Hess-Pierce B and Kader AA (2002) Antioxidant capacities, phenolic compounds, carotenoids and vitamin C contents of nectarine, peach and plum cultivars from California. J Agric Food Chem 50: 4976-4982.

79. Denev P, Ciz M, Ambrozova G, Lojek A, Yanakieva I, et al. (2010) Solid-phase extraction of berries' anthocyanins and evaluation of their antioxidative properties. Food Chem 123: 1055-1061.

80. Ou B, Hampsch-Woodill M and Prior R L (2001) Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescence probe. J Agric Food Chem 49(10): 4619-4626.

81. Ou B, Hampsch-Woodill M, Flanagan J, Deemer EK, Prior RL, et al. (2002) Novel fluorimetric assay for hydroxyl radical prevention capacity using fluorescein as the probe. J Agric Food Chem 50(10): 2772-2777.

82. Cízová H, Lojek A, Kubala L and Cíz M (2004) The effect of intestinal ischemia duration on changes in plasma antioxidant defense status in rats. Physiol Res 53: 523-531.

83. Slavíková H, Lojek A, Hamar J, Dušková M, Kubala L, et al. (1998). Total antioxidant capacity of serum increased in early but not in late period after intestinal ischemia in rats. Free Radic. Biol. Med 25: 9-18.

84. Meng J, Fang Y, Zhang A, Chen S, Xu T, et al. (2011) Phenolic content and antioxidant capacity of Chinese raisins produced in Xinjiang Province. Food Res Int.

85. Jayaprakasha GK, Girennavar B and Patil BS (2008) Radical scavenging activities of Rio Red grapefruits and Sour orange fruit extracts in different in vitro model systems. Bioresource Technol 99: 4484-4494.

86. Apak R, Guculu K G, Ozyurek M and Karademir SE (2004) Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric iron reducing capability in the presence of neocuproine: CUPRAC method. J Agric Food Chem 52: 7970-7981.

87. Magalhaes KF, Caires ARL and Oliveira SL (2011) Determination of Antioxidant Content in Biodiesel by Fluorescence Spectroscopy. Encontro de Fisica.

88. Borowski J, Szajdek A, Borowska E J, Ciska E and Zielinski H (2008) Content of selected bioactive components and antioxidant properties of broccoli (Brassica oleracea L.). Eur Food Res Technol 226: 459-465.

89. Arya SP, Mahajan M and Jain P (2000) Non-spectrophotometric methods for the determination of Vitamin C. Anal Chim Acta 417: 1-14.

90. Olsher M and Chong PLG (2008) Sterol superlattice affects antioxidant potency and can be used to assess adverse effects of antioxidants. Anal Biochem 382: 1-8.

91. Chong PL, Olsher M (2007) Fluorometric assay for detection of sterol oxidation in liposomal membranes. Methods Mol Biol 400: 145-158.

92. Zielinska D, Szawara-Nowak D and Zielinski H (2007) Comparison of spectrophotometric and electrochemical methods for the evaluation of the antioxidant capacity of buckwheat products after hydrothermal treatment. J Agric Food Chem 55: 6124-6131.

93. Scheller F and Schubert F (1992) Biosensors. Elsevier, Amsterdam.

94. Blum L and Coulet P (1991) Biosensor principles and application. Marcel Dekker Inc, New York.

95. Kellner R, Mermet JM, Otto M and Widmer (1998) Analytical Chemistry. Wiley–VCH Verlag, Weinheim

96. Milardovic S, Ivekovic D and Grabaric BS (2006) A novel amperometric method for antioxidant activity determination using DPPH free radical. Bioelectrochemistry 68: 175-180.

97. Tougas TP, Jannetti JM, Collier WG (1985) Theoretical and experimental response of a biamperometric detector for flow injection analysis. Anal Chem 57: 1377-1381.

98. Milardovic S, Ivekovic D, Rumenjak V, Grabaric BS (2005) Use of DPPH• / DPPH Redox Couple for Biamperometric Determination of Antioxidant Activity. Electroanalysis 17: 1847-1853.

99. Milardovic S, Kerekovic I, Derrico R, Rumenjak V (2007) A novel method for flow injection analysis of total antioxidant capacity using enzymatically produced ABTS•+ and biamperometric detector containing interdigitated electrode. Talanta 71: 213–220.

100. Milardovic S, Kerekovic I, Rumenjak V (2007) A flow injection biamperometric method for determination of total antioxidant capacity of alcoholic beverages using bienzymatically produced ABTS·+. Food Chem 105: 1688-1694.

101. Prieto-Simón B, Cortina M, Campàs M, Calas-Blanchard C (2008) Electrochemical biosensors as a tool for antioxidant capacity assessment. Sens Actuators B: Chem 129: 459-466.

102. Barroso MF, De-los-Santos-Álvarez N, Delerue-Matos C, Oliveira MBPP (2011) Towards a reliable technology for antioxidant capacity and oxidative damage evaluation: electrochemical (bio)sensors. Biosens Bioelectron 30: 1-12.

103. Cortina-Puig M, Noguer T, Marty JL, Calas-Blanchard CC (2010) Electrochemical Biosensors as a Tool for the Determination of Phenolic Compounds and Antioxidant Capacity in Foods and Beverages in Biosensor in Food Procesing, Safety and Quality Control, Cap 10 CRC Press

104. Mello LD, Kubota LT (2007) Biosensors as a tool for the antioxidant status evaluation. Talanta 72: 335-348.

105. Barroso MF, de-los-Santos-Álvareza N, Lobo-Castanón MJ, Miranda-Ordieres AJ, Delerue-Matos C, et al. (2011) DNA-based biosensor for the electrocatalytic determination of antioxidant capacity in beverages. Biosensors and Bioelectronics 26: 2396-2401.

106. Bonanni A, Campanella L, Gatta T, Gregori E, Tomassetti M (2007) Evaluation of the antioxidant and prooxidant properties of several commercial dry spices by different analytical methods. Food Chem 102: 751-758.

107. Gomes SA, Rebelo MJ (2003) A new laccase biosensor for polyphenols determination. Sensors 3:166-175.

108. Böyükbayram A, Kıralp S, Toppare L, Yağci Y (2006) Preparation of biosensors by immobilization of polyphenol oxidase in conducting copolymers and their use in determination of phenolic compounds in red wine. Bioelectrochemistry 69: 164-171.

109. Gil DMA, Rebelo MJF (2010) Evaluating the antioxidant capacity of wines: a laccase-based biosensor approach. Eur Food Res Technol 231:303-308.

110. Lopez M, Martinez F, Del Valle C, Orte C, Miro M (2001) Analysis of phenolic constituents of biological interest in red wines by highperformance liquid chromatography. J. Chromatogr. A 922: 359-363.

111. Mello LD, Sotomayor MT, Kubota LT (2003) HRP-based amperometric biosensor for the polyphenols determination in vegetables extract. Sens Actuators B Chem 96: 636-645.

112. Campanella L, Bonanni A, Finotti E, Tomassetti M (2004) Biosensors for determination of total and natural antioxidant capacity of red and white wines: comparison with other spectrophotometric and fluorimetric methods. Biosens Bioelectron 19: 641-651.

113. Kyo-Il Kim, Hee-Young Kang, Jae-Chan Lee, Seong-Ho Choi (2009) Fabrication of a Multi-Walled Nanotube (MWNT) Ionic Liquid Electrode and Its Application for Sensing Phenolics in Red Wines. Sensors 9: 6701-6714.

114. Cortina-Puig M, Muñoz-Berbel X, Rouillon R, Calas-Blanchard C, Marty JL (2009) Development of a cytochrome c-based screen-printed biosensor for the determination of the antioxidant capacity of orange juices. Bioelectrochemistry 76: 76-80.

115. Jayaprakasha GK, Jena BS, Negi PS, Sakariah KK (2002) Evaluation of Antioxidant Activities and Antimutagenicity of Turmeric Oil: A Byproduct from Curcumin Production. Z Naturforsch 57c: 828-835.

116. Jayaprakasha GK, Jaganmohan RL (2000) Phenolic constituents from lichen Parmotrema stup peum (Nyl.) Hale and their antioxidant activity. Z Naturforsch 55: 1018-1022.

117. Stalmach A, Mullen W, Nagai C, Crozier A (2006) On-line HPLC analysis of the antioxidant activity of phenolic compounds in brewed, paper-filtered coffee. Braz J Plant Physiol 18: 253-262.

118. Oishi M, Matsuda T, Nojiri S, Saito K (2002) Simultaneous determination of five antioxidants in food by HPLC with fluorescence detection. Food Hygiene and Safety Science (Shokuhin Eiseigaku Zasshi) 43: 104-109.

119. Dos Santos VA, Dos Santos DP, Castro-Gamboa I, Zanoni MV, Furlan M (2010) Evaluation of Antioxidant Capacity and Synergistic Associations of Quinonemethide Triterpenes and Phenolic Substances from Maytenus ilicifolia (Celastraceae). Molecules 15: 6956-6973.

120. Prior RL, Wu X, Schaich K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 53: 4290-4302.

121. Niki E (2010) Assessment of antioxidant capacity in vitro and in vivo. Free Radical Biology and Medicine. 49: 503-515.

122. Frankel EN, Meyer AS (2000) The problems of using onedimensional methods to evaluate multifunctional food and biological antioxidants. J Sci Food Agric 80: 1925-1941.

123. Cao G, Alessio HM, Cutler RG (1993) Oxygen-radical absorbance capacity assay for antioxidants. Free Radical Biol Med 14: 303-311.

124. Cao G, Prior RL (1999) Measurement of oxygen radical absorbance capacity in biological samples. Method Enzymol 299: 50-62.

125. Prior RL, Hoang H, Gu L, Wu X, Bacchiocca M, et al.(2003) Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. J Agric Food Chem 51: 3273-3279.

126. Apak R, Güçlü K, Demirata B, Özyürek M, Çelik SE, et al.(2007) Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC Assay. Molecules 12: 1496-1547.

127. Zieliński H, Zielińska D, Kostyra H (2012) Antioxidant capacity of a new crispy type food products determined by updated analytical strategies. Food Chem 130: 1098-1104.